REVIEW

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Melanoma Management

Targeting immune checkpoints in melanoma: an update

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Practice points

- Although curable when localized at diagnosis, melanoma is associated with a high risk of relapse and is a life-threatening condition at advanced stages.
- Multiple events orchestrate the interaction of the immune system, malignant cells and tumor microenvironment.
- Both stimulatory and inhibitory signals (or checkpoints) can modulate the antitumor immune response, and suppression of inhibitory receptors can lead to antitumoral effect.
- Monoclonal antibodies targeting CTLA-4 and PD-1 have been investigated in large, randomized trials and shown to improve survival in patients with locally advanced or metastatic melanoma.
- Ipilimumab, pembrolizumab and nivolumab are currently available for clinical use for the treatment of patients with advanced melanoma.
- Early recognition of immune-related adverse events and adequate treatment based on available guidelines is essential in managing the toxicities of these drugs.
- Responses to immune checkpoint blocking antibodies can have a different pattern and kinetics from those observed with conventional cytotoxic chemotherapy.
- The role of both prognostic and predictive biomarkers in this setting is not fully understood.
- Promising strategies to overcome resistance and improve outcomes include combinations of different compounds and novel immunotherapeutic agents.

Different treatment modalities encompassed under the term 'immunotherapy' have led to major breakthroughs in the treatment of melanoma. Immune checkpoint-blocking antibodies targeting CTLA-4 and PD-1 result in significant activity and prolonged survival in patients with advanced melanoma and are currently available for clinical use. Studies addressing novel immune checkpoint blocking antibodies, combined approaches and predictive/prognostic biomarkers are expected to broaden the applicability and efficacy of this approach. In this article, we will review clinically meaningful aspects of immune checkpoint blockade, promising strategies under development and the challenges faced in a continuous search to improve the outcomes of patients affected by this disease.

Melanoma: witnessing a change in paradigm

Although curable when diagnosed at early stages, melanoma is still associated with a significant risk of relapse and remains a fatal disease when diagnosed at advanced stages [1]. In 2015, approximately 74,000 new cases are expected to be diagnosed and almost 10,000 patients will die from this disease in the USA [2], and incidence continues to rise in many regions of the globe [3].

KEYWORDS

• CTLA-4 • immunotherapy • ipilimumab • melanoma • nivolumab • PD-1 • pembrolizumab • tremelimumab

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The term melanoma derives from the Greek *melas* (black) and *oma* (tumor), and alludes to a malignancy arising from melanocytes, which originate from the neural crest during embryogenesis [4]. Until the first decade of the 21st century, survival of patients with metastatic melanoma was historically poor, with less than 15% of the patients alive at 5 years after the initial diagnosis [4,5]. Treatments for patients with unresectable disease relied on conventional cytotoxic agents, particularly dacarbazine, and only a subset of patients achieved benefit with IL-2 [4,5].

A better understanding of melanoma biology and both host and tumor immunology led to significant advances in the treatment of this disease. Since 2011, six new agents were approved by the US FDA for the treatment of patients with advanced melanoma. In addition to the targeted agents, dabrafenib, trametinib and vemurafenib, the immune checkpoint blocking antibodies ipilimumab, pembrolizumab and nivolumab are currently available for clinical use. Antibodies targeting immune checkpoints revolutionized the way advanced melanoma is perceived and treated. Blockade of CTLA-4 with ipilimumab was the first strategy based on modulation of antitumor immune response to result in improvement in survival and durable responses in melanoma [6,7]. More recently, targeting the PD-1 showed significant efficacy in the same setting [8]. Long-term results of ipilimumab have been recently published, providing strong evidence that the historical survival curves for patients with melanoma are, finally, being redrawn [9].

Why are we doing this? A brief overview of the immune response in melanoma

The concept that immune system can result in antitumoral effects dates back to the 19th century, when tumor regressions were reported by Wilhelm Busch and William B Coley following infections of surgical wounds [10,11] In addition, extensive data in the literature support an association between immunosuppression and the development of malignancies other than melanoma. The knowledge generated during the following century enabled researches to understand the underlying mechanisms involved in these observations and to develop approaches that were proven to be therapeutically successful. The role of cellular immunity as a mechanism of prevention of cancer was termed 'immunosurveillance' [12], and the different steps correlating cancer progression and immune-mediated effects were described as 'immunoediting' [13]. Finally, the complex interactions leading to suppression of the antitumor immune response mediated by malignant cells and the tumor microenvironment are characterized as 'immune-evasion' [14,15].

The immune system can be divided into the innate and adaptive arms [15,16]. The innate immune system, which serves as the initial defense against foreign antigens, includes dendritic cells (DC), macrophages, neutrophils, basophils, eosinophils, natural killer (NK) cells and mast cells [15,16]. Once activated, macrophages and mast cells release stimulatory cytokines that recruit additional elements of the inflammatory response. DC act as major antigen-presenting cells (APC) through interaction with adaptive immune components in a process mediated by MHC classes I and II. The adaptive immune system includes B lymphocytes, $CD4^+$ helper T lymphocytes, and $CD8^+$ cytotoxic T lymphocytes (CTLs), and results in an antigen-specific response [15–17].

Effective immune-mediated immune response demands a complex chain of events that involves processing of tumoral antigens by APC, interaction with T lymphocytes, recruitment of effector cells, production of T-cell-mediated response and overcoming mechanisms of immunosuppression and negative regulation [15,17]. APC are central players in this process – they are responsible for processing tumoral antigens and presenting them, via MHC class I and II molecules, to T cells through the T-cell receptor (TCR).

In order to result in T-cell activation, proliferation and cytokine release, engagement of the TCR by MHC molecules requires accessory signals, which are modulated by the interaction of stimulatory and inhibitory receptors [18–20]. While co-stimulation leads to arousal of T-cell-mediated response, negative signals are essential in preventing autoimmunity and promoting T-cell tolerance. These regulatory molecules that are expressed by immune system cells, malignant cells and cells of the tumor microenvironment are termed immune checkpoints. Receptors encompassed under B7:CD28 family are key players in modulation of T-cell function. CD28 is a constitutive co-stimulatory receptor implicated in T-cell activation. The inter-related B7 family of ligands includes B7-1 (CD80), B7-2 (CD 86), PD-L1 (B7-H1), PD-L2 (B7-DC), ICOS ligand, B7-H3 and B7-H4 [21,22].

CTLA-4 is an inhibitory receptor induced upon activation of $CD4^+$ and $CD8^+$ T cells, but also expressed by memory and regulatory T cells. It competes with higher binding affinity with the stimulatory receptor CD28 for common ligands of the B7 family (B7-1 or CD80 and B7-2 or CD86), expressed by APCs. CTLA-4/B7 interaction results in suppression of T cell, a process that occurs early during the priming phase of T-cell stimulation and antigen exposure in lymphoid tissue [20–22].

PD-1 is also an inhibitory receptor expressed by effector T cells. PD-1 interacts with the B7-family ligands PD-L1 and PD-L2. PD-L1 is expressed in B, T, myeloid and dendritic cells, as well as in nonhematopoietic cells and is involved in evasion of T-cell-mediated responses by tumors, while PD-L2 expression is limited to macrophages and dendritic cells [20,21]. Differently from CTLA-4, PD-1 is expressed by antigen-exposed T cells during the effector phase of T-cell activation predominately in peripheral tissues and tumor microenvironment [20,21].

Other molecules with inhibitory effects include B- and T-cell attenuator (BTLA), a CD28 homolog and LAG3. More recently, a distinct subtype of inhibitory co-signaling molecules has been described independently by different authors, and named VISTA or PD-1H, expanding the milieu of targets to be explored in this increasingly complex, yet exciting landscape [23–25].

