Chinese Journal of Cancer

Review

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.

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doi: 10.5732/cjc.014.10123

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 immunosurveillance[5,6].

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)[7-10]. 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 cells[10]. 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 cells[10]. 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 research[10].

Cancer Immunoediting—Escape from Immune Control

The immunosurveillance concept gradually evolved into the "cancer immunoediting" 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 tumors[11]. Immunoediting 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 persistence[12]. 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 (TGF-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)/Gamelin-9, lymphocyte activation gene 3 (LAG3)/major histocompatibility complex class II molecules (MHC-II)], and the development of resistance to immune effectors.

In summary, immunoediting 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"[13].

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 immunoediting 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 function[14] and promotes the expansion of regulatory T (Treg) cells and myeloid-derived suppressor cells (MDSCs)[15,16]. IL-10 inhibits antitumor immunity[17]. PGs inhibits NK-cell-mediated toxicity, inhibits tumor necrosis factor (TNF) production, and suppresses B- and T-cell proliferation[18].

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 cells[19]. 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-L1[20]. It has been demonstrated

that significant functional crosstalk exists between Treg cells and MDSCs[21].

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[22,23]. 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[24].

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[1]. 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[25,26]. 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[27]. 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[28].

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[29-31]. 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 cytotoxicity (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[25]. 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 (34.8 vs. 25.5 months, P = 0.011)[32]. 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[34] and head and neck[35] cancers; trastuzumab (against HER2-neu) in breast[36,37] and gastroesophageal[38] cancers; ofatumumab (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)[50,51]. Trastuzumab emtansine (T-DM1) is another ADC that is approved for the treatment of HER2-positive metastatic breast cancer[52]. Examples of radioimmunoconjugates include ^{131}I-tositumomab and ^{90}Y-ibritumomab, which have demonstrated encouraging results in patients with NHL[53,54].

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[55]. 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[22]. 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[57]. 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[58]. 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[58]. Another phase III trial evaluated the combination of ipilimumab with dacarbazine (DTIC) in previously untreated metastatic melanoma patients[59]. 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[61]. 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, $P = 0.001$)[61]. 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 blockage 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*.[23] 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[64]. 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 [a]	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]	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 IpiliMUmab 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 [a]	Tremelimumab and CP-870,893 in Patients with Metastatic Melanoma
	NCT01621490 [a]	PH 1 Biomarker Study of Nivolumab and Ipilimumab and Nivolumab in Combination with Ipilimumab in Advanced Melanoma (PD-1)
	NCT01844505 [a]	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
	NCT00064129	Ipilimumab and Sargramostim in Treating Patients with Metastatic Prostate Cancer
Sarcoma	NCT01643278	Phase I Study of Ipilimumab (Anti-CTLA-4) in Children and Adolescents with Treatment–resistant Cancer
Solid tumors	NCT01975831 [a]	A Phase 1 Study to Evaluate MEDI4736 in Combination with Tremelimumab

[a]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.

