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Immunotherapy for Malignant Melanoma: Current Perspectives and Future Directions

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ABSTRACT

Malignant melanoma is a significant public health problem; according to 2013 SEER data, its average incidence rate rose 1.4% each year for the last decade, and it is now the sixth most common cancer diagnosis in the United States with 74,000 new cases each year. The rising incidence and historical poor response to chemotherapy have led to intense investigation of novel treatments for melanoma, including therapies to improve the immune-mediated destruction of cancer cells. Among the hallmarks of malignancy is the ability to evade this process: while early stages of tumor growth can induce functional CD8+ T cell responses, cancer cells become increasingly embedded in an immune-suppressive tumor stroma. In the tumor microenvironment, T-cell proliferation and effectors function are impaired due to engagement of cytosolic T-lymphocyte antigen 4 (CTLA4); furthermore, T-cell mediated cytotoxicity is impaired by engagement of T-cell programmed death receptor 1 (PD-1) with programmed death receptor ligand (PD-L1) expressed by cancer cells and antigen presenting cells. This receptor-ligand engagement thereby inhibits immunity, allows the tumor to continue to grow, and contributes to the phenomenon of "T-cell exhaustion." Inhibition of CTLA-4 and the interaction between PD-L1 and PD-1 overcomes a critical immune

checkpoint to facilitate destruction of cancer cells, and has led to robust clinical outcomes and long-term survival. In this chapter we discuss the rationale for CTLA-4 and PD-1/PDL1 inhibition in cancer, results of clinical trials targeting CTLA-4, PD-1 and PD-L1 in malignant melanoma, and the promise immunotherapy holds as a future anticancer treatment. These recent developments represent the fruits of years of preclinical and clinical research to better understand the complex mechanisms of immune regulation and the ways in which tumors exploit those mechanisms to evade and the antitumor immune response.

ABBREVIATIONS

CTLA4: Cytotoxic T-Lymphocyte Antigen-4; DCR: Disease Control Rate; DOR: Duration of Response; HR: Hazard Ratio; irAE: immune-related Adverse Event; OS: Overall Survival; PD1: Programmed Death Receptor 1; PDL1: Programmed Death Receptor Ligand 1; PFS: Progression Free Survival; TILs: Tumor-Infiltrating Lymphocytes

INTRODUCTION

As our knowledge and understanding of immune regulatory mechanisms such as immune checkpoints and co-stimulatory signals has improved, it has become clear that earlier approaches to immunotherapy for advanced cancer, which sought primarily to enhance activation of tumor-specific T cells and other immune effectors cells, were often inadequate to maintain effective disease control. The primary reason for the many disappointing results for immunotherapy in the past is that tumors evolve the ability to exploit regulatory mechanisms that suppress the antitumor immune response. The incredible growth in cancer immunotherapy has primarily been driven by the development and successful commercialization of immune checkpoint inhibitors against key regulatory ligands (CTLA-4, PD-1, and PDL-1) and the first oncolytic vaccine for the treatment of advanced melanoma.

Table 1: Key clinical trials of CTLA-4 checkpoint inhibitors in melanoma.

Agent	Phase	Indication	Treatment arms	N	Primary endpoint	Efficacy results	Grade 3/4 AEs	Status
Ipilimumab	III	Unresectable/ metastatic	Ipi vs Ipi + gp100 vs gp100	676	OS	10 months (Ipi + gp100) vs 10.1 months (Ipi alone) vs 6.4 months (gp100 only)	Ipi+gp100: derm (40%), GI (32.1%), endo (3.9%), other (3.2%), hepatic (2.1%)	Completed
						1-year OS: 43.6% vs 21.6%	Ipi alone: derm (43.5%), GI (29%), endo (7.6%), other (4.6%), hepatic (3.8%)	
						2-year OS: 25.3% vs 13.7%		
	III	Unresectable/ metastatic	Ipi + dacarbazine vs dacarbazine + placebo	502	OS	Median OS 11.2 months (Ipi + dacarbazine) vs 9.1 months (dacarbazine alone)	Diarrhea (32.8%); increase in ALT (29.1%); pruritus, increase in AST (26.7% each); rash (22.3%); colitis (4.5%); hepatitis (1.6%)	Completed
						1-year OS: 47.3% vs 36.3%		
						2-year OS: 28.5% vs 17.9%		
						3-year OS: 20.8% vs 12.2%		
	III	Resected, adjuvant	Ipi vs placebo	951	RFS	Median RFS: 26.1 months (ipi) vs 17.1 months (placebo)	Gastrointestinal (16% vs <1% in the placebo group), hepatic (11% vs <1%), and endocrine (8% vs none).	Completed
							Adverse events led to discontinuation of treatment in 245 (52%) of 471 patients who started ipilimumab (182 [39%] during the initial treatment period of four doses)	
						3-year RFS: 46.5% (ipi) vs 34.8% (placebo)		
	III	Resected, adjuvant	Ipi vs high-dose IFN					Ongoing

Tremelimumab	I/II	Unresectable/ metastatic	Tremilimumab	28+89	ORR	1 CR, 3 PR (phase 2)	12 (27%) at 15 mg/kg/ 6 (13%) at 10 mg/kg (Phase 2)	Completed
	II	Unresectable/ metastatic	Tremilimumab	246	ORR	6.6% ORR (16 PR)	Diarrhea (11%), colitis (4%), fatigue (2%)	Completed
	III	Unresectable/ metastatic	Tremilimumab vs standard of care chemotherapy	655	Response	10.7% ORR vs 9.8% (not significant) OS 12.6 vs 10.7 months (not significant)	Diarrhea (18%); fatigue (6%); nausea, vomiting, decreased appetite, abdominal pain, (4% each); dyspnea (3%); rash, peripheral edema (2% each); pruritus, thrombocytopenia, pirexia, neutropenia, constipation, cough, headache, weight decrease (≤1% each)	Completed
	I/II	Unresectable/ metastatic	Tremilimumab plus anti-CD40 antibody	24	Safety, response	ORR 27.3% Two patients with complete response; four with partial response	None noted. Grade 1/2 cytokine reléase síndrome seen with anti-CD40 infusion	Ongoing

Abbreviations: AEs: Adverse Events; ipi: ipilimumab; ORR: Objective Response Rate; OS: Overall Survival; RFS: Recurrence-Free Survival

Table 2: Key clinical trials of PD-1/PD-L1 checkpoint inhibitors in melanoma.

