



Update on systemic therapy for advanced cutaneous melanoma and recent development of novel drugs

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Received: 17 January 2018 / Accepted: 11 June 2018 / Published online: 17 July 2018
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Abstract

Malignant melanoma is generally chemo- and radio-resistant, and patients with advanced melanoma have a poor prognosis. However, with our increased understanding of the checkpoint immune molecules and genetic alterations of melanoma cells, more effective immunotherapy, such as anti CTLA4 antibody and anti PD-1 antibodies, and targeted drug therapy, such as BRAF inhibitors and MEK inhibitors, have been developed, resulting in improved overall survival and quality of life of patients with advanced melanoma. In addition, emerging technologies to develop prognostic and predictive biomarkers for response to systemic therapy could help clinicians make more accurate assessments of the disease and formulate more effective treatment plans. In this review, current standard systemic therapy options and recently developed novel drugs for advanced melanoma are discussed.

Keywords Melanoma · Targeted therapy · Checkpoint inhibitor · *BRAF* mutation · PD-1 · CTLA-4

Introduction

Melanoma is the most aggressive type of skin cancer, and the incidence of malignant melanoma has been increasing considerably over the last several decades. It is the fifth and sixth most common cancer among men and women, respectively, in the United States [1]. In 2017, approximately 87,110 new cases and 9730 deaths are estimated to occur in the United States [1]. Although melanoma occurs less frequently than other skin cancers, such as basal cell carcinoma and squamous cell carcinoma, it is responsible for the majority of skin cancer-related deaths [2]. The survival rate is largely associated with the stage of the disease at the time of diagnosis. Patients with early stage melanoma have an excellent chance of cure with surgery alone, whereas those with stage IV melanoma have a 5 year survival rate of 16% with conventional cytotoxic chemotherapy or cytokine therapies [3]. However, with recent advances in immunotherapy and targeted drug therapy, patients with metastatic melanoma now have more effective therapeutic options and are living longer than ever. We discuss here the current

standard systemic therapies and the recent development of novel therapies for advanced melanoma. Table 1 summarizes FDA-approved systemic therapy regimens for advanced melanoma.

Cytotoxic chemotherapy

Malignant melanoma is generally resistant to cytotoxic chemotherapy, and the clinical benefit of chemotherapy is modest at best. Dacarbazine was the first drug to be approved by the U.S. Food and Drug Administration (FDA) in 1975 for the management of advanced melanoma. However, the response rate of dacarbazine is less than 15%, and most recent phase III studies which used dacarbazine as a comparison drug frequently showed a response rate of less than 10% according to the RECIST response evaluation criteria [4–7]. In addition, dacarbazine has not been shown to have survival benefit in this patient population. Other cytotoxic drugs, such as platinum, taxanes and vinka alkaloids, have a similar single agent clinical activity [8]. Combination regimens of cytotoxic chemotherapeutic drugs, including interleukin-2-based biochemotherapy, have higher response rates, but the progression-free survival (PFS) duration is rather short, and no such regimens have been shown to prolong overall survival (OS) [8–11]. Therefore, cytotoxic chemotherapy is no longer the treatment of choice in the first-line

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Table 1 FDA-approved systemic therapy regimens for advanced melanoma

Drug name	Dosing regimen	Indications
Adjuvant therapy		
High-dose interferon- α -2b	20 MU/m ² IV 5 days a week for 4 weeks, then 10 MU/m ² SQ 3 times a week for 48 weeks	Lymph node metastasis (stage III) and/or > 4 mm Breslow thickness
Pegylated-interferon- α -2b	6 mcg/kg/week SC for 8 doses, followed by 3 mcg/kg/week SC for up to 5 years	Lymph node metastasis (stage III)
Ipilimumab	10 mg/kg IV every 3 weeks for 4 doses, followed by 10 mg/kg IV every 12 weeks for up to 3 years	Lymph node metastasis (stage III)
Nivolumab	240 mg IV Q2 weeks for 1 year	Stage III or IV melanoma
Dabrafenib + trametinib	Dabrafenib 150 mg PO BID + trametinib 2 mg PO daily for 1 year	Lymph node metastasis; V600E/K <i>BRAF</i> mutation (stage III)
Treatment for unresectable or metastatic melanoma		
Vemurafenib	960 mg PO BID	V600E/K <i>BRAF</i> mutation
Dabrafenib	150 mg PO BID	V600E/K <i>BRAF</i> mutation
Trametinib	2 mg PO daily	V600E/K <i>BRAF</i> mutation; not to be used if disease progressed on BRAF inhibitor
Dabrafenib + trametinib	Dabrafenib 150 mg PO BID Trametinib 2 mg PO daily	V600E/K <i>BRAF</i> mutation
Vemurafenib + cobimetinib	Vemurafenib 960 mg PO BID Cobimetinib 60 mg daily (21 days on; 7 days off) Q28 days	V600E/K <i>BRAF</i> mutation
Encorafenib + Binimetinib	Encorafenib 450 mg PO daily; Binimetinib 45 mg PO BID	Metastatic setting; V600E/K <i>BRAF</i> mutation
Nivolumab	240 mg IV Q2 weeks	All unresectable stage III or IV melanoma
Pembrolizumab	200 mg IV Q3 weeks	All unresectable stage III or IV melanoma
Ipilimumab	3 mg/kg IV Q3 weeks for a total of 4 doses	All unresectable stage III or IV melanoma
Nivolumab + ipilimumab	Nivolumab IV 1 mg/kg + ipilimumab IV 3 mg/kg for 4 doses, followed by nivolumab 240 mg IV Q2 weeks	All unresectable stage III or IV melanoma
Talimogene laherparepvec (T-VEC)	Up to 4 mL of 10 ⁶ PFU/mL intralesional injection on day 1; after 3 weeks, up to 4 mL of 10 ⁶ PFU/mL Q2 weeks	All unresectable stage III or IV melanoma; must have injectable cutaneous, subcutaneous, nodal or soft tissue metastasis
High-dose interleukin-2	600,000–720,000 units/kg IV Q8 hours for a maximum of 14 doses	All unresectable stage III or IV melanoma
Dacarbazine ^a	800–1000 mg/m ² IV Q3 weeks	All unresectable stage III or IV melanoma
Under FDA review (as of May 2018)		
Pembrolizumab	200 mg IV Q3 weeks for 1 year	Adjuvant setting; stage III

MU million units, IV intravenous, SQ subcutaneous, PO oral, BID twice daily, PFU plaque-forming units, IU international units

^aCytotoxic chemotherapy is no longer the treatment of choice in the first-line treatment setting, and is often considered as the last choice among the currently available standard therapies

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Interleukin-2

Interleukin-2 (IL-2) is an immunomodulatory cytokine, which activates natural killer cells and cytotoxic T-lymphocytes. A number of phase II studies of high-dose IL-2 demonstrated that a durable clinical response was achievable, and a majority of those who had a complete response had an OS duration longer than 5 years [12, 13]. Because of its ability to induce long-term durable response,

high-dose IL-2 was approved by the FDA in 1998. Unfortunately, only a small portion of patients respond to high-dose IL-2 therapy, and only approximately 4% of treated patients have long-term survival [12, 13]. In addition, high dose IL-2 therapy leads to severe toxicity, including significant capillary leak syndrome leading to hypoxia, hypotensive shock and renal failure. Accordingly, high dose IL-2 therapy requires intense cardiac monitoring in the intensive care unit setting or in hospitals specialized in this therapy. Therefore, the use of this treatment has fallen out of favor among most melanoma experts.