Conversely, stimulatory receptors involved in perpetuation and amplification of the immune response include inducible T-cell costimulator (ICOS), a distinct CD28 homolog and the TNF/TNF receptor (TNFR) family members, which comprises CD27/CD70, CD134 (OX40)/OX40L, CD137 (4-1BB)/4-1BBL, HVEM/LIGHT, CD30/CD30L and GITR/GITRL. These co-stimulatory molecules are involved in T-cell survival following initial activation, and therefore at distinct juncture from CD28 [15,17,26].

Blockade of both CTLA-4 and PD-1 using monoclonal antibodies has resulted in antitumoral effects in patients with melanoma and other malignancies, and results of clinical trials in patients with melanoma will be discussed in the following sections. Nevertheless, additional targets involved in modulation of antitumoral immune response are under development and could potentially improve the results.

Blocking CTLA-4

As discussed, CTLA-4 is a checkpoint molecule upregulated in activated $CD4^+$ and $CD8^+$ T cells a blockade of CTLA-4 leads to mitigation of T-cell suppressive signals [19,21,27].

Different agents targeting CTLA-4 were studied in clinical trials, with variable results. The most successful anti-CTLA agent, ipilimumab, a fully human IgG1 monoclonal antibody, resulted in overall survival benefit in both pretreated and treatment-naive patients with metastatic melanoma in different randomized, Phase III clinical trials [6,7]. ipilimumab was approved by the FDA for the treatment of patients with advanced melanoma in 2011 **(Table 1)**.

In the Phase III landmark study by Hodi *et al.*, 676 pretreated patients were randomized in this three-arm study to receive ipilimumab 3 mg/kg every 3 weeks for four doses (weeks 1, 4, 7, and 10; n = 137), ipilimumab in combination with gp100, a peptide vaccine (n = 403), or gp100 alone (n = 136) [6]. Patients with stable disease for at least 3 months after week 12 or a confirmed partial or complete response were offered additional courses of therapy (re-induction) with their assigned treatment regimen at the time of progression. ipilimumab alone resulted in superior overall response rates (ORR: 10.9% ipilimumab alone vs 5.7% ipilimumab + gp100 vs 1.5% gp100 alone) and overall survival (OS; 10.1 months ipilimumab alone vs 10.0 months ipilimumab + gp100 vs 6.4 months gp100 alone; HR for death in the comparison between ipilimumab alone and gp100 alone: 0.66; p = 0.003). There was no additional benefit from adding gp100 to ipilimumab alone (HR for death in the comparison between ipilimumab alone and ipilimumab + gp100: 1.04; p = 0.76). Of note, 21 out of 31 (68%) of the patients who met criteria for re-induction with ipilimumab achieved disease control (objective response or stable disease). Grade 3 or 4 immune-related adverse events occurred in 10–15% of patients treated with ipilimumab and in 3% treated with gp100 alone, and most often affected the skin and the GI tract. In total, 14 deaths (2.1%) occurred and were related to the study drugs, and seven were associated with immune-related adverse events [6]. Although positivity for HLA-A*0201 was mandatory for all patients included in this Phase III study due to the use of the HLA-A*0201-restricted gp100 vaccine, a subsequent analysis of patients receiving ipilimumab

Table 1. Select randomized trials investigating immune checkpoint blockers in melanoma.

Author	Year	Phase	Setting	n	Treatments	ORR (%)	mOS (months)	Ref.
Anti-CTLA-4 agents								
Hodi *et al.*	2010	III	Pretreated	676	Ipilimumab 3 mg/kg (A) vs ipilimumab 3 mg/kg + gp100 (B) vs gp100 (C)	10.9 (A) vs 5.7 (B) vs 1.5 (C)	10.1 (A) vs 10.0 (B) vs 6.4 (C)	[6]
Robert *et al.*	2010	III	Treatment-naive	502	Ipilimumab 10 mg/kg + DTIC vs placebo + DTIC	15.2 vs 10.3	11.2 vs 9.1	[7]
Ribas *et al.*	2013	III	Treatment-naive	655	Tremelimumab vs DTIC or TMZ	10.7 vs 9.8	12.6 vs 10.7	[28]
Anti-PD-1 agents								
Robert *et al.*	2014	I randomized	Pre-treated with anti-CTLA-4	173	Pembrolizumab 2 vs 10 mg/kg	26 vs 26	NR	[29]
Weber *et al.*	2014	III	Pre-treated with anti-CTLA-4	370	Nivolumab 3 mg/kg vs CT	32 vs 11	NA	[30]
Robert *et al.*	2015	III	Treatment-naive	418	Nivolumab 3 mg/kg vs DTIC	40 vs 13.9	NR vs 10.8	[8]
Robert *et al.*	2015	III	Up to one prior systemic treatment	834	Pembrolizumab 10 mg/kg every 2 weeks vs every 3 weeks vs ipilimumab 3 mg/kg	33.7 vs 32.9 vs 11.9	NR	[31]
Anti-PD-1 in combination with anti-CTLA-4								
Postow *et al.*	2015	II randomized	Treatment-naive	142 (109 *BRAF* wild-type)	Ipilimumab 3 mg/kg + nivolumab 1 mg/kg followed by nivolumab 3 mg/kg vs ipilimumab 3 mg/kg+ placebo	61 vs 11†	NR	[32]

†Among patients with *BRAF* wild-type tumors.

CT: Investigator's choice of chemotherapy; DTIC: Dacarbazine; mOS: Median overall survival; NA: Not available or not reported; NR: Not reached; ORR: Overall response rate (complete response + partial response); TMZ: Temozolomide.

in Phase II trials suggested that outcomes were similar regardless of HLA-A*0201 status [33].

A second Phase III study randomized treatment-naive patients in a 1:1 ratio to receive ipilimumab 10 mg/kg plus dacarbazine (n = 250) or placebo plus dacarbazine (n = 252) at weeks 1, 4, 7 and dacarbazine alone subsequently [7]. Maintenance doses of ipilimumab every 12 weeks starting at week 24 were recommended for those with stable disease or objective response during the induction phase and without dose-limiting adverse events at week 24. ipilimumab combined with dacarbazine resulted in prolonged overall survival in comparison to control (median OS: 11.2 months vs 9.1; HR for death: 0.72; $p < 0.001$). Although there were no differences in ORR (15.2 vs 10.3%; $p = 0.09$), median duration of response was significantly higher in the ipilimumab-containing arm (19.3 vs 8.1 months; $p = 0.03$). Grade 3 or 4 adverse events occurred in 56.3% of patients treated with ipilimumab plus dacarbazine, but there were no treatment-related deaths. A remarkably high incidence of elevations in alanine aminotransferase (33.2% any grade; 21.9% grade 3/4) and aspartate aminotransferase levels (29.1% any grade; 18.2% grade 3/4) was observed in the group receiving ipilimumab, which could be attributed to the concurrent use of dacarbazine [7]. Whether concomitant dacarbazine modified the occurrence of other immune-related adverse events is somewhat unclear, but some side effects, such as endocrinopathy, appear lower when dacarbazine was given with ipilimumab in this study.

Long-term results of Phase III clinical trial of ipilimumab in treatment-naive patients were recently published by Maio *et al.* [9]. The 5-year survival rate was 18.2% for patients treated with ipilimumab and dacarbazine and 8.8% for patients treated with placebo plus dacarbazine. Noteworthy, a plateau in the survival curve was seen beyond 3 years; in the original publication by Robert *et al.*, OS rates at 2 and 3 years for patients treated with ipilimumab were 28.5 and 20.8%, respectively [7] and, in the study by Hodi *et al.*, 21.6 and 23.5% at 2 years for ipilimumab plus gp100 and ipilimumab alone arms, respectively [6]. In a pooled analysis of 1861 patients treated with ipilimumab in 12 different studies, Hodi *et al.* reported patients with sustained responses extending through 10 years. In line with previously published data, median

OS for patients treated with ipilimumab was 11.4 months and a plateau in OS was observed after year 3, with a 3-year OS rate of 22% [34].

