REVIEW

Novel Immunologic Approaches to Melanoma Treatment ☆

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Abstract Approaches to treating melanoma have changed radically since the introduction of immunotherapy, and survival figures are now higher than possible with earlier therapies. The immunomodulators currently available mainly block CTLA-4 (cytotoxic T lymphocyte-associated molecule-4) and PD-1 (programed cell death protein 1) translocated to the cell surface, where they inhibit the antitumor immune response. Treatments blocking these molecules are being more widely used. Research now seeks new molecular targets, the best combinations of available drugs, and biomarkers that can identify ideal candidates for each one
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Novedades en inmunología del melanoma

Resumen Desde la introducción de la inmunoterapia el tratamiento del melanoma ha sufrido una revolución, consiguiéndose cifras de supervivencia superiores a las que se alcanzaban con los tratamientos previos. Los fármacos inmunomoduladores disponibles actualmente van dirigidos principalmente frente a las moléculas de superficie CTLA-4 y PD-1, que ejercen un efecto inhibidor sobre la respuesta inmune antitumoral. Se trata de un tratamiento en pleno proceso de expansión, y la investigación se dirige hacia el descubrimiento de nuevas moléculas, las combinaciones de los fármacos disponibles o la identificación de biomarcadores que permitan seleccionar a los pacientes idóneos para cada terapia.
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Introduction

Melanoma is the eighth most common cancer. It accounts for approximately 5% of all new cancer cases, and incidence is rising steadily.[1,2] The incidence of cutaneous melanoma in the Spanish population is 8.7 cases per 100 000 population.[3]

Survival rates for early-stage melanoma are higher than 95%, but prognosis worsens considerably for disseminated forms, which have a 5-year-survival rate of less than 20%.[2] Chemotherapy is the most widely accepted treatment for advanced cutaneous melanoma, but response rates are lower than 15% and the impact on survival outcomes is minimal.[4]

The emergence of immunotherapy marked an important change in the search for alternative treatments. The association between cancer cells and the immune system has been recognized for decades, and cutaneous melanoma is probably one of the most immunogenic tumors that exist. The interaction between the immune system and melanoma is dynamic, however, and tumor cells use various mechanisms to avoid recognition and destruction by the immune system, favoring disease progression.[5]

Treatment strategies aimed at boosting the immune response through vaccines and biochemotherapy were attempted for several years, but the results were disappointing.[6,7] A more recent approach, however, that of using monoclonal antibodies directed against immune checkpoint inhibitors, has been met with enthusiasm.[8]

Evidence Supporting the Immune Response in Melanoma

In contrast to other cancers, numerous findings, both clinical and histological, support the involvement of the immune system in the fight against melanoma.[9]

An inflammatory infiltrate within and around the tumor, sometimes accompanied by tumor regression, is observed in the majority of melanomas. Signs of regression are observed in up to 35% of cases, and tumor regression is actually 6 times more common in melanoma than in other types of tumors.[10]

Another more recently described finding is a correlation between the density of high endothelial venules and lymphocytic infiltration in association with greater tumor regression.[11] An association has also been observed between tumor regression and melanomas with a lower mitotic or proliferative rate, i.e., melanomas with a better prognosis[12] (Fig. 1).

The appearance of a halo around nevi provides further evidence of autoimmune reactivity and this halo can eventually cause the nevus to disappear completely. Vitiligo has also been observed in 2% to 7% of advanced melanomas following systemic therapy and has been linked to a better prognosis.[13]

Although the above findings and observations only serve to generate hypotheses, numerous studies have demonstrated the participation of the immune system in regressive melanoma. Findings include predominant infiltration of tumors by T lymphocytes, which are mediators of the innate immune system,[14] and oligoclonal expansion of T lymphocytes in melanomas with extensive areas of regression.[15] Adoptive cell therapy with T lymphocytes isolated from regressive melanomas has also been show to generate cytolytic activity against autologous melanomas.[16]

Whatever the case, the development of melanoma implies a breakdown in the body's defense system. This immune system failure is mediated by both factors that are intrinsic to the tumor cells and impaired immune defenses, which reduce the ability of the immune system to exert its effector function.

Suboptimal activation of specific T lymphocytes due to weak or absent expression of antigens in melanoma cells is known to result in immune response failure.[17] The expression of endothelial adhesion molecules in the tumor is also reduced, and in addition, certain cells in the tumor infiltrate, such as macrophages, release growth factors, cytokines, and suppressors of immune response.[18,19]

The antitumor immune response alone thus is not sufficient to produce the elimination of tumor cells, but it can favor response to immunotherapy.

Antigens That Trigger an Immune Response

Two signals are needed for the activation of T lymphocytes. First, the T-cell receptor (TCR) recognizes the antigen presented by dendritic cells through the major histocompatibility complex, and second, the cluster of differentiation (CD) antigen 28 expressed on the surface of T lymphocytes produces a costimulatory signal by binding to its ligands B7-1 or B7-2. Without this second signal, the T lymphocytes would not respond to the stimulus, resulting in anergy.[20]

Activated T lymphocytes must then migrate to the site of the tumor, where they will act to destroy the tumor cells.[21] These intratumoral cells, however, often exhibit decreased proliferation, cytokine production, and cytolytic activity, although outside the tumor microenvironment, they have been shown to mount powerful specific responses, suggesting the existence of signals in this microenvironment that block lymphocyte function.[22]

The activation of TCR also produces signals that stimulate immune checkpoint inhibitors. Cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) are cell surface receptors involved in the regulation of activated T lymphocytes. They belong to the B7-CD28 superfamily and by binding to their ligands, they trigger inhibitory pathways resulting in reduced T-lymphocyte activity.[23]

Cytotoxic T-Lymphocyte Associated Antigen

Activated T lymphocytes express CTLA-4. This receptor is expressed approximately 24 to 48 hours after activation and it binds to B7 with a greater infinity than to CD28, providing an inhibitory signal to T lymphocytes.[24]

CTLA-4 is a powerful coinhibitor that has a role in the development of tolerance from early on. Studies of CTLA-1$^{-/-}$ mice have shown that the absence of CTLA-4 results in a massive infiltrate of autoreactive T lymphocytes in diverse tissues, with death occurring within 2 to 3 weeks[25,26] (Fig. 2).

