# Checkpoint inhibitors in the treatment of cutaneous malignant melanoma

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> **Abstract:** Advanced malignant melanoma has historically been considered a uniformly lethal disease. Recent scientific strides have led to unprecedented understanding of both the molecular alterations and the mechanisms of immune evasion in this malignancy. The realization that an intense and dynamic interplay of stimulatory and inhibitory signals occurs in the "immune synapses" among T cells, tumor cells and dendritic cells, led to the development and subsequent clinical testing of agonist and antagonist monoclonal antibodies (mAb) that can modulate these signals. The resulting positive outcomes of the clinical trials utilizing CTLA-4, PD-1 and PD-L1 modulating drugs, has catapulted the field of immunotherapy into the realm of standard treatment. In this article we review the most important agents and clinical data feeding the ongoing paradigm change in the treatment of advanced melanoma

> **Keywords:** Melanoma; immunotherapy checkpoint modulation; cytotoxic T lymphocyte antigen-4 (CTLA-4); PD-1 PDL1

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### **Historical perspective**

Much has changed in the perennially limited panoply of options to treat malignant melanoma. Once touted as "the cancer that gives cancer a bad name", recent therapeutic advances have had such impact that melanoma treatment is now regarded as paradigm changing. These successes have occurred primarily in the areas of molecularly targeted therapeutics and immunotherapy. Molecularly targeted therapy of cutaneous melanoma is reviewed elsewhere in this issue. In this review, we will focus on the contemporary understanding of the mechanisms that mediate the T-cell immune response to tumors, the so-called immune checkpoints, and on the therapeutic applications resulting from this new knowledge.

Historically, immunotherapy has been extensively used to treat advanced melanoma; systemic treatment with cytokines and the use of a variety of tumor vaccines have been tested over the years. Nevertheless, until recently the only approved immunotherapy regimen for advanced melanoma was high-dose interleukin-2 (IL-2), based on a metaanalysis of treated patients with metastatic melanoma from 8 separate trials, with an objective response rate (ORR) of 16% and a complete response (CR) seen in 6% of patients, with 4% demonstrating very long-term durable remission, a small subset of patients likely cured of melanoma (1). Interferon alpha (IFN-alpha) has demonstrated modest antitumor activity in metastatic melanoma, although this agent is primarily used in the adjuvant setting, where it is approved unmodified as well as a pegylated product (2,3).

Biochemotherapy or chemoimmunotherapy have been used to treat advanced, metastatic melanoma. Various regimens have been tested, often combining drugs such as dacarbazine, cisplatin and vinblastine with IL-2 and IFN. While initial reports from single institution suggested high response rates and prolonged survival, large randomized trials have failed to confirm a survival benefit (4-8).

Adoptive immunotherapy for melanoma refers to the infusion of lymphocytes that have been manipulated to promote reactivity against tumor cells. These tumorinfiltrating lymphocytes (TIL) are generated ex vivo from the patient's tumor cells. Results in patients treated with prior lymphodepletion have been impressive, with response rates of 50% or higher being reported. However, the generation of TIL cells is cumbersome, time-consuming and dependent on the availability of viable tumor cells, the latter frequently failing to grow in culture. Recent strategies to eliminate these impediments have focused on the genetic engineering of patient's T-cells with retroviral vector insertion of the alpha and beta chains of the T-cell receptor (TCR) of highly reactive T-lymphocytes previously selected for mediating in vivo tumor regression (9,10). Another recent strategy is the infusion of expanded peptide-specific CD4+ T-cells co-cultured with a cancer-testis antigen (NY-ESO-1) peptide-pulsed autologous mononuclear cells. Infusion of a clonal population of these CD4+ T-cells has resulted in complete regression of metastatic melanoma, even when a significant percentage of the tumor cells did not express NY-ESO-1, presumably through the mechanism known as antigen spreading (11).