recurrent and metastatic HN cancer patients with positive PD-L1 expression into two cohorts (HPV- and non-HPV-associated)[65]. However, interim analyses presented at the American Society of Clinical Oncology (ASCO) 2014 annual meeting reported drug-related AEs in 46.7% patients[65]. The most common drug-related AEs included pruritus, fatigue, rash, and diarrhea. Tumor shrinkage was observed in several patients, but protocol-specified efficacy analyses were not available at the time of the interim analyses. Another phase I study evaluated the safety, tolerability, and clinical activity of MK-3475 as initial therapy in patients with locally advanced or metastatic NSCLC that expresses PD-L1[66]. Preliminary data indicate an ORR (confirmed and unconfirmed) of 36% by immune-related response criteria (irRC); 52% of the patients experienced a drug-related AE, usually grades 1–2, most commonly fatigue, pruritus, dermatitis acneiform, diarrhea, and dyspnea. Topalian *et al*.[63] evaluated the safety and activity of the fully human IgG4 anti-PD-1 mAb nivolumab (BMS-936558, MDX 1105, BMS-ONO) in patients with advanced melanoma, NSCLC, castration-resistant prostate cancer (CRPC), RCC, and colorectal cancer. Objective responses were observed in NSCLC, melanoma, and RCC, with ORRs ranging from 18% to 28%. No responses were observed in tumors lacking PD-L1, in contrast to the 36% ORR in tumors that expressed the ligand. Grade 3 or 4 AEs occurred in 14% of the patients, including 3 deaths from pulmonary toxicity. A phase I study is currently evaluating MPDL3280A, an engineered fully human IgG1 anti-PD-L1 mAb, in metastatic urothelial bladder cancer[67]. The results from the interim analysis were presented at the ASCO 2014 annual meeting, showing that of the 20 PD-L1-positive patients who were evaluable for efficacy at the time of the analyses, the ORR was 50% [1 CR and 9 partial responses (PRs)], with a median follow-up of 2.8 months[67]. Treatment-related grade 3-4 AEs occurred in 3.2% of the patients. Another ongoing phase I multicenter, open-label study is evaluating the safety, pharmacokinetics (PK), and antitumor activity of an engineered fully human IgG1 anti-PD-L1 mAb, MEDI4736, in a range of tumors including HN, pancreatic and gastric tumors, NSCLC, and melanoma[68]. Of the 26 evaluable patients in the dose escalation phase, 4 PRs (3 NSCLCs and 1 melanoma) and 5 additional patients with tumor shrinkage not meeting PR were observed. The disease control rate [PR + stable disease (SD) $\geqslant$ 12 weeks] was 46%. Treatment-related AEs occurred in 34% of the patients, all grades 1–2, and included diarrhea, fatigue, rash, and vomiting. Encouraged by the clinical activity of MEDI4736 in the initial phase, an expansion study was initiated in multiple cancer types, and 151 patients have been enrolled as of January 2014[69]. With a median follow-up of 6 weeks, tumor shrinkage is already detectable in various tumor types, including in patients with melanoma, pancreatic, HN, and gastroesophageal cancers. **Table 2** summarizes the major ongoing clinical trials with anti-PD-1 and anti-PD-L1 antibodies in various types of cancers.

The combination of two checkpoint inhibitors, anti-PD-1 with anti-CTLA-4, has also been explored and was associated with high response rates[70]. Wolchok *et al*.[70] conducted a phase I study of nivolumab combined with ipilimumab administered as concurrent and sequenced therapy in patients with advanced melanoma. Over 50% of the patients achieved greater than 80% reduction in the tumor size. 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 dentritic 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[73,74].

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[75-79]. 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 and 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
	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 Immunotherapy 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*)

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 Vemurafinib (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 1b 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.

effect of the tumor microenvironment[82]. 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.

Adoptive T-cell therapies

Adoptive cell transfer (ACT) is an immunotherapeutic approach that involves the *ex vivo* expansion and transfer of autologous

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[83]. 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[85]. 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[10].

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[93]. 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 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[105].

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 a 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.

Received: 2014-07-31; accepted: 2014-08-13.

References

[1] Coley WB. II. Contribution to the knowledge of sarcoma. Ann Surg, 1891,14:199–220.

[2] Clauberg KW. The immunobiological legacy of Emil von Behring and Paul Ehrlich. Dtsch Med J, 1954,5:138–146. [in undetermined language]

[3] Burnet M. Cancer; a biological approach. I. The processes of control. Br Med J, 1957,1:779–786.

[4] Thomas L, Lawrence HS. Cellular and humoral aspects of the hypersensitive states. New York: Hoeber-Harper, 1959:529–532.

[5] Knuth A, Wolfel T, Klehmann E, et al. Cytolytic T-cell clones against an autologous human melanoma: specificity study and definition of three antigens by immunoselection. Proc Natl Acad Sci U S A, 1989,86:2804–2808.

[6] Parker GA, Rosenberg SA. Serologic identification of multiple tumor-associated antigens on murine sarcomas. J Natl Cancer Inst, 1977,58:1303–1309.

[7] Cheever MA, Allison JP, Ferris AS, et al. The prioritization of cancer antigens: a National Cancer Institute pilot project for the acceleration of translational research. Clin Cancer Res, 2009,15:5323–5337.

