Harnessing the Body’s Natural Immune Response to Cancer

Immunological Pathways in Oncology
Abstract

The body’s immune system is often able to recognize malignant cells as “foreign,” and in many cases, is able to mount an effective antitumor response. However, most tumors eventually develop mechanisms for avoiding detection by the immune system, and this capacity is now recognized as one of the hallmarks of cancer. Tumor types vary with respect to susceptibility to immune-mediated treatment approaches depending on the degree of immunogenicity. A number of immune-mediated approaches for augmenting tumor regression have been investigated, including vaccines, oncolytic viral therapy, and adoptive cellular therapy.

T-cell activation and effector function are regulated through a balance of positive (activating) and negative (inhibiting) signals. The interleukin-2, interferon, and OX40 pathways represent examples of T-cell–activating signaling pathways, while the CTLA-4 and PD-L1/PD-1 pathways are inhibitory. These 2 checkpoint inhibitor pathways have been well studied, and both reduce T-cell activation and proliferation when stimulated. Agents that inhibit these pathways are currently available for the treatment of select malignancies, and are being developed further for others.

Recent clinical studies suggest that immune-mediated approaches may also be combined with more established treatments (such as radiotherapy, chemotherapy, and targeted therapy), with the goal of increasing effectiveness. Two different immune-mediated approaches also may be used in combination to achieve this goal. Determining the optimal use of immune-mediated approaches will be an important advance in the clinical application of these therapies.

Tumors can avoid detection by the immune system

Hallmarks of cancer are defined as the acquired capabilities of cancer cells that enable them to become tumors and ultimately malignant. Acquisition of these hallmarks is driven by genomic instability, which generates the genetic diversity that expedites their acquisition; and inflammation, which contributes to tumor progression through a variety of mechanisms. Inducing angiogenesis is also characteristic of cancer, and causes otherwise quiescent vasculature to continually sprout new vessels that supply required nutrients for tumor growth. Downregulation of molecules responsible for cell-to-cell and cell-to-extracellular matrix adhesion that increase the ability of tumor cells to migrate and spread, represents another key hallmark. More recently, the ability of cancers to prevent or limit the extent of immunological killing through immune response evasion has also been appreciated. The aforementioned defining features work together to allow for unchecked tumor growth and metastasis.

Historically, human cancer therapy has been centered on excisional surgery, as well as on the use of radiotherapy and chemotherapy. However, approaches...
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The body’s immune system responds to tumors in a series of steps

It is now appreciated that the immune response to cancer occurs in a series of stepwise events, and that effective immune-mediated therapies in some way initiate or reactivate these events (Figure 2).

Initially, tumor antigens are released by tumor cells and are captured and internalized by antigen-presenting cells (APCs) such as dendritic cells. Captured antigens are processed by the APCs and presented on major histocompatibility complex (MHC) molecules on the APC surface. Antigen-specific naïve T cells recognize the presented antigens via T-cell receptors (TCRs) and are activated, resulting in clonal expansion and enabling of effector T-cell function in what is referred to as the priming phase. Activated effector T cells then traffic to the tumor in the periphery, where they infiltrate the tumor and tumor microenvironment. Here, the activated T cells bind to specific antigens on the surface of tumor cells in the effector phase. Antigen

Figure 2. The antitumor immune response cycle consists of multiple steps.

Vaccines—which may be based on peptide and protein neoantigens, transfected cells, or transformed viruses—are being investigated as a means to evoke stronger antitumor immune responses. Oncolytic viral therapy, which is locally injected, is another such approach. However, characterization of the immune response induced by oncolytic viral therapy is ill-defined. Adoptive cellular therapy or CAR (chimeric antigen receptor) T-cell therapy, in which the patient’s own T cells are removed from the body, selected or engineered for antigen specificity, and then reintroduced into the patient, is actively being pursued as cancer treatment. Approaches that modify T-cell regulation also continue to be an important area of investigation and will be discussed in more detail below.
recognition triggers effector functions, including release of perforin and granzyme, leading to tumor cell death. Tumor cell death releases additional tumor antigens, increasing the breadth and depth of the response in subsequent revolutions of the cycle. Tumors may disrupt some of these steps, which can impede the cycle and dampen the immune response.