Although ipilimumab was approved by the FDA at a dose of 3 mg/kg given every 3 weeks for four induction doses, the optimal dose of ipilimumab and the role of maintenance therapy are still points of controversy. In the previously discussed Phase III trials, ipilimumab was used at doses of 3 mg/kg and 10 mg/kg and combined with different agents. Different doses of ipilimumab were investigated in a Phase II, dose-ranging study. Two hundred and seventeen pretreated patients were randomized to ipilimumab 10 mg/kg, 3 mg/kg or 0.3 mg/kg every 3 weeks for four cycles, followed by maintenance doses every 3 months [35]. A dose-dependent effect on response rates (primary end point) and incidence of immune-related adverse events was observed; objective responses occurred in 11.1% of patients treated at 10 mg/kg and only 4.2% at 3 mg/kg. In addition, median overall survival intervals were not significantly different across doses (11.4, 8.7 and 8.6 months, respectively), although the study was not designed to detect differences in overall survival [35]. Results of a randomized, Phase III trial comparing ipilimumab at 3 versus 10 mg/kg are expected (NCT01515189).

Ipilimumab was also investigated in the adjuvant setting for patients with completely resected melanoma in the EORTC 18071 Phase III trial [36]. Nine hundred and fifty one patients were randomized in a 1:1 ratio to receive ipilimumab 10 mg/kg (n = 475) or placebo (n = 476) every 3 weeks for four doses, then every 3 months for up to 3 years until completion, disease recurrence or unacceptable toxicity. Although immature for overall survival analysis, the study showed an improvement in the primary end point of recurrence-free survival (RFS) after a median follow-up of 2.7 years (median RFS: 26.1 vs 17.1 months; HR: 0.75; 95% CI: 0.64–0.90; p = 0.0013). Of note, while IFN is considered a valid option in the adjuvant setting, it was not included as a comparator arm in the EORTC 18071 study. How ipilimumab performs against high-dose IFN is being investigated in a separate clinical trial (NCT01274338).

Tremelimumab, an IgG2 anti-CTLA-4 monoclonal antibody, was also investigated in a Phase III clinical trial with 655 treatment-naive patients with melanoma [28]. Tremelimumab at a dose of 15 mg/kg given every 90 days was not superior to standard chemotherapy (temozolomide or dacarbazine) in terms of OS (median OS: 12.6 vs 10.7 months; p = 0.127) or response rate (10.7 vs 9.8%), although duration of response was significantly longer with tremelimumab (35.8 vs 13.7 months; p = 0.0011). Since the response rate and duration of response with tremelimumab resemble that of ipilimumab, it remains unknown as to why no overall survival benefit was seen in this study. It is possible that any difference in overall survival was not able to be detected due to patients initially assigned to the chemotherapy arm ultimately receiving ipilimumab in subsequent trials.

Blocking PD-1 & PD-L1

PD-1 or CD279 is an alternative T-cell costimulatory receptor expressed by activated T cells, B cells and myeloid cells [20,37–38]. PD-1 binds to two distinct specific ligands, PD-L1 and PD-L2. PD-1 ligands are expressed by the tumor microenvironment, malignant cells and APCs [14,15], and the interaction between PD-L1 and PD-1 results in dowregulation of T-cell activation, providing the rationale for the development of agents targeting both PD-1 and PD-L1 [39,40]. Based on unprecedented results across different trials, the anti-PD-1 monoclonal antibodies nivolumab (BMS-936558) and pembrolizumab (MK-3475) were approved by the FDA in 2014 (Table 1), and different compounds targeting PD-1 (pidilizumab) and PD-L1 (MDPL3280A; MEDI4736; BMS-936559) are under clinical development in melanoma and other malignancies.

In a Phase I study that enrolled 296 patients with distinct malignancies treated with nivolumab, objective responses occurred in 28% of the patients with advanced melanoma, and significant activity was also observed in patients with non-small-cell lung cancer and renal cell carcinoma [41]. In a subsequently published update limited to 107 patients with advanced melanoma treated with nivolumab, median overall survival was 16.8 months, with a 2-year overall survival rate of 43%. In total, 31% of the patients had an objective response and durable responses occurred across all nivolumab doses (0.1–10 mg/kg). Patients treated with the recommended dose for further development, 3 mg/kg given every 2 weeks, showed an impressive ORR of 41% [42]. Updated results presented by Hodi *et al.* suggested a median OS of 20.3 months in this cohort [43].

In an open-label, randomized, Phase III study involving patients with melanoma who

progressed on or after anti-CTLA-4 (and a BRAF inhibitor, in case of patients with tumors harboring BRAF mutations), nivolumab 3 mg/kg every 2 weeks (n = 268) was compared with investigator's choice of chemotherapy (n = 102) [30]. Nivolumab was associated with a higher rate of objective response in comparison to chemotherapy (32 vs 11%), with a median time to response of 2.1 months (range: 1.6–7.4 months). Reductions of ≥50% in target lesion burden occurred in 82% (31/38) of nivolumab responders and 60% (3/5) of responders in the investigator's choice of chemotherapy arm. Of note, nivolumab resulted in less grade 3 or 4 adverse events in comparison to chemotherapy (9 vs 31%), and there were no treatment-related deaths [30].

Results of a large Phase III, placebo-controlled trial including 418 treatment-naive patients were recently published by Robert *et al.* [8]. Patients with advanced melanoma without BRAF mutations were randomized to receive nivolumab 3 mg/kg every 2 weeks or dacarbazine 1000 mg/m^2 every 3 weeks. Nivolumab resulted in a 58% reduction in the risk of death, with 1-year overall survival rate of 72.9% as compared with 42.1% in the dacarbazine arm (HR for death: 0.42; 99.79% CI: 0.25–0.73; $p < 0.001$). Nivolumab also resulted in increased PFS (median PFS: 5.1 vs 2.2 months; HR: 0.43; 95% CI: 0.34–0.56; $p < 0.001$) and higher ORR (40 vs 13.9%; odds ratio: 4.06; $p < 0.001$). Of note, grade 3 or 4 adverse events were less common in patients treated with nivolumab (grade 3/4 AE rate 11.7 vs 17.6%) [8].

Pembrolizumab (previously named lambrolizumab and MK-3475), a distinct anti-PD-1 agent, was extensively studied in multicohort Phase I trials. Hamid *et al.* initially investigated pembrolizumab at doses of 2 mg/kg every 3 weeks and 10 mg/kg given every 2 or 3 weeks in a nonrandomized fashion in the KEYNOTE-001 trial [44]. Objective responses occurred in 38% of 135 included patients (both ipilimumab-naive and ipilimumab-pretreated). Grade 3 or 4 adverse events occurred in 13% of the patients. In a subsequent cohort of the same KEYNOTE-001 Phase I trial [29], 173 patients with prior progression on ipilimumab were randomized to receive pembrolizumab at 2 mg/kg every 3 weeks or 10 mg/mg every 3 weeks. ORR was 26% at both doses after a median follow-up of 8 months, with no difference in OS (estimated OS at 1 year: 58 vs 63%, 95% CI: 0.68–1.75). In the expansion cohort of the same trial including a total of 411 patients, pembrolizumab resulted in objective responses in 40% of the ipilimumab-naive patients and 28% in ipilimumab-pretreated patients [45]. Pembrolizumab was also shown to be superior to ipilimumab in the recently published KEYNOTE-006 study [31]. In this Phase III trial, 834 patients with unresectable stage III or IV melanoma and less than two prior systemic treatments (65.8% treatment-naive) were randomized to pembrolizumab 10 mg/kg every 2 weeks, pembrolizumab 10 mg/kg every 3 weeks or four doses of ipilimumab 3 mg/kg every 3 weeks. The estimated 6-month PFS rates were 47.3% for pembrolizumab every 2 weeks, 46.4% for pembrolizumab every 3 weeks and 26.5% for ipilimumab (HR for disease progression: 0.58; $p < 0.001$ for both pembrolizumab regimens vs ipilimumab); objective responses occurred in 33.7, 32.9 and 11.9% of patients in each treatment arm, respectively ($p < 0.001$ for both comparisons). More importantly, pembrolizumab also resulted in higher 12-month survival rates (74.1% for pembrolizumab every 2 weeks, 68.4% for pembrolizumab every 3 weeks and 58.2% for ipilimumab) and less grade 3–5 adverse events (13.3 and 10.1% with pembrolizumab, 19.1% with ipilimumab). The two pembrolizumab schedules resulted in similar efficacy and the trial was stopped after an interim analysis due to significant improvement in survival [31]. It is important to highlight that pembrolizumab was used at variable doses and schedules across different trials, ranging from 2 mg/kg every 3 weeks to 10 mg/kg every 2 weeks, apparently with very similar results, and the current dose approved by the FDA is 2 mg/kg every 3 weeks.