Targeted drug therapy

Targeted drug therapy utilizes agents that inhibit the growth or spread of cancer, by interfering with specific target molecules essential for proliferation, invasion and/or survival of cancer cells or tumor-associated vasculature. Interestingly, melanoma has the highest number of mutations among all cancers, likely because a majority of melanomas are induced by mutagenic ultraviolet exposure [14]. The most common mutation is a single substitution mutation in the *BRAF* gene, which occurs nearly 50% of cutaneous melanomas, followed by mutations in the *NRAS* (21%) [15]. However, in acral lentiginous and mucosal melanoma, mutations in *BRAF* and *NRAS* occur much less often; instead, mutations in the *KIT* gene play more important role [16]. In uveal melanoma, nearly 80% of tumors have mutations in either the *GNAQ* or *GNA11* gene, and rarely in *BRAF* and *NRAS* genes [17, 18]. All of these genetic alterations are kinase-activating mutations, a fact which has implications for the identification of focused and actionable drug targets. In fact, this therapeutic approach has led to successful drug development, exemplified by selective *BRAF* and *MEK* inhibitors currently used for the treatment of V600 *BRAF* mutant-melanoma.

BRAF-mutant melanoma

BRAF inhibitors

BRAF is a serine-threonine kinase protein substrate in the MAP kinase pathway, which plays an important role in proliferation and survival of melanoma cells. The vast majority of mutations in *BRAF* occur at codon 600, where a single amino acid substitution mutation replaces valine with glutamic acid (V600E). This variant accounts for nearly 80% of all *BRAF* mutations, followed by a single substitution from valine to lysine at the same codon (V600K), which accounts for ~15% [19]. These V600 *BRAF* mutations lead to constitutive activation of the MAP kinase pathway in melanoma, and inhibiting these mutations effectively leads to inactivation of this signaling pathway, resulting in cell growth arrest. One of the major breakthroughs in treatment of advanced melanoma was the development of selective *BRAF* inhibitors, namely vemurafenib and dabrafenib. A phase I, dose-escalation trial of vemurafenib demonstrated an overall response rate of 81% in 32 patients with metastatic V600 *BRAF* mutant-melanoma [20]. In a large, randomized phase III trial of vemurafenib, 675 patients with previously untreated metastatic melanoma harboring a V600E *BRAF* mutation were treated with either vemurafenib or dacarbazine [4, 21]. There was significant improvement in OS, PFS and response rate in the vemurafenib arm. A hazard ratio (HR) for OS was 0.70 (95% CI 0.57–0.87; $p=0.0008$), and median OS duration was 13.6 months for

the vemurafenib arm vs 9.7 months for the dacarbazine arm. A HR for PFS was HR 0.38 (95% CI 0.32–0.46; $p<0.0001$) with a median PFS duration of 6.9 months in the vemurafenib arm. Response rates were 48 and 5% in the vemurafenib and the dacarbazine arm, respectively. On the basis of the positive results of this phase III study, vemurafenib was approved by the FDA for treatment of advanced V600 *BRAF*-mutant melanoma in 2011.

Similarly, in a randomized phase III study which compared the clinical benefit of another selective *BRAF* inhibitor, dabrafenib, to that of dacarbazine, the response rate was higher (50 vs. 6%), and the median PFS duration was longer (5.1 vs. 2.7 months) in patients who received dabrafenib treatment [5]. A HR for PFS, which was the primary endpoint of the study, was 0.30 (95% CI 0.18–0.51; $p<0.0001$). This finding led to the approval of dabrafenib by the FDA in this patient population in 2013.

MEK inhibitors

MEK inhibitors have been evaluated in patients with metastatic melanoma harboring a V600 *BRAF* mutation because the *MEK* protein is the only known downstream substrate of *BRAF* kinase in the MAPK signaling transduction pathway. In the early 2000s, a number of *MEK* inhibitors, such as CI-1040 and PD0325901, were evaluated in patients with metastatic solid tumor. However, these drugs were too toxic or ineffective to be clinically useful [22]. In the late 2000s, trametinib, a specific inhibitor of the *MEK1* and *MEK2* proteins, was shown in phase I and II studies to have promising clinical activity in patients with V600 *BRAF*-mutant melanoma [23, 24]. In a randomized phase III study of 322 patients with metastatic V600E or K *BRAF*-mutant melanoma, trametinib had superior PFS (HR 0.45 [95% CI 0.33–0.63]; $p<0.001$) and response rate (22 vs. 8%) over cytotoxic chemotherapy [25]. The median PFS duration was 4.8 months among patients who were treated with trametinib. Interestingly, the clinical activity of trametinib was minimal in patients with V600 *BRAF* mutant melanoma whose disease had progressed on previous *BRAF* inhibitor treatment [24]. Although trametinib and selective *BRAF* inhibitors have not been compared head to head, most clinicians would choose either vemurafenib or dabrafenib over trametinib as a single agent therapy because of the higher response rate and longer median PFS duration observed with the *BRAF* inhibitors.

Combination of *BRAF* inhibitor and *MEK* inhibitor

Despite the high response rates observed with *BRAF* inhibitors, a vast majority of patients develop resistance to these drugs. Although a number of different mechanisms of resistance have been demonstrated, reactivation of the

MAP kinase pathway through development of new mutations in the *NRAS* and *MEK* genes, amplification of *CRAF*, or alternate splicing of *BRAF* appear to be the most common [26–30]. Preclinical studies demonstrated that addition of a MEK inhibitor to a BRAF inhibitor delays drug resistance to BRAF inhibitor treatment [26–30].

To test this hypothesis, three large, randomized phase III studies (COMBI-D, COMBI-V and co-BRIM) were conducted to evaluate the clinical benefit of a combination of a BRAF inhibitor and a MEK inhibitor compared with a BRAF inhibitor alone; The COMBI-V and COMBI-D trials evaluated a combination of dabrafenib and trametinib [31–33], and the co-BRIM trial evaluated a combination of vemurafenib and cobimetinib, another selective MEK1/2 inhibitor. All three studies demonstrated a significant clinical improvement over treatment with a single agent BRAF inhibitor, with respect to OS, PFS and response rate (Table 2).

The overall clinical benefit advantage of these two combination regimens over BRAF inhibitor alone were strikingly similar; HRs for PFS ranged from 0.56 to 0.67, and HRs for OS ranged from 0.69 to 0.71, indicating the clinical advantage of the combination regimens [31–33]. Median PFS and OS durations for the combination regimens were 11–12.3 months and 22.3–26 months, respectively. Response rates were 66–69% for the combination treatment vs 45–53% for BRAF inhibitor alone.