Target/ Agent	Phase	Indication	Treatment arms	N	Primary endpoint	Efficacy results	Grade 3/4 AEs	Status
PD-1/	I	Unresectable/ metastatic	Nivolumab	107	ORR	31% ORR; median OS 16.8 mos; 1-year OS 61%; 2-year OS 44%, 3-year OS 40%	21% pts (3% lymphopenia; 2% fatigue, increase lipase, diarrhea, hepatitis, and endocrine disorders)	Completed
Nivolumab	III	Unresectable/ metastatic PD after ipilimumab (Checkmate 037)	Nivolumab vs standard of care chemotherapy (dacarbazine or paclitaxel)	167	ORR	ORR: 31.7% (nivolumab) vs 10.6% in chemotherapy Group	Increased lipase, increased alanine aminotransferase, anemia, fatigue (1% each)	Completed

	III	Unresectable/ metastatic Previously untreated, BRAF wild type (Checkmate 066)	Nivolumab vs dacarbazine	418	ORR	ORR: 40% (nivo) vs 13.9% (dacarbazine) OS: 72.9% (nivo) vs 42.1% (dacarbazine) PFS: 5.1 months (nivo) vs 2.2 months (dacarbazine)	Fatigue, pruritus, nausea (11.7% nivolumab, 17.6% dacarbazine)	Completed
	III	Unresectable/ metastatic Previously untreated (Checkmate 067)	Nivolumab vs ipilimumab vs nivo plus ipi	945	PFS	PFS: 11.5 months (nivo + ipi), 6.9 months (nivo), 2.9 months (ipi)	Nivo alone: 16.3% Ipi alone: 27.3% Ipi + nivo: 55% (36.4% discontinuation rate)	Completed
PD-1/ Pembrolizumab	I/II	Unresectable/ metastatic in pre-treated and ipi-naïve patients	Pembrolizumab	135	Safety, response	ORR 38% ipi pre-treated, 37% ipi naive Median PFS > 7 months	13% pts, with 3 pts rash (2%), 1 pt pruritus (1%), 2 pts fatigue (1%)	Completed
	III	Unresectable/ metastatic in pre-treated and ipi-naïve patients	Pembrolizumab	365	ORR	ORR 40% ipi naive, 28% ipi pre-treated Median PFS: 24 weeks ipi naive, 23 weeks ipi pre-treated	Colitis, rash, diarrhea (12%)	Completed
	II	Unresectable/ metastatic in pre-treated and ipi-naïve patients (Keynote 002)	Pembrolizumab 2mg/kg or 10mg/kg vs investigator-choice chemotherapy	540	PFS	6-month PFS: 34% pembro 2mg/kg 38% pembro 10mg/kg 16% chemotherapy	11% in 2mg/kg group (fatigue) 14% in 10mg/kg group (fatigue) 26% in chemotherapy group	Completed
	III	Unresectable/ metastatic in ipi-naïve patients (Keynote 006)	Pembrolizumab 10mg/kg q2 wk or q3wk vs Ipilimumab	834		6-month PFS:47.3% pembro q2wk, 46.4% pembro q3wk, 26.5% ipi 12-month OS: 74.1%, 68.4%, 58.2%	13.3% q2 wk regimen, 10.1% q3 wk regimen, 19.9% ipi regimen	Completed
PD-L1/ BMS-936559	I	Unresectable/ metastatic	BMS-936559 1mg/kg, 3mg/kg, 10mg/kg	55	ORR	ORR (9 pts), 6% (1mg/kg), 29% (3mg/kg), 19% (10mg/kg) 27% SD (14 pts) at 24 weeks	3% (7 pts): 1 adrenaline insufficiency, 1 pancreatitis, 1 vomiting, 1 chest pain, 1 sarcoidosis, 1 endophthalmitis, 1 elevated aspartate, 1 myasthenia gravis	Completed

PD-L1/ MPDL3280A	I	Unresectable/ metastatic	MPDL3280A	45	ORR	26% ORR 24-week PFS 35%	Hyperglycemia (7%), elevated ALT (7%), elevated AST (4%)	Completed
PD-L1/ MPDL3280A	I	Unresectable/ metastatic patients with previously untreated BRAF mutant disease	MPDL3280A plus vemurafenib alone vs MPDL3280A plus vemurafenib and cobimetinib					Ongoing
MEDI4736	I	Unresectable/ metastatic patients with previously untreated BRAF mutant disease	MEDI4736 plus dabrafenib plus trametinib vs MEDI4736 plus trametinib alone					Ongoing

TUMOR AND T-CELL INTERACTION

One of the key mechanisms by which malignant cells suppress anti-tumor immunity lies in the interaction between tumor cells and infiltrating T-cells; despite expression of numerous antigens, tumor evasion of host immunity occurs, and a number of tumor-associated immune inhibitory mechanisms have been identified in the last decade [1]. They include expression of the ligand PD-L1 by tumor cells, which can engage the inhibitory receptor PD-1 on activated T cells; increased levels of the tryptophan-catabolizing enzyme Indoleamine-2,3-Dioxygenase (IDO), which starves T-cells of essential tryptophan; tumor infiltration with various subtypes of FoxP3+ regulatory T cells (Tregs), which mediate extrinsic T-cell suppression; and defective interleukin-2 (IL-2) production, driven in part through Early Growth Response Protein 2 (EGR2), causing T-cell energy [2-5]. These suppressive mechanisms have been demonstrated in solid tumor models in rodents, including melanoma, where increased expression of PD-L1, IDO, and FoxP3+ Tregs is seen in the tumor microenvironment [6].