Pidilizumab, a different PD-1 blocker, was also studied in patients with melanoma in a Phase II study with 106 patients (51% ipilimumab-pretreated). Though response rate was low at 5.9%, OS rates at 12 months were similar to pembrolizumab and nivolumab (64%) [46].

Blockade of PD-L1 was also shown to result in antitumoral activity in early-phase clinical trials [47,48]. MDPL3280A, a monoclonal antibody that blocks the ligand PD-L1, resulted in objective responses in multiple tumor types including melanoma in a Phase I, dose escalation study. The same drug was investigated in 45 patients with locally advanced or metastatic melanoma [49]. Objective responses occurred in nine out of 35 patients evaluable for efficacy

(ORR: 26%). Grade 3/4 adverse events occurred in 33% of the patients [49]. MEDI4736, also a monoclonal antibody targeting PD-L1, is currently being investigated in a Phase I clinical trial (NCT01693562) and preliminary results suggested a disease control rate (partial response + stable disease for at least ≥12 weeks) in 46% of the patients [50].

Incidence & management of toxicities associated with immune checkpoint blockers

Anti-CTLA-4 and anti-PD-1 agents result in adverse events that are diverse from those observed with conventional cytotoxic therapies. Since the physiologic purpose of immune checkpoints is to promote tolerance to self antigens and prevent autoimmunity [18,20], the use of immune checkpoint blockers can result in immune-mediated adverse events that mimic idiopathic auto-immune conditions and other T-cell-mediated disorders. These immune-related adverse events (irAE) occur at variable rates and severity (Table 2), and result from inflammatory infiltration of the skin (pruritus, rash), GI tract (enterocolitis), endocrine organs (thyroiditis, hypophysitis, pancreatitis), liver (hepatitis, elevated AST/ALT), lungs (pneumonitis), eyes (uveitis), among other less common irAE [51]. While most irAE are of grades 1 and 2, rare complications include bowel perforation due to colitis, death due to severe pneumonitis and permanent hypopituitarism requiring lifelong hormonal replacement therapy [52].

Although these irAEs can occur at any point during treatment with immune checkpoint blockers, the patterns of different manifestations of irAE are most familiar with ipilimumab; onset of cutaneous, gastrointestinal/hepatic and endocrine irAE usually occurs after 2–3 weeks, 6–7 weeks and 9 weeks, respectively [51]. In the Phase III trial by Hodi *et al.*, for example, all the irAE occurred during the induction or re-induction phases with ipilimumab, suggesting that these complications usually do not develop long periods after discontinuation of the drugs [6].

Since these are immune-mediated events, the use of steroids, antagonists of TNF-α (infliximab) and other immunosuppressive agents has been used with clinical success in some (but not all) situations [51,54]. In a cohort of 25 patients who developed ipilimumab-related hypophysitis, high-dose steroid therapy was not associated with increased resolution, suggesting that permanent hormone therapy may be required [55].

Table 2. Incidence of select adverse events related to anti-CTLA-4 and anti-PD-1 agents.

Grade 3/4 toxicities	Hodi *et al.* 2011 [6]; ipilimumab 3 mg/kg alone	Robert *et al.* 2011 [7]; ipilimumab 10 mg/kg + dacarbazine	Ribas *et al.* 2013 [28]; tremelimumab	Robert *et al.* 2014 [29]; pembrolizumab	Robert *et al.* 2015 [31]; pembrolizumab[†]	Robert *et al.* 2015 [8]; nivolumab 3 mg/kg	Wolchok *et al.* 2013 [53][‡]; nivolumab + ipilimumab	Postow *et al.* 2015 [32][‡]; nivolumab + ipilimumab
Any grade 3/4 (%)	45.8	56.3	52	6	13.3/10.1	11.7	53	54
Immune-mediated (%)	10.2	38.1	NA	2	NA	6.0	NA	NA
Fatigue (%)	6.9	10.9	19	3	0/0.4	0	0	5
Colitis (%)	5.3	4.9	18[§]	0	1.4/2.5	0.5	4	17
AST/ALT abnormalities or hepatitis (%)	0	31.6	1	0.6	1.1/1.8	1.5	24	18
Hypophysitis (%)	1.5	0.4	1	0	0.4/0.4	0.5	2	2
Hypothyroidism (%)	0	0.4	15	0	0.4/0	0	0	0
Rash (%)	0.8	1.2	2	<1	0	0.5	4	3

[†]Values for pembrolizumab 10 mg/kg every 2 weeks and every 3 weeks, respectively.
[‡]Combination treatment arm or cohort.
[§]Includes diarrhea.
NA: Not available under this description.

Guidelines are available to aim decisions and a detailed description is outside the scope of this review [51,54]; nevertheless, early detection followed by optimized medical management is essential in preventing serious immune-related complications. Of note, use of corticosteroids for the management of irAE has not been associated with changes in survival or duration of response to ipilimumab, but this observation needs further validation [56,57].

Patterns of response with immune-checkpoint blockers

Preliminary observations from patients in early-phase clinical trials investigating ipilimumab suggested that a subset of patients could develop patterns of response with immunotherapies distinct from those observed with conventional cytotoxic agents, with delayed responses occurring after variable intervals of stable disease or even progression [58].

Due to these atypical presentations, Wolchok *et al.* first proposed the immune-related response criteria (irRC) in an effort to standardize the assessment and capture atypical kinetics of response in these patients [59], which differ from the standard RECIST [60] or WHO response criteria for solid tumors (Table 3). One key component is the tolerance to new lesions and requirement of repeat imaging after at least 4 weeks for confirmation of response or progression. New lesions are added to the total sum of target lesion measurements, and are not sufficient to qualify as progression of disease. The irRC criteria were applied to 227 patients treated with ipilimumab in a Phase II study; 9.7% of the patients initially characterized as having progression of disease as per standard criteria eventually showed response to ipilimumab [59]. Similarly, in an analysis of long-term survivors following treatment with ipilimumab, 25% of the patients alive at 4 years had progression of disease as best response based on standard criteria [61].

Data in this regard are limited for patients treated with anti-PD-1 agents. Nevertheless, similar patterns of response have also been reported. For example, in the randomized cohort of the previously discussed KEYNOTE-001 trial, 19% of the patients treated with pembrolizumab and with progression of disease based on RECIST criteria were progression free at 6 months as per irRC [29].

Therefore, as per current standards and due to the possibility of responses of late onset, most guidelines and protocols allow for continuation of immune agents beyond initial progression as long as the patient remains clinically stable, with interval imaging after at least 4–6 additional weeks in order to confirm progression [59]. Most contemporary clinical trials incorporate irRC, in addition to standard criteria for response assessment, and more robust validation of irRC criteria should result from prospective trials. Patients that have symptomatic worsening of disease

Table 3. Criteria for response assessment.