In the phase 3 study of dabrafenib and trametinib vs dabrafenib alone (COMBI-D), the most common adverse event was pyrexia (52%) in the combination treatment group, and hyperkeratosis (33%) in the dabrafenib only group [31]. Grade 3 or 4 adverse events occurred in 32% patients in the combination treatment group and 31% patients in the dabrafenib only group [32]. A phase 3 trial of vemurafenib and cobimetinib (coBRIM) showed that the most common grade 3–4 adverse events were γ -glutamyl transferase increase (15%), blood creatine phosphokinase increase (12%), and alanine transaminase increase (11%) [33]. Serious adverse events occurred in 37 and 28% of patients in the combination treatment group and vemurafenib alone group.

With the clear survival advantage and acceptable safety profile, the combinations of BRAF inhibitor and MEK inhibitor have now become the standard for treatment of advanced V600 *BRAF* mutant-melanoma. In addition to the two combination regimens, another combination of encorafenib (a BRAF inhibitor) and binimetinib (a MEK inhibitor) was shown to have superior clinical benefit over encorafenib alone or vemurafenib alone [34]. Median PFS durations were 14.9, 9.6 and 7.3 months for the combination treatment, encorafenib alone and vemurafenib alone, respectively (HR 0.51 [for the combination treatment in relation to vemurafenib], [95% CI 0.39–0.67]; $p < 0.0001$). Overall survival was also longer with the combination treatment; Median

OS was 33.6 months for the combination vs 16.9 months for vemurafenib (HR 0.61 [95% CI 0.47–0.79]; $p < 0.0001$). Overall response rates were 64, 52 and 41% for the combination treatment, encorafenib alone and vemurafenib alone, respectively. On the basis of clear clinical advantage of the combination over the BRAF inhibitors alone, this regimen was approved by the FDA for treatment of V600 *BRAF*-mutant melanoma in June of 2018.

***KIT*-mutant melanoma**

KIT is a receptor tyrosine kinase that activates cellular signaling pathways via PI3K/AKT, the Janus kinase JAK2, and kinases of the Src family. Although *KIT* mutations are generally rare in melanoma, they occur more frequently in certain types of melanomas, such as mucosal (21%) and acral lentiginous melanomas (11%) [35]. In three phase II clinical trials of imatinib, a potent *KIT* inhibitor, a total of 118 patient with advanced melanoma containing a *KIT* mutation were treated, and the results showed response rates of 16–29% and a median PFS duration of 3–3.7 months [36–38]. Another potent *KIT* inhibitor, nilotinib, was also evaluated in a phase II study in 42 treatment-naïve patients with *KIT*-mutant melanoma; a response rate was 26%, and a median PFS duration was 4.2 months [39]. In all of the clinical trials of *KIT* inhibitors, greater clinical activity was observed in patients with *KIT* mutations occurring in exon 9 or 11. Although no *KIT* inhibitors have been approved by the FDA for the treatment of *KIT*-mutant melanoma, many oncologists consider imatinib as the treatment of choice for patients whose disease has progressed on checkpoint inhibitor therapy [14].

***NRAS*-mutant melanoma**

NRAS mutations are found in about 21% of all melanomas [15]. The most common *NRAS* mutations occur at codons 12, 13 and 61. *NRAS* activates the MAPK pathway and also other signaling pathways such as the PI3K/AKT pathway. *NRAS* mutations are associated with poorer prognosis in patients with melanoma [40, 41]. Unfortunately, there are no known effective targeted therapies for patients with *NRAS* mutations to date. Recently, a phase III study of binimetinib, a selective MEK inhibitor, versus dacarbazine was conducted to compare the clinical benefit of the MEK inhibition in this patient population. Four hundred and two patients with advanced *NRAS* mutant-melanoma were enrolled to receive either binimetinib or dacarbazine in a 2:1 ratio. In this study, patients who were treated with binimetinib had a longer PFS (HR 0.62 [95% CI 0.47–0.80]; $p < 0.001$), satisfying the primary endpoint of the trial [42]. However, a median PFS in the binimetinib group was only 2.8 months (95% CI 2.8–3.6), and there was no survival advantage. It is unlikely that binimetinib as a monotherapy will have a

Table 2 Results of randomized phase III clinical trials of targeted therapy drugs for metastatic V600E/K BRAF mutant-melanoma

Study	No. pts	Treatment	Primary endpoint	OS	PFS	ORR
Chapman et al. [4] McArthur et al. [21]	675	Vemurafenib vs dacarbazine	OS and PFS	HR 0.70 [95% CI 0.57–0.87]; p=0.0008 Median, 13.6 months for vemurafenib vs 9.7 months for dacarbazine	HR 0.38 [95% CI 0.32–0.46]; p<0.0001 Median, 6.9 months for vemurafenib vs 1.6 months for dacarbazine	57% for Vemurafenib vs 9% for dacarbazine
Hauschild et al. [5] [Clinical trial.gov (NCT01227889)]	250	Dabrafenib vs dacarbazine	PFS	HR 0.82 [95% CI 0.57–1.18] Median, 20.0 months for Dabrafenib vs 15.6 months for dacarbazine	HR 0.30 [95% CI 0.18–0.51]; p<0.0001 Median, 5.1 months for dabrafenib vs 2.7 months for dacarbazine	50% for Dabrafenib vs 6% for dacarbazine
Long et al. [31]	423	Dabrafenib+trametinib vs dabrafenib	PFS	HR 0.71 [95% CI 0.55–0.92]; p=0.0107 Median, 25.1 months for dabrafenib+trametinib vs 18.7 months for dabrafenib	HR 0.67 [95% CI 0.53–0.84]; p=0.0004 Median, 11 months for dabrafenib+trametinib vs 8.8 months for dabrafenib	69% for Dabrafenib+Trametinibvs 53% for dabrafenib
Robert et al. [32]	704	Dabrafenib+trametinib vs vemurafenib	OS	HR 0.69 [95% CI 0.53–0.89]; p=0.005 Median, 26 months for dabrafenib+trametinib vs 16.8 months for vemurafenib	HR 0.56 [95% CI 0.46–0.69]; p<0.0001 Median, 11.4 months for dabrafenib+trametinib vs 7.4 months for vemurafenib	64% for Dabrafenib+trametinibvs 51% for vemurafenib
Larkin et al. [33]	495	Vemurafenib+cobimetinib vs vemurafenib	PFS	HR 0.70 [95% CI 0.55–0.90]; p=0.005 Median, 22.3 months for vemurafenib+cobimetinib vs 17.4 months for vemurafenib	HR 0.58 [95% CI 0.46–0.72]; p<0.0001 Median, 12.3 months for vemurafenib+cobimetinib vs 7.2 months for vemurafenib	69.6% for vemurafenib+cobimetinib vs 50.0% for vemurafenib
Dummer et al. [34]	577	Encorafenib+binimetinib vs vemurafenib vs encorafenib	PFS	For encorafenib+binimetinib vs vemurafenib HR 0.61 [95% CI 0.47–0.79]; p<0.0001 Median, 33.6 months for encorafenib+binimetinib vs 16.9 months for vemurafenib	For encorafenib+binimetinib vs vemurafenib HR 0.51 [95% CI 0.39–0.67]; p<0.0001 Median, 14.9 months for encorafenib+binimetinib vs 7.3 months for vemurafenib	64% for encorafenib+binimetinib vs 52% for encorafenib vs 41% for vemurafenib

No. pts number of patients, OS overall Survival, PFS progression-free survival, ORR overall objective response rate, mo months, HR hazard ratio, CI confidence interval, p p-value

meaningful clinical impact in patients with *NRAS*-mutant melanoma, and for this reason, a new drug application for binimetinib was withdrawn in 2017.