PD-1 and PD Ligand 1

PD-1 is a transmembrane receptor of the CD28 family that is normally expressed in activated CD4$^+$ and CD8$^+$ lymphocytes.[27] It has an immunosuppressive function, which under normal conditions prevents an excessive immune response to autologous cells. PD-1 expression is induced by the activation of T lymphocytes and is interrupted when
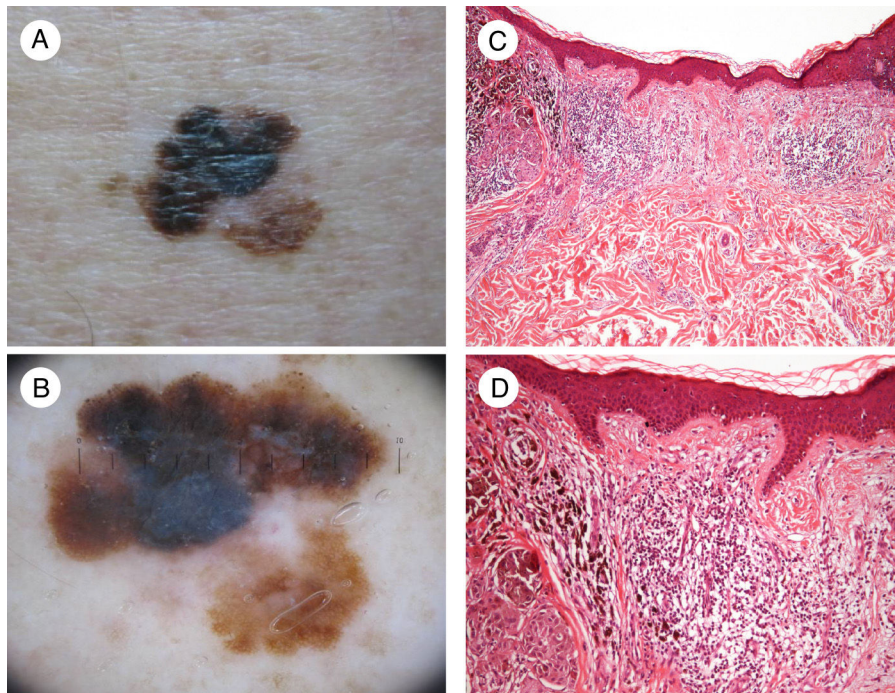
Figure 1   Image showing regression in melanoma. A, Clinical image showing a central region with loss of pigment. B, Dermoscopic features of the lesion showing a multicomponent pattern with a central white area. C and D, Histologic images showing a dense lymphocytic infiltrate in the dermis without epidermal involvement, together with melanophages and fibrosis in the dermis (hematoxylin-eosin, original magnification ×10 [C] and ×20 [D]).

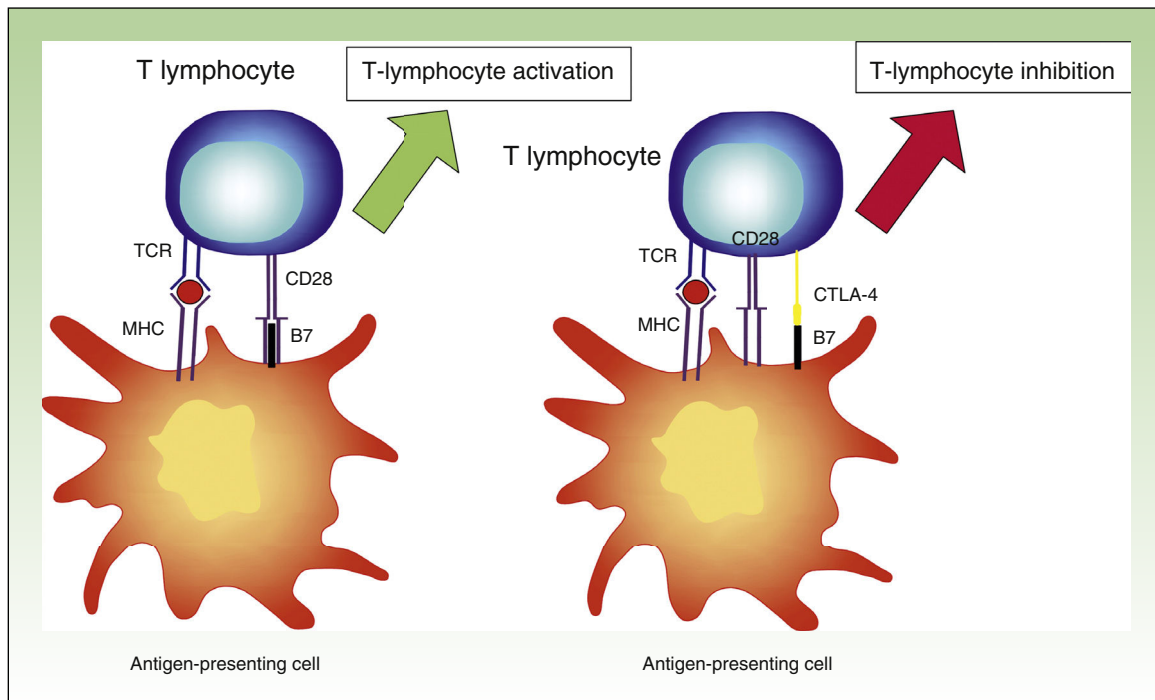**Figure 2**   Diagram showing T lymphocyte activation. Activation of T lymphocyte after interaction between TCR and HLA and between costimulatory molecules B7 and CD28 (left). Inhibition of response following binding of CTLA-4 to B7 (right).
TCR indicates T-cell receptor; CD28, cluster of differentiation 28; MHC, major histocompatibility complex; CTLA-4, cytoxic T-lymphocyte associated protein 4.
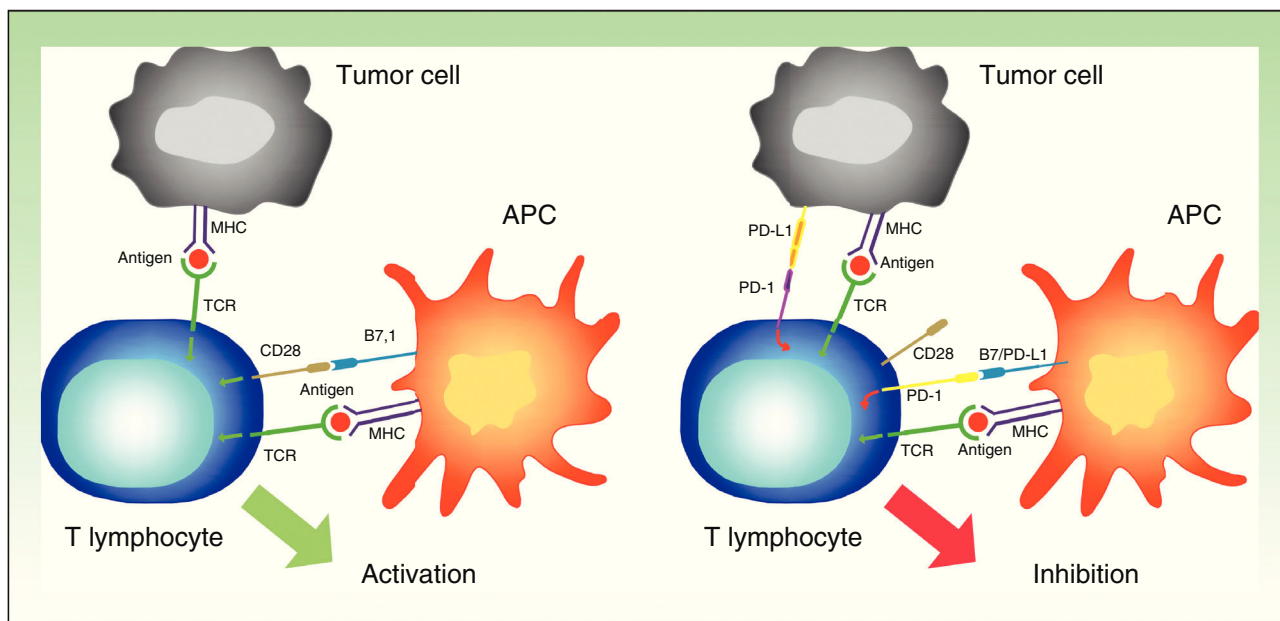
Figure 3   Diagram showing PD-1 and PD-L1 activity. Activation of T lymphocyte by interaction between stimulatory and costimulatory molecules (left). Inhibition of T cell following binding of PD-1 to PD-L1 (right).
APC indicates antigen-presenting cell; MHC, major histocompatibility complex; TCR, T-cell receptor; CD28, cluster of differentiation 28; PD-1, programmed death protein 1; PD-L1, programmed death protein ligand 1.