The body’s antitumor response can be dampened by cancer through activation of checkpoint inhibitors

During priming, TCR ligation to MHC-bound antigen is not sufficient for T-cell activation; a second signal is required (Figure 3). Cluster of differentiation (CD)28, another T-cell surface receptor, acts as the “master T-cell costimulator,” providing the required second signal when it is engaged. Either CD80 (B7.1) or CD86 can act as a costimulatory factor promoting T-cell activation when bound to CD28, and both are expressed on APCs. When TCR and CD28 are engaged on T cells, activation leads to clonal expansion and acquisition of effector function. Effector T cells specifically recognize and bind to tumor cells through interactions between their TCR and cognate antigen bound to MHC. This interaction leads to secretion of cytotoxic mediators required for destruction of tumor cells. These include perforin and granzyme, which cause lysis of tumor cells, and granulysin, which causes apoptosis of the tumor cells.

T-cell activation and effector function are also modulated through additional positive (activating) and negative (inhibiting) signals, with the overall extent of activation determined by the balance between the two (Figure 4). In addition to CD28, other examples of activating receptors include: GITR (glucocorticoid-induced TNFR-related protein), CD137, CD27 and HVEM (Herpesvirus entry mediator). OX40 is also a costimulatory receptor expressed on activated T cells that when bound to its ligand—OX40L—prolongs T-cell survival and memory generation, prevents T-cell tolerance, and reduces the immunosuppressive activity of regulatory T cells. Receptors that inhibit T-cell function when bound to ligand include: CTLA-4 (cytotoxic T-lymphocyte-associated protein-4), PD-1 (programmed cell death-1), TIM-3 (T-cell immunoglobulin and mucin-domain containing-3), BTLA (B- and T-lymphocyte attenuator), VISTA (V-domain immunoglobulin [Ig]-containing suppressor of T-cell activation), and LAG-3 (lymphocyte-activation gene 3).

CTLA-4—an immune checkpoint, cell surface receptor—represents a key negative modulator of effector T-cell activation. It limits activating signals delivered through CD28 by competing for shared
ligands CD80 and CD86 (Figure 5 on page 3).\textsuperscript{7} CTLA-4 represents the primary coinhibitory check of T-cell activation, particularly during priming.\textsuperscript{7,8,14} Its expression is upregulated after TCR activation and enhanced by CD28 costimulation.\textsuperscript{7,14} CTLA-4 sets a threshold for activation, playing a crucial role in regulating self-immunity.\textsuperscript{7,9,14} Disruption of CTLA-4 may lower the threshold of activation for tumor-specific T cells, allowing for effective priming and clonal expansion of tumor-specific T cells.\textsuperscript{7,14} CTLA-4 disruption may also enhance the formation and function of memory T cells, which aid in subsequent tumor antigen recognition and response.\textsuperscript{15}

PD-1 is another immune checkpoint receptor expressed on active T cells that restrains effector T-cell function when bound to cognate ligands such as PD-ligand 1 (PD-L1) (Figure 6).\textsuperscript{7,8} PD-L1 is expressed by a wide range of cell types, including epithelial cells and numerous tumor cells, and its expression can be upregulated in response to inflammatory signals.\textsuperscript{7,8} For example, tumor-infiltrating T cells directly induce PD-L1 expression on tumor cells via IFN-gamma (IFN-\gamma).\textsuperscript{7} As a means of immune evasion, tumors may exploit the PD-1/PD-L1 pathway to reduce effector T-cell function and prevent immune-mediated destruction.\textsuperscript{7} Conversely, disruption of the PD-1/PD-L1 pathway can restore effector T-cell function in the tumor microenvironment, leading to a greater antitumor immune response.\textsuperscript{7}