Checkpoint inhibitor immunotherapy

Since the 1990s, researchers have identified several important checkpoint molecules in cytotoxic T-lymphocytes and have elucidated their role in suppression of T-cells. The most understood checkpoint molecules are the cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed cell death-1 (PD-1) receptors, and development of antibodies against these checkpoint receptors has dramatically changed the treatment for advanced melanoma (Table 3).

Anti CTLA-4 antibody therapy

CTLA-4 is a component of the CD28:B7 immunoglobulin family, and it is an important checkpoint molecule, which suppresses T cell activation to prevent autoimmunity and allows tolerance to self-antigens [43]. Ipilimumab, a monoclonal anti CTLA-4 antibody, blocks binding of CTLA-4 to its ligand, B7 protein, in antigen-presenting cells (i.e. dendritic cells), resulting in activation and proliferation of cytotoxic T cells and ultimately leading to antitumor immune response [43]. In a pivotal phase III study, in which 676 patients with unresectable stage III or IV melanoma (HLA-A2 positive) were randomized to receive ipilimumab, gp100 peptide vaccine, or a combination of ipilimumab and gp100 vaccine, patients treated with ipilimumab (or the combination) had superior overall survival and PFS duration as compared to those treated with gp100 vaccine [44]. The median overall survival duration was 10.0 months for patients who received ipilimumab (95% CI 8.5–11.5) in contrast to 6.4 months (95% CI 5.5–8.7) among patients who received gp100 alone (HR for OS, 0.68; $p < 0.001$) [40]. A 36% reduction in risk of disease progression was observed with ipilimumab treatment alone as compared with gp100 alone (HR 0.64, $p < 0.001$). This positive survival data led to FDA approval of ipilimumab at 3 mg/kg for the treatment of advanced melanoma in 2011. The clinical benefit of ipilimumab can be durable. A meta-analysis of 1861 melanoma patients treated with ipilimumab in various clinical trials demonstrated that 22% of patients were alive at 3 years, and a vast majority of those who were alive at 3 years were still alive at 5 years and beyond [45].

However, treating physicians and patients must be aware of potentially severe immune-related toxicity associated with ipilimumab treatment. The common immune-related adverse events include dermatitis, colitis, hepatitis, endocrinopathy and neuropathy, and grade 3 or 4 immune-related toxicity occurs in 15–28% of patients treated with ipilimumab [44, 46, 47]. If a patient develops severe immune-related toxicity,

it is essential to treat promptly with a high-dose steroid, as death has been reported as a consequence of toxicity, resulting from adverse effects such as Guillain–Barre syndrome and bowel perforation secondary to colitis [48].

Anti-PD-1 antibody therapy

Presence of the PD-1 receptor is essential in immune regulation. PD-1 binds to its ligands, PD-L1 and PD-L2, resulting in suppression of cytotoxic T lymphocytes. Accordingly, anti PD-1 antibodies have shown promising preclinical antitumor activity by interfering with this PD-1 receptor/ligand binding [49–51].

Nivolumab is a human IgG4 monoclonal antibody against the PD-1 receptor, and it blocks the binding of PD-1 to its ligands, PD-L1 and PD-L2. In a randomized phase III study, 418 previously untreated patients with metastatic BRAF wild type melanoma were assigned to receive either nivolumab (3 mg/kg every 2 weeks) or chemotherapy [52]. With 16.7 months of follow-up, patient who were treated with nivolumab had a longer OS (HR 0.42 [95% CI 0.25–0.73]; $p < 0.001$) and PFS (HR 0.43 [95% CI 0.34–0.56]; $p < 0.001$) than those treated with chemotherapy. Twelve-month OS rates were 72.9 and 42.1% in the nivolumab group and the dacarbazine group, respectively. The overall response rate of nivolumab was 40.0%. In another phase III study, the response rate for nivolumab was 31.7% among 120 melanoma patients whose disease had progressed after anti CTLA-4 antibody therapy, once again higher than found with cytotoxic chemotherapy (10.6%) [6].

Pembrolizumab is another monoclonal anti PD-1 antibody. A randomized phase II clinical trial compared the clinical activity of pembrolizumab (2 or 10 mg/kg every 3 weeks) with that of chemotherapy in patients with ipilimumab-refractory metastatic melanoma [7]. This study showed that patients who received either dose of pembrolizumab had a longer PFS than those who were treated with chemotherapy (HR with 2 mg/kg pembrolizumab 0.57 [95% CI 0.45–0.73]; $p < 0.0001$). In addition, a higher overall response rate was noted in the pembrolizumab group than the chemotherapy group (21 vs. 4%). In another phase III study of 834 previously untreated patients with advanced melanoma who were randomized to either pembrolizumab (given at 1 of 2 different dosing schedules) or ipilimumab, patients in the pembrolizumab group had longer OS and PFS over ipilimumab [46]. The hazard ratios for OS and PFS were 0.69 (95% CI 0.52–0.90; $p = 0.0036$) and 0.69 (95% CI 0.52–0.90; $p = 0.0036$), respectively, favoring pembrolizumab (2 mg/kg every 3 week dosing) over ipilimumab. The two dosing schedules of pembrolizumab, every 2 weeks or every 3 weeks, showed similar results in both PFS and OS.

Anti PD-1 antibody therapies are well tolerated. The safety profiles of nivolumab and pembrolizumab are more

Table 3 Results of randomized phase II/III clinical trials of novel immunotherapy for metastatic melanoma