the antigen is eliminated by the immune response. If this response is inadequate, however, PD-1 expression persists, generating a phenotype of ineffective T lymphocytes.[28]

PD-1 has 2 ligands: PD-ligand 1 (PD-L1), also known as *B7-H1* and *CD274*, and PD-L2 (B7-DC or CD273). PD-L1 is expressed in more cells than PD-L2. When PD-L1 is expressed on the surface of tumor cells and binds to PD-1 on T lymphocytes, it activates a signaling cascade that suppresses TCR activation, thereby preventing the release of growth factors and survival signals.[29] Evidently, thus, binding of PD-1 expressed on the surface of T lymphocytes to its ligand has a key role in blocking the immune response (Fig. 3).

The Mechanism of Action of Immunomodulators

Numerous treatment strategies aimed at boosting the antitumor immune response have been developed since the 1980s, but they have had limited success.[16] Recent years, however, have witnessed the emergence of a new strategy based on the blockage of receptors that inhibit the antitumor immune response rather than on the direct reactivation of the immune system. The antibodies used in these treatments bind not to the tumor cells but to the lymphocytes in order to stimulate a response.

CTLA-4 Inhibition

T lymphocytes express CTLA-4 on their surface within 2 to 3 days of activation and as a consequence exhibit reduced activity.[30] In theory, thus, CTLA-4 blockade would allow specific T cells to maintain their activity.

The above theory was proven by the use of fully humanized monoclonal antibodies targeting CTLA-4, such as ipilimumab. By interfering with CTLA-4-B7 binding, the inhibitory stimulus disappears, and the lymphocytes maintain their activity.[31] While this interaction means that the immune system will continue to fight against the tumor cells, it also means that it will target nontumor cells (Fig. 4).

PD-1 and PD-L1 Blockade

PD-1 needs to bind to its ligands in order to exert its inhibitory effect. Most tissues, including tumor cells, express PD-L1. PD-L2, by contrast, is expressed only on dendritic cells, macrophages, mast cells, and B lymphocytes.[32] PD-L1 expression on tumor cells can be mediated by oncogenic processes secondary to activating mutations, or activated by interferon released by specific T lymphocytes infiltrating the tumor.[33,34] PD-L1 expression, however, can apparently only be induced if T lymphocytes expressing PD-1 exist. This phenomenon is known as *adaptive immune resistance*[35] (Fig. 5).

The above pathway can be inhibited by blocking PD-1, preventing it from binding to PD-L1 or PD-L2, or blocking PD-L1, impeding thus its interaction with PD-1.

Unlike CTLA-4-deficient mice, PD-1- and PD-L1-deficient mice do not die early, but rather progressively develop multiple autoimmune disorders.[36,37] This would explain the wide range of adverse effects observed in patients treated with immunomodulators.

Oncolytic Viruses

Oncolytic viral therapy is a new tool that has proven effective in the treatment of multiple solid tumors, including

Table 1 Commercial Data, Characteristics, and Administration Route for Currently Available Immunomodulatory Drugs.

Agent	Previous Names	Commercial Name	Pharmaceutical Company	Therapeutic Target	Type of Molecule	Administration Route	Dose
Ipilimumab	MDX-010 MDX-101	Yervoy	Bristol-Myers Squibb	CTLA-4	Humanized recombinant IgG1 antibody	Intravenous	3 mg/kg every 3 wk up to 4 doses
Nivolumab	MDX-1106, ONO-4538 BMS-936558	Opdivo	Bristol-Myers Squibb	PD-1	Fully humanized IgG4 antibody	Intravenous (for 60 min)	3 mg/kg every 2 wk
Pembrolizumab	MK-3475, Lambrolizumab	Keytruda	Merck	PD-1	Humanized IgG4 antibody	Intravenous	2 mg/kg every 3 wk
Pidilizumab	CT-011	–	Medivation	PD-1	Humanized IgG1 antibody	Intravenous	1.5 mg/kg
Atezolizumab	MPDL-3280A	–	Roche	PD-L1	Humanized IgG1 antibody	Intravenous	15 mg/kg every 3 wk
Talimogene laherparepvec	JS1/34.5-/47-/(GM-CSF); Onco-Vex^{GM-CSF}	Imlygic	Amgen	Tumor cells	Attenuated HSV-1	Subcutaneous	First visit: Vial containing 10^6 PFU/mL Subsequent visits: Vial containing 10^8 PFU/mL (maximum 4 mL/visit)

Abbreviations: CTLA-4, cytoxic T-lymphocyte associated protein 4; HSV-1, herpes simplex virus type 1; Ig, immunoglobulin; PD-1, programmed death protein 1; PFU, plaque-forming units; PD-L1, programmed death protein ligand 1

melanoma.[38] The best results to date have been obtained with oncolytic herpes simplex virus type 1 (HSV-1) vectors. HSV-1 is a strongly oncolytic genetically modified virus that selectively infects and replicates in tumor cells, but spares healthy cells.[39]

Immunomodulatory Drugs

Anti-CTLA-4 Antibodies

Ipilimumab
Ipilimumab was the first immunomodulatory drug with proven survival benefits in patients with metastatic melanoma.[31,40] It was approved by the US Food and Drug Administration (FDA) in 2011 and by the European Medicines Agency (EMA) just a few months later[41] (Table 1).

A randomized controlled trial comparing ipilimumab and dacarbazine showed longer overall survival for patients treated with ipilimumab.[42] Median survival was just 9.5 months, but what was remarkable was that 22% of patients survived long term.[43]

Because of its mechanism of action, ipilimumab is associated with a large number of adverse effects, most of which are of an autoimmune nature. Adverse effects have been reported in approximately 60% of patients treated

with this drug.[44] The most common effects are diarrhea, colitis, dermatitis, hypophysitis, and thyroiditis. Just 10% to 15% of adverse effects are moderate or severe (grade 3 or 4) and they tend to resolve within 4 to 6 weeks with corticosteroids or in a relatively short time with other immunosuppressants[45] (Table 2).