[8] Nukaya I, Yasumoto M, Iwasaki T, et al. Identification of HLA-A24 epitope peptides of carcinoembryonic antigen which induce tumor-reactive cytotoxic T lymphocyte. Int J Cancer, 1999,80:92–97.

[9] Rosenberg SA, Kawakami Y, Robbins PF, et al. Identification of the genes encoding cancer antigens: implications for cancer immunotherapy. Adv Cancer Res, 1996,70:145–177.

[10] Wayteck L, Breckpot K, Demeester J, et al. A personalized view on cancer immunotherapy. Cancer Lett, 2014,352:113–125.

[11] Mittal D, Gubin MM, Schreiber RD, et al. New insights into cancer immunoediting and its three component phases—elimination, equilibrium and escape. Curr Opin Immunol, 2014,27C:16–25.

[12] Teng MW, Vesely MD, Duret H, et al. Opposing roles for IL-23 and IL-12 in maintaining occult cancer in an equilibrium state. Cancer Res, 2012,72:3987–3996.

[13] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell, 2011,144:646–674.

[14] Bodmer S, Strommer K, Frei K, et al. Immunosuppression and transforming growth factor-beta in glioblastoma. Preferential production of transforming growth factor-beta 2. J Immunol, 1989,143:3222–3229.

[15] Bierie B, Moses HL. Transforming growth factor beta (TGF-beta) and inflammation in cancer. Cytokine Growth Factor Rev, 2010,21:49–59.

[16] Wan YY, Flavell RA. TGF-beta and regulatory T cell in immunity and autoimmunity. J Clin Immunol, 2008,28:647–659.

[17] Fortis C, Foppoli M, Gianotti L, et al. Increased interleukin-10 serum levels in patients with solid tumours. Cancer Lett, 1996,104:1–5.

[18] Huang M, Sharma S, Mao JT, et al. Non-small cell lung cancer-derived soluble mediators and prostaglandin E2 enhance peripheral blood lymphocyte IL-10 transcription and protein production. J Immunol, 1996,157:5512–5520.

[19] Zou W. Regulatory T cells, tumour immunity and immunotherapy. Nat Rev Immunol, 2006,6:295–307.

[20] Fujimura T, Mahnke K, Enk AH. Myeloid derived suppressor cells and their role in tolerance induction in cancer. J Dermatol Sci, 2010,59:1–6.

[21] Fujimura T, Kambayashi Y, Aiba S. Crosstalk between regulatory T cells (Tregs) and myeloid derived suppressor cells (MDSCs) during melanoma growth. Oncoimmunology, 2012,1:1433–1434.

[22] Finn OJ. Cancer immunology. N Engl J Med, 2008,358:2704–2715.

[23] Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med, 2012,366:2455–2465.

[24] Lee H, Pal SK, Reckamp K, et al. STAT3: a target to enhance antitumor immune response. Curr Top Microbiol Immunol, 2011,344:41–59.

[25] Kirkwood JM, Strawderman MH, Ernstoff MS, et al. Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. J Clin Oncol, 1996,14:7–17.

[26] Rosenberg SA, Yang JC, Topalian SL, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer

using high-dose bolus interleukin 2. JAMA, 1994,271:907–913.
[27] Lamm DL, Blumenstein BA, Crawford ED, et al. A randomized trial of intravesical doxorubicin and immunotherapy with Bacille Calmette-Guerin for transitional-cell carcinoma of the bladder. N Engl J Med, 1991,325:1205–1209.
[28] Foa R, Guarini A, Gansbacher B. IL2 treatment for cancer: from biology to gene therapy. Br J Cancer, 1992,66:992–998.
[29] Fisher RI, Rosenberg SA, Fyfe G. Long-term survival update for high-dose recombinant interleukin-2 in patients with renal cell carcinoma. Cancer J Sci Am, 2000,6 Suppl 1:S55–57.
[30] Fyfe G, Fisher RI, Rosenberg SA, et al. Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. J Clin Oncol, 1995,13:688–696.
[31] Atkins MB, Lotze MT, Dutcher JP, et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J Clin Oncol, 1999,17:2105–2116.
[32] Eggermont AM, Suciu S, Santinami M, et al. Adjuvant therapy with pegylated interferon alfa-2b versus observation alone in resected stage III melanoma: final results of EORTC 18991, a randomised phase III trial. Lancet, 2008,372:117–126.
[33] Grillo-Lopez AJ, White CA, Dallaire BK, et al. Rituximab: the first monoclonal antibody approved for the treatment of lymphoma. Curr Pharm Biotechnol, 2000,1:1–9.
[34] Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med, 2004,351:337–345.
[35] Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med, 2006,354:567–578.
[36] Dahabreh IJ, Linardou H, Siannis F, et al. Trastuzumab in the adjuvant treatment of early-stage breast cancer: a systematic review and meta-analysis of randomized controlled trials. Oncologist, 2008,13:620–630.
[37] von Minckwitz G, Schwedler K, Schmidt M, et al. Trastuzumab beyond progression: overall survival analysis of the GBG 26/BIG 3–05 phase III study in HER2-positive breast cancer. Eur J Cancer, 2011,47:2273–2281.
[38] Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet, 2010,376:687–697.
[39] Wierda WG, Kipps TJ, Mayer J, et al. Ofatumumab as single-agent CD20 immunotherapy in fludarabine-refractory chronic lymphocytic leukemia. J Clin Oncol, 2010,28:1749–1755.
[40] Hillmen P, Skotnicki AB, Robak T, et al. Alemtuzumab compared with chlorambucil as first-line therapy for chronic lymphocytic leukemia. J Clin Oncol, 2007,25:5616–5623.
[41] Lundin J, Hagberg H, Repp R, et al. Phase 2 study of alemtuzumab (anti-CD52 monoclonal antibody) in patients with advanced mycosis fungoides/Sezary syndrome. Blood, 2003,101:4267–4272.
[42] Dearden CE, Matutes E, Cazin B, et al. High remission rate in T-cell prolymphocytic leukemia with CAMPATH–1H. Blood, 2001,98:1721–1726.
[43] Hallek M, Fischer K, Fingerle-Rowson G, et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet, 2010,376:1164–1174.
[44] Robak T, Dmoszynska A, Solal-Celigny P, et al. Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia. J Clin Oncol, 2010,28:1756–1765.
[45] Van Cutsem E, Peeters M, Siena S, et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. J Clin Oncol, 2007,25:1658–1664.
[46] Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med, 2004,350:2335–2342.
[47] Friedman HS, Prados MD, Wen PY, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol, 2009,27:4733–4740.
[48] Rini BI, Halabi S, Rosenberg JE, et al. Phase III trial of bevacizumab plus interferon alfa versus interferon alfa monotherapy in patients with metastatic renal cell carcinoma: final results of CALGB 90206. J Clin Oncol, 2010,28:2137–2143.
[49] Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med, 2006,355:2542–2550.
[50] Younes A, Gopal AK, Smith SE, et al. Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin´s lymphoma. J Clin Oncol, 2012,30:2183–2189.
[51] Pro B, Advani R, Brice P, et al. Brentuximab vedotin (SGN-35) in patients with relapsed or refractory systemic anaplastic large-cell lymphoma: results of a phase II study. J Clin Oncol, 2012,30:2190–2196.
[52] Verma S, Miles D, Gianni L, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med, 2012,367:1783–1791.
[53] Kaminski MS, Zelenetz AD, Press OW, et al. Pivotal study of iodine I 131 tositumomab for chemotherapy-refractory low-grade or transformed low-grade B-cell non-Hodgkin´s lymphomas. J Clin Oncol, 2001,19:3918–3928.
[54] Witzig TE, Gordon LI, Cabanillas F, et al. Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin´s lymphoma. J Clin Oncol, 2002,20:2453–2463.
[55] Parry RV, Chemnitz JM, Frauwirth KA, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol, 2005,25:9543–9553.
[56] Simpson TR, Li F, Montalvo-Ortiz W, et al. Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. J Exp Med, 2013,210:1695–1710.