Therapeutic vaccines have been utilized to treat melanoma for several decades. There are many possible vaccination strategies. Autologous or allogeneic intact tumor cells or antigen-supplemented tumor cells have been frequently utilized. Defined antigen vaccines include purified peptides, proteins, gangliosides and anti-idiotypes. Genetic manipulation of tumor cells, viruses, or dendritic cells transfected with cytokines or with antigen genes also constitute a major area of focus in cancer vaccine development arms (12-14). Promising results from direct injection into melanoma lesions of an oncolvtic herpes simplex virus type 1 encoding GM-CSF has led to a randomized phase III trial in patients with unresectable stage III and stage IV melanoma that has reached its primary endpoint of durable response rate, and may become the first approved melanoma vaccine in the US (15).

It is within this therapeutic landscape that the concept of interfering with the regulation of certain newly identified molecules on T-cells, antigen presenting cells (APCs) and tumor cells has been tested.

# **Biology of immune checkpoints**

The immune system is traditionally divided into innate and adaptive components. The innate immune system is the first line of defense against external threats such as infectious agents and toxins although some of its components, such as natural killer cells (NK cells) can also target cancer cells. The adaptive immune system requires proliferation of antigen-specific T-cells as well as antibody-producing B cells. The development of an immune response against cancer cells relies mostly on adaptive immune responses. These responses consist of an activation arm and an effector arm (16-18).

The activation of T-cells is a complex and balanced process, which until recently had been incompletely understood. T-cell activation is a multistep process; at least two signals are required. The first signal consists of antigen recognition by naïve T-cells through their interaction with APCs. This is mediated by the T-cell receptor on the cell membrane of T-cells and the major histocompatibility complex (MHC) receptor, on the membrane of APCs. The second signal emanates from co-stimulation, the result of the interplay of a series of stimulatory and inhibitory interactions between different ligands and receptors located on the surface of both cells. The principal and sine qua non co-stimulatory signal is provided by the interaction between CD28 receptors on the surface of T-cells and their ligands B7.1 (CD80) and B7.2 (CD86) on the surface of APCs. This results in T-cell activation, cytokine secretion, and expansion of clones with the capacity to recognize the presented antigen(s) (19,20).

Many of the components of this "immune synapse" and their specific role in T-cell activation or inhibition have been recently elucidated (21) (*Figure 1*).

CTLA-4 (Cytotoxic T Lymphocyte Antigen-4) and PD-1 (Program Death-1 or CD279) are among some recently described receptors with the ability to negatively regulate the T-cell activation process.

# Cytotoxic T lymphocyte antigen-4 (CTLA-4)

The CTLA-4 molecule was originally described in 1987, its function largely unknown at the time (22). Initial studies with knockout mice unveiled its important role in immune homeostasis, as mice developed severe autoimmune disease and died with extensive lymphoproliferative infiltration (23). As previously mentioned, the T-cell surface molecule CD28 interacts with its ligands (B7-1/CD80, B7-2/CD81) on the surface of the APCs to provide a co-stimulatory signal that is essential for T-cell activation. CTLA-4 up-regulation and cell membrane expression begins 48 hours after T-cell activation. CTLA-4, which has greater binding affinity for the B7 surface molecules found on the APC than CD28, effectively induces T-cell activation. The physiologic

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**Figure 1** Interactions occurring in the lymph nodes between tumor antigen bearing antigen presenting cells (APCs) and naïve T cells leading to T cell activation, proliferation and circulation to the peripheral tissues, where the now activated T cells interact with tumor cells. Agonistic/stimulatory interactions are represented in red and antagonistic/inhibitory interactions in black.

function of CTLA-4 is to act as an immune brake to prevent T-cell overstimulation and its consequences, e.g., autoimmune disease. Inhibition of CTLA-4 by administration of blocking anti-CTLA-4 monoclonal antibodies (mAb) can shift the immune system balance toward T-cell activation. CTLA-4 receptors are frequently expressed in tumor cells, contributing to their immune system evasion. Tumor responses were first demonstrated in mice by Leach *et al.* in 1996 (24).