Impairment of PD-1 or PD-L1 leads to disruption of different sets of interactions that may have differential consequences for immune regulation of an antitumor response.\textsuperscript{16,17} PD-ligand 2 (PD-L2) is a second ligand for PD-1 that is primarily expressed on immune cells, but it is also expressed on nonimmune cells, including vascular endothelia, lung epithelia, and placental cells.\textsuperscript{18} Expression of PD-L2 can be upregulated in response to inflammatory cytokines such as IFN-\gamma and tumor necrosis factor-alfa (TNF-\alpha).\textsuperscript{19} PD-L2 can be expressed by endothelial cells as a means to inhibit effector T-cell activity and reduce tissue damage.\textsuperscript{19} PD-1 impairment prevents its interaction with both PD-L1 and PD-L2, whereas PD-L1 impairment leaves the PD-L2 pathway intact.\textsuperscript{20-24}

Similar to CTLA-4, PD-L1 can also bind to CD80 on naïve T cells, and this interaction allows it to exert effects during the priming phase of T-cell activation.\textsuperscript{8,25,26} Interaction of PD-L1 with CD80 on naïve T cells causes inhibitory intracellular signaling.\textsuperscript{25,26} PD-L1 expressed on T cells has also been found to bind CD80 on APCs. This interaction may also limit T-cell activation by preventing CD80 on APCs from interacting with CD28 on T cells.\textsuperscript{8} PD-L1 impairment, thus, prevents PD-L1 interaction with CD80, potentially maximizing its availability to activate T cells.\textsuperscript{26}
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Key Points

- Immune evasion is one of the hallmarks of cancer; multiple approaches for engaging an antitumor immune response are under investigation.
- TCR ligation is not sufficient for T-cell activation; a second signal is required.
- Immune response is regulated by a balance of costimulation and coinhibition acting at different steps.
- PD-1/PD-L1 and CTLA-4 are 2 checkpoint inhibitor pathways that have been well studied; they decrease T-cell function when stimulated.
- CTLA-4 binding to CD80 and CD86 may lower the threshold for T-cell activation and trigger an immune response.
- The PD-1/PD-L1 pathway plays a role in the tumor periphery and in the lymph nodes:
  - In the tumor periphery (effector phase)
    • Tumors can upregulate PD-L1 to evade immune surveillance.
    • In normal tissue, PD-L2 expression is increased in response to inflammation and may prevent tissue damage.
  - In the lymph nodes (priming phase)
    • The PD-L1 pathway is thought to play an important role in T-cell activation.

Evidence suggests that immune-mediated approaches could complement conventional cancer therapies

Commonly used treatments for cancer, namely radiotherapy, chemotherapy, and targeted therapies, can impact the antitumor immune cycle, providing a rationale for combination with immune therapies (Figure 7).\(^\text{27,28}\) Radiotherapy may modulate tumor immune characteristics by enhancing tumor antigen presentation on tumor cells via the upregulation of both MHC and tumor-associated antigens (TAAs)\(^\text{29,30}\) Some TAAs, such as CT (cancer testis)-antigens, are antigens that are expressed in a large variety of malignancies but absent from healthy tissue.\(^\text{30}\) Radiotherapy can also increase PD-L1 expression on tumor cells, which may actually work to counter the effectiveness of radiotherapy.\(^\text{31,32}\) Chemotherapeutic agents may have similar immune-modulatory effects, upregulating the expression of both TAAs and MHC on tumor cells.\(^\text{33,34}\) Some chemotherapeutic agents can also modulate the expression of CD80 and PD-L1 on tumor cells.\(^\text{33,35}\)

Figure 7. Combination strategies may complement immune-directed approaches.
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Targeting proliferative signals and angiogenesis may also have effects on the immune character of the tumor microenvironment. Impairing proliferative signaling has been shown to increase TAA and MHC expression on tumor cells, increase T-cell infiltration, and modulate PD-L1 expression on tumor cells. Targeting angiogenesis has also been demonstrated to relieve impairment of APC maturation. The effects described above suggest that conventional and targeted therapies may complement immune-directed approaches, and combination of the two may lead to increased antitumor activity.