Category	RECIST v1.1 criteria	WHO criteria	irRC criteria
Measurement of target lesions	Unidimensional	Bidimensional	Bidimensional
Complete response	Disappearance of all lesions (target and nontarget); lymph nodes must regress to <10 mm short axis; requires confirmation	Disappearance of all lesions, requires confirmation	Disappearance of all lesions, requires confirmation
Partial response	≥30% decrease in tumor burden compared with baseline; no new lesions or progression of nontarget lesions; requires confirmation	≥50% decrease in tumor burden relative to baseline; no new lesions or progression of nontarget lesions; requires confirmation	≥50% decrease in tumor burden relative to baseline; requires confirmation
Progressive disease	≥20% absolute increase in tumor burden relative to nadir; progression of nontarget lesions; appearance of new lesions	≥25% increase in tumor burden relative to nadir, unequivocal progression of nontarget lesions; appearance of new lesions	≥25% increase in tumor burden relative to nadir; new lesions added to tumor burden; requires confirmation
Stable disease	Any response that does not meet criteria for complete response, partial response or progressive disease	Any response that does not meet criteria for complete response, partial response or progressive disease	Any response that does not meet criteria for complete response, partial response or progressive disease

Adapted with permission from [52].

should be offered alternative treatment modalities since the possibility of late benefit to immune checkpoint blockade after obvious progression unfortunately remains the exception.

Role of prognostic/predictive markers

Although several variables have been linked to response following treatment with immune checkpoint blockers and serve as potential biomarkers, no factors can clearly predict response to these agents and tools to select patients who could benefit the most from these therapies remain needed.

In some trials of immunotherapeutic agents, immune-related adverse events have been associated with improved clinical outcomes [56,62–63]. In a recently published meta-analysis, Teulings *et al.* found an overall incidence of vitiligo 3.4% among 5737 patients treated with 139 different immune therapies (not limited to immune checkpoint blockers) since 1995 [64]. Despite the low incidence, vitiligo development was associated with superior PFS (HR: 0.51; 95% CI: 0.32–0.82; $p \leq 0.005$) and OS (HR: 0.25; 95% CI: 0.10–0.61; $p \leq 0.003$) in 27 studies reporting individual patient data [64].

In the peripheral blood of patients treated with anti-CTLA-4 therapy, a mild increase in absolute lymphocyte count above 1000 cells/μl following two doses of ipilimumab (at week 7) was associated with improved outcomes ($p = 0.01$ for clinical benefit and $p<0.001$ for survival) [65], and a similar correlation was found for a lymphocyte count ≥1000 cells/μl at baseline [66]. Eosinophils have similarly been positively associated with ipilimumab outcomes [67]. CTLA-4 blockade was also associated with upregulation of markers of $CD4^+$ and $CD8^+$ T-cell activation [68]. More recently, Chakravarti *et al.* reported that increased CTLA-4 expression on tumor cells and on tumor-infiltrating lymphocytes (TIL) analyzed by immunohistochemistry on pretreatment of tumor samples correlated with poor outcomes in patients treated with ipilimumab [69].

In patients treated with anti-PD-1 agents, PD-L1 has been most extensively investigated as a possible biomarker [70]. Correlative studies in the Phase I trial of nivolumab published by Topalian *et al.* initially showed no objective responses in patients with PD-L1 negative tumors ($p = 0.006$) [41]. Similarly, Herbst *et al.* recently reported responses to MDPL3280A, an anti-PD-L1 agent, were associated with high expression levels of PD-L1, particularly in tumor-infiltrating immune cells [48]. However, larger analyses, while generally showing more favorable outcomes to PD-1 agents for patients whose tumors express PD-L1, indicate that PD-L1 is not a predictive biomarker. Patients with PD-L1 negative tumors can achieve substantial benefits from PD-1 approaches [8,70–71]. In a randomized Phase III trial, patients with PD-L1 negative tumors still achieved improved overall survival with nivolumab compared with dacarbazine chemotherapy. In addition, in a randomized, Phase II trial comparing nivolumab in combination with ipilimumab to ipilimumab alone, objective response rate was independent of tumor PD-L1 status in the combination group (58 vs 55%) [32]. Hence, although this a topic of ongoing research, neither PD-1 nor PD-L1 expression levels in tumoral or immune cells can be used as dichotomous indicators for selection of patients for immune checkpoint blockade as per current standards. PD-L1 expression is inducible, and there may be great heterogeneity, even within an individual patient [72]; moreover, there is no validated cut-off for positivity of PD-L1 expression and distinct antibodies have been used for immunohistochemical analyses, further complicating our understanding of this potential biomarker and limiting our ability to extract robust conclusions.

Significant efforts were made to characterize the intratumoral inflammatory infiltrate in patients treated with immune checkpoint blockade, particularly qualitative, quantitative and spatial variables of TIL. More recently, van Rooji *et al.* were able to correlate a tumoral neoantigen-specific T-cell activity with response to ipilimumab using whole-exome sequencing of tumor samples and TIL [73]. Using the same rationale, Snyder *et al.* used massive parallel sequencing of the exomes of patients treated with CTLA-4 blockade and demonstrated that the somatic mutational load was associated with improved benefit from therapy ($p = 0.01$). The authors also showed that specific tumor neoantigens were able to activate T cells [74].

Moreover, in a recently published study, high-throughput sequencing of the variable β-chain of the TCR was used to characterize the expansion and clonality of the T-cell repertoire and the effects of CTLA-4 and PD-1 blockade [75]. In patients treated with anti-CTLA-4 agents, there was a significant increase in number and complexity of TCR variants in peripheral blood

mononuclear cells during treatment, which was associated with toxicity. No obvious changes in the peripheral blood in TCR diversity were seen in patients treated with pembrolizumab [75].

In a broad analysis encompassing some of the variables and biomarkers previously described, Tumeh *et al.* analyzed samples of 46 patients treated with pembrolizumab and were able to demonstrate that responses correlated with proliferation of intratumoral $CD8^+$ T cells following treatment, and also higher numbers $CD8^+$, $PD\text{-}1^+$ and $PD\text{-}L1^+$ cells in baseline biopsies, but not with levels of CD4 expression at baseline [76]. Using similar techniques of next-generation sequencing of the variable β-chain of TCR, the authors also showed that a development of an intratumoral T-cell population less diverse in repertoire and more clonal in nature also correlated with response to PD-1 blockade [76].

Future perspective

As previously highlighted, despite the great excitement that emerged following the publication of recent studies, more than half of the patients with metastatic melanoma are expected to attain no significant reduction in the tumor burden when immune checkpoint blockers are used as single agents.

Different strategies to overcome resistance and enhance the activity of immunotherapies are under clinical development. In addition to the previously discussed monoclonal antibodies targeting PD-L1, agents targeting distinct checkpoints and molecules involved in immune response are being studied, including the co-stimulatory receptors OX40 (NCT01644968) [77], CD137 (NCT01471210), GITR (NCT01239134), CD27 (alone – NCT01460134 or in combination with nivolumab – NCT02335918). Results of a Phase I trial investigating the anti-CD137 agonist monoclonal antibody urelumab (BMS-663513) were reported in 2008, showing three partial responses among 54 patients with advanced melanoma. Most frequent adverse events included fatigue, elevation of liver enzymes and neutropenia [78].