The effector arm of the adaptive immune system is comprised of expanded activated T-cells permeating the peripheral tissues, and the corresponding memory cells. The interactions of these effector cells with their targets (in this context, tumors cells) have been found to be affected by mediators expressed in the tumor cells and T-cells. These mediators may promote or inhibit tumor cell destruction and modulation of their activity has resulted in potent clinical benefit.

# PD-1 and PD-L1

PD-1 is an immunoglobulin superfamily member expressed in a subpopulation of CD4/CD8-normal thymocytes and induced in peripheral lymphocytes following their activation. PD-1 knockout mice grow normally but develop splenomegaly, augmented proliferative B cell responses and autoimmune diseases (25). Finally, while CTLA-4 seems to influence early activation of T-cells, the PD-1 pathway is more influential in T-cell exhaustion in peripheral tissues.

The PD-1 protein is a co-inhibitory receptor expressed on B cells and activated or exhausted T-cells. PD-1 has two known ligands: PD-L1 (B7-H1) and PD-L2 (B7-DC). PD-1 has greater affinity for PD-L1. PD-L1 is widely expressed in hematopoietic and nonhematopoietic tissues as well as in tumor cells (26-28). PD-L2 is expressed on dendritic cells, mast cells, macrophages and B cells. When PD-1 binds to PD-L1-expressing tumor cells, T-cell activity is suppressed, leading to T-cell exhaustion. Expression of the PD-1/ PD-L1 pathway protects tumor cells from immunological responses mediated by T-cells (29,30). Inhibition of the PD-1/PD-L1 pathway reverses immune evasion by replenishing the pool of activated non-exhausted T-cells. Recently, a molecular interaction between PD-L1 and CD80 was discovered, in which CD80 can deliver inhibitory signals when engaged by PD-L1. This interaction may have implications in tumor immune resistance (31). The development and clinical testing of anti-PD-1 and anti-PD-L1 blocking antibodies has resulted in hitherto unseen clinical activity in a variety of malignancies including melanoma.

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# Clinical results with immune checkpoint inhibitors

# CTLA-4 inbibitors

The two mAb targeting CTLA-4 that have been investigated are ipilimumab and tremelimumab.

# Ipilimumab

Ipilimumab (MDX-010, Bristol Myers Squibb, New York, NY, USA) is a fully humanized IgG1kappa monoclonal antibody against CTLA-4. Data from phase II studies suggest a long-term survival effect of ipilimumab monotherapy. In an analysis of three of these studies the median follow up was from 10.1 to 16.3 months, with a range reaching up to 37.5 months, the 12-month survival rates were >47%, the 18-month survival rates were >34% and the 24-month survival rates were  $\geq$ 30%. Even for previously treated patients, 24 months survival rates ranged from 24% to 33%. A meaningful proportion of patients continued to survive beyond the updated follow-up period. Long-term survivors included patients with progressive disease (32-35).

The pivotal phase III trial in previously treated patients was published in 2010 (36). A total of 676 HLA-A\*0201positive patients with unresectable stage III or IV melanoma with progressive disease, were randomly assigned, in a 3:1:1 ratio, to receive ipilimumab plus gp100, ipilimumab alone, or gp100 alone. Ipilimumab, at a dose of 3 mg/kg, was administered with or without gp100 every 3 weeks for up to four treatments. This phase III study showed that ipilimumab, either alone or with gp100, improved overall survival (OS) compared with gp100 alone in previously treated patients with metastatic melanoma; more than 70% of the patients had visceral metastases and more than 36% had elevated LDH, both poor prognostic factors. The median OS in the ipilimumab-plus-gp100 group was 10.0 months as compared with 6.4 months in the gp100alone group (hazard ratio for death, 0.68; P<0.001). The median OS in the ipilimumab-alone group was 10.1 months with ipilimumab alone as compared with gp100 alone (hazard ratio 0.66; P=0.003). OS in the ipilimumabplus-gp100 group, the ipilimumab-alone group, and the gp100-alone group, respectively, were 43.6%, 45.6%, and 25.3% at 12 months, 30.0%, 33.2%, and 16.3% at 18 months, and 21.6%, 23.5%, and 13.7% at 24 months. The effect of ipilimumab on OS was independent of age, gender, baseline LDH levels, stage of disease, and previous IL-2 therapy.