CTLA-4 and T Cell Response

CTLA-4 (cytosolic T-lymphocyte antigen-4), an inhibitory receptor expressed by tumor-infiltrating T cells and regulatory T cells, was the prototypical immune checkpoint first described by Allison and colleagues in the mid-1990s [7]. CTLA-4 is expressed on activated T cells and counters the co-stimulatory signal provided by CD28, thereby down-regulating the extent of T-cell activation. CTLA-4 engagement by antibody cross-linking or binding to B7 inhibits proliferation and accumulation of the primary T cell growth factor, IL-2, by cells stimulated with anti-CD3 and anti-CD28. Initial studies showing the efficacy of anti CTLA-4 antibodies in the treatment of tumor models in mice assumed that the effect was due to the blockade of CTLA-4 inhibitory signaling in effectors T cells [8,9]. The discovery of FoxP3+ regulatory Tregs expressing high levels of CTLA-4 on their surface suggested Tregs themselves were also a potential target for anti-CTLA4 therapy [10,11], and recent studies have shown blockade of CTLA-4 on both T-effectors cells and Tregs is required for optimal tumor immunity [12]. A summary of the clinical trials of anti-CTLA4 antibodies in malignant melanoma may be found later in this chapter.

PD-1 and PD-L1 in Tumor Survival

An intense focus in immunotherapy has been on PD-1, a 55 KD type I transmembrane protein with two known ligands: PD-L1 (B7-H1/CD274) and PD-L2 (B7-DC/CD273) [13]. When bound to PD-1 on T cells, both PD-L1 and PD-L2 has been shown to down-regulate T-cell activation in both murine and human models [14-16]. PD-1-deficient mice develop autoimmune phenomena, including dilated cardiomyopathy [17] and a lupus-like syndrome with arthritis and nephritis [18]. Additional mouse models have showed that antibody-mediated abrogation of PD-1 led to encephalomyelitis [19] and graft-versus-host disease [20], suggesting that its blockade has the potential to activate auto-inflammatory T-cell responses. Importantly, inhibition of PD-1 activity also enhances anti-tumor immune responses [21-23].

PD-L1 can be found on neutrophils [24], antigen-presenting cells (macrophages and dendritic cells), myeloid-derived suppressor cells [25], and activated T cells. It is also found on a number of solid tumors, including non-small cell lung cancer (NSCLC) [26], renal cell carcinoma [27,28], esophageal cancer [29], and oral squamous cell carcinoma [30,31]. In melanoma, PD-L1 is expressed both in melanoma and also on stroma cells, and its expression increases with disease progression [32].

PD-L1 binding enhances apoptosis of activated tumor-specific T cells in vitro [33], and may protect tumor cells from the induction of apoptosis by effectors T cells [34]. Retrospective analyses in several human tumor types suggest that tumor expression of PD-L1 may permit immune evasion by tumors; in renal cell carcinoma, high surface expression of PD-L1 on tumor cells is related to tumor aggressiveness. Patients with high tumor and/or lymphocyte PD-L1 levels were 4.5 times more likely to die from their cancer than those exhibiting low levels of PD-L1 [35]. In a multivariate analysis, high expression of PD-L1 in melanoma was independently associated with a worse OS rate compared to low expression levels [36], conferring an adaptive resistance to immune attack and release of IFN-γ by Tumor Infiltrating Lymphocytes (TILs) [37]. Tumor over expression of PD-L1 may explain how melanomas escape immune destruction, with preclinical data leading to the suggestion that therapies directed at this pathway may benefit patients.

Various experimental systems have been tested to design strategies to block the PD-1/ PD-L1 interaction, including DNA vaccination against the extracellular region of PD-1 [38], genetic ablation of the PD-1 gene [39], and recombinant antibodies directed at the extracellular region of PD-1 and PD-L1 [40]. Of these, monoclonal antibodies have been the most studied in patients, with results of recent clinical trials of PD-1 and PD-L1 antibodies discussed later in this chapter.

CLINICAL TRIALS OF CHECKPOINT INHIBITION

Anti-CTLA4 (Ipilimumab) for Aadvanced Melanoma

Ipilimumab is a monoclonal Antibody (mAb) targeting cytosolic T-lymphocyte-associated antigen 4 (CTLA-4), the development of which has spanned nearly 2 decades [41].

One of the pivotal trials of ipilimumab in previously treated patients with metastatic melanoma was MDX010-20, a multinational, randomized, double-blind, Phase III trial. In this study, patients were randomized (3:1:1) to ipilimumab plus the gp100 peptide vaccine (n = 403), ipilimumab alone (n = 137), or gp100 alone (n = 136). Ipilimumab (3 mg/kg) with or without gp100 was administered once every 3 weeks for up to 4 treatments. Treatment with ipilimumab plus gp100 demonstrated a statistically significant OS benefit compared with gp100 alone (median survival 10.0 *vs.* 6.4 months; Hazard Ratio [HR] = 0.68; P < .001) [42]. Additionally, 1- and 2-year survival rates were nearly double for patients treated with ipilimumab plus gp100 (43.6% and 25.3%, respectively) versus gp100 alone (21.6% and 13.7%, respectively). Results were also consistently in favor of ipilimumab plus gp100 regardless of disease state at presentation, baseline lactate dehydrogenase levels, or age.

