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Review Article

Immune checkpoint blockade opens an avenue of cancer immunotherapy with a potent clinical efficacy

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Key words

Anti-PD-L1 Ab, cancer immunoediting, cancer immunosurveillance, immune checkpoint molecules, PD-1/PDL-1 pathway

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Recent progress in tumor immunology has revealed that tumors generate immunologically restrained milieu during the process of their growth, which facilitates the escape of tumors from host immune systems. Immune checkpoint molecules, which transduce co-inhibitory signals to immuno-competent cells, are one of the most important components conferring the immunosuppressive capacity in the tumor microenvironment. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death-1 (PD-1) are typical immune checkpoint molecules intimately involved in the suppression of anti-tumor immunity. Antibodies against those molecules have been developed, such as ipilimumab (anti-CTLA-4 antibody), nivolumab and pembrolizumab (anti-PD-1 antibody), and have been approved by regulatory agencies and used in some countries. Treatment with these antibodies demonstrates previously unobserved clinical efficacies superior to the conventional therapies. In this review, we first discuss the escape mechanisms of cancer from host immune systems, and then focus on the recent advances in immune checkpoint blockade therapy and on the new findings of related immune reactions, aiming to provide a better understanding of the novel cancer immunotherapies.

Conventionally, surgical therapy, chemotherapy and radiotherapy have been applied in the treatment of cancer and saved many lives. Meanwhile, immunotherapy has begun to be explored as a fourth therapy option for intractable or advanced cancer that cannot be treated by the conventional therapies. To date, several kinds of immune therapies, including cancer peptide vaccines, dendritic cell vaccines and adoptive transfer of cytotoxic T lymphocytes (CTL), have been clinically applied.[1,2] A common theme in the previously explored immunotherapies has been to aim for therapeutic benefit by "evoking or reinforcing the host immune reactions against cancer." Although certain immunotherapeutic approaches, including tumor-infiltrating lymphocytes (TIL) therapy and chimeric antigen receptor (CAR) T cells therapy, are reported to demonstrate therapeutic efficacies in some cancers,[3–5] achievement of satisfactory clinical response rates and/or superior curative effects has been uncommon, even when induction of anti-tumor T cell responses is observed in peripheral blood. The immunosuppressive condition in the tumor microenvironment is among the most crucial factors that account for this issue, because tumoricidal effects of tumor-reactive T cells, which are evoked or reinforced in the host by immunotherapies, are attenuated when they make contact with tumors.[6–8] Immune checkpoint molecules transduce co-inhibitory signals to immune cells, including T cells, and inherently work to maintain immunological homeostasis and tolerance by preventing overactivation of the host immune system.[9] It has been revealed that some immune checkpoint molecules are highly expressed in tumor tissues and can be utilized to generate immunosuppressive conditions in the microenvironment around the tumor.[6–8,10,11] Based on these findings, the research and development of novel immunotherapies, so-called "immune checkpoint blockade therapies," has been intensive. In contrast to the previous approaches, the concept of an immune checkpoint blockade is to induce therapeutic benefit by "cancelling the immunosuppressive machineries generated in the tumor microenvironment." The most representative immune checkpoint molecules underlying the mechanisms of tumor-associated immunosuppression are CTLA-4 (cytotoxic T-lymphocyte-associated protein-4, CD152)[12–14] and PD-1 (programmed cell death-1, CD279).[15–17] The antibodies (Abs) against those molecules (i.e. ipilimumab [anti-CTLA-4 Ab],[18] nivolumab[19,20] and pembrolizumab [anti-PD-1 Ab]),[21] have been developed and approved as drugs in some countries, including the USA and Japan. Abs against PD-L1 (programmed cell death ligand-1) are also under development. It has been reported that, compared to the traditional therapies, those Abs display superior clinical efficacies, including prolongation of overall survival and increase of objective response rates in some types of can-

cers, including melanoma, non-small cell lung cancer, renal cell carcinoma and urothelial bladder cancer.[49,50]