Study (phase)	No. pts	Treatment	Primary endpoint	OS	PFS	ORR
Hodi et al. [44] [Clinical trial.gov (NCT01721746)] (phase III)	676	Ipilimumab + gp100 vs Ipilimumab vs gp100	OS	For ipilimumab vs gp100 HR 0.66 [95% CI 0.51–0.87]; p = 0.003 Median, 10.1 months for ipilimumab vs 6.4 months for gp100	For ipilimumab vs gp100 HR 0.64 [95% CI 0.50–0.83]; p < 0.001 Median, 2.8 months for ipilimumab vs 2.7 months for gp100	10.9% for ipilimumab vs 1.5% for gp100
Weber et al. [6, 93] (phase II)	405 ^a	Nivolumab vs Chemotherapy	ORR, OS	HR 0.95 [95.5% CI 0.73–1.24]; p = 0.7164 Median 15.7 months for Nivolumab vs 14.3 for chemotherapy	HR 1.0 [95.1% CI 0.78–1.436] Median 3.1 months for Nivolumab vs 3.7 for chemotherapy	31.7% for Nivolumab vs 10.6% for chemotherapy
Robert et al. [52] (phase III)	418	Nivolumab vs Dacarbazine	OS	HR 0.42 [95% CI 0.25–0.73]; p < 0.001 Median Not reached for Nivolumab vs 10.8 months for Dacarbazine	HR 0.43 [95% CI 0.34–0.56]; p < 0.001 Median 5.1 months for Nivolumab vs 2.2 months for Dacarbazine	40% for Nivolumab vs 13.9% for Dacarbazine
Wolchok et al. [47] (phase III)	945	Nivolumab + Ipilimumab vs Ipilimumab	OS, PFS	HR 0.55 [95% CI 0.42–0.72]; p < 0.001 Median, not reached for Nivolumab + Ipilimumab vs 19.9 for ipilimumab	HR 0.43 [95% CI 0.35–0.52]; p < 0.001 Median 11.5 months for Nivolumab + ipilimumab vs 2.9 for Ipilimumab	58% for Nivolumab + Ipilimumab vs 19% for Ipilimumab
Robert et al. [46] (phase III)	834	Pembrolizumab Q2 week vs Pembrolizumab 3Q weeks vs Ipilimumab	OS, PFS	HR 0.63 [98% CI 0.48–0.81]; p < 0.001 Median, 37.6 months for Nivolumab vs 2.9 for Ipilimumab	HR 0.55 [95% CI 0.45–0.66]; p < 0.001 Median, 6.9 months for Nivolumab vs 2.9 for Ipilimumab	44% for Nivolumab vs 19% for Ipilimumab
Ribas et al. [7, 94] (phase II)	540 ^b	Pembrolizumab 2 mg/kg vs Pembrolizumab 10 mg/kg vs Chemotherapy	PFS, OS	For Pembrolizumab Q3 weeks vs Ipilimumab HR 0.69 [95% CI 0.52–0.90]; p = 0.0036 12 months survival rate 68.4% for Pembrolizumab vs 58.2% for Ipilimumab	For Pembrolizumab Q3 weeks vs Ipilimumab HR 0.58 [95% CI 0.47–0.72] p < 0.001 Median, 4.1 months for Pembrolizumab vs 2.8 for Ipilimumab	32.9% for Pembrolizumab Q3 weeks vs 11.9% for Ipilimumab
				For Pembrolizumab 2 mg/kg vs chemotherapy HR 0.86 [95% CI 0.67–1.10] p = 0.1173 Median 13.4 months for Pembrolizumab 2 mg/kg vs 11.0 months for chemotherapy	For Pembrolizumab 2 mg/kg vs chemotherapy HR 0.57 [95% CI 0.45–0.73]; p < 0.0001 Median, 2.9 months vs 2.7 months for chemotherapy 6-month PFS 34% for pembrolizumab 2 mg/kg vs 16% for chemotherapy	21% for Pembrolizumab 2 mg/kg vs 4% for chemotherapy

Table 3 (continued)

Study (phase)	No. pts	Treatment	Primary endpoint	OS	PFS	ORR
Andtbacka et al. [58] (phase III)	436	Talimogene Laherparepvec (T-VEC) vs GM-CSF SQ	Durable ORR (≥ 6 months)	HR 0.79 [95% CI 0.62–1.00]; $p = 0.051$ Median, 23.3 months for T-VEC vs 18.9 months for GM-CSF	Time to treatment failure HR 0.42 [95% CI 0.32–0.54] Median, 8.2 months for T-VEC vs 2.9 months for GM-CSF	26.4% for T-VEC vs 5.7% for GM-CSF Durable response rate, 16.3% for T-VEC vs 2.1% for GM-CSF

No. pts number of patients, OS overall Survival, PFS progression-free survival, ORR overall objective response rate, mo months, HR hazard ratio, CI confidence interval, p p-value, SQ subcutaneous

^aPatients disease had progressed after anti CTLA-4 antibody therapy

^bIpilimumab-refractory patients [5]

favorable than either chemotherapy or ipilimumab [6, 7, 46, 52]. Grade 3 or 4 immune-related toxicity, including colitis, dermatitis, hepatitis and endocrinopathy, occurs in approximately 12% of patients, and less than 7% of patients require treatment discontinuation due to the toxicity [6, 7, 46, 52]. In 2014, both nivolumab and pembrolizumab were approved by the FDA for the treatment of metastatic or unresectable advanced melanoma regardless of *BRAF* mutation status.

Combination therapy with PD-1 inhibitor and CTLA 4 inhibitor

In preclinical mouse models, the combination of CTLA-4 and PD-1 blockade appeared to lead to synergistic antitumor activity, leading to clinical development of this combination therapy [53, 54]. In a phase I study of the combination of nivolumab and ipilimumab in patients with advanced melanoma, the response rate was 53%, and all patients with a response had tumor reduction of $> 80\%$ [55]. This encouraging result promptly led to a randomized phase III study which compared the combination regimen with ipilimumab alone, and also single agent nivolumab with ipilimumab [47]. The study showed that the HR for death with the combination treatment vs ipilimumab alone was 0.55 [98% CI 0.42–0.72] ($p < 0.001$), indicating a clear OS advantage of the combination regimen. Three-year OS rates were 58 and 34% in the combination group and ipilimumab group, respectively. A median PFS was 11.5 and 2.9 months in the combination treatment group and the ipilimumab group, respectively (HR 0.43 [95% CI 0.35–0.052]; $p < 0.001$), and a response rate was also higher in the combination treatment arm (58 vs. 19% in the ipilimumab arm) [47]. This study was not designed to directly compare the clinical benefit of the combination regimen and that of nivolumab alone. However, when a descriptive analysis was performed to compare these two treatment arms, there were numerically superior PFS and overall response results with the combination treatment; A HR for risk of progression or death was 0.78 (95% CI 0.64–0.96) (a median PFS of 6.9 months in the nivolumab arm), and an overall response rate for the nivolumab arm was 44%. On the basis of the significant clinical benefit over ipilimumab, the combination of nivolumab and ipilimumab was approved by FDA for the treatment of metastatic melanoma in 2015.

Unfortunately, combination therapy is associated with more severe toxicity. Grade 3 or 4 immune-related adverse events occurred in 59% of patients in the combination group, including increased lipase level (11%), diarrhea (9%), increased alanine aminotransferase level (9%), colitis (8%), and 39% required treatment discontinuation because of the intolerable side effects [47].

Therefore, a treatment decision between single agent anti PD-1 antibody therapy and the combination therapy must

be made only after a thorough discussion with the patient regarding both clinical activity and potential severe toxicity.