Another multinational, randomized, double-blind, Phase III trial compared ipilimumab (10 mg/kg) plus dacarbazine (850 mg/m2; n = 250) with dacarbazine alone (850 mg/m2; n = 252) with placebo, given at weeks 1, 4, 7, and 10 followed by dacarbazine alone every 3 weeks through week 22, in treatment-naïve patients with advanced melanoma [43]. Median OS was significantly longer for patients treated with ipilimumab plus dacarbazine compared with dacarbazine alone (11.2 *vs.* 9.1 months), and survival rates were higher with ipilimumab and dacarbazine at 1 year (47.3% *vs.* 36.3%), 2 years (28.5% *vs.* 17.9%), and 3 years (20.8% *vs.* 12.2%; HR = 0.72, P < .001). The addition of ipilimumab to dacarbazine also resulted in a 24% reduction in the risk of progression (HR = 0.76, P = .006). In these trials, approximately 10% of patients experienced prolonged tumor regression. In fact, some patients from the initial phase 2 trials have remained free of progression for more than 7 years [44]. In 2011, ipilimumab was approved in 2011 by the United States Food and Drug Administration (FDA) for the treatment of advanced melanoma, considered a milestone event as it was the first therapy shown to improve OS in a randomized trial in this patient population [45]. Recently, pooled data from 4846 patients who received ipilimumab within a clinical study or expanded access program was analyzed to provide an estimation of long-term survival: a plateau in survival began approximately 3 years after treatment for 21% of patients, and in some continued for up to 10 years. The durability of long-term survival did not appear to be impacted by prior therapy, dose or treatment regimen [46].

Ipilimumab in the Adjuvant Setting

The success of ipilimumab in patients with metastatic melanoma led to the investigation of its use in the adjuvant setting. EORTC 18071 was a randomized, double-blind, Phase III trial of ipilimumab versus placebo in patients with Stage III coetaneous melanoma (excluding lymph node metastasis ≤1 mm or in-transit metastasis) with adequate resection of lymph nodes. Patients were randomly assigned (1:1) to receive intravenous infusions of 10 mg/kg ipilimumab or placebo every 3 weeks for four doses, then every 3 months for up to 3 years. Of 951 patients enrolled, 475 were assigned to ipilimumab and 476 to placebo, all of whom were included in the intention-to-treat analyses. At a median follow-up of 2.74 years, median Recurrence-Free

Survival (RFS) was 26.1 months [95% CI 19.3-39.3] in the ipilimumab group versus 17.1 months [95% CI 13.4-21.6] in the placebo group (Hazard Ratio [HR] 0.75 [95% CI 0.64–0.90]; p=0•0013); 3-year RFS was 46.5% [95% CI 41.5–51.3] in the ipilimumab group versus 34.8% [95% CI 30.1–39.5] in the placebo group [47]. Based on the outcome of the EORTC trial, ipilimumab was granted FDA approval for the adjuvant treatment of melanoma in November 2015. Ipilimumab is also being evaluated at a lower dose (3mg/kg) in the ECOG 1609 trial, a Phase III randomized study of adjuvant ipilimumab versus high-dose interferon a-2b for resected high-risk melanoma [NCT01274338].

Immune-related Adverse Events with Ipilimumab

The unprecedented activity of ipilimumab in patients with metastatic melanoma is exciting but not without side effects. Because it "loosens the reins" on T cell activation and proliferation, ipilimumab can potentially allow self-reactive T cells to proliferate, and this has been associated with characteristic side effects collectively referred to as immune-related Adverse Events (irAEs). The most common irAEs include diarrhea, colitis, pneumonitis, endocrinopathies including hypophysitis and thyroiditis, hepatitis, vitiligo, and rash [48]. Approximately 60% of ipilimumab-treated patients in study MDX010-20 experienced irAEs [42] and in the adjuvant trial of ipilimumab at 10mg/kg, of Grade 3-4 irAEs 16% were gastrointestinal, 11% were hepatic, and 8% were endocrine [47]. More importantly, irAEs led to discontinuation of treatment in 52% of patients who started ipilimumab (39% during the initial treatment period of four doses), and of five deaths, three patients died because of colitis (two with gastrointestinal perforation), one from myocarditis, and one from multiorgan failure with Guillain-Barre syndrome. Close monitoring is essential during anti-CTLA-4 therapy, and management guidelines recommend symptomatic treatment for mild irAEs and dose delay/omission and intense monitoring for moderate or persistent mild irAEs [49]. In patients with persistent or high-grade irAEs, ipilimumab should be permanently discontinued and high-dose systemic corticosteroid therapy started. The most common irAEs typically resolve within 4 to 9 weeks of onset, depending on the organ system involved [50].

