Cancer immunotherapy in clinical practice—the past, present, and future

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Abstract

Considerable progress has been made in the field of cancer immunotherapy in recent years. This has been made possible in large part by the identification of new immune-based cellular targets and the development of novel approaches aimed at stimulating the immune system. The role played by the immunosuppressive microenvironment in the development of tumors has been established. The success of checkpoint-inhibiting antibodies and cancer vaccines has marked the beginning of a new era in cancer treatment. This review highlights the clinically relevant principles of cancer immunology and various immunotherapeutic approaches that have either already entered mainstream oncologic practice or are currently in the process of being evaluated in clinical trials. Furthermore, the current barriers to the development of effective immunotherapies and the potential strategies of overcoming them are also discussed.

Key words Cancer immunotherapy, immunoediting, checkpoint inhibitors, cancer vaccines, adoptive cell transfer

Cancer therapy is a continuously evolving field, and novel immunotherapeutic approaches are now emerging as effective treatment options against various types of cancers. Cancer immunotherapy relies on the principle of mobilizing the host immune system to fight against cancer cells. Various approaches have been attempted during the last several decades to harness the innate powers of the immune system to fight cancer. Despite the best efforts, however, only limited success has been achieved in developing effective antitumor immunotherapies. The inability to overcome the immunosuppressive behavior of the tumor microenvironment is considered a major hurdle in the development of effective immunotherapies. With the identification of new immune-based targets, cancer immunotherapy is now beginning to resurface as a promising treatment strategy. The immunotherapeutic approach has tremendous potential for application in various types of cancers, ranging from a preventive vaccine in cervical cancer to potent therapeutic options in melanoma. In this review, we present an overview of clinically relevant immunology and immunotherapy principles, and various immunotherapeutic approaches that are being integrated into current oncologic practice.

Historical Overview

The antitumor potential of the immune system has been recognized for a long time. The first known attempt to use the power of the immune system in treating cancer was made by William B. Coley in 1891[1]. Coley observed a case of unresectable neck sarcoma that went into complete remission after an episode of erysipelas, a bacterial skin infection, and he hypothesized that the patient’s response to the infection led to the regression of the tumor[1]. Coley subsequently prepared a mixture of bacterial toxins and treated bone and soft-tissue sarcoma patients, with varying degrees of success. A major limitation of his approach was the lack of consistency and reproducibility. More than 6 decades later, building on the idea that the immune system has a protective effect against cancer, Paul Erlich proposed the concept of “immunosurveillance”[2]. The immunosurveillance concept was later expanded and formally introduced by Burnet[3] and Thomas et al.[4] in the early 1970s. They proposed a model in which the immune system of immunocompetent individuals played a critical role in preventing cancer development by eliminating tumor cells that are recognized as being foreign[3,4]. These initial milestones played a crucial role in our current understanding of the mechanisms of tumor immunology and the overall development of cancer immunotherapy as a field.
Role of Tumor Antigens in Immunotherapy—Tumor Antigen Classes/Categories

Later, it was identified that tumor cells express immunogenic antigens (now called tumor antigens), which can elicit potent humoral and T-cell immune responses and are central to the concept of immunosurveillance10.11.

Tumor antigens generally include five different classes of antigens, namely, tissue-differentiation antigens [Melan-A/melanoma antigen recognized by T cells (MART-1), tyrosinase-related protein-2 (TRP-2), glycoprotein 100 (gp100), prostate-specific antigen (PSA), and prostatic acid phosphatase (PAP)], overexpressed antigens [carcinoembryonic antigen (CEA), survivin, and telomerase], cancer-testes antigens (CTAs) that are derived from epigenetic changes [melanoma antigen family A, 3 (MAGE-A3) and NY-ESO-1], antigens derived from mutated genes (P53 and RAS), and viral antigens derived from human papillomavirus (HPV) and Epstein-Barr virus (EBV)10.12. A number of these antigens are currently under clinical evaluation as potential immunotherapeutic targets using cancer vaccines. Tumor antigens can also be broadly divided into two categories of “self-antigens” (CTAs, differentiation and overexpressed antigens), which are present on both tumor cells and normal tissues, and “tumor-specific antigens,” which are restricted to tumor cells10.13. Immunotherapies against self-antigens are associated with a high incidence of “on-target, off-tumor” adverse effects, which is explained by that these antigens are expressed on both tumor cells and normal cells10.13. Tumor-specific antigens therefore appear to be better immunotherapeutic targets in this regard because their expression is restricted to tumor cells. However, only limited success has been achieved thus far with the development of effective tumor-specific antigen-based therapies, partly due to the lack of in-depth research13.

Cancer Immunooediting—Escape from Immune Control

The immunosurveillance concept gradually evolved into the “cancer immunooediting” concept, which provides critical insight into the interaction of the immune system with cancer. It became known that the immune system not only plays a key role in the prevention of tumor formation but also contributes to the development of tumors and in shaping the immunogenicity of emerging tumors11. Immunooediting is a dynamic triphasic process that comprises the “elimination,” “equilibrium,” and “escape” phases.

In the elimination phase, tumor cells are eliminated by immunosurveillance. Stimulation of the immune system, which involves T-cell recognition of tumor-associated antigens, is the underlying principle of immune surveillance. This is followed by the equilibrium phase or the period of immune-mediated tumor dormancy, in which the immune system is in balance with tumor cells. A tumor is believed to be maintained in equilibrium by opposing forces acting in the tumor microenvironment, such as interleukin (IL)-12, which promotes the elimination of tumor cells, and IL-23, which promotes their persistence10.13. In the final escape phase, cancer cells escape the immune restraints, resulting in tumor growth. This is mediated through the down-regulation of tumor-associated antigens, a decrease in the secretion of inflammatory cytokines, increases in the production of suppressive cytokines/soluble factors (vascular endothelial growth factor (VEGF), transforming growth factor-β (TGFB-β), and prostaglandin E2 (PGE2)) and immunoinhibitory checkpoint pathways (cytotoxic T-lymphocyte-associated protein-4 (CTLA-4)/B7, programmed cell death-1 (PD-1)/programmed cell death ligand-1 (PD-L1), T-cell immunoglobulin- and mucin domain-containing molecule-3 (TIM-3)/Gmelin-9, lymphocyte activation gene 3 (LAG3)/major histocompatibility complex class II molecules (MHCI-I)), and the development of resistance to immune effectors.