Mechanisms by which Cancer Cells Evade Host Immune System

How does cancer develop in an immunocompetent host? Interactions between tumor and immune system at the initial stage of carcinogenesis. The process from the emergence of neoplastic cells to the organization of tumor tissue is one of the most pivotal subjects that has been investigated actively in the field of cancer biology. Regarding tumor–immune system interaction at the initial stage of carcinogenesis (i.e. when the cancerous cells emerge), the following concept has been proposed and widely accepted: gene mutations are unremittingly induced with a constant probability by endogenous or environmental stimuli, so that mutant cells with a potential of carcinogenesis are thought to emerge routinely *in vivo*. Yet, the host immune system constantly monitors and detects these mutated carcinogenic cells and eliminate them through the mechanism referred to as "cancer immunosurveillance."[22–24] However, through accumulated emergence of the mutated cells, some of them incidentally acquire the capacity to evade immunosurveillance (i.e. avoiding a clearance by the host immune system), and continue their expansion to establish the organization of tumor tissue. Those changes in the immunogenicity of tumor cells, which result from continuous pressure against the tumors by the host immune system and the consequent occurrence of the mutants resistant to the immunosurveillance, are referred to as "cancer immunoediting" (Fig. 1).[23–25] In other words, cancers that we observe in clinical settings as a detectable mass have already evaded anti-tumor immunity by editing immunogenicity from the initial stage of carcinogenesis, while the frequency of the mutations (i.e. the number and/or the repertoire of neoantigens) varies among the tumor types.[26,27] Accordingly, immune resistance is inherent in the nature of established cancers.

Why is the effect of the conventional cancer immunotherapy often limited? Immunosuppressive mechanisms in the tumor microenvironment. It has been well-documented that tumor cells express tumor-specific and/or tumor-associated antigens (Ags; e.g. cancer-testis Ags and tumor-related mutated Ags), which can be recognized by T cells as immunogenic targets.[28,29] Therefore, it seems a plausible approach to evoke or reinforce T cell responses against these tumor Ags through vaccination and/or promotion of immune-stimulatory mechanisms. However, even though tumor Ag-specific T cell responses are induced and detected in peripheral blood by such approaches, they do not necessarily lead to clinically appreciated therapeutic benefits, such as shrinkage of tumor mass or prolongation of survival. One of the major reasons accounting for this issue is the immunosuppressive tumor microenvironment, which is developed as a result of cancer immunoediting. In the tumor microenvironment, the cancer-specific milieus are formed by several kinds of cellular populations, including tumor cells, stromal cells and infiltrating immune cells. Those milieus have potent immunosuppressive potential, by which tumoricidal functions of tumor-specific T cells are massively prohibited. The pivotal mechanisms underlying the immunosuppressive functions in the tumor microenvironment can be summarized in three categories as described below. It is noteworthy that these mechanisms affect and coordinate one another and synergistically exert their potential.

1 Existence of immunosuppressive cellular populations: massive infiltration of regulatory T cells (Treg cells) and myeloid-derived suppressor cells are observed in certain types of tumors.[30,31]
2 Production of immunosuppressive humoral factors: tumor cells and neighboring stromal cells produce suppressive cytokines, such as transforming growth factor-β and interleukin-10, as well as enzymes such as indoleamine 2,3-dioxygenase.[30,32,33]
3 Expression of immune checkpoint molecules: CTLA-4 and PD-L1 are highly expressed on Treg cells and tumor cells, respectively. Expression of PD-L1 is also detected on tumor stromal cells and infiltrating immune cells.[34]

In spite of these immunosuppressive mechanisms in the tumor microenvironment, adoptive transfer of TIL and CAR-T cells has demonstrated clinical efficacy in some cancers, including melanoma and hematological malignancies. Although precise reasons for the efficacy remain unclear, it might be associated with a hypothesis that adoptive T cell therapy does not require the induction phase *in vivo* where the immunosuppressive mechanisms mainly operate, or a hypothesis that *in vitro* activated T cells are resistant to immunosuppression in the effector phase in the tumor microenvironment. These points are important in exploring for the development of effective cancer immunotherapies.