Early Trials of Tremelimumab in Advanced Melanoma

Tremelimumab (CP-675,206, Pfizer) is another anti-CTLA-4 mAb in development. Consistent with ipilimumab, tremelimumab demonstrated encouraging antitumor activity with durable responses in phase 1 and phase 2 studies in patients with metastatic melanoma [51,52]. Based on these findings, a Phase III study comparing tremelimumab (15 mg/kg once every 90 days) with physician's choice of chemotherapy (temozolomide or dacarbazine) was conducted in patients with newly diagnosed metastatic melanoma [53]. However, tremelimumab failed to improve OS compared with chemotherapy (12.6 *vs.* 10.7 months; HR = 0.88; p = .127), and clinical development as a single agent in melanoma was discontinued. Tremelimumab is currently under investigation in combination with an anti-CD40 antibody and showed preliminary high Objective

Response Rate (ORR) in patients with advanced melanoma [54]. Among 24 patients treated, ORR was 27.3%; two patients (9.1%) had complete responses and four (18.2%) had partial responses. The median follow-up was 22 months with a median PFS of 2.5 months and a median OS of 26.1 months. Cytokine Release Syndrome (CRS), Grade 1-2, was the most common treatment-related toxicity occurring in 19 (79.2%) patients. CRS symptoms were controlled with standard supportive care. All episodes of CRS resolved within 24 hours of anti-CD40 administration.

Anti-PD1 (nivolumab and pembrolizumab) in Advanced Melanoma

In 2013, anti-PD-1/PD-L1 antibodies were named "drug of the year" [55], due in large part to the exciting data reported from early phase trials of anti-PD1 antibodies nivolumab (BMS-936558, Bristol-Meyers Squibb) and pembrolizumab (MK-3475, Merck) in patients with advanced melanoma. These studies demonstrated impressive rates of response and prolonged Duration of Response (DOR), and are summarized below.

Nivolumab

Nivolumab, a fully human immunoglobulin G4 (IgG4) anti-PD-1 mAb, was the first to be assessed in patients with metastatic melanoma. Nivolumab was evaluated in a Phase I study in patients with a variety of malignancies (n = 296), including 107 with advanced melanoma [56,57]. The ORR for the melanoma cohort was 31% (33 of 106), and OS rates were 61% at 1 year, 44% at 2 years, and 40% at 3 years. Grade 3 or 4 drug-related AEs occurred in 21% of patients, with the most common being lymphopenia (3%), and fatigue, increased lipase, diarrhea, and endocrine disorders (2% each); no Grade ≥ 3 drug-related pneumonitis was reported in the melanoma cohort.

The Checkmate-037 trial evaluated the efficacy and safety of nivolumab compared with Investigator's Choice of Chemotherapy (ICC) as a second-line or later-line treatment in patients with advanced melanoma. In this randomized open-label Phase III trial, patients with unrespectable or metastatic melanoma with disease progression after ipilimumab (or ipilimumab and a BRAF inhibitor if they were BRAFV 600 mutation-positive) were randomly assigned 2:1 to receive an intravenous infusion of nivolumab 3 mg/kg every 2 weeks or ICC (dacarbazine 1000 mg/m2 every 3 weeks or paclitaxel 175 mg/m2 combined with Ccarboplatin AUC 6 every 3 weeks) until progression or unacceptable toxic effects [58]. Confirmed ORR was reported in 38 patients (31.7% [95% CI 23.5-40.8]) of the first 120 patients in the nivolumab group versus five (10.6% [95% CI 3.5-23.1]) of 47 patients in the ICC group. Grade 3–4 adverse events related to nivolumab included increased lipase, increased alanine aminotransferase, anemia, and fatigue (1% each). No treatment-related deaths occurred.

The Checkmate-066 trial evaluated nivolumab versus dacarbazine in 418 previously untreated BRAF wild-type patients with metastatic melanoma [59]. Patients were assigned nivolumab 3mg/kg every two weeks plus dacarbazine-matched placebo every 3 weeks or dacarbazine (1000 mg/m2) every 3 weeks and nivolumab-matched placebo every 2 weeks. At 1 year, the OS was 72.9%

[95% CI 65.5-78.9] in the nivolumab group versus 42.1% [95% CI 33- 50.9] in the dacarbazine group (HR for death 0.42 [99.79% CI 0.25-0.73], p<0.001). The median PFS was 5.1 months in the nivolumab group versus 2.2 months in the dacarbazine group (HR for death or progression of disease 0.43 [95% CI 0.34-0.56], p<0.001). The ORR was 40.0% [95% CI 33.3-47.0] in the nivolumab group versus 13.9% [95% CI 9.5-19.4] in the dacarbazine group (odds ratio, 4.06; P<0.001). Common adverse events associated with nivolumab included fatigue, pruritus, and nausea. Drug-related adverse events of Grade 3 or 4 occurred in 11.7% of the patients treated with nivolumab and 17.6% of those treated with dacarbazine.

Nivolumab was approved for the treatment of patients with metastatic melanoma who failed prior ipilimumab therapy in December 2014, and received approval for first-line use in combination with ipilimumab (October 2015) as well as first-line use as a single agent in November 2015.

Pembrolizumab

Pembrolizumab (formerly MK-3475), a humanized IgG4 anti-PD-1 mAb, has been studied in both ipilimumab-naïve melanoma patients and those previously treated with ipilimumab. The results of a Phase I/II clinical trial of pembrolizumab in 135 patients with advanced melanoma showed a confirmed RECIST 1.1 response rate of 38% across all dose cohorts [60], with the highest confirmed response rate observed in the cohort that received 10 mg/kg every 2 weeks (52% [95% CI 38-66]). The response rate did not differ significantly between patients who had received prior ipilimumab treatment and those who had not (ORR 38% [95% CI 23-55] and 37% [95% CI 26-49], respectively). Responses were durable in the majority of patients (median follow-up, 11 months among patients with a response); 81% of the patients who had a response (42 of 52) were still receiving treatment at the time of analysis. The median PFS among 135 patients was greater than 7 months. Constitutional symptoms (fatigue, fever, chills, myalgias, headaches) were reported frequently but were of low grade in more than 95% of the cases. Inflammatory phenomena including diarrhea (20% of patients), hypothyroidism (8% of patients), and pneumonitis (4% of patients) were also Grade 1 or 2, and responded well to glucocorticoids. Recently presented data on 365 evaluable patients treated with pembrolizumab showed an ORR of 40% (95% CI 32%-48%) in ipilimumab-naïve patients, and ORR of 28% (95% CI 22%-35%) in patients previously treated with ipilimumab [61]. Median PFS was 24 weeks and 23 weeks, respectively, with demonstrated activity in all major subgroups irrespective of number and type of prior therapy. Overall, 12% of pts experienced drug-related grade 3/4 AEs and 4% discontinued due to a drug-related AE. There were no drug-related deaths.