In summary, immunooediting is a critical process that promotes tumor progression via the evasion of tumor cells from the immune system. Indeed, escape from immune control is now also recognized as one of the “Hallmarks of Cancer”11.

Role of Tumor Microenvironment in Promoting Cancer—Tumor Immunosuppressive Microenvironment

As previously discussed, tumor cells can evade the host immune system during the escape phase of the immunooediting process through several mechanisms, including the down-regulation of tumor-associated antigens, immunoinhibitory checkpoint pathways, and the development of resistance to immune effectors. In addition, tumor cells create an immunosuppressive microenvironment via the elaboration of various cytokines and chemokines, such as TGF-β, IL-10, prostaglandins (PGs), chemokine (C-C motif) ligand 2 (CCL-2), and VEGF. TGF-β inhibits T- and natural killer (NK)-cell proliferation and function14,15 and promotes the expansion of regulatory T (Treg) cells and myeloid-derived suppressor cells (MDSCs)16.17. IL-10 inhibits antitumor immunity18. PGs inhibits NK-cell-mediated toxicity, inhibits tumor necrosis factor (TNF) production, and suppresses B- and T-cell proliferation19.

Treg cells are FOXP3+CD25+CD4+ immunosuppressive cells that are capable of discriminating between self-antigens and non-self-antigens and thereby play an important role in maintaining immunologic self-tolerance by inhibiting self-reactive effector T cells. Treg cells are increased in several types of cancers and have been shown to inhibit tumor antigen-specific effector T cells and immune responses against tumor cells20.21. Some proposed mechanisms for Treg cell-mediated immunosuppression include immunoinhibitory molecule CTLA-4 activation, direct T-cell killing, indoleamine 2,3-dioxygenase (IDO) induction, IL-10 production, TGF-β secretion, and PD-L1 expression. MDSCs are a heterogeneous group of cells derived from the myeloid lineage pathway that promote the immunosuppressive environment within tumors through the production of suppressive cytokines [TGF-β, IL-10, PGE2, nitric oxide (NO)] and the expression of PD-L120. It has been demonstrated
that significant functional crosstalk exists between Treg cells and MDSCs.

Co-inhibitory signaling pathways mediated via immunoinhibitory checkpoints such as CTLA-4, PD-1, TIM-3, and LAG3 also play important roles in tumor-induced immune suppression. Signal transducer and activator of transcription 3 (STAT3) is another important regulator of immunoinhibitory molecule expression, and aberrant STAT3 signaling is associated with a decreased antitumor immune response.

The immunosuppressive tumor microenvironment poses a significant barrier to the effectiveness of cancer immunotherapies. It promotes tumor growth and also prevents tumors from eliciting the effective endogenous immunity that is required for their eradication. As a result, it is now well recognized that successful immunotherapeutic modalities would have not only potent antitumor activity but also the ability to reverse tumor-induced immunosuppression.

Cancer Immunotherapeutic Approaches

The goal of cancer immunotherapy is to induce antitumor responses by the host immune system. This is achieved by approaches that are aimed at augmenting immune surveillance and relieving immune suppression. Various modalities have been explored, ranging from the immunostimulation of non-specific cytokine to the development of highly specific genetically engineered T cells. Cancer immunotherapy can be broadly divided into “active” and “passive” immunotherapeutic strategies. Passive immunotherapeutic approaches include non-specific immune stimulation using cytokines, monoclonal antibodies (mAbs), and checkpoint inhibitors and adoptive cell transfer (ACT) approaches using tumor-infiltrating lymphocytes (TILs) or genetically engineered T cells. Active immunotherapy includes the induction of the tumor-directed immune response through the vaccination of patients with tumor antigens.

Non-specific immunostimulation techniques

The first ever attempt to use this approach was made by Coley when he demonstrated the ability of bacterial toxins to cause tumor regression in sarcoma patients. It is now well understood that this is mediated through the stimulation of the innate arm of the immune system in response to pathogen-derived nucleic acids. IL-2 and interferon (IFN)-α also cause non-specific immunostimulation and were among the first Food and Drug Administration (FDA)-approved immunotherapies for solid cancers in the 1990s. The use of Bacillus Calmette-Guerin (BCG) in the treatment of superficial bladder cancer is another successful application of non-antigen-specific immune stimulation against tumor cells in clinical practice. However, barring these few exceptions, non-specific immune stimulants have not demonstrated significant clinical activity in treating cancers. Moreover, their widespread clinical use is limited by toxicity, heterogeneous and unpredictable responses, and the lack of a specific antitumor effect.

IL-2 is an immune-stimulating cytokine that promotes the activation, proliferation, survival, and effector functions of antitumor T cells. The results from clinical trials involving patients with metastatic renal cell carcinoma (RCC) and melanoma have shown that high-dose (HD) IL-2 monotherapy (aldesleukin) is associated with an overall response rate (ORR) of approximately 15% and an impressive durable complete response (CR) in a small number of patients.

These findings led to the FDA approval of HD IL-2 for the treatment of patients with metastatic RCC and melanoma in 1992 and 1998, respectively. However, the clinical use of HD IL-2 is limited by its high toxicity and the inability to identify a specific patient subpopulation expected to derive maximum benefit from the treatment. Toxicity related to HD IL-2 is due to capillary leak syndrome, which is characterized by increased vascular permeability and decreased microcirculatory perfusion, eventually leading to multiorgan failure.