Pseudoprogression is a common phenomenon following the introduction of immunomodulatory drugs. It is due to the activation of T lymphocytes and the release of cytokines, which produce chemotaxis of inflammatory mediators, edema, and even local necrosis, causing a temporary increase in tumor volume.[44] Pseudoprogression is more common in melanoma than in other tumors, and it is something that clinicians need to be familiar with in order not to interrupt treatment in patients who could ultimately benefit from immunotherapy, despite the initial increase in tumor size.

Pseudoprogression is also a limiting factor for the use of immunomodulatory drugs in patients with advanced metastatic disease in high-risk locations, such as metastases to the central nervous system. An increase in tumor volume, albeit temporary, would be life-threatening in such cases.

Tremelimumab
Tremelimumab is an IgG2 anti-CTLA-4 antibody. Despite the successful results reported in early-phase trials, the phase III trial showed no significant differences between
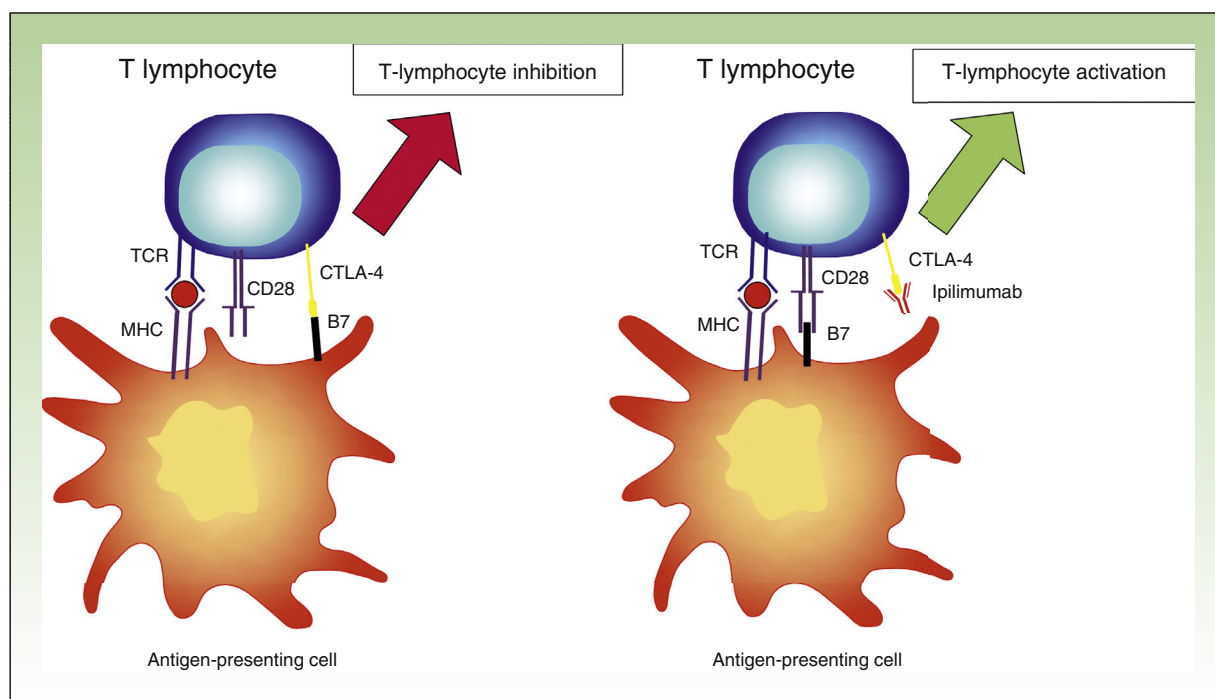
Figure 4   Diagram showing activity of ipilimumab. T-lymphocyte inhibition through binding between CTLA-4 and B7 (left). Activation of T lymphocyte through CTLA-4 blockade following binding between CTLA-4 and the monoclonal antibody ipilimumab. TCR indicates T-cell receptor; CD28, cluster of differentiation 28; MHC, major histocompatibility complex; CTLA-4, cytoxic T-lymphocyte associated protein 4.

Table 2   Classification of Adverse Effects Associated With Pharmacological Treatment Based on the Common Terminology Criteria for Adverse Events (Version 4.0) Issued by the National Cancer Institute.

Grade	Category	Description
I	Mild	Asymptomatic or mild symptoms. Intervention not indicated.
II	Moderate	Symptomatic, limits instrumental activities of daily living.[a] Local or noninvasive intervention indicated.
III	Severe	Not immediately life-threatening but limits self-care activities of daily living.[b] Hospitalization indicated.
IV	Very Severe	Life-threatening adverse effects. Urgent intervention indicated.
V	Death	Death related to administration of drug.

Source: National Cancer Institute.[45]
 [a] Preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
 [b] Bathing, dressing and undressing, feeding self, using the toilet, taking medication, not bedridden.

tremelimumab and chemotherapy, and the drug was therefore not approved for use in advanced melanoma.[46]

Anti-PD-1 Antibodies

Nivolumab
Nivolumab was the first anti-PD-1 antibody that showed good tolerance and significant response in several patients with solid tumors.[47] It was approved for use in Spain at the end of 2015, and in February of the following year, was made available for patients with unresectable or metastatic melanoma, regardless of *BRAF* mutation status. It is a monoclonal antibody with high affinity for PD-1. It blocks interactions between this receptor and PD-L1 and PD-L2, increasing specific T lymphocyte proliferation and cytokine production.[47,48]

The results of the first phase III trial showed nivolumab to be superior to chemotherapy, and this superiority was confirmed in later trials[48,49] (Table 3). The main treatment-related adverse effects are fatigue, diarrhea, nausea, anorexia, and fever. Practically all the adverse effects resolve spontaneously or with the administration of systemic corticosteroids. Autoimmune-type effects include cutaneous effects (exanthema, pruritus, vitiligo), gastrointestinal effects (diarrhea, colitis), elevated liver enzymes, hypothyroidism or hyperthyroidism, and pneumonitis.[51] Although adverse effects are relatively uncommon, careful monitoring of patients, together with initiation of treatment where necessary, is essential.

Pembrolizumab
Pembrolizumab is the monoclonal antibody with the highest affinity for PD-1. The results from phase I and II

Table 3 Diagram Summarizing Some of the Most Significant Clinical Trials and Their Results for Response Rates, Survival, and Adverse Effects.