Pembrolizumab was compared to investigator's choice chemotherapy in ipilimumab-refractory patients in the KEYNOTE-002 trial [62], which showed a superior PFS in patients assigned to pembrolizumab 2 mg/kg (HR 0.57 [95% CI 0.45-0.73]; p<0•0001) and pembrolizumab 10 mg/kg (HR 0.50 [95% CI 0.39-0.64]; p<0•0001) compared with those assigned to chemotherapy. Six-

month PFS was 34% [95% CI 27-41] in the pembrolizumab 2 mg/kg group, 38% [95% CI 31-45] in the 10 mg/kg group, and 16% [95% CI 10–22] in the chemotherapy group. Treatment-related Grade 3–4 adverse events occurred in 20 (11%) patients in the pembrolizumab 2 mg/kg group, 25 (14%) in the pembrolizumab 10 mg/kg group, and 45 (26%) in the chemotherapy group. The most common treatment-related Grade 3–4 adverse event in the pembrolizumab groups was fatigue.

Pembrolizumab was also compared to ipilimumab in patients with advanced melanoma in the KEYNOTE-006 trial [63]. This Phase III study randomized 834 patients with advanced melanoma in a 1:1:1 ratio to receive pembrolizumab (10 mg/kg) every 2 weeks or every 3 weeks or four doses of ipilimumab (at 3 mg per kilogram) 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 0.58; P<0.001 for both pembrolizumab regimens versus ipilimumab; 95% CIs 0.46-0.72 and 0.47-0.72, respectively). Estimated 12-month survival rates were 74.1%, 68.4%, and 58.2%, respectively (HR for death for pembrolizumab every 2 weeks, 0.63 [95% CI 0.47-0.83]; p=0.0005 and HR every 3 weeks, 0.69 [95% CI 0.52-0.90]; p=0.0036). The response rate was improved with pembrolizumab administered every 2 weeks (33.7%) and every 3 weeks (32.9%), as compared with ipilimumab (11.9%) , p<0.001 for both comparisons. Responses were ongoing in 89.4%, 96.7%, and 87.9% of patients, respectively, after a median follow-up of 7.9 months. Efficacy was similar in the two pembrolizumab groups. Rates of treatment-related adverse events of grade 3 to 5 severity were lower in the pembrolizumab groups (13.3% and 10.1%) than in the ipilimumab group (19.9%).

In September 2014, pembrolizumab was approved for the treatment of patients with metastatic melanoma who failed prior ipilimumab therapy. No first-line indication has yet been approved.

Anti-PDL1 Therapy

BMS-936559

A high-affinity, fully human, anti-PD-L1 IgG4 monoclonal antibody that blocks the binding of PD-L1 to both PD-1 and CD80 (BMS-936559) has been tested in patients with advanced solid tumors, with preliminary evidence of clinical activity against melanoma, kidney cancer and NSCLC [64]. In a study of BMS-936559 published in 2012, 207 patients received treatment (55 with melanoma). Efficacy was analyzed in 160 patients in whom a response could be evaluated, with objective responses (confirmed complete or partial responses) observed in patients with melanoma, NSCLC, renal-cell cancer, and ovarian cancer [65]. There were 9 objective responses among 52 melanoma patients receiving 1mg/kg, 3mg/kg, and 10mg/kg doses (response rates of 6%, 29%, and 19%, respectively) with 3 patients achieving complete responses and 27% with stable disease lasting at least 24 weeks. Adverse events of any grade were reported in 188 of 207 patients (91%), with the most common drug-related adverse events being fatigue, infusion reactions, diarrhea, arthralgia, rash, nausea, pruritus, and headache. Most events were low grade,

with treatment-related grade 3 or 4 events noted in 19 of 207 (9%). Drug-related immunogenic adverse events were observed in 81 of 207 patients (39%) and included rash, hypothyroidism, hepatitis, and one case each of sarcoidosis, endophthalmitis, diabetes mellitus, and myasthenia gravis; these events were managed with glucocorticoids, treatment interruption, or trial discontinuation. Of the nine patients treated with high-dose glucocorticoids, 4 maintained disease controls.

Atezolizumab (MPDL3280A)

A chimeric IgG1 anti-PD-L1 antibody, atezolizumab (formerly MPDL3280A), has shown excellent clinical activity in patients with metastatic melanoma. In a Phase I trial, 45 patients with metastatic melanoma (64% of which had received prior systemic therapy) were treated with doses of 1 to 20mg/kg; of patients evaluable for efficacy, there was an ORR of 26% (9/35) and a 24-week PFS of 35% [66]. Several additional patients had delayed antitumor activity after apparent radiographic progression. Grade 3/4 adverse events included hyperglycemia (7%), elevated ALT (7%), and elevated AST (4%), with no high-grade pneumonitis reported. MPDL3280A is currently being explored in combination with vemurafenib compared to vemurafenib plus cobimetinib in patients with previously untreated BRAF V600 mutant metastatic melanoma [NCT01656642].