IFNs can affect all phases of the innate and adaptive immune responses. IFNs mediate a wide spectrum of immune mechanisms, such as promoting T-cell responses, enhancing NK-cell cytotoxicity, up-regulating Fc receptors, promoting antibody-dependent cell-mediated cytoligogenic (ADCC), and regulating B-cell proliferation and immunoglobulin production. IFN-α has been approved for use in melanoma, RCC (in combination with bevacizumab), Kaposi’s sarcoma, chronic myeloid leukemia (CML), hairy cell leukemia (HCL), and follicular lymphoma. However, except for melanoma, the use of IFN in most of these diseases has been replaced by more efficacious treatments that were gradually identified over a period of time. In melanoma, the randomized phase III ECOG-1684 study demonstrated that the adjuvant HD IFN-α2b was associated with a significant improvement in median relapse-free survival (RFS; 1.7 vs. 1 years, P = 0.002) and median overall survival (OS; 3.8 vs. 2.8 years, P = 0.024) when compared with the observation arm. Consequently, HD IFN-α2b was approved by the FDA in 1995 and is still regarded as the standard adjuvant treatment for patients who have a high risk of disease recurrence after surgery. Adjuvant therapy with pegylated IFN was also approved recently in 2011 by the FDA for the treatment of stage III melanoma, as based on the results of the EORTC-18991 study. This trial showed that weekly subcutaneous pegylated IFN-α2b was associated with a 9.4-month improvement in RFS in comparison to the observation arm (P = 0.011). However, no significant difference in OS or distant metastasis–free survival (DMFS) was observed between the treatment groups in this trial. The important toxicities with IFN include flu-like symptoms, depression, hepatic transaminase elevation, and neutropenia.

Antibody-based immunotherapies

This approach involves the use of antibodies, antibody fragments, antibody-drug conjugates (ADCs), and radioimmunoconjugates to inhibit “tumor-associated biological targets” or “immune checkpoints.”

Blockade of tumor target-associated ligand-receptor binding

mAbs block tumor target-associated ligand-receptor binding, and thus lead to the inhibition of downstream signaling. mAbs may...
also induce other mechanisms such as ADCC, antibody-dependent phagocytosis (ADPh), and complement-dependent cytotoxicity (CDC). The first therapeutic mAb to demonstrate significant clinical activity and obtain FDA approval was rituximab, a human/mouse chimeric IgG1 directed against CD20, which was approved in 1997 for the treatment of relapsed or refractory, CD20+, B-cell, low-grade or follicular non-Hodgkin’s lymphoma (NHL)[33]. Since then, several other chimeric, partially or fully human mAbs have been FDA-approved for use in a wide range of clinical indications. Some examples are as follows: cetuximab [against epidermal growth factor receptor (EGFR)] in colorectal[44] and head and neck[33] cancers; trastuzumab (against HER2-neu) in breast[36,37] and gastroesophageal[38] cancers; otatuzumab (against CD20) in chronic lymphocytic leukemia (CLL)[39], alemtuzumab (against CD52) in CLL[40], cutaneous T-cell lymphoma (CTCL)[41], and T-prolymphocytic leukemia[42]; rituximab in CLL[43,44], panitumumab (against EGFR) in colorectal cancer[45]; and bevacizumab (against VEGF) in colorectal cancer[46], glioblastoma[47], RCC[48], and non–small cell lung carcinoma (NSCLC)[49].

In addition, immunoconjugates that are composed of mAbs linked to a biologically active cytotoxic drug (ADC) or a radioisotope (radioimmunoconjugate) have been developed for use in clinical practice. ADCs combine the cancer-killing properties of the cytotoxic agent with the targeted action of mAbs, resulting in a selective destruction of tumor cells. Brentuximab vedotin is an ADC generated by conjugating the humanized anti-CD30 mAb SGN-30 to the cytotoxic agent monomethyl auristatin E (MMAE); it is approved for relapsed Hodgkin’s lymphoma (HL) and relapsed systemic anaplastic large cell lymphoma (ALCL)[55,56]. Trastuzumab emtansine (T-DM1) is another ADC that is approved for the treatment of HER2-positive metastatic breast cancer[50]. Examples of radioimmunoconjugates include [131]I-tositumomab and [90Y]-ibritumomab, which have demonstrated encouraging results in patients with NHL[51,52].

However, these mAbs, although considered a form of immune-based therapy, do not increase host immunity against cancer. Such treatment modalities are used as a tool to prevent ligand-receptor binding (naked antibodies) or to guide the delivery of target-oriented therapies (i.e., ADCs).

**Targeting immune checkpoints**

CTLA-4 (CD152) and PD-1 (CD279) are critical checkpoint molecules that negatively regulate T-cell activation via distinct mechanisms[32]. Antibodies targeted against these immune checkpoints can activate antitumor T cells and have revolutionized the field of immunotherapy in recent years. Nonetheless, the effectiveness of these agents is restricted to tumors that are able to induce endogenous antitumor T cells[10]. Targeting immune checkpoints with mAbs is associated with auto-immune sequelae and inflammatory damage to normal parenchyma.

CTLA-4 is a co-inhibitory molecule expressed on activated T cells and Treg cells[23]. Interaction of CTLA-4 on T cells with the B7-1/B7-2 ligands on antigen-presenting cells (APCs) results in attenuation and inhibition of the CD28-mediated T-cell stimulatory signal. The inhibition of CTLA-4 results in the reactivation and proliferation of T cells[22] and also decreases the number of suppressive Treg cells in tumor tissues[56], thereby shifting the tumor microenvironment from immunosuppressive to inflammatory[37]. Ipilimumab is a first-in-class humanized IgG1 mAb against CTLA-4 that was approved by the FDA in 2011 for the treatment of advanced melanoma[56]. In a phase III trial for stage IV melanoma, ipilimumab administered with or without a gp100 peptide vaccine was compared with gp100 alone. The trial demonstrated that ipilimumab use was associated with an increase in median OS to 10 months compared with the 6.4 months in the gp100-only arm (P < 0.001); grade 3 or 4 toxicity was observed in 10%–15% of the patients treated with ipilimumab[56]. Another phase III trial evaluated the combination of ipilimumab with dacarbazine (DTIC) in previously untreated metastatic melanoma patients[56]. The OS was significantly better in the DTIC plus ipilimumab arm than in the control arm (11.2 vs. 9.1 months, P < 0.001), and grade 3 or 4 adverse events (AEs) occurred in 56.3% and 27.5% of the patients in the two arms, respectively. The spectrum of AEs encountered with ipilimumab is consistent with its immune mechanism of action and most commonly include skin reaction, colitis, uveitis, hepatitis, and endocrinopathies such as hypophysitis and thyroiditis[60]. Recent data suggest that ipilimumab, when administered at a high dose, is also effective in the adjuvant treatment of stage III melanoma[51]. In a phase III clinical trial, ipilimumab as adjuvant therapy provided a clinically and statistically significant improvement in RFS compared with placebo for patients with stage III melanoma at a high risk of recurrence (26.1 vs. 17.1 months, P = 0.001)[53]. Several other clinical trials are currently evaluating the role of CTLA-4 blockade in various other solid tumors, including malignant mesothelioma, hepatocellular carcinoma (HCC), neuroblastoma, sarcoma, and prostate, pancreatic, colorectal, and lung cancers (Table 1).