Combining immune-mediated approaches that exert complementary effects may invoke greater antitumor immune responses

Scientific rationale exists for combining immune-mediated approaches as a means to invoke more potent antitumor responses. Although both CTLA-4 and the PD-1/PD-L1 pathway serve coinhibitory functions in T-cell regulation, their functions are distinct, having nonredundant effects on T cells (Figure 8). Inhibition of early activation and priming of T cells in lymph nodes are mainly mediated by CTLA-4, whereas the PD-1/PD-L1 pathway is largely responsible for modulating effector T-cell function in peripheral tissue and tumor.

The increased T-cell activity associated with CTLA-4 disruption may, in part, be offset by increased PD-1/PD-L1 signaling that may facilitate tumor immune evasion. CTLA-4 disruption increases the activation of tumor-specific T cells, which leads to clonal expansion and subsequent migration to tumor sites. These activated T cells increase inflammatory signaling through release of cytokines such as IFN-γ into the tumor microenvironment, in turn, increases expression of PD-L1 on tumor cells, which may restrain effector T-cell function through interaction with PD-1 on T cells. Disruption of the PD-1/PD-L1 pathway may allow the tumor-specific T cells activated by CTLA-4 disruption to restore their effector function in the periphery. In addition, since CTLA-4 and PD-L1 bind to the same molecule—CD80—simultaneous disruption of both inhibitor pathways may have an additional effect in the priming phase.

Disruption of immune regulatory pathways may have unintended consequences for healthy tissue. Approaches that primarily act to disrupt interactions early in the immune cycle may have different consequences than approaches that act late in the immune cycle.
Key Points

- Simultaneous disruption of multiple immunological pathways has the potential for additive antitumor responses
- Combining immune-mediated approaches that target pathways with distinct regulatory roles is under investigation
- Conventional and targeted therapies may change tumors and their microenvironment, and may render the tumor more susceptible to immune-mediated approaches

Tumor types differ in their immunogenicity

Tumors that are potentially sensitive to immune-mediated approaches rely on the functional competence of multiple immunological elements, and may be recognized by key traits (Figure 9). Tumor antigenicity represents one such trait, and it is fundamental to the ability of the immune system to recognize a tumor and initiate an immune response. Tumor antigenicity may be associated with mutational burden, since the quantity of neoantigens per tumor correlates with mutational load, which varies from tumor type and within tumor types. There is evidence to suggest that tumor mutational load and frequency of neoantigen formation may be associated with sensitivity to immune-mediated approaches. T-cell infiltration into tumor tissue is also necessary for successful immune-mediated tumor elimination. Tumor PD-L1 expression correlates with the presence of infiltrating T cells, and potentially with sensitivity to immune-mediated approaches. Moreover, strength of PD-L1 expression may be associated with sensitivity to disruption of the PD-1/PD-L1 axis. PD-L1 expression can vary across and within tumor types, although some of the variability may be due to the lack of standardization for determining PD-L1 positivity, given that there are multiple PD-L1 tests using different antibodies and different scoring criteria. (Figure 10 on page 8).

The presence of infiltrating effector T cells may be indicative of a tumor that has provoked an immune response, and this has been shown to be prognostic for several types of cancer. The presence of high densities of tumor-infiltrating effector and memory T cells has also been reported in numerous tumor types; a more favorable prognosis has been noted in these tumors. Consistent with these findings, higher density of immune cells may be indicative of susceptibility to immune-mediated approaches. Tumor types differ with regard to their immune characteristics and, thus, differ in their sensitivity.

![Figure 9](image_url)

**Figure 9.** Immune-sensitive tumor types may have some common immune characteristics.
to immune-mediated approaches. Melanoma is considered very sensitive to immune-mediated approaches based on its having one of the highest median frequencies of somatic mutations (Table 1).\textsuperscript{42,55,56}

Moreover, melanoma has a relatively high density of tumor-infiltrating effector T cells, and initial density is associated with response to PD-1 disruption.\textsuperscript{58} In addition, a relatively high percentage of patients with melanoma possess PD-L1–positive tumors.\textsuperscript{59-61} In contrast to melanoma, pancreatic cancer appears less sensitive to immune-mediated approaches. Pancreatic cancer has a relatively low median frequency of somatic mutations.\textsuperscript{42,55,56} A lower density of tumor-infiltrating effector T cells is also seen in pancreatic cancer compared with other cancers.\textsuperscript{62,63} Depending on