Immune checkpoint molecules: A mechanism to restrain T cell responses in the tumor microenvironment. Although various innate and adaptive immune cells contribute to anti-tumor immunity, it is generally considered that T cells specific to

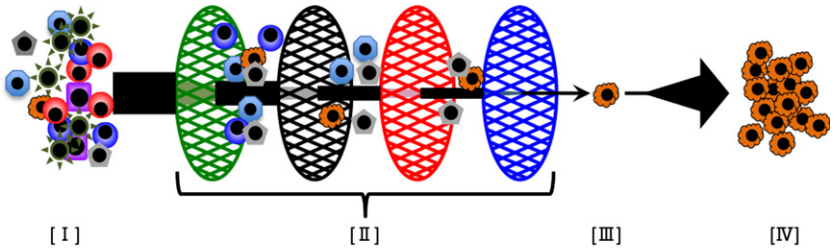

Fig. 1. Immunosurveillance and cancer immunoediting. Although gene mutations and resultant generations of cancerous mutant cells routinely occur in a body, the immunosurveillance system detects and eliminates these mutant cells in most cases by trapping them into immunological filters. However, the strong selective pressure by the immune system itself engenders further oncogenic cells, which are inherently immune-resistant and, thus, slip through the immunological filters. Such changes in the immunogenicity of tumors are referred to as cancer immunoediting. Once the mutant cells procure the features with which they prevail over the tumoricidal effects of host immunity, those cells can expand and generate tumors.

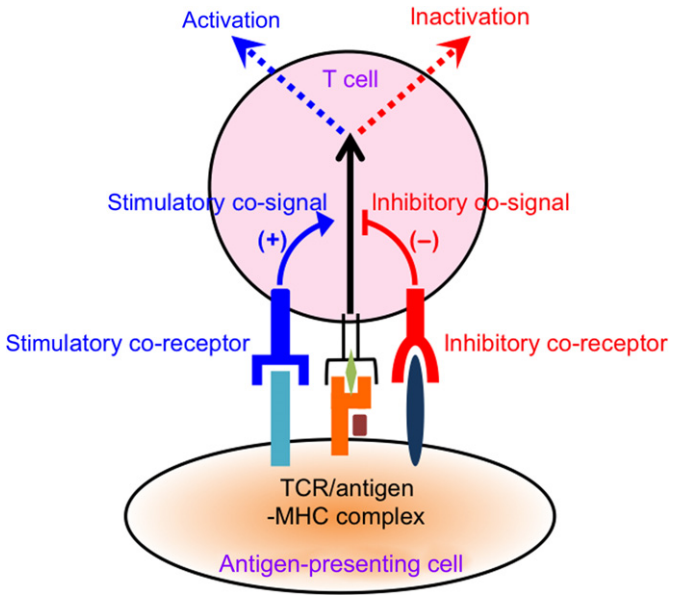

Fig. 2. Regulation of T cell responses by stimulatory and inhibitory co-signals. While T cell receptor (TCR) transduces "the first signal" into T cells, co-signaling receptors deliver "the second signal." When stimulatory co-signals are predominant over inhibitory co-signals, the T cells activate to proliferate, produce cytokines and/or exert cytotoxic activities. In contrast, when inhibitory co-signals are predominant, T cells are rendered inactivated and become unresponsive to Ags, a status referred to as immune tolerance or exhaustion. A fine regulation of T cell functions by balancing stimulatory and inhibitory co-receptors routinely takes place in hosts to maintain immunological homeostasis.