PD-1 is another co-inhibitory receptor expressed on the surface of activated T cells, Treg cells, and monocytes[23,62]. PD-1 has two ligands, PD-L1 and PD-L2. The predominant ligand, PD-L1, is expressed on many tumor cells and suppressive immune cells in the tumor microenvironment and participates in tumor immune evasion. Interaction of PD-1 with PD-L1 results in the inhibition of T-cell functioning. As a result, T cells have a decreased ability to produce cytokines, proliferate, or cause tumor lysis. Antibody-mediated blockade of PD-1 or PD-L1 results in the inhibition of this checkpoint, leading to T-cell functional activation and enhanced antitumor activity[23,63]. Brahmer et al[3] demonstrated that PD-1/PD-L1 axis blockade with a fully human IgG4 anti-PD-L1 mAb (BMS-936559) is a safe and effective immunotherapy target in a phase I study that enrolled selected patients with advanced NSCLC, melanoma, RCC, colorectal, ovarian, pancreatic, gastric, and breast cancers. Treatment-related grade 3 or 4 AEs occurred in 9% of the patients, and ORRs ranging from 6% to 17% were observed in various malignancies. Another phase I study evaluated the engineered humanized IgG4 anti-PD-1 mAb pembrolizumab (MK-3475, formerly lambrolizumab) in patients with advanced melanoma[54]. The ORR was 38%, with a median progression-free survival (PFS) of 7 months. Most AEs were low grade and included fatigue, rash, pruritus, and diarrhea. The single agent MK-3475 is also being evaluated in patients with other tumor types, such as head and neck (HN) cancer and NSCLC. A multicenter, non-randomized ongoing trial is enrolling
Table 1. Summary of major ongoing clinical trials evaluating the role of cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) blockade in various tumor types

| Cancer type                  | Trial identifier | Study title                                                                                                                                                                                                 |
|------------------------------|------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Colorectal cancer            | NCT00313794      | Study of Ticilimumab in Patients with Metastatic Colorectal Cancer Whose Disease Had Progressed after Treatment                                                                                             |
| Gastrointestinal stromal tumor | NCT01643278    | Dasatinib and Ipilimumab in Treating Patients with Gastrointestinal Stromal Tumors or Other Sarcomas that Cannot Be Removed by Surgery or Are Metastatic                                                              |
| Hematologic malignancies     | NCT01592370      | Safety Study of Nivolumab and Ipilimumab in Hematologic Malignancy                                                                                                                                           |
| Hepatocellular carcinoma     | NCT01008358      | Anti-CTLA-4 Human Monoclonal Antibody CP-675,206 in Patients with Advanced Hepatocellular Carcinoma                                                                                                          |
|                              | NCT01853618      | Tremelimumab with Chemoembolization or Ablation for Liver Cancer                                                                                                                                             |
| Lung cancer                  | NCT01331525      | The Addition of Ipilimumab to Carboplatin and Etoposide Chemotherapy for Extensive Stage Small Cell Lung Cancer (ICE)                                                                                           |
|                              | NCT02000947      | A Phase 1b Study of MEDI4736 in Combination with Tremelimumab in Subjects with Advanced Non-small Cell Lung Cancer (D4190C00006)                                                                           |
|                              | NCT02046733      | Small Cell Lung Cancer Trial with IpilimUb in Limited Disease (STIMULI)                                                                                                                                     |
| Melanoma                     | NCT00610857      | Safety and Efficacy of Combination HDI and Anti-CTLA4 for Recurrent Inoperable Stage III or Stage IV Melanoma                                                                                                |
|                              | NCT01740401      | CTLA-4 Blockade and Low-dose Cyclophosphamide in Patients with Advanced Malignant Melanoma                                                                                                                     |
|                              | NCT01216696      | Ipilimumab in Patients with Advanced Melanoma and Spontaneous Preexisting Immune Response to NY-ESO-1 (CTLA4 NY-ESO-1)                                                                                           |
|                              | NCT01274338      | Ipilimumab or High-dose Interferon Alfa-2b in Treating Patients with High-risk Stage III-IV Melanoma That Has Been Removed by Surgery                                                                           |
|                              | NCT01940809      | Ipilimumab with or without Dabrafenib and/or Trametinib in Treating Patients with Melanoma That Is Metastatic or Cannot Be Removed by Surgery                                                                      |
|                              | NCT01103635      | Tremelimumab and CP-870,893 in Patients with Metastatic Melanoma                                                                                                                                            |
|                              | NCT01621490      | PH 1 Biomarker Study of Nivolumab and Ipilimumab and Nivolumab in Combination with Ipilimumab in Advanced Melanoma (PD-1)                                                                                     |
|                              | NCT01844505      | Phase 3 Study of Nivolumab or Nivolumab Plus Ipilimumab vs. Ipilimumab Alone in Previously Untreated Advanced Melanoma (CheckMate 067)                                                                  |
| Mesothelioma                 | NCT01655888      | The Anti-CTLA-4 Monoclonal Antibody Tremelimumab in Malignant Mesothelioma                                                                                                                                   |
|                              | NCT01843374      | Randomized, Double-blind Study Comparing Tremelimumab to Placebo in Subjects with Unresectable Malignant Mesothelioma                                                                                           |
|                              | NCT01649024      | A Clinical Study with Tremelimumab as Monotherapy in Malignant Mesothelioma                                                                                                                                    |
| Neuroblastoma                | NCT01445379      | Phase I Study of Ipilimumab (Anti-CTLA-4) in Children and Adolescents with Treatment-resistant Cancer                                                                                                        |
| Pancreatic cancer            | NCT01896869      | A Phase 2, Multicenter Study of FOLFIRINOX followed by Ipilimumab with Allogenic GM-CSF Transfected Pancreatic Tumor Vaccine in the Treatment of Metastatic Pancreatic Cancer                                           |
|                              | NCT01473940      | Ipilimumab and Gemcitabine Hydrochloride in Treating Patients with Stage III-IV or Recurrent Pancreatic Cancer that Cannot Be Removed by Surgery                                                                  |
| Prostate cancer              | NCT00050596      | Comparison Study of MDX-010 (CTLA-4) Alone and Combined with Docetaxel in the Treatment of Patients with Hormone-refractory Prostate Cancer                                                                    |
|                              | NCT01498978      | Ipilimumab in Combination with Androgen Suppression Therapy in Treating Patients with Metastatic Hormone-resistant Prostate Cancer                                                                           |
|                              | NCT01804465      | A Randomized Phase 2 Trial of Combining Sipuleucel-T with Immediate vs. Delayed CTLA-4 Blockade for Prostate Cancer                                                                                           |
| Sarcoma                      | NCT00064129      | Ipilimumab and Sargramostim in Treating Patients with Metastatic Prostate Cancer                                                                                                                           |
| Solid tumors                 | NCT01975831      | A Phase 1 Study to Evaluate MEDI4736 in Combination with Tremelimumab                                                                          |