Study[a]	Drug	Phase	Population	Patients, No.	Arms	Response Rates	DFS, mo	Adverse Effects (Grade ≥3)
Hodi et al. (2010)[40]	Ipilimumab	III	Previously treated advanced melanoma	676	Ipilimumab + vaccine gp100 Ipilimumab Vaccine gp100	Combination: 5% Ipilimumab: 10.9% Vaccine gp100: 1.5%	DFS combination: 10 DFS ipilimumab: 10.1 DFS vaccine gp100: 6.4	Ipilimumab and combination: 10%-15% Vaccine gp100: 3%
Topalian et al. (2012)[48]	Nivolumab	I	Advanced melanoma	104	Nivolumab as monotherapy	28%	DFS > 11.1	14%
Robert et al. (2015)[49]	Nivolumab	III	Previously untreated advanced melanoma BRAF-WT	418	Nivolumab DCZ	Nivolumab: 40% DCZ: 13.9%	DFS: 5.1	Nivolumab: 11.7% DCZ: 17.6%
Weber et al. (2015)[50] [Checkmate-037]	Nivolumab	III	Advanced melanoma after ipilimumab or anti-BRAF	405	Nivolumab QT DCZ Paclitaxel	Nivolumab: 31.7% QT: 10.6%	DFS Nivolumab: 4.7 DFS QT: 4.2	Nivolumab: 9% QT: 31%
Hamid et al. (2013)[52]	Pembrolizumab	I	Advanced melanoma	135	Pembrolizumab as monotherapy	38%	DFS > 7	13%
Ribas et al. (2015)[55] [Keynote-002]	Pembrolizumab	II	Advanced melanoma in patients resistant to ipilimumab	540	Pembrolizumab 0.2 mg/kg 0.10 mg/kg QT	Pembrolizumab: 21% (25% 10 mg/kg) QT: 4%	DFS pembrolizumab: 5.4 (5.8 in 10 mg/kg arm) DFS QT: 3.6	Pembrolizumab: 11% (14% 10 mg/kg) QT: 26%
Robert et al. (2015)[56] [Keynote-006]	Pembrolizumab	III	Advanced melanoma	834	Pembrolizumab Ipilimumab	Pembrolizumab: 33% Ipilimumab: 12%	DFS pembrolizumab: 5.5 DFS ipilimumab: 2.8	Pembrolizumab: 10% Ipilimumab: 20%
Andtbacka et al. (2015)[64]	T-VEC	III	Advanced melanoma	436	Intralesional T-VEC Subcutaneous GM-CSF	T-VEC: 26% GM-CSF: 5.7	DSF T-VEC: 8.2 DFS GM-CSF: 2.9	T-VEC: 36% (2% grade > 3) GM-CSF: 21%

Table 3 (*Continued*)

Study[a]	Drug	Phase	Population	Patients, No.	Arms	Response Rates	DFS, mo	Adverse Effects (Grade ≥3)
Wolchok et al. (2013)[67]	Ipilimumab + nivolumab	I	Advanced melanoma	53	Ipilimumab + nivolumab Sequential treatment	Combination: 40% Sequential: 20%	DFS combination > 5 DFS sequential > 2	Combination: 53% Sequential: 18%
Postow et al. (2015)[68]	Ipilimumab + nivolumab	I	Previously untreated advanced melanoma	142	Ipilimumab + nivolumab Ipilimumab only	Combination: 60% (22% complete response) Ipilimumab: 11% (partial response)	DFS combination > 11 (study cutoff) BRAF-WT/8.5 BRAF+ DFS ipilimumab: 4.4 for BRAF-WT/2.7 for BRAF+	Combination: 54% Ipilimumab: 24%
Larkin et al. (2015)[69]	Ipilimumab + nivolumab	III	Previously untreated advanced melanoma	945	Ipilimumab + nivolumab Ipilimumab Nivolumab	Combination: 57.6% Ipilimumab: 19% Nivolumab: 43.7%	DFS combination: 11.9 DFS ipilimumab: 2.9 DFS Nivolumab: 6.9	Combination: 55% Ipilimumab: 27.3% Nivolumab: 16.3%

Abbreviations: BRAF+, patients with *BRAF* mutation; BRAF-WT, patients without *BRAF* mutation (wild-type); DCZ, dacarbazine; DFS, disease-free survival; T-VEC, talimogene laherparepvec; QT, chemotherapy.

[a] The name of the study is shown between parentheses under the name of the authors.
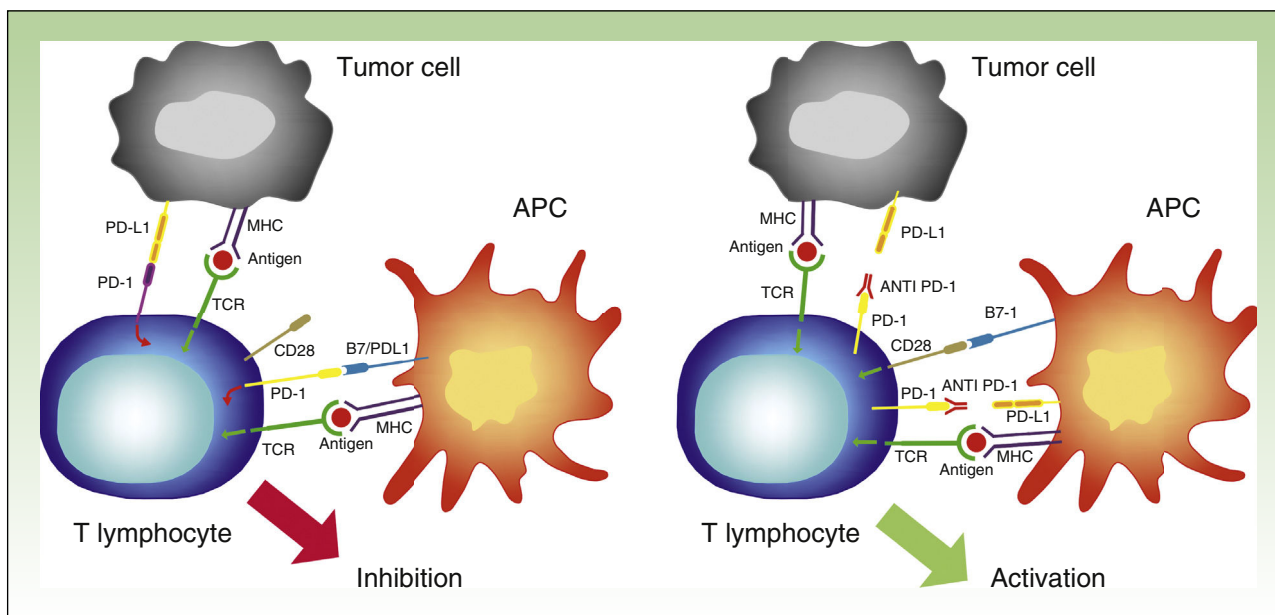
Figure 5   Interaction between PD-1 and PD-L1 leading to T-lymphocyte inhibition (left). Binding between anti PD-1 drugs such as nivolumab or pembrolizumab to PD-1 on the surface of the lymphocytes, resulting in their activation and hence the generation of the antitumor response (right).
APC indicates antigen-presenting cell; MHC, major histocompatibility complex; TCR, T-cell receptor; CD28, cluster of differentiation 28; PD-1, programmed death protein 1; PD-L1, programmed death protein ligand 1.

trials led the authorities to grant accelerated approval for the use of this drug in patients with advanced melanoma who did not respond to ipilimumab.[52,53] Pembrolizumab is associated with better responses and survival rates than either chemotherapy or ipilimumab.[54–56] The most common adverse effects are asthenia, skin rash, pruritus, and diarrhea[52,55] (Table 3).