The most common adverse events related to the study drugs were immune-related events, which occurred in approximately 60% of the patients treated with ipilimumab and 32% of the patients treated with gp100. The frequency of grade 3 or 4 immune-related adverse events was 10% to 15% in the ipilimumab groups and 3.0% in the gp100alone group. The immune-related adverse events most often affected the skin and gastrointestinal tract. The most common immune-related adverse event was diarrhea, which occurred at any grade in 27% to 31% of the patients in the ipilimumab groups. There were 14 deaths related to the study drugs (2.1%), of which 7 were associated with immune-related adverse events.

The second phase III trial with this agent was carried out in 502 patients with previously-untreated metastatic melanoma (37). Patients were randomized 1:1 to ipilimumab 10 mg/kg in combination with dacarbazine or to dacarbazine alone. Patients treated with ipilimumab and dacarbazine had an OS of 11.2 months compared with 9.2 months in the dacarbazine-alone arm. Both the overall and long-term survivals were improved. OS at one, two and three years in the ipilimumab-containing arm were 47%, 29% and 21% respectively versus 36%,18% and 12% in the dacarbazine alone arm. Hepatic toxicity was reported more frequently in patients given combination therapy of ipilimumab plus dacarbazine than in those in the other phase III trial who received ipilimumab alone. However, the occurrence of colitis, rash, and hypophysitis was less frequent than observed in patients receiving ipilimumab alone. Based on the results of these phase III trials, ipilimumab at a dose of 3 mg/kg was approved by the Food and Drug Administration (FDA) for the treatment of metastatic melanoma.

Unusual patterns of tumor response were noted early in the clinical development of CTLA-4 antibodies. These patterns included apparent initial worsening of the disease, sometimes with the appearance of new lesions, followed by a response or stabilization, occurring over a period of several weeks to months (38). Awareness of these patterns is important in order to avoid early treatment discontinuation. These findings led to the establishment of immune related response criteria (irRC) that are presently being validated in immunotherapy trials (39,40).

Management guidelines with algorithms for management of immune-related adverse events (irAEs) have been developed (41,42). These events require close patient followup and timely administration of corticosteroids and infliximab.

Patients who have previously benefited from treatment and later relapse can be successfully retreated with up to 55% of patients regaining disease control (43,44).

Recently, in an analysis of ipilimumab survival including 4,846 patients (1,861 patients from 12 pooled trials and 2,985 from an expanded access trial), Schadendorf et al. showed a 3-year survival rate of 21%. The primary analysis of the 1,861 patients showed that the median OS was 11.4 months and 254 patients (22%) were still alive after three years. There were no deaths among patients who survived beyond seven years, at which time the OS rate was 17%. The longest OS follow-up in the database was 9.9 years. The plateau, which started at three years and continued through to ten years, was observed regardless of dose (3 or 10 mg/kg), whether the patients had received previous treatment or not, and whether or not they had been kept on a maintenance dose of the drug. While these data are not conclusive regarding differences between the doses or the populations because of its retrospective nature, they do demonstrate that ipilimumab can lead to longlasting tumor control in metastatic melanoma (45).

The search for reliable surrogate markers that could help identify patients most likely to respond checkpoint modulation is under intensive investigation. Retrospective analyses of large studies have suggested better clinical outcomes in patients treated with anti-CTLA-4 antibodies who experienced irAEs; this effect appeared to be more pronounced in patients who had more severe irAEs (46-48). Another possible surrogate marker for response is the absolute lymphocyte count (ALC). Some studies have shown that an ALC >1,000/µL with an increase in the ALC after two ipilimumab treatments correlates with clinical benefit (complete and partial response or stable disease 24 weeks after beginning of the treatment) and OS (49). Other potentially predictive factors include increased expression of inducible costimulator molecule (ICOS) on T-cells, neutrophil/lymphocyte ratios and a multitude of components of the immune microenvironment including regulatory T-cells, myeloid suppressor cells, cytokines and chemokines. Evidence of prior immune response against certain peptide antigens such NY-ESO-1 has been correlated with a higher likelihood of response to ipilimumab (50).