**Table 1. Mutational burden for several tumor types.\textsuperscript{42,55-57}**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Median frequency of mutations/megabase</th>
<th>Mutations per tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>&gt;100</td>
<td>~200</td>
</tr>
<tr>
<td>NSCLC</td>
<td>10-100</td>
<td>~200</td>
</tr>
<tr>
<td>Bladder</td>
<td>~10</td>
<td>~302*</td>
</tr>
<tr>
<td>HNSCC</td>
<td>~10</td>
<td>~75</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>&lt;1</td>
<td>~50</td>
</tr>
</tbody>
</table>

*Exonic mutations.

**Figure 10.** PD-L1 is expressed in a range of tumor types. Examples of tumor types with strong PD-L1 staining ($\geq$10% of cells).
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Other tumor types possess immunogenic characteristics that fall somewhere between melanoma and pancreatic cancer. Bladder cancer possesses a high frequency of somatic mutations that is lower than only melanoma or lung cancer. In addition, high intramural effector T-cell density has been detected in bladder cancer and is associated with sensitivity to immune-mediated approaches for invasive bladder cancer. PD-L1 expression has been detected by immunohistochemistry in bladder cancer tumor samples.

Non–small cell lung cancer (NSCLC, including squamous NSCLC and adenocarcinoma) is another tumor type with potentially high immunogenicity, as it has one of the highest median frequencies of somatic mutations, second only to melanoma. PD-L1 can be highly expressed in metastatic NSCLC and is expressed in both squamous and non-squamous histologies. In a clinical trial, NSCLC patients with tumors bearing high levels of PD-L1 expression were more sensitive to the immune-mediated approach under investigation, compared with patients with lower levels of PD-L1 expression in their tumors.

Head and neck squamous cell carcinoma (HNSCC) tumors, regardless of etiology, demonstrate multiple characteristics associated with higher immunogenicity. PD-L1 can be highly expressed in HNSCC, and expression occurs in primary, recurrent, and metastatic tumors. PD-L1 is expressed in both human papillomavirus virus (HPV)-positive and HPV-negative HNSCC tumors, although expression can be higher in HPV-positive tumors, where it can create an immune-privileged environment for continuous HPV infection.

Conclusions

Cancer cells must acquire biologic capabilities, referred to as hallmarks of cancer, that enable tumor growth and metastasis. Immune evasion is now recognized as a hallmark of cancer, an insight that has led to the investigation of multiple approaches for engaging an antitumor immune response, including T-cell regulation. These immune-mediated approaches are being investigated individually or in combination with commonly used cancer treatments such as chemotherapy and radiation therapy, or with different immune-mediated approaches. Disruption of checkpoint inhibitors—exemplified by the CTLA-4 and PD-1/PD-L1 pathways—represents a means to “remove the brakes” on T-cell inhibition, and represents one of the approaches that is actively being investigated as an oncology treatment option. These 2 pathways have nonredundant effects on effector T cells, and use of agents with different mechanisms of action may lead to stronger antitumor responses when used in combination. Generally speaking, early T-cell activation is inhibited by CTLA-4, whereas the PD-1/PD-L1 pathway modulates effector T-cell function, although it can also exert effects on T-cell priming. Disruption of PD-1 prevents interactions with both PD-L1 and PD-L2 ligands, whereas the blocking of PD-L1 leaves PD-1 free to interact with PD-L2.

Immunogenic tumor types may have some common immune characteristics, including relatively high mutational loads and levels of neoantigens. Tumor PD-L1 expression can vary across and within tumor types and correlates with the presence of infiltrating T cells. Continued studies of checkpoint inhibitors, and the tumor types most responsive to their disruption, are areas of current research.

Key Points

- Immunogenic tumor types may have some common immune characteristics, including relatively high mutational loads and levels of neoantigens, T-cell infiltration, and expression of PD-L1.
- Tumor types vary with respect to their immunogenicity.
- Several tumor types, including melanoma, bladder, NSCLC, and HNSCC demonstrate multiple characteristics associated with immune sensitivity.
References


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