*Trials evaluating the combination of CTLA-4 and programmed cell death-1 (PD-1)/programmed cell death ligand-1 (PD-L1) blockade. Source: http://www.clinicaltrials.gov; Accessed on July 26, 2014.*
Role of immunotherapy in current oncologic practice

The ORR was 40% in the concurrent regimen group and 20% in the sequenced regimen group. Therapy-related and generally reversible grade 3 or 4 AEs occurred in 53% and 20% of the patients in the concurrent and sequential regimen groups, respectively. A three-arm randomized phase III trial is currently comparing the efficacy of nivolumab and ipilimumab monotherapies with their combination (NCT01844505). Unfortunately, no perfect predictive biomarkers have been identified to date for either anti-PD-1 or anti-CTLA-4 mAbs.

Therapeutic cancer vaccines

Vaccine-based therapies promote an induction of the immune response with a high specificity against the presented antigens. Various vaccination strategies including immunization with whole tumor cells, tumor lysates, peptides, proteins, recombinant viruses, or DNA/mRNA encoding tumor antigens have been explored. The tumor-associated antigens are either delivered alone or can be loaded ex vivo onto APCs such as dendritic cells (DCs) by genetic engineering using viral vectors[71]. Clinical trials evaluating ex vivo mRNA-transfected DCs were first published in 2002[72]. Over the last decade, this approach has been evaluated in a wide range of cancer patients, including those with melanoma, colorectal, lung, breast, prostate, and pancreatic cancers[73-79].

Several vaccines have been developed thus far against various tumor types, including breast (HER2), lung (MUC1), pancreatic (telomerase peptides), and prostate (PAP) cancers, but the majority have failed to demonstrate any significant clinical benefit[75-78]. An exception to this is the sipuleucil-T vaccine that contains autologous peripheral blood mononuclear cells (PBMCs) activated ex vivo with a recombinant fusion protein, PA2024. This fusion protein comprises PAP granulocyte-macrophage colony-stimulating factor (GM-CSF), an immune cell activator. The phase III Immunotherapy for Prostate AdenoCarcinoma Treatment (IMPACT) trial in minimally symptomatic metastatic CRPC patients demonstrated that sipuleucil-T was associated with a modest but statistically significant improvement in OS by 4.1 months compared with placebo[80]. Consequently, sipuleucil-T was approved by the FDA in 2010 for clinical use in metastatic CRPC patients. Talimogene laherparepvec (T-VEC) is an oncolytic immunotherapy derived from Herpes simplex virus (HSV) type-1 and is designed to selectively replicate in tumors and produce GM-CSF to enhance systemic antitumor immune responses[81]. The results from the OncoVEX Pivotal Trial in Melanoma (OPTIM), a randomized phase III trial evaluating T-VEC or GM-CSF in patients with unresected melanoma with regional or distant metastases, were recently reported[81]. The trial met its primary endpoint of a statistically significant improvement in durable response rate (DRR) with T-VEC (16% vs. 2%, P < 0.001), with a strong trend toward improved OS in patients treated with T-VEC (23.3 vs. 18.9 months, P = 0.051). The most common AEs associated with T-VEC included fatigue, chills, and pyrexia.

Phase III clinical trials evaluating DC-based cancer vaccines in glioma (NCT00045968), RCC (NCT01582672), and melanoma (NCT01875653) are underway. It has been postulated that the critical barrier to the efficacy of antitumor vaccines is the suppressive
### Table 2. Summary of major ongoing clinical trials evaluating the role of PD-1/PD-L1 blockade in various tumor types