The development of metastatic disease to the brain is a major cause of morbidity and mortality in patients with advanced melanoma and the role and efficacy of ipilimumab in melanoma involving the brain has been examined (51-54).

Another subject of discussion is the issue of which is the most efficacious dose of ipilimumab. The recently closed randomized study CA184-169, investigating ipilimumab at 3 versus 10 mg/kg, will hopefully determine the optimal dose of ipilimumab in metastatic melanoma.

The approval of highly effective BRAF and MEK targeted agents in BRAF-mutated metastatic melanoma has led to the question of how to best combine these agents with immunotherapy, in particular, with ipilimumab. Phase I trials combining BRAF inhibitors with ipilimumab were met with unexpected toxicity, reiterating the need for formal safety oriented combination trials and redirecting the focus of the research towards sequential than concomitant drug administration (55). The best clinical data available is retrospective (56,57). In an effort to provide guidance to clinical oncologists, the Society for Immunotherapy of Cancer (SITC) has recently published consensus guidelines on this matter (58). A soon to be activated intergroup study comparing the two possible sequences of ipilimumab and a combination of a BRAF and a MEK inhibitor will address this question in a prospective and randomized fashion.

Combinations of ipilimumab with other forms of immunotherapy such as IL-2 (59), GM-CSF (60) and oncolytic viral vaccines (61) have been reported. The encouraging results of a recent report on the combination of ipilimumab plus nivolumab will be discussed later in this article.

Postow *et al.* reported on the occurrence of an abscopal effect of radiation therapy in a patient previously treated with ipilimumab (62). The abscopal effect is a phenomenon in which local radiotherapy is associated with the regression of metastatic cancer at a distance from the irradiated site, which is likely mediated by activation of the immune system. The role of concomitant or sequential radiation therapy in ipilimumab-treated patients has recently been reviewed (63).

Tremelimumab (CP-675,206, Pfizer/Medimmune, New York, NY, USA/Gaithersburg, MD, USA) is a fully human IgG2 anti-CTLA-4 mAb that has been studied in clinical trials as a single agent and in combination in patients with advanced melanoma (64,65). The promising clinical activity of tremelimumab in phase I and II trials in advanced melanoma led to a phase III clinical trial in which patients with treatment naive advanced melanoma were randomized patients to single-agent tremelimumab (15 mg/kg IV every 3 months) or standard-of-care chemotherapy with either dacarbazine or temozolomide. The primary endpoint was OS. The trial was halted for futility based on the recommendations of the Data Safety Monitoring Board. Median survival in the tremelimumab arm was 12.02 months and in the chemotherapy arm 10.45 months, with the majority of responses to tremelimumab being durable (66). Additional follow up and retrospective analyses of prognostic factors

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identified a low C-reactive protein (CRP) level as a prognostic surrogate for response. Tremelimumab development has recently resumed under a new pharmaceutical company ownership.

# CTLA-4 use in the adjuvant setting

The demonstration of clinical activity of ipilimumab in advanced melanoma led to investigation of its use in the adjuvant setting, after complete surgical resection of highrisk melanoma. A study of 75 patients with high-risk disease were treated with ipilimumab plus a peptide vaccine and provided safety data in this patient group (67). The European Organization for Research and Treatment of Cancer (EORTC) has completed a phase III trial in patients with high-risk stage III disease (EORTC 18071, NCT00636168). In this trial, following complete resection of high risk melanoma, patients were randomly assigned to ipilimumab (dose 10 mg/kg every three weeks for four cycles then every 12 weeks for a total of three years treatment) or to placebo to determine whether ipilimumab prevents disease recurrence. The trial has completed accrual and initial results are expected this year.

In addition, the Eastern Cooperative Oncology Group (ECOG) is also evaluating ipilimumab in the adjuvant setting with the E1609 intergroup trial. Patients with resected stage IIIB, IIIC, or IV disease are randomized to receive 3 or 10 mg/kg of ipilimumab versus standard high dose interferon-alpha. This study is currently still accruing patients (NCT01274338).