Both nivolumab and pembrolizumab were approved for use in advanced (unresectable or metastatic) melanoma by the Spanish Agency for Medicines and Medical Devices (AEMPS) in January 2016.[57,58]

Another anti-PD-1 antibody, pidilizumab, was found to be inferior to both nivolumab and pembrolizumab and was not granted approval.[59]

Anti PD-L1 Antibodies

Atezolizumab
The first results reported for atezolizumab in melanoma showed response rates of up to 29%. When combined with vemurafenib, however, an objective response rate (for complete and partial responses) of 76% was observed.[60]

BMS-936559
BMS-936559 is a fully humanized IgG4 anti-PD-L1 antibody. Preliminary studies have shown response rates of over 17% and a low rate of adverse effects (9%).[61]

MED14736
MED14736 is a fully humanized IgG1 anti-PD-L1 antibody. It is currently being investigated in a combined study together with dabrafenib and trametinib. Provisional results have

shown response rates of over 65% and severe adverse effects in 35% to 40% of patients.[62]

Oncolytic Viruses

Talimogene Laherparepvec
Talimogene laherparepvec was approved for use in patients with unresectable melanoma and regional and distance metastases (stages IIIB, IIIC, and IVM1a) without visceral involvement at the end of 2015.[63] It is an attenuated HSV-I vector produced by genetic recombination, designed to specifically replicate inside tumor cells and produce human granulocyte-macrophage colony stimulating factor (GM-CSF) with the aim of stimulating the systemic antitumor response.

It is administered subcutaneously and must be injected directly into cutaneous melanoma lesions. In patients with multiple lesions, the largest lesions must be treated first

Treatment with talimogene laherparepvec has shown response rates of 26%, together a favorable adverse effect profile and increased in long-term survival.[64] Compared with ipilimumab and vemurafenib, talimogene laherparepvec showed comparable or slightly superior overall survival outcomes, particularly in patients with visceral metastasis.[65]

Drug Combinations

CTLA-4 and PD-1 exert an inhibitory function through different pathways, and therefore blockade of both receptors should favor the destruction of tumor cells by the immune system. This theory was first proven in animal models[66] and later in humans. To date, randomized clinical trials have investigated the combined use of nivolumab and ipilimumab

and shown higher response rates than those achieved with either treatment alone.[67–69] Nonetheless, over half of the patients treated developed serious adverse effects. The most common serious effects were colitis, diarrhea, and elevated liver enzymes, and most cases required treatment with systemic corticosteroids.[68,69]

The combined use of nivolumab and ipilimumab was approved by the FDA in September 2015 and is currently pending approval by the EMA.

Biomarkers of Response to Immunotherapy

Following the success of immunotherapy, research has now turned to the search for biomarkers capable of identifying patients with a greater chance of responding to treatment.

Expression of PD-L1 in pretreatment tumor samples has shown the clearest association with response to date, with an increased response to anti PD-1 and anti PD-L1 antibodies observed in PD-L1-positive patients.[34,70,71] No association has been found between PD-L1 expression and response to ipilimumab and nivolumab combination therapy.[68]

PD-L1 has several disadvantages in terms of its potential as a biomarker. Its expression is highly variable, even in samples from the same patient, and in addition, although greater clinical benefits have been observed in PD-L1-positive patients, a considerable number of PD-L1-negative patients also respond to treatment.[60,70]

Interferon released by CD8+ cells infiltrating the tumor margin and parenchyma is another potential confounder, as it induces PD-L1 expression in tumor cells (adaptive immune response). PD-L1 expression is thus an indirect marker of antigen-specific T lymphocyte activation. Tumors with higher levels of PD-L1 and PD-1 respond better to treatment, but specific CD8+ lymphocytes must first be present.[72]

Generally speaking, the best biomarker currently available for predicting response to any immunomodulatory drug is the presence of CD8+ at the tumor margin.[72]

Future Prospects

Future combinations

Multiple trials are underway investigating combinations of immunomodulatory drugs and other treatments for use in patients with advanced melanoma.

Both CTLA-4 and PD-1 inhibit immune response through different pathways, and it is therefore essential to consider their synergistic use. In the case of targeted therapies and oncolytic viral therapy, the antigens released as a result of the death of tumor cells are presented to the lymphocytes by antigen-presenting cells, which also release certain cytokines and chemokines. These events all induce an antitumor immune response, and in this sense anti-BRAF drugs, oncolytic viruses, and peptidic vaccines could favor response to immunomodulatory drugs.[73]

Adjuvant Therapy

Preventive treatment in patients with a greater risk of metastasis is one of the key challenges in the management of melanoma. The options currently used to control locally advanced tumors are surgery, sentinel lymph node biopsy, and interferon α. Nonetheless, the possibility of effectively treating these patients with other agents could change the course of disease, as well as management approaches by dermatologists.

In October 2015, the FDA, following the results of a clinical trial, approved ipilimumab for use as adjuvant therapy in patients with locally advanced melanoma, i.e., in patients with stage III melanoma, lymph node metastases > 1 mm, and complete lymph node dissection.[74] The trial showed a 5-year disease-free survival rate of 40.8% versus 30.3% for the placebo group.[75] Nevertheless, 45% of patients experienced grade 3 or 4 adverse effects, although the severity of these effects can be considerably reduced through adequate management. The most common adverse effects associated with ipilimumab are diarrhea, colitis, endocrine effects (hypophysitis with hypopituitarism, hypothyroidism or hyperthyroidism, and adrenal dysfunction), vitiligo, exanthema, pruritus, and fatigue.

Multiple studies are currently investigating the use of immunomodulatory drugs other than ipilimumab as adjuvant therapy for patients with high-risk tumors following complete excision, and their results will become available in the coming years.[76]

Conclusions

Treatment of melanoma has changed significantly since the introduction of immunotherapy scarcely 5 years ago, and survival rates are much higher than those obtained with previous treatments.

Nevertheless, the discovery of any new treatment necessitates new studies to better understand the underlying mechanisms of action. Research in the field of immunotherapy and melanoma should focus on identifying criteria for selecting the most suitable candidates for treatment and exploring which drugs or combinations of drugs produce the best responses. The effectiveness of immunomodulatory drugs could be even further enhanced by combining their use with other approaches, such as targeted therapy and oncolytic viral therapy.