tumor Ags play a crucial role in tumor elimination. To evoke T cell activation, two signals are indispensable (Fig. 2).[9] One is the signal through T cell receptor (TCR) induced by the complex of antigenic peptide and major histocompatibility complex (MHC or HLA in human), and the other is the signals through the surface molecules termed stimulatory co-receptors, such as CD28, 4-1BB and OX-40. CD28 engages CD80 (B7-1) and CD86 (B7-2) expressed on professional antigen-presenting cells and transduce the stimulatory co-signal into T cells. Meanwhile, as mentioned above, immune checkpoint molecules, which transduce inhibitory co-signals, also exist to counteract stimulatory co-signals and prevent overactivation of immune systems. CTLA-4 and PD-1 are the most representative immune checkpoint molecules. Whether T cells are activated or inactivated upon TCR ligation depends on the balance between stimulatory and inhibitory co-signals. Thus, in the tumor microenvironment where immune checkpoint molecules are highly expressed, the balance of co-signals is greatly biased toward the inhibition-dominant side, so that anti-tumor responses are strikingly restrained (Fig. 3a). The aim of cancer immunotherapies is to make the balance biased toward the stimulation-dominant side, especially in tumor tissues. In conventional immunotherapy, the aim is sought by "putting the weights on the stimulatory side" (Fig. 3b). The reasons why this approach is less effective in inducing clinical benefits include the difficulty to provide enough stimulatory co-signals to exceed heavily overweighted inhibitory conditions in the tumor microenvironment. In addition, even if extremely potent stimulations are given to re-balance toward the stimulation-dominant side, such methods are difficult to perform in patients in practice because of adverse events associated with overactivation of immune cells in non-tumor organs. In contrast, the aim of immune checkpoint blockade therapy is to "decrease or remove the weights from the inhibitory side," so as to re-balance anti-tumor immunity toward the stimulation-dominant side in the tumor microenvironment (Fig. 3c). It has been reported that the objective response rates of immune checkpoint blockade therapies are approximately 30% in melanoma[35] and 20% in non-small cell lung cancer.[36] In addition, immune checkpoint blockade therapy is less effective in some types of cancers. Some patients and certain types of cancers, unfortunately, do not respond to these therapies as a result of insufficient numbers and/or repertoires of neoantigens to evoke host immunity. Although it has yet to be refined as a therapy, immune checkpoint blockade therapy provides a major breakthrough in oncology as it can save cancer patients who are not cured by conventional therapies. In the following section, we discuss immune checkpoint blockade therapy for cancer by focusing on anti-PD-L1 Abs, which are currently

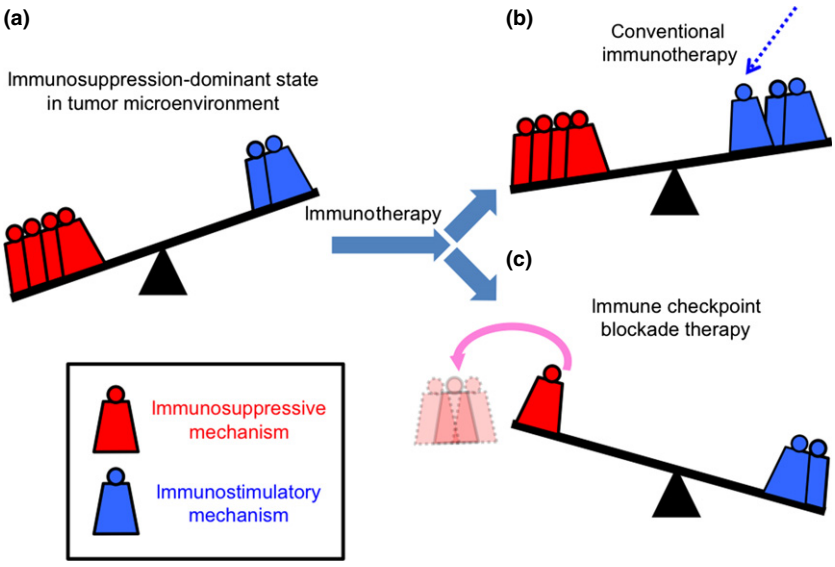

Fig. 3. Conceptual diagram of cancer immunotherapy with immune checkpoint blockade. (a) The aim of cancer immunotherapies is to make the balance of the host immunity biased toward the stimulation-dominant side while the balance is strikingly biased toward the inhibition-dominant side in the tumor microenvironment. (b) In conventional immunotherapy, the immunological balance is readjusted by "putting the weight on the stimulatory side." Yet, in many cases, such approaches cannot overcome the potent immunosuppressive mechanisms in the tumor microenvironment. (c) In immune checkpoint blockade therapy, the balance is readjusted by "decreasing or removing the weight from the inhibitory side."

under clinical trials and will possibly be the next drugs approved for use in this therapeutic approach.