### PD-1/PD-L1 inhibitors

PD-1/PD-L1 binding is an important tumor evasion mechanism, inducing immune tolerance through apoptosis of the activated effector T-lymphocytes. Preclinical data supported further therapeutic exploration of PD-1/PDL-1 modulation (68,69).

Blocking mAb directed to PD-1 and PD-L1 are among the most promising immunotherapeutic approaches recently developed.

Nivolumab (MDX-1106, BMS 9368558, Bristol Myers Squibb, New York, NY, USA) is a fully human IgG4 monoclonal antibody that targets the human PD-1 molecule. A recent report on the 107 melanoma patient cohort from a 296 patient phase I trial of nivolumab demonstrated a median OS of 16.8 months, and 1- and 2-year survival rates of 62% and 43%, respectively. Among 33 patients with objective tumor regressions (31%), the Kaplan-Meier estimated median response duration was 2 years. An additional 7% of patients (seven of 107) experienced stable disease lasting for 24 weeks or more. Seventeen patients discontinued therapy for reasons other than disease progression, and 12 (71%) of 17 maintained responses offtherapy for at least 16 weeks (range, 16 to 56+ weeks). The maximum-tolerated dose of nivolumab was not reached within the tested dose range. The most common events of any grade that occurred in patients with melanoma were fatigue (32%), rash (23%), and diarrhea (18%). Twentyfour (22%) of 107 patients with melanoma experienced grade 3 to 4 treatment-related adverse events. Treatmentrelated select adverse events of any grade were observed in 58 (54%) of 107 patients with melanoma, the most common of which included skin disorders (36%), GI events (18%), and endocrinopathies (13%). Grade 3 to 4 treatmentrelated select events were seen in five patients (5%). There were no drug-related deaths in the population of patients with melanoma, although there were three mortalities following treatment-related adverse events in the overall phase I patient population (1%): two patients with nonsmall-cell lung cancer and one with colorectal cancer died of pneumonitis. Most adverse events occurred within the first 6 months of therapy, and cumulative toxicities were not observed with prolonged drug exposure (70).

Lambrolizumab (MK3475, Merck, Whitehouse Station, NJ, USA) is another humanized IgG4 kappa antibody against PD-1 currently in clinical trials. A total of 135 patients with advanced melanoma were treated at a dose of 10 mg/kg every 2 or 3 weeks or 2 mg/kg every 3 weeks. Common adverse events attributed to treatment were fatigue, rash, pruritus, and diarrhea; most of the adverse events were low grade. The confirmed RECIST response rate across all dose cohorts was 38% with a 52% confirmed response rate observed in the cohort that received 10 mg/kg every 2 weeks. The response rate was unaffected by prior ipilimumab treatment. Responses were durable in most patients with a median follow-up of 11 months among responding patients. The overall median progression-free survival (PFS) among the 135 patients was longer than 7 months (71).

Further development of lambrolizumab has included a randomized phase II trial in 510 patients treated with low dose lambrolizumab, high dose lambrolizumab or standard chemotherapy in advanced melanoma progressing after prior therapy. This trial has completed accrual in late 2013.

A pivotal trial phase III trial in 645 ipilimumabnaïve patients with unresectable or metastatic melanoma

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randomized to evaluate the safety and efficacy of two dosing schedules of lambrolizumab compared to ipilimumab has also recently completed accrual.

Pidilizumab (CT-011, CureTech, Yavne, Israel) is an IgG1 kappa humanized antibody targeting the PD-1 protein. Phase I data has included mostly patients with hematologic malignancies. Two phase II studies in patients with metastatic colorectal cancer and metastatic melanoma have been completed but the results not yet reported (72).