| Cancer type                  | Trial identifier | Study title                                                                                                           |
|------------------------------|-----------------|-----------------------------------------------------------------------------------------------------------------------|
| Colon cancer                 | NCT02060188     | A Study of Nivolumab and Nivolumab Plus Ipilimumab in Recurrent and Metastatic Colon Cancer (CheckMate 142)           |
| Glioblastoma multiforme      | NCT01952769     | Anti-PD1 Antibody in Diffuse Intrinsic Pontine Glioma and Relapsed Glioblastoma Multiforme                             |
| Hematologic malignancies     | NCT01096602     | Blockade of PD-1 in Conjunction with the Dendritic Cell/AML Vaccine Following Chemotherapy-induced Remission          |
|                              | NCT01067287     | Blockade of PD-1 in Conjunction with the Dendritic Cell/Myeloma Vaccines Following Stem Cell Transplantation         |
|                              | NCT02077959     | Lenalidomide and Pidilizumab in Treating Patients with Relapsed or Refractory Multiple Myeloma (KEYNOTE-013)           |
|                              | NCT01953692     | A Trial of Pembrolizumab (MK-3475) in Participants with Blood Cancers (MK-3475-013) (KEYNOTE-013)                     |
|                              | NCT02036502     | A Study of Pembrolizumab (MK-3475) in Combination with Lenalidomide and Dexamethasone in Participants with Multiple Myeloma (MK-3475-023/KEYNOTE-023) |
| Hepatocellular carcinoma    | NCT01658878     | Dose Escalation Study of Nivolumab (Anti-PD-1; BMS-936558; ONO-4538) in Patients (Pts) with Advanced Hepatocellular Carcinoma (HCC) with or without Chronic Viral Hepatitis (Anti-PD-1 HCC) |
| Lung cancer                  | NCT01928576     | Phase II Anti-PD1 Epigenetic Priming Study in NSCLC (NA_00084192)                                                      |
|                              | NCT02039674     | A Study of Pembrolizumab (MK-3475) in Combination with Chemotherapy or Immuno therapy in Participants with Lung Cancer (MK-3475-021/KEYNOTE-021) |
|                              | NCT01673867     | Study of BMS-936558 (Nivolumab) Compared to Docetaxel in Previously Treated Metastatic Non-squamous NSCLC (CheckMate 057) |
|                              | NCT02041533     | An Open-label, Randomized, Phase 3 Trial of Nivolumab Versus Investigator’s Choice Chemotherapy as First-line Therapy for Stage IV or Recurrent PD-L1+ Non-small Cell Lung Cancer (CheckMate 026) |
|                              | NCT02088112     | MEDI4736 (Anti-PD-L1) Combined with Gefitinib in Subjects with Non–small Cell Lung Cancer (NSCLC)                   |
|                              | NCT01846416     | A Phase 2 Study of MPDL3280A (an Engineered Anti-PDL1 Antibody) in Patients with PD-L1-positive Locally Advanced or Metastatic Non-small Cell Lung Cancer — "FiR"|
|                              | NCT02031458     | A Phase 2 Study of MPDL3280A (an Engineered Anti-PDL1 Antibody) in Patients with PD-L1-positive Locally Advanced or Metastatic Non-small Cell Lung Cancer — "BiRCH"|
|                              | NCT02007070     | Study of Pembrolizumab (MK-3475) in Participants with Advanced Non-small Cell Lung Cancer (MK-3475-025/KEYNOTE-025) |
|                              | NCT02087423     | A Global Study to Assess the Effects of MEDI4736 in Patients with Locally Advanced or Metastatic Non-small Cell Lung Cancer (ATLANTIC) |
|                              | NCT01903993     | A Randomized Phase 2 Study of MPDL3280A (an Engineered Anti-PDL1 Antibody) Compared with Docetaxel in Patients with Locally Advanced or Metastatic Non-small Cell Lung Cancer Who Have Failed Platinum Therapy — "POPLAR" |
|                              | NCT02008227     | A Randomized Phase 3 Study of MPDL3280A (an Engineered Anti-PDL1 Antibody) Compared to Docetaxel in Patients with Locally Advanced or Metastatic Non-small Cell Lung Cancer Who Have Failed Platinum Therapy — "OAK" |
|                              | NCT02125461     | A Global Study to Assess the Effects of MEDI4736 Following Concurrent Chemoradiation in Patients with Stage III Unresectable Non-small Cell Lung Cancer (PACIFIC) |
|                              | NCT01642004     | Study of BMS-936558 (Nivolumab) Compared to Docetaxel in Previously Treated Advanced or Metastatic Squamous Cell Non-small Cell Lung Cancer (NSCLC) (CheckMate 017) |
| Melanoma                     | NCT01176474     | Multiple Class I Peptides & Montanide ISA 51VG with Escalating Doses of Anti-PD-1 Antibody BMS936558                 |
|                              | NCT01866319     | Study to Evaluate the Safety and Efficacy of Two Different Dosing Schedules of Pembrolizumab (MK-3475) Compared to Ipilimumab in Participants with Advanced Melanoma (MK-3475-006/KEYNOTE-006) |
|                              | NCT01704287     | Study of Pembrolizumab (MK-3475) Versus Chemotherapy in Participants with Advanced Melanoma (P08719/KEYNOTE-002) |

(To be continued)
The effect of the tumor microenvironment. Combining cancer vaccines with other immunomodulatory agents or techniques, such as depletion of Treg cells, to overcome the immunosuppressive tumor microenvironment is likely to result in improved clinical outcomes.

**Table 2. Summary of major ongoing clinical trials evaluating the role of PD-1/PD-L1 blockade in various tumor types (continued)**