Immune Checkpoint Blockade Therapy by Anti-PD-L1 Ab

Functions of PD-1/PD-L1 inhibitory co-signaling pathway. PD-1 is expressed on the cell surface of activated T cells, B cells and natural killer cells, and transduces an inhibitory signal upon the ligation with PD-L1/PD-L2.[15–17] Because PD-1-deficient mice suffer from spontaneous autoimmune diseases, PD-1 is considered to function as an immune checkpoint molecule that is indispensable for immunological homeostasis.[16,17] Although CTLA-4 is also a critical immune checkpoint molecule, as mentioned above, phenotypes of the gene knockout mice are quite different. Deficiency of CTLA-4, but not PD-1, leads to lethal autoimmune diseases in mice, and the symptoms observed in PD-1-deficient mice are much milder than those in CTLA-4-deficient mice.[37–39] The mechanisms how CTLA-4 and PD-1 molecules display their immune checkpoint functions would explain the phenotypic differences between the mice deficient of these molecules. The expression of CTLA-4 is induced at the early stage of T cell activation, whereas PD-1 is expressed at the later stage, particularly after the differentiation into effector cells. Thus, in anti-tumor immunity, CTLA-4 plays an important role as an immune regulator during the priming of T cells in the draining lymph nodes of tumors, while PD-1 is the pivotal immune checkpoint molecule in the tumor microenvironment where tumor-specific T cells exert their tumoricidal functions (Fig. 4).

PD-L1, a ligand of PD-1, is expressed on certain immune cell types, including macrophages and activated T cells.[40,41] Unlike PD-1-deficient mice, PD-L1-deficient mice do not exhibit spontaneous autoimmune diseases.[42] However, the reactivities of PD-L1-deficient $CD4^+$ and $CD8^+$ T cells were strikingly augmented *in vitro* and *in vivo* as compared with those of wild-type T cells, confirming a crucial role of PD-L1 in the suppression of T cell activation.[43] Importantly, strong expression of PD-L1 has been detected in various types of tumor samples.[11,44] It has been reported that the expression levels of PD-L1 correlate with advanced stage of cancer and with poor prognosis of patients.[45] In the tumor microenvironment, the expression of PD-L1 is induced on tumor cells and stromal cells in response to inflammatory cytokines, such as interferon-γ (IFN-γ), produced by T cells infiltrating into tumor tissue. Thus, the expression of PD-L1 in tumor lesions is an essential mechanism of cancer immunoediting.

Antibody against PD-L1 as a therapeutic agent for cancer. As mentioned above, PD-1/PDL-1 pathway is an important immune checkpoint mechanism for limiting the overactivation of immune responses. Based on the differences between PD-1/PD-L1 and CTLA-4 in the expression patterns and in the phenotypes of the gene-knockout mice, anti-PD-L1 Ab is considered as a therapeutic agent for cancer which likely possesses the potential to inhibit the immunosuppressive effects in the tumor microenvironment with less adverse effects than anti-CTLA-4 Ab. The therapeutic efficacy of anti-PD-L1 Ab for cancer was initially demonstrated with experiments using mouse models.[11,46,47] Subsequently, Bristol-Myers Squibb developed BMS-936559, a fully human anti-PD-L1 monoclonal IgG4 Ab, and started clinical trials for patients with advanced cancers, including melanoma, non-small cell lung cancer and renal cell carcinoma.[48] As a result, the objective response rates were observed in 17% of patients with melanoma, 12% with renal cell carcinoma and 10% with non-small cell lung cancer. The rates of stable disease longer than 24 weeks were 27% in patients with melanoma, 41% in renal cell carcinoma and 12% in non-small cell lung cancer. Drug-related adverse events of grade 3 or 4 were detected in 9% of subjects, showing gastrointestinal symptoms, hyperglycemia and general fatigue.