Combination strategies involving immune checkpoint inhibition with both anti-CTLA4 and anti-PD-1 agents are also being extensively explored, and may shape the future of treatment for patients with advanced melanoma. In a Phase I study, 53 patients were treated with the combination of nivolumab and ipilimumab administered simultaneously every 3 weeks for four doses in dose-escalation cohorts, followed by single-agent nivolumab every 3 weeks for four doses and maintenance infusions every 12 weeks [53]. Overall response rate of the combination among all drug levels was 40%; among patients who received the maximum acceptable doses (nivolumab 1 mg/kg and ipilimumab 3 mg/kg, objective responses occurred in nine of 17 patients (53%). Of note, the combination resulted in significant toxicity, and 53% of the patients developed grade 3 or 4 adverse events [53]. Updated results presented in 2014 showed a 2-year overall survival rate of 75% [79]. More recently, the combination of nivolumab and ipilimumab was shown to result in higher response rates (61 vs 11%; $p < 0.001$) and prolonged PFS (not reached vs 4 months; HR: 0.40; 95% CI: 0.23–0.68; $p < 0.001$) in comparison to ipilimumab alone in *BRAF* V600 wild-type tumors in a randomized, double-blind, Phase II trial [32]. The study included 142 previously untreated patients with metastatic melanoma (109 with *BRAF* wild-type and 33 with *BRAF* V600E mutation-positive tumors). Response rates were also higher among patients with *BRAF* mutation-positive tumors (52 vs 10%), with 22% of complete responses. Drug-related grade 3 or 4 adverse events occurred more frequently in the combination group (54 vs 24%) [32]. Results of a large, randomized trial comparing nivolumab plus ipilimumab to nivolumab or ipilimumab alone are eagerly awaited (NCT01844505), and it is crucial to determine whether the concurrent administration of these agents will result in prolongation of survival.

The role of harnessing additional components and steps of the immune response is also being explored, and the modulation of mechanisms of response mediated by natural killer cells (NK) with monoclonal antibodies that target the KIR is also a promising strategy. The anti-KIR monoclonal antibody lirilumab is currently being studied in combination with ipilimumab (NCT01750580) and nivolumab (NCT01714739).

Talimogene laherparepvec (T-VEC) is an oncolytic immunotherapy derived from herpes simplex virus type-1 (HSV-1) capable of selectively replicating within tumors and producing GM-CSF [80,81]. In a Phase III trial, T-VEC + GM-CSF compared with GM-CSF alone showed an increased durable response rate in patients treated with T-VEC [82]. Updated survival analysis suggested a trend toward improved survival, although the difference did not reach statistical significance (median OS: 23.3 vs 18.9 months; $p = 0.06$) [83]. In a Phase IB trial,

T-VEC was given intralesionally to 18 patients at weeks 1, 4 and then every 2 weeks in association with ipilimumab every 3 weeks starting at week 6. Objective responses occurred in 41% of the patients, including 24% complete responses. Only two patients had possible immune-related grade 3 or 4 adverse events. Correlative studies showed increased activated $CD8^+$ T cells during T-VEC + ipilimumab treatment [84].

A randomized Phase II trial compared ipilimumab 10 mg/kg to ipilimumab combined with subcutaneous GM-CSF [85]. ipilimumab plus GM-CSF resulted in improved overall survival (HR: 0.65; stratified log rank p1 = 0.016; p2 = 0.033) in patients with melanoma, despite lack of improvement in response rates (11.3 vs 4.7%; not significant) or median PFS (3 vs 3.2 months; not significant) [85]. Toxicity of ipilimumab + GM-CSF was also lower than ipilimumab alone in this study, but whether these findings are generalizable to patients receiving 3 mg/kg of ipilimumab remains unknown. Ipilimumab was also combined with peg-IFN alfa-2b in a Phase I study. Among 30 patients evaluable for response, ORR was 46.7% and median OS was 16.6 months, with 55.8% if the patients alive at 1 year [86].

It has also been shown that antigen release resulting from cell death and modulation of the tumor microenvironment could potentially enhance the antitumoral effect of immune checkpoint blockers, providing a rationale for the combination of anti-CTLA-4 and anti-PD-1 agents with conventional cytotoxic chemotherapies and targeted agents in patients with mutations involved in activation of the MAP kinase pathway, particularly BRAF mutations [87–89]. Unfortunately, the combination of ipilimumab and vemurafenib and ipilimumab with dabrafenib and trametinib resulted in significant side effects [90,91], but less is known about the safety and efficacy of regimens containing nivolumab or pembrolizumab in combination with BRAF-targeted agents.

Our group previously reported the regression of distant metastases in a patient receiving radiation therapy during maintenance treatment with ipilimumab [92]. In this particular case, radiation therapy induced a significant increment in the titer of epitope-specific antibodies and $CD4^+$ T-cell activation in the setting of concurrent immune checkpoint blockade. This so-called 'abscopal effect' could result from an increased antigen presentation within the tumor stroma result from direct damage induced by radiation therapy, providing a model in which complementary steps of the immune-mediated response are stimulated in a concurrent fashion. Additional prospective evaluation is underway, and at this time, it remains unclear whether adding radiotherapy enhances clinical efficacy of immune checkpoint blockade.

In conclusion, despite the significant advances in the use and understanding of immune checkpoint blockers, several topics remain unclear, including whether these preliminary results of combined approaches will lead to long-term benefits, the optimal duration of agents targeting PD-1 and the role of re-induction therapy for patients who had initial response. In addition, biomarkers and, more importantly, predictors of response are still far from standard clinical practice. Ongoing studies and the development of novel therapies targeting immune checkpoints could help solve some of these questions and also expand the magnitude of benefit achieved with these strategies.

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References

Papers of special note have been highlighted as:
• of interest

1 Wolchok JD. *Scientific American* 310, 40–45 (2014).

2 Siegel RL, Miller KD, Jemal A. Cancer statistics 2015. *CA Cancer J. Clin.* 65, 5–29 (2015).

3 Erdmann F, Lortet-Tieulent J, Schüz J *et al.* International trends in the incidence of malignant melanoma 1953–2008: are recent generations at higher or lower risk? *Int. J. Cancer* 132(2), 385–400 (2013).

4 Gorantla VC, Kirkwood JM. State of melanoma: an historic overview of a field in transition. *Hematol. Oncol. Clin. North Am.* 28(3), 415–435 (2014).

5 Miller AJ, Mihm MC. Melanoma. *N. Engl. J. Med.* 355, 51–65 (2006).

6 Hodi FS, O'Day SJ, McDermott DF *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* 363(8), 711–723 (2010).

7 Robert C, Thomas L, Bondarenko I *et al.* ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N. Engl. J. Med.* 364, 2517–2526 (2011).

8 Robert C, Long GV, Brady B *et al.* Nivolumab in previously untreated melanoma

without *BRAF* mutation. *N. Engl. J. Med.* 372, 320–330 (2015).

9 Maio M, Grob JJ, amdal S *et al.* Five-year survival rates for treatment-naive patients with advanced melanoma who received ipilimumab plus dacarbazine in a Phase III trial. *J. Clin. Oncol.* 33(10), 1191–1196 (2015).

10 Curiel TJ. Historical perspectives and current trends in cancer immunotherapy. In: *Cancer Immunotherapy: Paradigms, Practice and Promise.* Curiel TJ (Ed.). Springer, New York, NY, USA, (2012).

11 Coley WB. Contribution to the knowledge of sarcoma. *Ann. Surg.* 14, 199–220 (1891).

12 Burnet FM. The concept of immunological surveillance. *Prog. Exp. Tumor Res.* 13, 1–27 (1970).

13 Dunn GP, Bruce AT, Ikeda H *et al.* Cancer immunoediting: from immunosurveillance to tumor escape. *Nat. Immunol.* 3(11), 991–998 (2002).

14 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 144(5), 646–674 (2011).

15 Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* 39(1), 1–10 (2013).

• **Excellent review addressing the interaction of tumor/microenvironment/immune system.**

16 De Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. *Nat. Rev. Cancer* 6(1), 24–37 (2006).

17 Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature* 480(7378), 480–489 (2011).

18 Peggs KS, Quezada SA, Korman AJ, Allison JP. Principles and use of anti-CTLA-4 antibody in human cancer immunotherapy. *Curr. Opin. Immunol.* 18, 206–213 (2006).

19 Robert C, Ghiringhelli F. What is the role of cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma? *Oncologist* 14, 848–861 (2009).