| Cancer type            | Trial identifier | Study title                                                                                                                                 |
|------------------------|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| Melanoma               | NCT01656642     | A Phase 1b Study of MPDL3280A (an Engineered Anti-PDL1 Antibody) in Combination with Vemurafenib (Zelboraf®) in Patients with Previously Untreated BRAFV600–Mutation Positive Metastatic Melanoma |
|                        | NCT02027961     | Phase 1 Safety and Tolerability of MEDI4736 in Combination with Dabrafenib and Trametinib or with Trametinib Alone                                |
|                        | NCT01721746     | A Study to Compare BMS-936558 to the Physician’s Choice of Either Dacarbazine or Carboplatin and Paclitaxel in Advanced Melanoma Patients That Have Progressed Following Anti-CTLA-4 Therapy (CheckMate 037) |
|                        | NCT01721772     | Study of BMS-936558 vs. Dacarbazine in Untreated, Unresectable or Metastatic Melanoma (CheckMate066)                                          |
| Merkel cell carcinoma  | NCT02155647     | MSB0010718C in Subjects with Merkel Cell Carcinoma                                                                                         |
| Pancreatic cancer      | NCT01313416     | Gemcitabine and CT-011 for Resected Pancreatic Cancer                                                                                        |
| Prostate cancer        | NCT01420965     | Sipuleucel-T, CT-011, and Cyclophosphamide for Advanced Prostate Cancer                                                                      |
| Renal cell carcinoma   | NCT01358721     | Phase I Biomarker Study (BMS-936558)                                                                                                          |
|                        | NCT01441765     | PD-1 Alone or with Dendritic Cell/Renal Cell Carcinoma Fusion Cell Vaccine                                                                  |
|                        | NCT01668784     | Study of Nivolumab (BMS-936558) Vs. Everolimus in Pre-treated Advanced or Metastatic Clear-cell Renal Cell Carcinoma (CheckMate025)            |
| Solid tumors           | NCT01629758     | Safety Study of IL-21/Anti-PD-1 Combination in the Treatment of Solid Tumors                                                                   |
|                        | NCT01714739     | A Phase I Study of an Anti-KIR Antibody in Combination with an Anti-PD1 Antibody in Patients with Advanced Solid Tumors                          |
|                        | NCT01968109     | Safety Study of Anti-LAG-3 with and without Anti-PD-1 in the Treatment of Solid Tumors                                                        |
|                        | NCT02179918     | A Study of 4-1BB Agonist PF-05082566 Plus PD-1 Inhibitor MK-3475 in Patients with Solid Tumors (B1641003/KEYNOTE–0036)                        |
|                        | NCT02013804     | A Phase 1 Study to Evaluate AMP-514                                                                                                          |
|                        | NCT01295827     | Study of Pembrolizumab (MK-3475) in Participants with Progressive Locally Advanced or Metastatic Carcinoma, Melanoma, or Non–small Cell Lung Carcinoma (P07990/MK–3475–001/KEYNOTE–001) |
|                        | NCT00836888     | ONO-4538 Phase I Study in Patients with Advanced Malignant Solid Tumors in Japan                                                              |
|                        | NCT01352884     | Study to Assess the Safety, Tolerability, and Pharmacokinetics of AMP-224 in Patients with Advanced Cancer                                          |
|                        | NCT01375842     | A Phase 1 Study of MPDL3280A (an Engineered Anti-PDL1 Antibody) in Patients with Locally Advanced or Metastatic Solid Tumors                   |
|                        | NCT01633970     | A Phase 1 Study of MPDL3280A (an Engineered Anti-PDL1 Antibody) in Combination with Avastin (Bevacizumab) and/or with Chemotherapy in Patients with Locally Advanced or Metastatic Solid Tumors |
|                        | NCT01772004     | MSB0010718C in Solid Tumors                                                                                                                  |
|                        | NCT01943461     | MSB0010718C in Metastatic or Locally Advanced Solid Tumors                                                                                   |
|                        | NCT01938612     | A Phase I, Open-label, Multicentre Study to Evaluate the Safety, Tolerability and Pharmacokinetics of MEDI4736 in Japanese Patients with Advanced Solid Tumors |
|                        | NCT01848834     | Study of Pembrolizumab (MK-3475) in Participants with Advanced Solid Tumors (MK-3475-012/KEYNOTE-012)                                           |
|                        | NCT00729664     | Multiple Ascending Dose (MDX1105-01) (Anti-PDL1)                                                                                               |
| Urothelial bladder cancer | NCT02108652   | A Study of MPDL3280A in Patients with Locally Advanced or Metastatic Urothelial Bladder Cancer                                                  |

Source: http://www.clinicaltrials.gov; Accessed on July 26, 2014.

Adoptive T-cell therapies

Adoptive cell transfer (ACT) is an immunotherapeutic approach that involves the ex vivo expansion and transfer of autologous T cells.
lymphocytes with antitumor activity into cancer patients. This approach increases the number of antigen-specific T-cell populations, which leads to an enhanced antitumor immune response through cytokine release and tumor cell lysis\(^{[88-91]}\). ACT requires either the isolation (from tumor or peripheral blood) or production of autologous lymphocytes with antitumor activity.

One approach is to isolate TILs with antitumor activity from the tumor samples of the patient and then culture and expand them ex vivo to therapeutic levels. However, the difficulty in expanding TILs to sufficient numbers has hampered the applicability of this approach to non-melanoma tumors. The other more direct approach is the production of highly specific genetically engineered autologous T cells that express tumor antigen-specific T-cell receptors (TCR) or immunoglobulin-based fusion protein, known as chimeric antigen receptors (CARs). To produce TCR-engineered T cells, the antigen-specific TCR genes are transferred into lymphocytes isolated from the peripheral blood. These genetically engineered designer T cells (dTcs) are then cultured and expanded in vitro for clinical use. The success of this approach was demonstrated when TCR-based dTcs with specificity for melanoma antigens (MART-1 and gp100) were shown to cause tumor regression in otherwise treatment-refractory melanoma patients\(^{[84]}\). dTcs specific for NY-ESO-1 CTA resulted in objective responses in 5 out of 7 synovial cell carcinoma patients\(^{[86]}\). dTcs specific for GD2 led to objective responses in 3 out of 11 neuroblastoma patients\(^{[86]}\). CAR-modified T cells are composed of an extracellular targeting site, most commonly the antigen-reactive portions of immunoglobulin light and heavy chains, which is fused with the T-cell intracellular signaling domain. CARs were originally developed by Gross et al.\(^{[87]}\). CAR-modified T cells have been designed against a wide range of tumor antigens, including GD2 in neuroblastoma, CD19 \(^{+}\) B-cell in NHL and CLL, and KIT \(^{+}\) in gastrointestinal stromal tumor (GIST)\(^{[88-91]}\). A major difference between TCR- and CAR-engineered T cells is in the ability to recognize human leukocyte antigens (HLAs). TCRs are specific for certain HLA-peptide complexes, whereas CARs recognize antigens in a non-HLA-dependent way. As a consequence, CARs have the advantage of a broader clinical application in patients with different HLA haplotypes\(^{[89]}\).