Talimogene laherparepvec

Talimogene laherparepvec (also called T-VEC or OncoVex^{GM-CSF}) is an oncolytic herpes simplex type 1 virus, which is genetically modified to express granulocyte macrophage colony-stimulating factor (GM-CSF) upon invasion into tumor cells [56]. T-VEC is injected intralesionally to exert the optimal local and systemic immunologic effects. In a phase I/II study of T-VEC, viral replication and GM-CSF expression were observed at the injection sites, confirming its biological effects. Interestingly, tumor regression was observed in the injected lesions as well as in uninjected lesions [57]. In a phase III trial, intralesional T-VEC treatment was compared with subcutaneous injection of recombinant GM-CSF in 436 patients with unresectable or metastatic melanoma who had injectable tumor lesions in the skin or lymph nodes [58]. The results revealed that patients who were treated with T-VEC had a higher durable response rate (16.3 vs. 2.1% of GM-CSF) and overall response rate (26.4 vs. 5.7%). Survival duration in the T-VEC arm was also superior (median OS 23.3 vs. 18.9 months with HR of 0.79 [95% CI 0.62–1.00]; $p=0.051$). However, patients with lung or visceral organ metastasis did not benefit from T-VEC treatment. The treatment was well tolerated; fatigue, chills and pyrexia were common (43–50% of the patients), but only 11% of patients who received T-VEC injections experienced grade 3 or 4 treatment-related adverse events, including fatigue, injection site pain, vomiting, and cellulitis. Because of the evidence of durable response rate and the favorable safety profile, T-VEC was approved by the FDA in 2015 for the treatment of melanoma lesions in the skin and lymph nodes, which are not amenable to curative surgical resection.

Systemic therapy for brain metastasis

Historically, melanoma patients with brain metastases have poor outcomes and very limited treatment options. The median survival of patients with brain metastasis is approximately 4 months [59, 60]. Temozolomide had been the systemic therapy of choice for many oncologists for years until recently because of its ability to cross blood brain barrier. However, the clinical response of temozolomide in the brain is minimal. In a phase II study of temozolomide in patients with brain metastasis, the intracranial response rate was only 7% among 151 treated patients, and a median OS duration was 3.5 months [61].

However, with the development of novel targeted drug therapy and checkpoint inhibitor therapy, there are now more effective therapeutic options. In a phase II study of

ipilimumab, 16% of patients ($N=51$) with asymptomatic brain metastasis who were not previously treated with steroids had a clinical response [62], and a median OS duration was 7 months. When nivolumab is combined with ipilimumab, a clinical benefit is more obvious. There were two phase II studies examining the clinical activity of combination therapy in melanoma patients with brain metastasis [63], and intracranial response rates ranged from 53 to 56% among the 101 neurologically-asymptomatic patients in the two studies combined. Intracranial complications, such as intracranial hemorrhage or edema, were rarely observed with this regimen.

For patients with V600 *BRAF*-mutant melanoma with brain metastasis, *BRAF* inhibitor-based targeted drug therapy is also a great systemic therapeutic option. In a phase II study of dabrafenib, an intracranial response rate was 39.2% among 74 patients with V600E mutant-melanoma, and a median PFS duration was approximately 4 months [64]. More recently, a result of a phase II study of the combination of dabrafenib and trametinib in patients with brain metastasis were published [65]. In this study, among 76 patients with V600E *BRAF*-mutant asymptomatic brain metastasis, 44 (58%) had an intracranial clinical response, and a median PFS duration was 5.6 months. Similar to the results of the checkpoint inhibitor trials, severe intracranial complications with the combination of dabrafenib and trametinib were rare.

Although these trials have a short follow up duration and overall survival assessment is not available, the results of the combination of nivolumab and ipilimumab, as well as the combination of dabrafenib and trametinib, are very encouraging. Because of the high response rates in the brain, these regimens could be used in conjunction with, or possibly replace, stereotactic radiosurgery as the treatment of choice, especially in patients with concurrent extracranial metastasis.

Adjuvant systemic therapy

Approved by the FDA in 1995, high-dose interferon-alpha-2b had long been the only available treatment option for patients with a high risk of recurrence following surgical lymph node resection/dissection. However, despite early reports of the survival advantage of adjuvant high-dose interferon-alpha treatment over observation in patients with stage III regional nodal metastasis and/or thick primary melanoma (> 4 mm Breslow thickness) [66], a long-term evaluation of the clinical efficacy demonstrated a loss of overall survival benefit of the interferon treatment [67]. In addition, this therapy is associated with significant toxicity, such as flu-like symptoms, severe fatigue and hepatic dysfunction.

In 2011, pegylated interferon-alpha-2b was approved by the FDA on the basis of significant improvement in relapse-free survival over observation in patients with resected

stage III melanoma [68]. The reduction in risk of disease recurrence or death was 18% (HR 0.82 [95% CI 0.71–0.96]; $p=0.01$). However, overall survival benefit was not observed in patients who were treated with pegylated interferon-alpha-2b compared to those who were observed. Therefore, this treatment has not been widely accepted by medical oncologists and surgeons.

Subsequently, ipilimumab was evaluated for clinical benefit in the adjuvant setting. In a large phase III study of high-dose ipilimumab (10 mg/kg) in patients with stage III melanoma who underwent regional lymph node dissection, 475 patients were treated with ipilimumab, and 476 patients were treated placebo [69]. The treatment schedule of ipilimumab was 10 mg/kg every 3 weeks for 4 doses, followed by once every 3 months for up to 3 years or until disease recurrence or unacceptable toxicity. Recurrence-free survival, the primary endpoint of the trial, was significantly longer in the ipilimumab arm (HR 0.76 [95% CI 0.64–0.89]; $p<0.001$), and overall survival was also superior (HR 0.72 [95.1% CI 0.58–0.88]; $p=0.001$). These positive results led to FDA approval of high-dose ipilimumab as an adjuvant therapy in 2015. Unfortunately, toxicity associated with high-dose ipilimumab is severe; nearly 42% of patients experienced grade 3 or 4 immune-related adverse events, and 1.1% of patients in the ipilimumab arm died due to treatment-induced adverse events.

Most recently, very promising results from a phase III study of nivolumab vs high-dose ipilimumab in an adjuvant setting were published [70]. In a large, randomized, double-blind phase III study, 906 patients with stage IIIB, IIIC and IV melanoma were randomized to receive either nivolumab (3 mg/kg every 2 weeks) or ipilimumab (10 mg/kg every 3 weeks for 4 cycles, followed by 10 mg/kg every 12 weeks) for 1 year following complete lymph node dissection or metastectomy [70]. Patients in the nivolumab arm had significantly longer recurrence-free survival over those in the ipilimumab arm (HR 0.65 [97.56% CI 0.51–0.83]; $p<0.001$), meeting the study's primary endpoint. A 1-year rate of recurrence-free survival was 70.5% in the nivolumab arm and 60.8% in the ipilimumab arm. The follow-up duration of this study was too short to evaluate the overall survival benefit of nivolumab. As expected, immune-related adverse events were much less frequent with nivolumab treatment; Grade 3 or 4 toxicity occurred in 14.4 and 45.9% of patients who were treated nivolumab and ipilimumab, respectively. Accordingly, a fewer percentage of patients in the nivolumab arm required treatment discontinuation due to the toxicity (9.7 vs. 42.6%). In December of 2017, nivolumab was approved by the FDA for treatment of resected stage III or IV melanoma with high risk for recurrence.