20 Ott PA, Hodi FS, Robert C. CTLA-4 and PD-1/PD-L1 blockade: new immunotherapeutic modalities with durable clinical benefit in melanoma patients. *Clin. Cancer Res.* 19(19), 5300–5309 (2013).

21 Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu. Rev. Immunol.* 23, 515–548 (2005).

• **Comprehensive review addressing the background/rationale involved in immune checkpoint blockade.**

22 Linsley PS, Ledbetter JA. The role of the CD28 receptor during T cell responses to antigen. *Annu. Rev. Immunol.* 11, 191–212 (1993).

23 Wang L, Rubinstein R, Lines JL *et al.* VISTA, a novel mouse Ig superfamily ligand that negatively regulates T cell responses. *J. Exp. Med.* 208(3), 577–592 (2011).

24 Flies DB, Wang S, Xu H, Chen L. Cutting edge: a monoclonal antibody specific for the programmed death-1 homolog prevents graft-versus-host disease in mouse models. *J. Immunol.* 187(4), 1537–1541 (2011).

25 Flies DB, Han X, Higuchi T *et al.* Coinhibitory receptor PD-1H preferentially suppresses $CD4^+$ T cell-mediated immunity. *J. Clin. Invest.* 124(5), 1966–1975 (2014).

26 Watts TH. TNF/TNFR family members in costimulation of T cell responses. *Annu. Rev. Immunol.* 23, 23–68 (2005).

27 O'Day SJ, Hamid O, Urba WJ. Targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4): a novel strategy for the treatment of melanoma and other malignancies. *Cancer* 110, 2614–2627 (2007).

28 Ribas A, Kefford R, Marshall MA *et al.* Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. *J. Clin. Oncol.* 31(5), 616–622 (2013).

29 Robert C, Ribas A, Wolchok JD *et al.* Antiprogrammed- death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a Phase I trial. *Lancet* 384, 1109–1117 (2014).

30 Weber JS, Minor D, D'Angelo SP *et al.* A Phase III randomized, open-label study of nivolumab (anti-PD-1; BMS-936558; ONO-4538) versus investigator's choice chemotherapy (ICC) in patients with advanced melanoma with prior anti-CTLA-4 therapy. Presented at: *2014 European Society of Medical Oncology Congress, Madrid, Spain.* 26–30 September 2014 (Abstract 728).

31 Robert C, Schachter J, Long GV *et al.* Pembrolizumab versus ipilimumab in advanced melanoma. *N. Engl. J. Med.* doi:10.1056/NEJMoa1503093 (2015) (Epub ahead of print).

32 Postow MA, Chesney J, Pavlick AC *et al.* Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N. Engl. J. Med.* doi:10.1056/NEJMoa1414428 (Epub ahead of print) (2015).

33 Wolchok JD, Weber JS, Hamid O *et al.* ipilimumab efficacy and safety in patients with melanoma: a retrospective analysis of HLA subtype from four trials. *Cancer Immun.* 10, 9–14 (2010).

34 Shadendorf D, Hodi FS, Robert C *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.* doi:10.1200/JCO.2014.56.2736 (2015) (Epub ahead of print).

35 Wolchok JD, Neyns B, Linette G *et al.* Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, Phase 2, dose-ranging study. *Lancet Oncol.* 11(2),155–164 (2010)

36 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.* 32(Suppl. 5) Abstract LBA9008 (2014).

37 Freeman GJ, Long AJ, Iwai Y *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.* 192(7), 1027–1034 (2000).

38 Nishimura H, Honjo T. PD-1: an inhibitory immunoreceptor involved in peripheral tolerance. *Trends Immunol.* 22(5), 265–268 (2001).

39 Carter L, Fouser LA, Jussif J *et al.* PD-1:PD-L inhibitory pathway affects both $CD4^+$ and $CD8^+$ T cells and is overcome by IL-2. *Eur. J. Immunol.* 32(3), 634–643 (2002).

40 Latchman Y, Wood CR, Chernova T *et al.* PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat. Immunol.* 2(3), 261–268 (2001).

41 Topalian SL, Hodi FS, Brahmer JR *et al.* Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* 366, 2443–2454 (2012).

42 Topalian SL, Sznol M, McDermott DF *et al.* Survival, durable tumor remission and long-term safety in patients with advanced melanoma receiving nivolumab. *J. Clin. Oncol.* 32, 1020–1030 (2014).

43 Hodi FS, Sznol M, Kluger HM *et al.* Long-term survival of ipilimumab-naive patients with advanced melanoma treated with nivolumab (anti-PD-1, BMS-936558, ONO-4538) in a Phase I trial. *J. Clin. Oncol.* 32(Suppl. 5), Abstract 9002 (2014).

44 Hamid O, Robert C, Daud A *et al.* Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N. Engl. J. Med.* 369, 134–144 (2013).

45 Ribas A, Hodi FS, Kefford R *et al.* Efficacy and safety of the anti-PD-1 monoclonal antibody MK-3475 in 411 patients with melanoma. *J. Clin. Oncol.* 32(Suppl. 5), Abstract LBA9000 (2014).

46 Atkins MB, Kudchadkar RR, Sznol M *et al.* Phase II, multicenter, safety and efficacy study of pidilizumab in patients with metastatic melanoma. *J. Clin. Oncol.* 32(Suppl. 5), Abstract 9001 (2014).

47 Herbst RS, Gordon MS, Fine GD *et al.* A study of MPDL3280A, an engineered PD-L1 antibody in patients with locally advanced or metastatic tumors. *J. Clin .Oncol.* 31(Suppl.) Abstract 3000 (2013).

48 Herbst RS, Soria JC, Kowanetz M *et al.* Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 515(7528), 563–567 (2014).

49 Hamid O, Sosman JA, Lawrence DP *et al.* Clinical activity, safety, and biomarkers with MPDL32801A, an engineered anti-PDL1 antibody in patients with locally advanced or metastatic malignant melanoma. *J. Clin. Oncol.* 31(Suppl.) Abstract 9010 (2013).

50 Lutzky J, Antonia SJ, Blake-Haskins A *et al.* A Phase I study of MEDI4736, an anti-PD-L1 antibody, in patients with advanced solid tumors. *J. Clin. Oncol.* 32(Suppl. 5), Abstract 3001 (2014).

51 Weber JS, Kahler KC, Hauschild A. Management of immune-related adverse events and kinetics of response with ipilimumab. *J. Clin. Oncol.* 30, 2691–2697 (2012).

52 Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. *J. Clin. Oncol.* 33(17), 1974–1980 (2015).

53 Wolchok JD, Kluger H, Callahan MK *et al.* Nivolumab plus ipilimumab in advanced melanoma. *N. Engl. J .Med.* 369, 122–133 (2013).

54 Bristol-Myers Squibb: Yervoy (ipilimumab): Immune-mediated adverse reaction management guide. www.hcp.yervoy.com

55 Min L, Hodi FS, Giobbie-Hurder A *et al.* Systemic high-dose corticosteroid treatment does not improve the outcome of ipilimumab-related hypophysitis. A retrospective cohort study. *Cancer Res.* 21(4), 749–755 (2014).

56 Downey SG, Klapper JA, Smith FO *et al.* Prognostic factors related to clinical response in patients with metastatic melanoma treated by CTL-associated antigen-4 blockade. *Clin. Cancer Res.* 13, 6681–6688 (2007).

57 Ascierto PA, Simeone E, Sileni VC *et al.* Clinical experience with ipilimumab 3mg/kg: real-world efficacy and safety data from an expanded access programme cohort. *J. Transl. Med.* 12, 116–122 (2014).

58 Saenger YM, Wolchok JD. The heterogeneity of the kinetics of response to ipilimumab in metastatic melanoma: patient cases. *Cancer Immun.* 8, 1–7 (2008).

59 Wolchok JD, Hoos A, O'Day S *et al.* Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin. Cancer Res.* 15, 7412–7420 (2009).