AMP-224 (Amplimmune/Medimmune, Gaithersburg, MD, USA) is an IgG1 chimeric antibody comprised of the extracellular domain of the PD-1 ligand programmed cell death ligand 2 (PD-L2) and the Fc region of human IgG. It blocks interaction between PD-1 and its ligands B7-DC (PD-L2 or programmed cell death-1 ligand 2) and B7-H1 (PD-L1 or CD274 molecule), thereby inhibiting the subsequent activation of PD-1. AMP-224 is thought to promote depletion of PD-1<sup>high</sup> expressing T-cells (exhausted effector cells) with subsequent replenishment of the T-cell pool with functional T-cells. In a phase I trial, 35 of the 42 patients had advanced melanoma. Infusion reactions were common with no evidence of pneumonitis or GI toxicities. Partial responses and stable disease were documented in a minority of patients (73).

BMS-936559 (Bristol Myers Squibb, New York, NY, USA) is a human IgG4 blocking antibody targeting PD-L1. In a phase I study, durable tumor regression was observed in patients with advanced cancer, including non-small cell lung cancer, melanoma, and renal cell cancer treated with MDX-1105. A complete or partial response was reported in 9 out of 52 patients with melanoma (17%). The most common drug-related toxicities were fatigue, infusion reactions, diarrhea, arthralgia, rash, nausea, pruritus, and headache. Most events were of low grade, with treatment-related grade 3 or 4 events observed in 9% of patients. Both the response and toxicity rates appeared to be lower compared to anti-PD-1 antibodies (74).

MEDI4736 (Medimmune, Gaithersburg, MD, USA) is an IgG1 kappa blocking anti-PD-L1 antibody which is currently being investigated in early clinical trials. Preliminary data suggests activity in various advanced malignancies including melanoma and a favorable toxicity profile (75).

MPDL3280A (RG7446, Genentech, South San Francisco, CA, USA) is a human IgG1 monoclonal antibody containing an engineered Fc-domain that binds to PD-L1. In a phase I trial 45 melanoma patients were treated, most having received prior systemic therapy. The incidence of all grade 3 and grade 4 adverse events was 33%, including hyperglycemia (7%), elevated ALT (7%) and elevated AST (4%). No grade 3-5 pneumonitis was reported and no treatment-related deaths occurred on study. Thirty-five patients were evaluable for efficacy. An ORR of 26% (9/35) was observed, with all RECIST responses ongoing or improving, some with tumor shrinkage within days of initial treatment. The 24-week PFS was 35%. Archival tumors were mandatory and their analyses showed a correlation between PD-L1 status and efficacy (76).

MSB0010718C (Merck Serono, Darmstadt, Germany) is yet another monoclonal antibody with specificity for PD-L1 in an early phase I trial (77).

Although ipilimumab and antibodies against PD-L1/ PD-1 have not been directly compared in a clinical trial, the side effect profile of the latter, particularly the irAEs appears to be more favorable than those associated with CTLA-4 antibodies.

Preclinical data has suggested that the combination of CTLA-4 and PD-1 blocking antibodies could result in synergistic clinical activity (78).

Wolchock et al. studied the safety and anti-tumor activity of CTLA-4 and PD-1 blockade in a concurrent and sequential fashion (79). In their report, 86 patients with advanced melanoma were treated with concurrent ipilimumab at 3 mg/kg every 3 weeks for 4 doses and continued every 12 weeks for up to 8 doses (53 patients). Thirty-three patients received a sequential regimen consisting of ipilimumab followed by nivolumab every 2 weeks for up to 48 doses. Of the 53 patients receiving the concurrent regimen, 93% experienced treatment related adverse events. The most common side effects were rash (55%), pruritus (47%), fatigue (38%), and diarrhea (34%). Treatment related severe adverse events were observed in 49% of these patients, and the most common were liver enzyme abnormalities (15%). The 33 patients treated sequentially experienced a lower number of treatment related adverse events (73%). These included pruritus (18%) and lipase elevation (12%). Toxicities were generally manageable and reversed with immunosuppressive drugs. Twenty-one patients (40%) in the concurrent regimen experienced objective antitumor responses by modified WHO criteria (3 complete responses); the authors also reported 65% clinical activity for patients in the concurrent group. In contrast, only six patients (20%) in the sequential group experienced objective responses. The clinical activity was also lower in this group (43%). In summary, the concurrent combination of ipilimumab and nivolumab results in an increased response rate compared with monotherapies with either agent while demonstrating consistent durability