Meanwhile, Genentech developed MPDL3280A, a fully human Fc-engineered anti-PD-L1 monoclonal IgG1 Ab, and initiated clinical trials in various solid tumors, including metastatic urothelial bladder cancer (UBC).[49] In recent studies administering MPDL3280A in UBC patients, the resected tumor tissues were subjected to immunohistochemistry for PD-

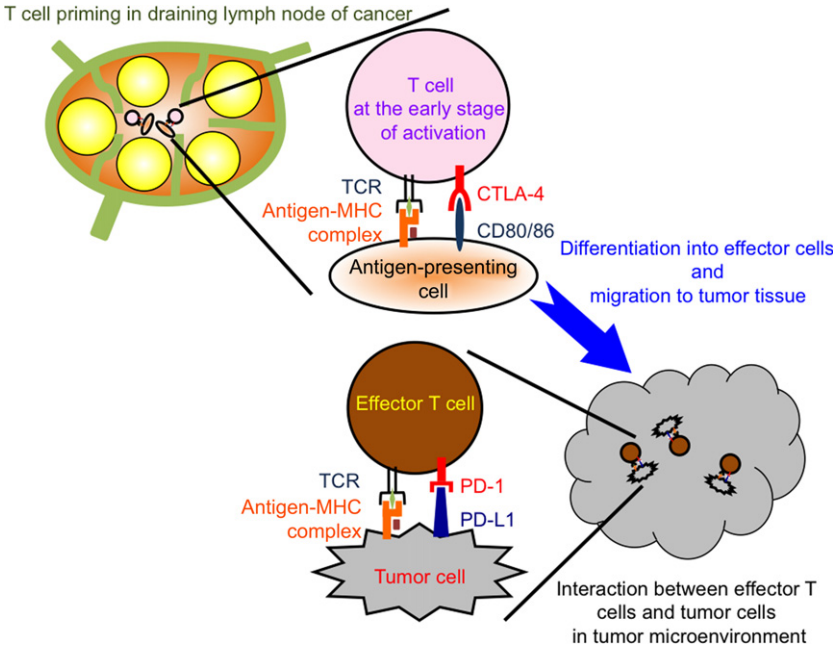

Fig. 4. Distinct phases in which CTLA-4 and PD-1 express immune checkpoint functions. When T cells are primed by interactions with antigen-presenting cells in the lymph node, expression of CTLA-4 is upregulated to prevent or shut down excessive T cell responses. Meanwhile, the activated T cells differentiate into effector cells, migrate to tumor tissue, and attack the target cells. During this process, T cells upregulate PD-1 expression, which renders them susceptible to the immune inhibition by PD-L1, which is highly expressed in the tumor microenvironment. Collectively, CTLA-4 is the immune checkpoint molecule working at the early phase of T cell activation, while PD-1 is the one at the later phase when the effector functions are exerted.

L1, and the patients were stratified by the expression levels of PD-L1 in the tumor lesions. The objective responses were observed in 43% of patients with high expression of PD-L1, including 7% complete response, whereas only 11% of patients with low or no expression of PD-L1 showed objective responses. Interestingly, the therapeutic effects of MPDL3280A were associated with the PD-L1 expression on immune cells infiltrating into the tumor, but not with PD-L1 levels on tumor cells.[49] It is noteworthy that the clinical responses were rapidly induced after the first treatment (median 42 days), and the reduction of tumor burden was observed in 55% of the patients. In other types of tumor, MPDL3280A achieved objective response rates at 23% in non-small cell lung cancer, 30% in melanoma and 14% in renal cell carcinoma.[50] Treatment-related adverse events of Grade 3 or 4 were observed in 12.6% of patients receiving MPDL3280A, including gastrointestinal symptoms, respiratory symptoms and liver dysfunction. Based on the results of these recent clinical trials, MPDL3280A was designated as a breakthrough therapy by the US Food and Drug Administration for the treatment of UBC and non-small cell lung cancer.