60 Eisenhauer EA, Therasse P, Bogaerts J *et al.* New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur. J. Cancer* 45(2), 228–247 (2009).

61 Wolchok JD, Weber JS, Maio M *et al.* Four-year survival rates for patients with metastatic melanoma who received ipilimumab in Phase II clinical trials. *Ann. Oncol.* 24, 2174–2180 (2013).

62 Attia P, Phan GQ, Maker AV *et al.* Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. *J. Clin. Oncol.* 23, 6043–6053 (2005).

63 Gogas H, Ioannovich J, Dafni U *et al.* Prognostic significance of autoimmunity during treatment of melanoma with interferon. *N. Engl. J. Med.* 354, 709–718 (2006).

64 Teulings H, Limpens J, Jansen SN *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.* 33(7), 773–781 (2015).

65 Ku GY, Yuan J, Page DB *et al.* Single-institution experience with ipilimumab in advanced melanoma patients in the compassionate use setting: lymphocyte count after 2 doses correlates with survival. *Cancer* 116, 1767–1775 (2010).

66 Postow MA, Chasalow SD, Yuan J *et al.* Evaluation of the absolute lymphocyte count as a biomarker for melanoma patients treated with the commercially available dose of ipilimumab (3 mg/kg). *J. Clin. Oncol.* 30(Suppl.), Abstract 8575 (2012).

67 Schindler K, Harmankaya K, Postow MA *et al.* Pretreatment levels of absolute and relative eosinophil count to improve overall survival (OS) in patients with metastatic melanoma under treatment with ipilimumab, an anti-CTLA-4 antibody. *J. Clin. Oncol.* 31(Suppl.) Abstract 9024 (2013).

68 Wang W, Yu D, Sarnaik AA *et al.* Biomarkers on melanoma patient T cells associated with ipilimumab treatment. *J. Transl. Med.* 10, 146–157 (2012).

69 Chakravarti N, Glitza IC, Trinh VA *et al.* Biomarker analysis predicts antitumor activity of ipilimumab therapy in patients with refractory metastatic melanoma. *Cancer Res.* 74: CT332 (2014).

70 Taube JM, Klein AP, Brahmer JR *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.* 20, 5064–5074 (2014).

71 Weber JS, Kudchadkar RR, Yu B *et al.* Safety, efficacy, and biomarkers of nivolumab with vaccine in ipilimumab-refractory or -naive melanoma. *J. Clin. Oncol.* 31, 4311–4318 (2013).

72 Madore J, Vilain RE, Menzies AM *et al.* PD-L1 expression in melanoma shows marked heterogeneity within and between patients: implications for anti-PD-1/PD-L1 clinical trials. *Pigment Cell Melanoma Res.* 28(3), 245–253 (2014).

73 van Rooij N, van Buuren MM, Philips D *et al.* Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. *J. Clin. Oncol.* 31(32), e439–e442 (2013).

74 Snyder A, Makarov V, Merghoub T *et al.* Exome sequencing reveals a genetic basis underlying clinical response to CTLA-4 blockade. *N. Engl. J. Med.* 371, 2189–2199 (2014).

- **Very interesting work correlating the response to ipilimumab to specific neo-antigens.**

75 Robert L, Harview C, merson R *et al.* Distinct immunological mechanisms of CTLA-4 and PD-1 blockade revealed by analyzing TCR usage in blood lymphocytes. *Oncoimmunology* 3, e29244 (2014).

76 Tumeh PC, Harview CL, Yearley JH *et al.* PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 515(7528), 568–571 (2014).

- **Very interesting work addressing biomarkers in patients treated with pembrolizumab.**

77 Curti BD, Kovacsovics-Bankowski M, Morris N *et al.* OX40 is a potent immune-stimulating target in late-stage cancer patients. *Cancer Res.* 73, 7189–7198 (2013).

78 Sznol M, Hodi FS, Margolin K *et al*. Phase I study of BMS-663513, a fully human anti-CD137 agonist monoclonal antibody, in patients (pts) with advanced cancer (CA). *J. Clin. Oncol.* 26(15 Suppl.), 3007 (2008).

79 Sznol M, Klugger HM, Callahan MK *et al*. Survival, response duration, and activity by *BRAF* mutation (MT) status of nivolumab (NIVO, anti-PD-1, BMS-936558, ONO-4538) and ipilimumab (IPI) concurrent therapy in advanced melanoma (MEL). *J. Clin. Oncol.* 32(Suppl. 5), Abstract LBA9003 (2014).

80 Liu BL, Robinson M, Han ZQ *et al*. ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. *Gene Ther.* 10(4), 292–303 (2003).

81 Toda M, Martuza RL, Rabkin SD. Tumor growth inhibition by intratumoral inoculation of defective herpes simplex virus vectors expressing granulocyte-macrophage colony-stimulating factor. *Mol. Ther.* 2(4), 324–329 (2000).

82 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.* 32(Suppl. 5), Abstract 9008a (2014).

83 Andtbacka RHI, Collichio FA, Amatruda T *et al*. Final planned overall survival (OS) from OPTiM, a randomized Phase III trial of talimogene laherparepvec (T-VEC) versus GM-CSF for the treatment of unresected stage IIIB/C/IV melanoma (NCT00769704). *J. Immunother. Cancer* 2(Suppl. 3), P263 (2014).

84 Puzanov I, Milhem MM, Andtbacka RHI *et al*. Primary analysis of a Phase 1b multicenter trial to evaluate safety and efficacy of talimogene laherparepvec (T-VEC) and ipilimumab (ipi) in previously untreated, unresected stage IIIB-IV melanoma. *J. Clin. Oncol.* 32(Suppl. 5), Abstract 9029 (2014).

85 Hodi S, Lee SJ, McDermott DF *et al*. Multicenter, randomized Phase II trial of GM-CSF (GM) plus ipilimumab (Ipi) versus Ipi alone in metastatic melanoma: E1608. *J. Clin. Oncol.* 31(Suppl.), Abstract CRA9007 (2013).

86 Kudchadkar RR, Gibney GT, Dorman D *et al*. A Phase IB study of ipilimumab with peginterferon alfa-2b for patients with unresectable stages IIIB/C/IV melanoma. *J. Clin. Oncol.* 32(Suppl. 5), Abstract 9098 (2014).

87 Agarwala SS. Novel immunotherapies as potential therapeutic partners for traditional or targeted agents: cytotoxic T-lymphocyte antigen-4 blockade in advanced melanoma. *Melanoma Res.* 20(1), 1–10 (2010).

88 Maker AV, Phan GQ, Attia P *et al*. Tumor regression and autoimmunity in patients treated with cytotoxic T lymphocyte associated antigen 4 blockade and interleukin 2: a Phase I/II study. *Ann. Surg. Oncol.* 12(12), 1005–1016 (2005).

89 Ozao-Choy J, Ge M, Kao J *et al*. The novel role of tyrosine kinase inhibitor in the reversal of immune suppression and modulation of tumor microenvironment for immune-based cancer therapies. *Cancer Res.* 69(6), 2514–2522 (2009).

90 Ribas A, Hodi FS, Callahan M *et al*. Hepatotoxicity with combination of vemurafenib and ipilimumab. *N. Engl. J. Med.* 368, 1365–1366 (2013).

91 Puzanov I, Callahan MK, Linette GP *et al*. Phase 1 study of the BRAF inhibitor dabrafenib (D) with or without the MEK inhibitor trametinib (T) in combination with ipilimumab (Ipi) for V600E/K mutation-positive unresectable or metastatic melanoma (MM). *J Clin. Oncol.* 32(Suppl. 5s), Abstract 2511 (2014).

92 Postow MA, Callahan MK, Barker CA *et al*. immunologic correlates of the abscopal effect in a patient with melanoma. *N. Engl. J. Med.* 366, 925–931 (2012).