[57] Curran MA, Montalvo W, Yagita H, et al. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci U S A, 2010,107:4275–4280.
[58] Hodi FS, O´Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med, 2010,363:711–723.
[59] Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med, 2011,364:2517–2526.
[60] Fecher LA, Agarwala SS, Hodi FS, et al. Ipilimumab and its toxicities: a multidisciplinary approach. Oncologist, 2013,18:733–743.
[61] Eggermont AM, Chiarion-Sileni V, Grob JJ, et al. Ipilimumab versus placebo after complete resection of stage III melanoma: initial efficacy and safety results from the EORTC 18071 phase III trial. J Clin Oncol, 2014,32:5s.
[62] Wang L, Pino-Lagos K, de Vries VC, et al. Programmed death 1 ligand signaling regulates the generation of adaptive Foxp3+CD4+ regulatory T cells. Proc Natl Acad Sci U S A, 2008,105:9331–9336.
[63] Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med, 2012,366:2443–2454.
[64] Hamid O, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med, 2013,369:134–144.
[65] Seiwert TY, Burtness B, Weiss J, et al. A phase Ib study of MK-3475 in patients with human papillomavirus (HPV)-associated and non-HPV-associated head and neck (H/N) cancer. J Clin Oncol, 2014,32:5s (suppl, abstr 6011).
[66] Rizvi NA, Garon EB, Patnaik A, et al. Safety and clinical activity of MK-3475 as initial therapy in patients with advanced non–small cell lung cancer (NSCLC). J Clin Oncol, 2014,32:5s (suppl, abstr 8007).
[67] Powles T, Vogelzang NJ, Fine GD, et al. Inhibition of PD-L1 by MPDL3280A and clinical activity in pts with metastatic urothelial bladder cancer (UBC). J Clin Oncol, 2014,32:5s (suppl, abstr 5011).
[68] Lutzky J, Antonia SJ, Blake-Haskins A, et al. A phase 1 study of MEDI4736, an anti-PD-L1 antibody, in patients with advanced solid tumors. J Clin Oncol, 2014,32:5s (suppl, abstr 3001).
[69] Segal NH, Antonia SJ, Brahmer JR, et al. Preliminary data from a multi-arm expansion study of MEDI4736, an anti-PD-L1 antibody. J Clin Oncol, 2014,32:5s (suppl, abstr 3002).
[70] Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med, 2013,369:122–133.
[71] Galluzzi L, Senovilla L, Vacchelli E, et al. Trial watch: dendritic cell-based interventions for cancer therapy. Oncoimmunology, 2012,1:1111–1134.
[72] Heiser A, Coleman D, Dannull J, et al. Autologous dendritic cells transfected with prostate-specific antigen RNA stimulate CTL responses against metastatic prostate tumors. J Clin Invest, 2002,109:409–417.
[73] Suso EM, Dueland S, Rasmussen AM, et al. hTERT mRNA dendritic cell vaccination: complete response in a pancreatic cancer patient associated with response against several hTERT epitopes. Cancer Immunol Immunother, 2011,60:809–818.
[74] Van Nuffel AM, Benteyn D, Wilgenhof S, et al. Intravenous and intradermal TriMix-dendritic cell therapy results in a broad T-cell response and durable tumor response in a chemorefractory stage IV-M1c melanoma patient. Cancer Immunol Immunother, 2012,61:1033–1043.
[75] Bernhardt SL, Gjertsen MK, Trachsel S, et al. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: a dose escalating phase I/II study. Br J Cancer, 2006,95:1474–1482.
[76] Butts C, Murray N, Maksymiuk A, et al. Randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer. J Clin Oncol, 2005,23:6674–6681.
[77] Czerniecki BJ, Koski GK, Koldovsky U, et al. Targeting HER-2/neu in early breast cancer development using dendritic cells with staged interleukin-12 burst secretion. Cancer Res, 2007,67:1842–1852.
[78] Disis ML, Schiffman K, Guthrie K, et al. Effect of dose on immune response in patients vaccinated with an her-2/neu intracellular domain protein-based vaccine. J Clin Oncol, 2004,22:1916–1925.
[79] Gould P. Sipuleucel-T shows partial advantage in prostate cancer. Lancet Oncol, 2006,7:710.
[80] Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med, 2010,363:411–422.
[81] Kaufman HL, Andtbacka RHI, Collichio FA, et al. Primary overall survival (OS) from OPTiM, a randomized phase III trial of talimogene laherparepvec (T-VEC) versus subcutaneous (SC) granulocyte-macrophage colony-stimulating factor (GM-CSF) for the treatment (tx) of unresected stage IIIB/C and IV melanoma. J Clin Oncol, 2014,32:5s (suppl, abstr 9008a).
[82] Saied A, Pillarisetty VG, Katz SC. Immunotherapy for solid tumors—a review for surgeons. J Surg Res, 2014,187:525–535.
[83] Zigler M, Shir A, Levitzki A. Targeted cancer immunotherapy. Curr Opin Pharmacol, 2013,13:504–510.
[84] Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science, 2006,314:126–129.
[85] Overwijk WW, Theoret MR, Finkelstein SE, et al. Tumor regression and autoimmunity after reversal of a functionally tolerant state of self-reactive CD8+ T cells. J Exp Med, 2003,198:569–580.
[86] Louis CU, Savoldo B, Dotti G, et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. Blood, 2011,118:6050–6056.
[87] Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc Natl Acad Sci U S A, 1989,86:10024–10028.
[88] Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med, 2013,368:1509–1518.
[89] Katz SC, Burga RA, Naheed S, et al. Anti-KIT designer T cells for the treatment of gastrointestinal stromal tumor. J Transl Med, 2013,11:46.