In conclusion, boosting the immune response is a key factor in the treatment of melanoma and will probably form part of all treatment regimens yet to come.

Ethical Disclosures

Protection of humans and animals. The authors declare that no tests were carried out in humans or animals for the purpose of this study.

Confidentiality of data. The authors declare that they have followed their hospital's protocol on the publication of data concerning patients.

Right to privacy and informed consent. The authors declare that no private patient data appear in this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

References

1. American Cancer Society. Cancer Statistics Center (cited 20 Nov 2016). Available from: http://cancerstatisticscenter.cancer.org. LASR.

2. Guy G, Thompson T, Watson M, Massetti GM, Richardson LC. Vital signs: melanoma incidence and mortality trends and projections-United States, 1982-2030. Centers for Disease Control and Prevention (CDC). MMWR Morb Mortal Wkly Rep. 2015;64:591–6.

3. Tejera-Vaquerizo A, Descalzo-Gallego MA, Otero-Rivas MM, Posada-García C, Rodriguez-Pazos L, Pastushenko I, et al. Skin cancer incidence and mortality in Spain: A systematic review and meta-analysis. Actas Dermosifiliogr. 2016;107:318–28.

4. Middleton MR, Grob JJ, Aaronson N, Fierlbeck G, Tilgen W, Seiter S, et al. Randomized phase III study of temozolomide versus dacarbazine in the treatment of patients with advanced metastatic malignant melanoma. J Clin Oncol. 2000;18:158–66.

5. Hoenicke L, Zender L. Immune surveillance of senescent cells-biological significance in cancer- and non-cancer pathologies. Carcinogenesis. 2012;33:1123–6.

6. Legha SS, Ring S, Eton O, Bedikian A, Buzaid AC, Plager C, et al. Development of a biochemotherapy regimen with concurrent administration of cisplatin, vinblastine, dacarbazine, interferon alfa, and interleukin-2 for patients with metastatic melanoma. J Clin Oncol. 1998;16:1752–9.

7. Eton O, Legha SS, Bedikian AY, Lee JJ, Buzaid AC, Hodges C, et al. Sequential biochemotherapy versus chemotherapy for metastatic melanoma: Results from a phase III randomized trial. J Clin Oncol. 2002;20:2045–52.

8. Wieder T, Brenner E, Braumüller H, Röcken M. Immunotherapy of melanoma: Efficacy and mode of action. J Dtsch Dermatol Ges. 2016;14:28–36.

9. Finn OJ. Cancer immunology. N Engl J Med. 2008;358:2704–15.

10. High WA, Stewart D, Wilbers CRH, Cockerell CJ, Hoang MP, Fitzpatrick JE. Completely regressed primary cutaneous malignant melanoma with nodal and/or visceral metastases: A report of 5 cases and assessment of the literature and diagnostic criteria. J Am Acad Dermatol. 2005;53:89–100.

11. Avram G, Sánchez-Sendra B, Martín JM, Terrádez L, Ramos D, Monteagudo C. The density and type of MECA-79-positive high endothelial venules correlate with lymphocytic infiltration and tumour regression in primary cutaneous melanoma. Histopathology. 2013;63:852–61.

12. Shen S, Wolfe R, McLean CA, Haskett M, Kelly JW. Characteristics and associations of high-mitotic-rate melanoma. JAMA Dermatol. 2014;150:1048–55.

13. Teulings H-E, Limpens J, Jansen SN, Zwinderman AH, Retisma JB, Spuls PL, et al. Vitiligo-like depigmentation in patients with stage III-IV melanoma receiving immunotherapy and its association with survival: a systematic review and meta-analysis. J Clin Oncol. 2015;33:773–81.

14. Mackensen A, Ferradini L, Carcelain G, Triebel F, Faurel F, Viel S, et al. Evidence for in situ amplification of cytotoxic T-lymphocytes with antitumor activity in a human regressive melanoma. Cancer Res. 1993;53:3569–73.

15. Ferradini L, Mackensen A, Genevée C, Bosq J, Duvillard P, Avril MF, et al. Analysis of T cell receptor variability in tumor-infiltrating lymphocytes from a human regressive melanoma. Evidence for in situ T cell clonal expansion. J Clin Invest. 1993;91:1183–90.

16. Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: A clinical path to effective cancer immunotherapy. Nat Rev Cancer. 2008;8:299–308.

17. Marincola FM, Jaffee EM, Hicklin DJ, Ferrone S. Escape of human solid tumors from T-cell recognition: Molecular mechanisms and functional significance. Adv Immunol. 2000;74:181–273.

18. Anichini A, Vegetti C, Mortarini R. The paradox of T-cell-mediated antitumor immunity in spite of poor clinical outcome in human melanoma. Cancer Immunol Immunother. 2004;53:855–64.

19. Allavena P, Sica A, Solinas G, Porta C, Mantovani A. The inflammatory micro-environment in tumor progression: The role of tumor-associated macrophages. Crit Rev Oncol Hematol. 2008;66:1–9.

20. Smith-Garvin JE, Koretzky GAJM. T cell activation. Annu Rev Immunol. 2009;27:591–619.

21. Mahoney KM, Freeman GJ, McDermott DF. The next immune-checkpoint inhibitors: PD-1/PD-L1 blockade in melanoma. Clin Ther. 2015;37:764–82.

22. Harlin H, Kuna TV, Peterson AC, Meng Y, Gajewski TF. Tumor progression despite massive influx of activated CD8(+) T cells in a patient with malignant melanoma ascites. Cancer Immunol Immunother. 2006;55:1185–97.

23. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. Annu Rev Immunol. 2005;23:515–48.

24. Perkins D, Wang Z, Donovan C, He H, Mark D, Guan G, et al. Regulation of CTLA-4 expression during T cell activation. J Immunol. 1996;156:4154–9.

25. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity. 1995;3: 541–7.

26. McCoy KD, Le Gros G. The role of CTLA-4 in the regulation of T cell immune responses. Immunol Cell Biol. 1999;77:1–10.

27. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. 1992;11: 3887–95.

28. Vibhakar R, Juan G, Traganos F, Darzynkiewicz Z, Finger LR. Activation-induced expression of human programmed death-1 gene in T-lymphocytes. Exp Cell Res. 1997;232:25–8.

29. Freeman GJ, Long a J, Iwai Y, Bourke A, Chernova T, Nishimura H, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med. 2000;192:1027–34.

30. Lindsten T, Lee KP, Harris ES, Petrinyak B, Craighead N, Reynolds PJ, et al. Characterization of CTLA-4 structure and expression on human T cells. J Immunol. 1993;151:3489–99.

31. Phan GQ, Yang JC, Sherry RM, Hwo P, Topalian SL, Schwatzentruber DJ, et al. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc Natl Acad Sci U S A. 2003;100:8372–7.

32. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. Curr Opin Immunol. 2012;24:207–12.

33. Parsa AT, Waldron JS, Panner A, Crane CA, Parney IF, Barry JJ, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat Med. 2007;13:84–8.

34. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, et al. Association of PD-1, PD-1 Ligands, and other features of the

tumor immune microenvironment with response to anti-PD-1 therapy. Clin Cancer Res. 2014;20:5064–74.

35. Ribas A. Adaptive immune resistance: How cancer protects from immune attack. Cancer Discov. 2015;5:915–9.

36. Okazaki T, Tanaka Y, Nishio R, Mitsuiye T, Mizoguchi A, Wang J, et al. Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1 deficient mice. Nat Med. 2003;9:1477–83.

37. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity. 1999;11:141–51.

38. Freeman AI, Zakay-Rones Z, Gomori JM, Linetsky E, Rasooli L, Greenbaum E, et al. Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme. Mol Ther. 2006;13:221–8.

39. Hu JCC, Coffin RS, Davis CJ, Graham NJ, Groves N, Guest PJ, et al. A phase I study of OncoVEXGM-CSF, a second-generation oncolytic herpes simplex virus expressing granulocyte macrophage colony-stimulating factor. Clin Cancer Res. 2006;12:6737–47.

40. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363:711–23.

41. About the center for drug evaluation and research. Ipilimumab. U.S. Food and Drug Administration. 27 Nov 2015 [cited 12 May 2016]. Available from: http://www.fda.gov/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm248478.htm.

42. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011;364:2517–26.

43. Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, et al. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. J Clin Oncol. 2015;33:1889–94.

44. Chiou VL, Burotto M. Pseudoprogression and immune-related response in solid tumors. J Clin Oncol. 2015;33:3541–3.

45. National Cancer Institute. Common terminology criteria for adverse events. Version 4.9. 20 mayo, 2009 [cited 10 May2016]. Available from: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5(7.pdf.

46. Ribas A, Kefford R, Marshall MA, Punt CJ, Haanen JB, Marmol M, et al. Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. J Clin Oncol. 2013;31:616–22.

47. Brahmer JR, Drake CG, Wollner I, Powder JD, Picus J, Sharfman WH, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: Safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol. 2010;28:3167–75.

48. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366:2443–54.

49. Robert C, Long GV, Brady B, Dutiraux C, Mario M, Mortier L, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015;372:320–30.

50. Weber JS, Kudchadkar RR, Gibney GT, DeConti RC, Yu B, Wang W, et al. Phase I/II trial of PD-1 antibody nivolumab with peptide vaccine in patients naive to or that failed ipilimumab. ASCO Meet Abstr. 2013;31 15 Suppl:9011.

51. Larkin J, Lao CD, Urba WJ, McDermott DF, Horak C, Jiang J, et al. Efficacy and safety of nivolumab in patients with BRAF V600 mutant and BRAF wild-type advanced melanoma: A pooled analysis of 4 clinical trials. JAMA Oncol. 2015;1:433–40.

52. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med. 2013;369:134–44.

53. Robert C, Ribas A, Wolchok JD, Hodi FS, Hamid O, Kefford R, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: A randomised dose-comparison cohort of a phase 1 trial. Lancet (London, England). 2014;384:1109–17.

54. Ribas A, Hamid O, Daud A, Hodi FS, Hamid O, Kefford R, et al. Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. JAMA. 2016;315:1600.

55. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): A randomised, controlled, phase 2 trial. Lancet Oncol. 2015;16:908–18.

56. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus ipilimumab in advanced melanoma. N Engl J Med. 2015;372:2521–32.

57. Agencia española de medicamentos y productos sanitarios. Nuevos medicamentos: nivolumab, 19 de junio de 2015 [cited 10 May 2016]. Available from: http://www.aemps.gob.es/medicamentosUsoHumano/informesPublicos/docs/IPT-nivolumab-Opdivo.pdf.

58. Agencia española de medicamentos y productos sanitarios. Nuevos medicamentos: pembrolizumab, 19 de junio de 2015 [cited 10 May 2016]. Available from: http://www.aemps.gob.es/medicamentosUsoHumano/informesPublicos/docs/IPT-pembrolizumab-Keytruda.pdf.

59. Atkins MB, Kudchadkar RR, Sznol M, et al. Phase 2 multicenter, safety and efficacy study of pidilizumab in patients with metastatic melanoma. J Clin Oncol. 2014;32 (suppl;abstr 9001).

60. Hamid O. Preliminary clinical safety, tolerability and activity of atezolizumab (anti-PD-L1) combined with Zelboraf in BRAFv600 metastatic melanoma. Pigment Cell Melanoma Res. 2015;28:778.

61. Brahmer JR, Tykodi SS, Chow LQM, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366:2455–65.

62. Ribas A, Butler M, Lutzky J, Lawrence DP, Robert C, Miller W, et al. Phase I study combining anti-PD-L1 (MEDI4736) with BRAF (dabrafenib) and/or MEK (trametinib) inhibitors in advanced melanoma. ASCO Meet Abstr. 2015;33 15 Suppl:3003.

63. About the center for drug evaluation and research. Imlygic. U.S. Food and Drug Administration, 27 Oct 2015 [cited 12 May 2016]. Available from: http://www.fda.gov/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/ucm469411.htm.

64. Andtbacka RHI, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. J Clin Oncol. 2015;33:2780–8.

65. Quinn C, Ma Q, Kudlac A, Palmer S, Barber B, Zhao Z. Indirect treatment comparison of talimogene laherparepvec compared with ipilimumab and vemurafenib for the treatment of patients with metastatic melanoma. Adv Ther. 2016;33:643–57.

66. Curran MA, Montalvo W, Yagita H, Allison JP. 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;107:4275–80.

67. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369:122–33.

68. Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, et al. Nivolumab and ipilimumab ver-

sus ipilimumab in untreated melanoma. N Engl J Med. 2015;372:2006–17.

69. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015;373:23–34.

70. Ribas A, Hodi FS, Kefford R, Hamid O, Daud A, Wolchok JD, et al. Efficacy and safety of the anti-PD-1 monoclonal antibody MK-3475 in 411 patients (pts) with melanoma (MEL). ASCO Meet Abstr. 2014.

71. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515:563–7.

72. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2015;515:568–71.

73. Sumimoto H, Imabayashi F, Iwata T, Kawakami Y. The BRAF-MAPK signaling pathway is essential for cancer-immune evasion in human melanoma cells. J Exp Med. 2006;203:1651–6.

74. Eggermont AMM, Chiarion-sileni V, Grob J, Dummer R, Wolchock JD, Schmidt H, et al. Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): A randomised, double-blind, phase 3 trial. 2015;2045(Eortc 18071):1-9.

75. Eggermont AMM, Chiarion-Sileni V, Grob J-J, Dummer R, Wolchok JD, Schmidt H, et al. Prolonged survival in stage III melanoma with ipilimumab adjuvant therapy. N Engl J Med. 2016;375:1845–55.

76. Adjuvant immunotherapy melanoma (cited 15 Jan 2017). Available from: https://clinicaltrials.gov/.