Biomarkers correlated with therapeutic efficacy of anti-PD-L1 Ab in cancer patients. Identifying predictive biomarkers for the safety, efficacy or lack of responses of drugs is one of the most important and pressing subjects in the field of clinical cancer research so as to accomplish personalized medicine. In 2014, Genentech demonstrated biomarkers correlated with the responses to MPDL3280A in patients of several cancers, including non-small cell lung cancer, melanoma, renal cell carcinoma, and head and neck squamous cell carcinoma.[49,50] Resembling the aforementioned case of UBC, the clinically beneficial responses to MPDL3280A treatment were significantly correlated with the expression levels of PD-L1 on the immune cells infiltrating into tumor tissues. In contrast, PD-L1 expression levels on tumor cells showed little correlation with the clinical responses to MPDL3280A. This observation is inconsistent with those demonstrated in studies using anti-PD-1 Ab, where significant correlation between PD-L1 expression on tumor cells and the clinical response was appreciated.[19,51] The reason why such discrepancy was observed between anti-PD-1 and anti-PD-L1 Ab therapies remains unclear.

Analysis of the gene expression signature in tumor tissues prior to the MPDL3280A treatment indicated a significant correlation between CTLA-4 gene expression and the clinical responses.[50] Moreover, in melanoma patients, gene expressions of IFN-γ and the IFN-γ-inducible genes (e.g. indoleamine 2,3-dioxygenase 1 [IDO1] and monokine induced by gamma interferon [MIG or CXCL9] in pre-treated tumor tissues) demonstrated a strong correlation with the regression of the tumors by MPDL3280A treatment.[50] Such associations were found to be specific in melanoma, but much weaker or no association was observed in patients with NSCLC or renal cell carcinoma.[50] These findings suggest important biological markers which predict clinical efficacy in anti-PD-L1 Ab therapy, and also indicate the concept that, for the best clinical benefit by anti-PD-L1 Ab, anti-tumor immunity is generated but simultaneously suppressed by the PD-L1/PD-1 immune checkpoint mechanism when the subjects receive the treatment. Administration of MPDL3280A in such patients cancels the suppression and releases the "ready-to-go" status of the anti-tumor immunity.

Future Perspective of Immune Checkpoint Blockade Therapy against Cancer

To date, the antibodies against CTLA-4 and PD-1 have been developed as highly effective drugs for advanced melanoma. In this review, we first explained molecular and cellular mechanisms underlying immune checkpoint blockade therapy, and then focused on anti-PD-L1 Ab, a drug recently developed to attenuate the PD-L1/PD-1 immune checkpoint, to describe its therapeutic efficacy, adverse events and the predictive biological markers associated with clinical responses. Immune checkpoint molecules other than CTLA-4 and PD-1/PD-L1, including lymphocyte-activation gene-3, T cell immunoglobulin mucin-3, and B and T lymphocyte attenuator (BTLA), are deemed to be potential clinical targets, and research and development of those molecules are actively carried out at present.[52–54] For better clinical application of immune checkpoint blockade therapy, the following points need to be explored in future studies:

1 Immune checkpoint molecules which execute predominant immunosuppressive effects would vary among distinct cancers and individual cases. Therefore, diagnostic tools to identify the most appropriate target manipulated for the treatment should be developed.
2 Therapeutic drugs with the least adverse events should be designed by elucidating the molecular and cellular mechanisms underlying the immune inhibitory function of each immune checkpoint molecule.
3 Combined immunotherapies where Abs against distinct immune checkpoint molecules are combined, or immune checkpoint blockade is combined with non-immunotherapies, including chemotherapies, tyrosine kinase inhibitors and irradiation therapy, should be explored to further augment therapeutic efficacies.
4 Predictive biomarkers that accurately correlate with clinical responses or adverse events in immune checkpoint therapies should be identified.

It is highly anticipated that, by solving these issues, immune checkpoint blockade therapies can be applied on a broader range of cancers with more effective and safer protocols.

Disclosure Statement

All authors have no conflict of interest to declare.

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