Likewise, pembrolizumab was investigated in an adjuvant setting and showed a significant clinical benefit [71].

A total of 1019 patients with stage IIIA (at least one micrometastasis measuring >1 mm), IIIB or IIIC melanoma were randomized in a phase III study to either pembrolizumab treatment 200 mg every 3 weeks or placebo for a total of 18 doses after a complete lymph node dissection [71]. Patients who were treated with pembrolizumab had a significantly longer RFS duration (HR 0.57 [98.4% CI 0.43–0.74]; $p<0.001$); A RFS rate at 18 months was 71.4% for the pembrolizumab arm and 53.2% for the placebo arm. The clinical benefit was observed regardless of the PD-L1 expression status. Treatment-related grade ≥ 3 adverse events occurred in 14.7% of patients who were treated with pembrolizumab, and 13.0% of patients discontinued treatment due to drug-related toxicity. At the time of this review, the results of the phase III trials of pembrolizumab are under FDA review for potential approval in the treatment of patients with resected melanoma with high-risk for recurrence.

At the same time that the results from the phase III adjuvant nivolumab trial were published, a positive result from another phase III adjuvant study were also published. In this large, double-blind, randomized study, 870 patients with stage IIIA (>1 mm metastatic tumor load)—IIIC V600E or V600K *BRAF*-mutant melanoma were treated for 1 year with either a combination of dabrafenib (150 mg twice a day) and trametinib (2 mg a day) or placebo [72]. The primary endpoint was relapse-free survival. At the time of the analysis, patients in the combination treatment arm had significantly longer relapse-free survival compared with those with the placebo arm (HR 0.47; [95% CI 0.39–0.58]; $p<0.001$). In addition, 3-year overall survival rate was superior in the treatment group (86 vs. 77%, HR 0.57; [95% CI 0.42–0.79]; $p=0.0006$). In April of 2018, the FDA approved the combination of dabrafenib and trametinib for treatment of resected stage III V600 *BRAF*-mutant melanoma with high risk for recurrence.

In another phase III adjuvant study of a *BRAF*-targeting drug, 498 patients with stage IIC or III melanoma were randomized to receive either 960 mg vemurafenib twice a day or placebo for 1 year after complete melanoma resection and for a stage III disease, lymph node dissection [73]. The study failed to meet the primary objective of prolongation of disease-free survival (DFS) with vemurafenib treatment in patients with stage IIIC melanoma (cohort 2) (HR 0.80 [95% CI 0.54–1.18]; $p=0.2598$), but in patients with stage IIC-IIIB (cohort 1), there was significant improvement in DFS duration in the vemurafenib arm (HR 0.54 [95% CI 0.37–0.78]; $p=0.001$). However, because of the study's original statistical design which prespecified a hierarchical analysis of cohort 2 data prior to cohort 1, the study was deemed negative, and vemurafenib is not a standard treatment as an adjuvant therapy.

Experimental novel therapeutic approaches

Indoleamine 2,3-dioxygenase inhibitor

Indoleamine 2,3-dioxygenase (IDO) is a catalytic enzyme which converts L-tryptophan to N-formylkynurenine. It has been shown to limit T cell function and mediate immune tolerance [74]. Accordingly, inhibition of the IDO enzyme has been shown to synergize the anti-tumor effect of checkpoint inhibitors. In a phase I/II study of the combination of an IDO inhibitor, epacadostat, and nivolumab, 25 of 40 patients (63%) with metastatic melanoma had an objective response. In a phase II study of another IDO inhibitor, indoximod, in combination with pembrolizumab, 31 of 51 patients (61%) with advanced melanoma had a clinical response, including 10 (20%) with a complete response, and a median PFS duration was 12.9 months [75]. Despite the promising results in the early phase clinical trials, however, a phase III study of the combination of pembrolizumab and epacadostat failed to demonstrate improvement in PFS duration over pembrolizumab alone in patients with advanced melanoma in the first-line treatment setting. As a result, a future of IDO inhibitor treatment in advanced melanoma is not clear.

Adoptive T cell therapy

Adoptive T cell therapy (ACT) aims to maximize the cytotoxic immune response against tumor cells via the reintroduction of either patient-derived or genetically engineered tumor infiltrating T cells. Adoptive tumor infiltrating lymphocytes (TIL) transfer therapy, which utilizes autologous tumor reactive T cells harvested from a patient's own tumor lesion, is administered after lymphodepletion chemotherapy and is followed by high-dose IL-2 therapy. Clinical experience with this approach is encouraging; clinical response rates of nearly 50% have been reported, and a majority of these responses are durable [76, 77]. Unfortunately, TIL isolation and expansion are labor intensive, and only a limited number of academic centers are capable of managing the TIL preparation and administration. Ongoing collaborations between academic institutions and the biopharmaceutical industry will likely make this treatment more widely available.

Combination of BRAF/MEK inhibitor and checkpoint inhibitor

For patients with advanced V600 *BRAF* mutant-melanoma, combinations of a *BRAF* inhibitor and a MEK inhibitor generally have higher response rates than checkpoint inhibitor therapy, but it appears that clinical responses may be more durable with checkpoint inhibitor therapy. Therefore, it has been widely speculated whether a combination of targeted

drug therapy and checkpoint inhibitor therapy could improve durable response rates and lead to prolonged patient survival.

In addition to inducing constitutive activation of the MAP kinase signaling pathway, a V600 *BRAF* mutation plays a significant role in immune escape, and inhibition of this mutation can enhance CD8 + T cell-mediated cytotoxicity, especially when T cells are further stimulated with the checkpoint inhibitor [78–80]. In a phase I study of atezolizumab (anti PD-L1 antibody) in combination with vemurafenib and cobimetinib, the safety profile of the triple combination was acceptable, and 29 of 34 treated patients (85%) had a clinical response (confirmed and unconfirmed combined) [81]. At this time, there are at least 2 ongoing phase III studies comparing the clinical benefit of a triple combination, *BRAF* inhibitor, MEK inhibitor and checkpoint inhibitor, to the benefit of a *BRAF* inhibitor and MEK inhibitor combination alone (Table 4), and the results of these studies are eagerly anticipated.

Molecular biomarkers for melanoma

A number of tumor-based biomarkers have been evaluated and validated as prognostic and predictive biomarkers for melanoma. Examples of widely accepted prognostic biomarkers for primary melanoma which are already included in the AJCC staging system include certain distinct tumor characteristics, such as Breslow thickness, presence of ulceration, mitotic rate and presence of nodal metastasis. Likewise, the presence of somatic mutations in the tumor can be a prognostic biomarker. Patients with melanoma harboring either a V600 *BRAF* or a *NRAS* mutation, for example, have a more aggressive disease course and a poor prognosis unless they are treated with effective systemic therapy [41, 82]. In addition, the V600 *BRAF* mutation is also an excellent predictive biomarker for clinical response to *BRAF* inhibitors and MEK inhibitors as described above [4, 5, 25]. Recently, there have been great strides made in the discovery of novel predictive or prognostic biomarkers.