Despite the encouraging results observed with TCR- and CAR-based therapies, this approach needs to be further optimized to reduce toxicity, and ways to escape the inhibition of the immunosuppressive tumor microenvironment need to be devised\(^{[92]}\). IL-2 administration and lymphodepletion of the host (using combinations of cyclophosphamide, fludarabine, and total body irradiation) prior to adoptive T-cell transfer of TILs or TCR/ CAR-engineered T cells can improve the therapeutic efficacy of this approach. Lymphodepletion is believed to exert its beneficial effect by eradicating immunosuppressive Treg cells from the tumor microenvironment. Based on preliminary data, when a combination of TILs, IL-2, and an intensive lymphodepletion regimen was administered to metastatic melanoma patients, response rates ranging from 50% to 70% were observed\(^{[88]}\). An ongoing clinical trial is evaluating the ability of HPV E6- and E7-reactive TILs (HPV-TILs) to treat metastatic HPV-positive cancers\(^{[94]}\). The HPV-TIL infusion was preceded by non-myeloablative conditioning and was followed by the administration of bolus HD IL-2. An interim analysis of the cervical cancer cohort showed that of the 9 women treated in the study, 2 achieved CR that was ongoing at 18 and 11 months after treatment. One patient had a PR, whereas the others showed no response.

Allogeneic stem cell transplantation (AlloSCT) can also be regarded as a form of unselected ACT therapy, in which engrafting donor-derived lymphocyte populations stimulate a graft-versus-tumor (GVT) effect, leading to long-term cancer control\(^{[95]}\).

**Other immunotherapeutic strategies**

Classical cytotoxic chemotherapy has been traditionally considered to be associated with generalized immune suppression. However, it is now being recognized that cytotoxic chemotherapeutic agents and targeted compounds can also modulate the immune system and promote antitumor immunity, either by inducing the immunogenic death of tumor cells or by engaging immune effector mechanisms\(^{[96]}\). The ability of cyclophosphamide to suppress Treg cells was demonstrated more than two decades ago\(^{[97]}\). Gemcitabine has been shown to reduce the number of MDSCs and increase MHC-I expression\(^{[98]}\). Treatment with oxaliplatin is associated with cytokine secretion in the tumor microenvironment, which can promote the maturation of DCs, leading to enhanced T-cell responses\(^{[99]}\). The small-molecule multi-kinase inhibitors sunitinib and sorafenib result in decreased levels and activity of Treg cells and MDSCs. Ionizing radiation has also been shown to promote systemic antitumor activity, likely through the recruitment of effector T cells to tumor sites and by promoting tumor antigen recognition\(^{[100,101]}\).

Denileukin diftitox is a recombinant immunotoxin composed of the diphtheria toxin-IL-2 fusion protein that has been explored as an immunotherapeutic strategy. This therapy is aimed at depleting immunoinhibitory Treg cells from the tumor microenvironment and exploits the principle that Treg cells constitutively express IL-2 receptor on their surface\(^{[102]}\). Denileukin diftitox was approved by the FDA in 1999 for the treatment of CTCL.

Glucocorticoid-induced TNF-receptor family related protein (GITR) and OX40 are two co-stimulatory molecules constitutively expressed on Treg cells and MDSCs. Treatment with oxaliplatin is associated with cytokine secretion in the tumor microenvironment, which can promote the maturation of DCs, leading to enhanced T-cell responses\(^{[99]}\). The small-molecule multi-kinase inhibitors sunitinib and sorafenib result in decreased levels and activity of Treg cells and MDSCs. Ionizing radiation has also been shown to promote systemic antitumor activity, likely through the recruitment of effector T cells to tumor sites and by promoting tumor antigen recognition\(^{[100,101]}\).

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Glucocorticoid-induced TNF-receptor family related protein (GITR) and OX40 are two co-stimulatory molecules constitutively expressed on Treg cells and can be targeted with anti-GITR mAb and anti-OX40 mAb, respectively, to inhibit immunosuppressive Treg cells\(^{[103,104]}\). Therapies against these targets are currently under evaluation.

It is now well established that tumor development involves multiple immunoinhibitory pathways. Thus, combining cytotoxic and targeted agents with other immunotherapeutic approaches, such as vaccines and ACT, might be another effective strategy to enhance the antitumor efficacy of immune-based therapies and improve clinical outcomes\(^{[109]}\).

**Conclusions and Future Challenges**

Considerable progress has been made in the field of cancer...
immunotherapy during the past three decades. Clinically effective immune-based antitumor approaches such as vaccines (sipuleucil-T) and checkpoint inhibitors (ipilimumab, anti-PD-1 and anti-PD-L1 antibodies) are gradually making headway into the mainstream oncologic practice. Although these advancements represent a significant accomplishment, the path is still riddled with numerous obstacles and challenges. The tumor microenvironment continues to remain a significant challenge in the development of effective anticancer immunotherapies, largely due to the inability of the current approaches to effectively overcome the tumor-induced immunosuppression. Moreover, the presence of tumor heterogeneity contributes to inter- and intra-patient differences in response to these immunotherapies. Immune-based therapies are also frequently associated with auto-immune adverse effects to by-stander organs due to the non-specific immunostimulation. Therefore, adopting a personalized approach in delivering immunotherapy would help to reduce toxicity and costs while improving efficacy. The development of novel tumor antigen specificity-based immunotherapies and the identification of predictive biomarkers are critical to attaining this goal. Successful immunotherapeutic approaches will likely involve the combination of agents that target multiple pathways, including the inhibition of suppressive cells and immunoinhibitory molecules. Such combinatorial approaches are more likely to produce synergistic effects and the induction of durable immunologic memory. Indeed, the frequent and durable response observed with the combination of ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1) in melanoma is an excellent illustration of this principle. Finally, elucidating new tumor-promoting pathways and the development of novel therapeutic strategies to inhibit those targets will be the key to achieving continued success in this promising field of cancer immunotherapy.

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