MEDI4736

MEDI4736 is another PD-L1 inhibitor that has shown promising early activity in NSCLC; interim results of a Phase I trial reported no colitis or pneumonitis of any grade, with several durable remissions [67]. MEDI4736 is also currently under investigation in combination with dabrafenib plus trametinib or with trametinib alone in subjects with metastatic or unrespectable melanoma [NCT02027961].

VACCINE THERAPY IN MELANOMA

Talimogene Laherparepvec (TVEC)

Talimogene Laherparepvec (T-VEC) is a herpes simplex virus type 1–derived oncolytic immunotherapy designed to selectively replicate within tumors and produce Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) to enhance systemic antitumor immune responses. The OPTIM trial compared T-VEC to GM-CSF in patients with unselected stage IIIB to IV melanoma [68]. This Phase III trial included patients with injectable melanoma that was not surgically respectable who were randomly assigned 2:1 to intraregional T-VEC or subcutaneous GM-CSF. The primary end point was durable response rate (DRR; objective response lasting continuously ≥ 6 months) per independent assessment. Key secondary end points included OS and ORR. Among 436 patients randomly assigned, DRR was significantly higher with T-VEC (16.3% [95% CI 12.1-20.5] than GM-CSF (2.1% [95% CI 0-4.5]; OR 8.9; p< .001). ORR was also higher in the T-VEC arm (26.4% [95% CI 21.4-31.5] versus GM-CSF (5.7% [95% CI, 1.9-9.5]). Median OS was 23.3 months [95% CI 19.5-29.6] with T-VEC and 18.9 months [95% CI 16-23.7] with GM-CSF (HR

0.79 [95% CI 0.62-1.00], p=.051). T-VEC efficacy was most pronounced in patients with stage IIIB, IIIC, or IVM1a disease and in patients with treatment-naive disease. The most common Adverse Events (AEs) with T-VEC were fatigue, chills, and pyrexia. The only grade 3 or 4 AE occurring in ≥ 2% of T-VEC–treated patients was cellulitis (2.1%). No fatal treatment-related AEs occurred.

TVEC was approved in October 2015 for the local treatment of unrespectable coetaneous, subcutaneous, and nodal lesions in patients with melanoma recurrent after initial surgery.

COMBINATION STRATEGIES

Ipilimumab and Nivolumab in Advanced Melanoma

As PD-1/PD-L1 and CTLA-4 have distinct biologic properties, preclinical studies suggested that the combination of CTLA-4 and PD 1 blockade is more effective than either alone. This approach was explored in the Checkmate-067 trial, which evaluated 945 previously untreated patients with melanoma in a double-blind, randomized Phase III trial of nivolumab alone, ipilimumab alone, or nivolumab plus ipilimumab in combination [69]. Patients were randomly assigned in a 1:1:1 ratio to receive one of the following regimens: nivolumab 3mg/kg IV every 2 weeks (plus ipilimumab-matched placebo); nivolumab 1mg/kg every 3 weeks plus ipilimumab 3mg/kg every 3 weeks for 4 doses, followed by nivolumab 3mg/kg every 2 weeks for cycle 3 and beyond; or ipilimumab 3mg/kg every 3 weeks for 4 doses plus nivolumab-matched placebo. The median PFS was 11.5 months [95% CI 8.9-16.7] with nivolumab plus ipilimumab, as compared with 2.9 months [95% CI 2.8-3.4] with ipilimumab (HR for death or disease progression, 0.42 [99.5% CI 0.31-0.57], p<0.001) and 6.9 months [95% CI 4.3-9.5] with nivolumab (HR compared with ipilimumab, 0.57 [99.5% CI 0.43-0.76], p<0.001). In patients with tumors positive for PD-L1, the median PFS was 14 months in the nivolumab-plus-ipilimumab group and in the nivolumab group, but in patients with PD-L1–negative tumors, PFS was longer with the combination therapy than with nivolumab alone (11.2 months [95% CI 8.0-not reached] *vs.* 5.3 months [95% CI 2.8-7.1]). While clinical efficacy was seen, the combination therapy yielded a greater frequency of irAEs: treatment-related adverse events of Grade 3 or 4 occurred in 16.3% of the patients in the nivolumab group, 55% of those in the nivolumab-plus-ipilimumab group, and 27.3% of those in the ipilimumab group. Drug discontinuation occurred in 7.7% of the patients in the nivolumab group, 36.4% of those in the nivolumab-plus-ipilimumab group, and 14.8% of those in the ipilimumab group, with the most common events being diarrhea (in 1.9%, 8.3%, and 4.5%, respectively) and colitis (in 0.6%, 8.3%, and 7.7%, respectively).

FUTURE DIRECTIONS

Biomarkers

The growing body of data on expression of PD-1/PD-L1 as potential prognostic and predictive biomarkers also adds to the rising interest in targeting this pathway for cancer therapy [70]. An association between PD-L1 expression and tumor response has yet to be fully validated, but is

being closely examined based on preclinical and clinical data. A recent immunohistochemical analysis of the tumor microenvironment in 41 patients treated with anti PD-1 antibody showed that PD-L1 expression was geographically associated with infiltrating immune cells ($p<0.001$), and that PD-L1 and PD-L2 expression were closely associated with response to anti-PD-1 therapy [71]. Interestingly, PD-L1+ cell lines isolated from 83 melanoma patients treated at a single institution demonstrated enhanced invasion and growth in immunocompromised mouse models [72], suggesting that PD-L1 expression may be associated with a more aggressive phenotype. In primary tumors and paired metastases from 83 consecutive melanoma patients treated at a single institution, PD-L1 membrane expression correlated with a highly invasive phenotype and enhanced ability to grow in vivo using immunocompromised mouse models, and may be proposed as a novel prognostic marker in metastatic melanoma [72].