[90] Porter DL, Levine BL, Kalos M, et al. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med, 2011,365:725–733.
[91] Pule MA, Savoldo B, Myers GD, et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nat Med, 2008,14:1264–1270.
[92] Curran KJ, Pegram HJ, Brentjens RJ. Chimeric antigen receptors for T cell immunotherapy: current understanding and future directions. J Gene Med, 2012,14:405–415.
[93] Dudley ME, Yang JC, Sherry R, et al. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol, 2008,26:5233–5239.
[94] Hinrichs CS, Stevanovic S, Draper L, et al. HPV-targeted tumor-infiltrating lymphocytes for cervical cancer. J Clin Oncol, 2014,32:5s (suppl, abstr LBA3008).
[95] Parmar S, Ritchie DS. Allogeneic transplantation as anticancer immunotherapy. Curr Opin Immunol, 2014,27C:38–45.
[96] Galluzzi L, Senovilla L, Zitvogel L, et al. The secret ally: immunostimulation by anticancer drugs. Nat Rev Drug Discov, 2012,11:215–233.
[97] Berd D, Mastrangelo MJ. Active immunotherapy of human melanoma exploiting the immunopotentiating effects of cyclophosphamide. Cancer Invest, 1988,6:337–349.
[98] Suzuki E, Kapoor V, Jassar AS, et al. Gemcitabine selectively eliminates splenic Gr-1+/CD11b+ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. Clin Cancer Res, 2005,11:6713–6721.
[99] Liu WM, Fowler DW, Smith P, et al. Pre-treatment with chemotherapy can enhance the antigenicity and immunogenicity of tumours by promoting adaptive immune responses. Br J Cancer, 2010,102:115–123.
[100] Slovin SF, Higano CS, Hamid O, et al. Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study. Ann Oncol, 2013,24:1813–1821.
[101] Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: a paradigm shift. J Natl Cancer Inst, 2013,105:256–265.
[102] Turturro F. Denileukin diftitox: a biotherapeutic paradigm shift in the treatment of lymphoid-derived disorders. Expert Rev Anticancer Ther, 2007,7:11–17.
[103] Ko K, Yamazaki S, Nakamura K, et al. Treatment of advanced tumors with agonistic anti-GITR mAb and its effects on tumor-infiltrating Foxp3+CD25+CD4+ regulatory T cells. J Exp Med, 2005,202:885–891.
[104] Piconese S, Valzasina B, Colombo MP. OX40 triggering blocks suppression by regulatory T cells and facilitates tumor rejection. J Exp Med, 2008,205:825–839.
[105] Vanneman M, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. Nat Rev Cancer, 2012,12:237–251.