Table 1 Important immune chee	ckpoint targets under develo	opment (80)	
Target (signal)	Antibody	Drug company	Function
OX40 (agonist)	9B12 MFDI6469	AgonOx Medimmune	T-cell expansion, cytokine production, and cell survival. OX40 is a co-stimulatory molecule on the surface of T cells. Agonistic antibodies have antitumor activity
CD40 (agonist)	ChiLob7/4 CP-870,893	Cancer Res UK Pfizer	Co-stimulatory molecule expressed on dendritic cells, B cells and monocytes. CD40L is expressed on CD4+ T-cells. The interaction of CD40-CD40L triggers T-cell
	Dacetuzumab Lucatumumab	Seattle Genetics Novartis	activation
CD137/4-1BB (agonist)	Urelumab/BMS663513	BMS	CD137 signaling enhances T-lymphocyte proliferation and T-helper-1 cytokine production, protecting CD8+ T-lymphocytes from apoptosis
LAG-3 (lymphocyte activation gene-3) (antagonist)	BMS986016	BMS	Transmembrane protein that binds MHC class II molecules, enhances regulatory T-cell activity, and negatively regulates cellular proliferation, activation, and homeostasis of T cells. Synergy between PD-1 and LAG-3 pathways to induce tolerance to self and tumor antigens
TIM-3 (T cell lg mucin-3) (antagonist)			Inhibitory receptor expressed on tumor reactive T cells. TIM-3 is frequently co- expressed with PD-1
CD27 (agonist)	IF5	Celldex	Enhances the activation and survival of CD8+ T-cells and their subsequent differentiation into memory cells; promotes the differentiation of CD4+ T cells into IFN $\gamma$ -secreting cells and the proliferation and effect or activity of NK cells
KIR antagonist	IPH2101 BMS-986015	Innate BMS	KIR are immune receptors that down-regulate NK cell activity; anti-KIR antibodies potentiate inmate immunity by blocking signaling through inhibitory KIRs
GITR (Glucocorticoid-induced TNFR-related protein) (agonist)	TRX518	Tolerex	GITR induces T-cell activation by providing a costimulatory signal that increases proliferation, activation, and cytokine production of CD4+ and CD8+ T cells after TCR engagement

of these responses. The apparent enhanced clinical activity of the concurrent combination comes with increased attendant toxicity. A phase III clinical trial randomizing advanced melanoma patients previously untreated with ipilimumab to concurrent nivolumab plus ipilimumab, nivolumab alone or ipilimumab alone (2:1:1 ratio) has been recently completed accrual.

# Other agents currently under development

The discovery and understanding of the growing number of molecules mediating the immune response has led to the clinical development of modulators for other immune checkpoint targets. Some of the most important are listed on *Table 1*.

Several studies suggest a relationship between PD-L1 expression on tumor cells and objective response to both anti-PD-1 antibodies and anti-PD-L1 antibodies (77,81-83). Further work is required to develop a sensitive and highly predictive assay that might be useful for patient selection. It is likely that in the future other immune checkpoint regulatory molecules such as those listed on *Table 1* as well as other components of the tumor microenvironment may turn out to be predictors of response to immunotherapy (80).

#### Conclusions

The long path taken by immunotherapy to finally deliver on its promise of effective therapies and durable responses to patients suffering from advanced melanoma has been a frustrating and elusive, even quixotic ride for most of the last three decades. There are now many opportunities to achieve further breakthroughs in the treatment of melanoma and other "untreatable" malignancies in the near future. Surrogate markers will allow us to select which patients would be appropriate for specific interventions, and the wide spectrum of immune adverse events will be managed more effectively. Careful study of the basic mechanisms and clinical evaluation of the yet unexplored or undiscovered immune pathways, along with the combined use of molecularly targeted agents will bring about long term tumor control and possibly curative treatments.

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