Patients with stage I or II melanoma generally have an excellent prognosis, with a 5 year overall survival rate of $\geq 90\%$ [83]. However, it is difficult to foresee who among those patients with early stage melanoma will develop recurrence and die of disease. The quantitative reverse transcription polymerase chain reaction (PCR)-based 31-gene expression profile (GEP) analysis was evaluated as a potential biomarker for high recurrence risk [84–86]. In this analysis, RNA is isolated from formalin-fixed paraffin-embedded cutaneous melanoma specimens and converted to complementary DNA (cDNA), with which 31 gene targets are tested. The analysis stratifies the molecular profiles into 2 classes: class I with a low risk for recurrence and class II with a higher risk for recurrence. In retrospective multicenter studies, the 31-gene signature

Table 4 Currently enrolling or recently completed randomized phase III studies for advanced melanoma

Clinical trial	Treatment	Patient population ^a	Primary endpoint	No. of patients	Status
Adjuvant therapy					
NCT01502696	Pegylated-Interferon-alpha2b vs Observation	Ulcerated primary Stage II; [T(2–4)bN0M0] ^a	RFS	1200	Completed accrual
NCT01274338	High-dose Ipilimumab vs Low-dose Ipilimumab vs High-dose Interferon- α -2b	Stage IIIB/C-IV ^a	OS, RFS	1500	Completed accrual
NCT02506153	Pembrolizumab vs High-dose Ipilimumab or High-dose Interferon- α -2b	Stage IIIA(N2a)/B/C-IV ^a	OS, RFS	1378	Completed accrual
NCT03068455	Nivolumab + Ipilimumab vs Nivolumab + Placebo	Stage IIIB/C/D-IV ^b	PFS	900	Completed accrual
Treatment for unresectable or metastatic melanoma					
NCT02752074	Pembrolizumab + Epcadostat vs Pembrolizumab + Placebo	Previously untreated for metastatic disease	OS, PFS	706	Completed accrual
NCT02263508	Pembrolizumab + T-VEC vs Pembrolizumab + Placebo	Previously untreated for metastatic disease	OS, PFS	660	Completed accrual
NCT02339571	Nivolumab + Ipilimumab + Sargramostim vs Nivolumab + Ipilimumab	Previously untreated for metastatic disease	OS	400	Enrolling
NCT02905266	Nivolumab + Ipilimumab vs Dabrafenib + Trametinib	Previously untreated for metastatic disease; V600 <i>BRAF</i>	2 year OS rate	300	Enrolling
NCT02908672	Vemurafenib + Cobimetinib + Atezolizumab vs Vemurafenib + Cobimetinib + Placebo	Previously untreated for metastatic disease; V600 <i>BRAF</i>	PFS	500	Enrolling
NCT02967692	Dabrafenib + Trametinib + PDR001 (PD-1 antibody) vs Dabrafenib + Trametinib + Placebo	Previously untreated for metastatic disease; V600 <i>BRAF</i>	PFS	538	Enrolling

OS overall survival, RFS recurrence-free survival, PFS progression-free survival

^aAccording to the 7th edition of AJCC staging

^bAccording to the 8th edition of AJCC staging

was prognostic in patients with cutaneous melanoma, and metastatic risk was predicted with relatively high accuracy. When multivariate analyses of known prognostic factors, including those currently in the AJCC staging system, were performed, the GEP expression profile was one of the most significant prognostic factors, along with Breslow thickness, mitotic rates and presence of ulceration in patients with stage I or II melanoma [84–86]. Similarly, in an analysis of 322 patients with cutaneous melanoma in two prospective registry studies using the 31-gene GEP expression profile, 1.5 year RFS rates were 97 and 77%, 1.5 year DFMS rates were 99 and 89%, and 1.5 year OS rates were 99 and 92% among those with class I and class II molecular profiles, respectively ($p < 0.0001$ for each) [86]. Although initial limited data suggests that the GEP signature can accurately predicts the risk for recurrence and distant metastasis, the additive value of this analysis in clinical practice is an open question at this time. If long-term follow-up studies with larger numbers of patients

demonstrate a similar degree of the positive prognostic stratification, the GEP expression profile may have a significant clinical impact.

Another important recent development is analysis of circulating free tumor-derived DNA (ctDNA) for prognostic and predictive biomarkers for clinical response. ctDNA is released as a result of cell apoptosis or necrosis during the high tumor cell turnover, and can provide genetic information on the tumor in real time. It is less invasive to obtain samples for ctDNA analysis, since this involves a blood draw rather than the tumor biopsy or surgical resection required for tumor genetic sequencing analysis. ctDNA analysis can also detect genetic heterogeneity when there is more than one progressing tumor clone in a patient. In melanoma, plasma ctDNA level has been shown to be associated with a clinical response to BRAF inhibitor therapy and immunotherapy, including anti PD-1 antibody therapies, in small series of retrospective analyses [87–90]. In addition, ctDNA levels may indicate tumor burden, because a larger tumor volume or a higher number

of metastatic foci are likely to result in increased shedding of ctDNA into the blood stream. ctDNA level was found to be more predictive of tumor volume compared to other potential serological biomarkers, such as LDH, S100 calcium-binding protein B (S100B) and melanoma inhibitory activity (MIA) [90–92]. Accordingly, ctDNA levels have shown to be correlated with radiographic measures of tumor response to targeted drug therapy [87, 90]. Furthermore, ctDNA analyses can theoretically be used to elucidate mechanisms of resistance to targeted therapy by detecting new genetic mutations which are developed during the course of therapy [90]. Thus far, the clinical utility of ctDNA analysis in patients with metastatic melanoma has not been widely accepted, due to the lack of large, prospective studies. Therefore, prospective clinical trials evaluating ctDNA as a prognostic and predictive biomarker for melanoma are highly desired.

Conclusion

Within the past decade, great advances have been made in the development of novel targeted drug and checkpoint inhibitor treatments for patients with advanced melanoma. As a result, patients with advanced melanoma are now living longer than ever; however, a majority of patients with metastatic or unresectable melanoma still do not survive longer than 5 years. Further, many of the current therapies come with significant toxicity. Therefore, there is still a critical unmet need for more effective and better tolerated therapy regimens which both increase the survival and cure rate and improve the quality of life for these patients. There are many ongoing preclinical and clinical investigations to search for new actionable drug targets, identify suitable predictive biomarkers for therapeutic efficacy, and optimize the sequence or concurrent combination of promising drugs. These research efforts will surely reshape the systemic therapy landscape in melanoma in the coming years.

Acknowledgements Editorial assistance to the authors was provided by Ms. Lesley C. Scott-Skye at California Pacific Medical Center Research Institute.

Compliance with ethical standards

Conflict of interest Kevin B. Kim has received honoraria from Genentech Inc. Incyte Corp, Novartis Pharmaceuticals, Bristol-Myers Squibb, Array BioPharma for serving as a member of advisory board meeting and honoraria from Bristol-Myers Squibb, Novartis Pharmaceuticals and Merck for serving as a member of speakers bureau.

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