In the Phase Ib study of pembrolizumab in 71 patients with advanced melanoma, those with PD-L1 expressing tumors (n=53) had an ORR of 53 percent [95% CI; range 38-61] compared to 13 percent in non-expressing tumors [95% CI; range 4-31], and also had an improved PFS (10.6 months compared to 2.9 months) [73]. In a subset analysis of melanoma patients treated with nivolumab, higher ORR, longer PFS, and longer OS were seen in PD-L1 positive patients [74].

irAEs and Clinical Response

It is possible that irAEs may be associated with durable response and clinical benefit, and that association has previously been studied with anti-CTLA-4 therapy [75], although contradictory reports exist [76]. A retrospective analysis of irAEs in 148 melanoma patients treated with nivolumab showed a statistically significant OS difference amongst patients with any grade of irAE versus those without ($p=<0.001$), and OS benefit was noted in patients who reported 3 or more irAE events ($p=<0.001$) [77]. Subset analyses showed statistically significant OS differences with rash ($p=0.001$ [HR 0.423, 95% CI 0.243-0.735]) and vitiligo ($p=0.012$ [HR 0.184, 95% CI 0.036 to 0.94]). Rash and vitiligo also correlated with statistically significant OS differences in patients with metastatic disease ($p=0.004$ and $p=0.028$, respectively). No significant survival differences were seen with other irAEs (endocrinopathies, colitis, or pneumonitis).

Similar correlations have been studied in patients treated with pembrolizumab [78], who found that patients with Grade 0-1 irAEs had ORR of 19.2%, while patients with Grade 2 or higher irAEs had an ORR of 28.6%. The investigators found DCR was slightly higher but not statistically significant among those patients who experienced irAEs (52.4%) compared with those who did not (38.5%) ($p = 0.34$). Another study of pembrolizumab in melanoma patients found that the 42% of patients who developed cutaneous AEs (maculopapular eruption 29%, pruritus 12%, and hypopigmentation 8%), had significantly longer progression-free intervals in all 3 groups (pembrolizumab 10 mg/kg every 3 weeks, $p < .001$; 10 mg/kg every 2 weeks, $p = .04$; and 2 mg/kg every 3 weeks, $p = .007$) compared to patients who did not develop cutaneous AEs [79].

Vitiligo has been previously reported with both anti-CTLA-4 and anti-PD1 therapy [80]; as the PD-L1: PD1 pathway likely mediates peripheral tolerance of melanosomal proteins (including tyrosinase and TRP-2), interference with PD-1 signaling may induce autoimmune vitiligo [81].

This provides a plausible explanation for the onset and persistence of depigmentation in patients treated with immunotherapy, as enhancing immune recognition of melanocyte-associated proteins in patients with completely resected or advanced unrespectable melanoma may be a surrogate for clinical benefit. Further evidence can be found in a recently published meta-analysis of immunotherapy in melanoma, where vitiligo was significantly associated with both PFS and OS, and patients with vitiligo had two- to four-times lower risk of disease progression and death, respectively, compared to patients without vitiligo [82].

Novel Combination Strategies

There is now a proliferation of trials looking at combination immunotherapy strategies for the treatment of advanced melanoma. Recently, a combination of anti-CTLA4, anti-PD-L1 and IDO inhibition in vivo showed a synergistic diminution of tumor growth and proliferation of tumor-infiltrating CD8+ T cells [83] and a combination trial of IDO inhibitor plus ipilimumab is currently underway [NCT 2073123]. Development of other combinations including those with vaccines and Tumor-Infiltrating Lymphocytes (TILs) are also forthcoming. A trial of vaccine combined with escalating doses of nivolumab or ipilimumab plus nivolumab in patients with resected Stage IIIC/IV disease is ongoing [NCT 01176474], as is neoadjuvant ipilimumab plus high-dose interferon [NCT 01608594], vemurafenib plus adoptive cell transfer and high-dose IL2 [NCT 01659151], ipilimumab plus or minus TVEC [NCT 01740297], pembrolizumab in combination with trametinib and dabrafenib [NCT 02130466]. Adjuvant nivolumab is being compared to ipilimumab in patients with resected Stage III C melanoma [NCT 02388906], and sequencing with targeted agents is being studied with dabrafenib plus trametinib followed by ipilimumab and nivolumab, or the reverse sequence, in patients with Stage III/IV BRAF V600 mutant melanoma [NCT 02224781].

CONCLUSION

As discussed here, clinical trials have demonstrated that PD-1 and PD-L-1 blockade results in impressive progression-free and overall survival in metastatic melanoma, making it an important treatment option for this lethal cancer. Indeed, the concept of melanoma being an “incurable” disease is increasingly challenged by long-term survival outcomes seen with immunomodulatory agents, in particular the plateau observed of 2- and 3-year survival seen in metastatic melanoma patients treated with anti-PD1 therapy [84]. Such advances are due to important discoveries made in the fields of immunology and molecular biology, and future therapies will depend upon successful clinical trial translation of ongoing developments. Much interest has been shown in this rapidly moving field of immunotherapeutic drug development, with the potential for lasting and profound clinical responses in a variety of cancers. As it stands, immune checkpoint inhibition is an important therapeutic advance in oncology, with a very promising future. The regulatory approvals of ipilimumab, nivolumab, pembrolizumab, and TVEC have brought immunotherapy to the forefront of cancer therapeutics, and made the goal of long-term tumor control seem achievable. Results from long-term survival data in addition to the early phase studies in development are eagerly awaited.

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