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MELANOMA (RJ SULLIVAN, SECTION EDITOR)

The Role of BRAF-Targeted Therapy for Advanced Melanoma in the Immunotherapy Era

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Abstract

Purpose of Review The treatment of advanced melanoma has changed dramatically in recent years with several new drugs having been approved for the treatment of melanoma since 2011. This review aims to evaluate the role of BRAF-targeted therapy for advanced melanoma in the immunotherapy era.

Recent Findings Currently, in patients with BRAF wild-type advanced melanoma, anti-PD-1 (nivolumab or pembrolizumab) is the main treatment. The combination of nivolumab and ipilimumab (anti-CTLA-4) is also an important option for these patients, resulting in a better outcome, but with less favorable toxicity profile. In patients with BRAF mutations, three regimens of BRAF plus MEK inhibitors are now approved (vemurafenib plus cobimetinib, dabrafenib plus trametinib, and encorafenib plus binimetinib), which achieve rapid antitumor responses and a significant survival benefit. In these patients, as well as in BRAF wild-type patients, immunotherapy can be also effective and is regularly used.

Summary Immunotherapy and targeted therapy have become the new standards of care, substantially improving survival rates. However, many questions still remain unanswered, such as what is the best first- and second-line treatment and the best treatment sequence. New combinations of drugs, targeted therapy combined with immunotherapy, and sequencing approaches are now underway in many ongoing clinical trials.

Keywords Melanoma · Immunotherapy · Targeted therapy · CTLA-4 · PD-1 · Ipilimumab · Nivolumab · Pembrolizumab · Vemurafenib · Cobimetinib · Dabrafenib · Trametinib · Encorafenib · Binimetinib · Combination · Sequencing

Introduction

The treatment of metastatic melanoma has been revolutionized by advances in molecular targeted therapy and immunotherapy. Before 2011, the only therapeutic options for patients with unresectable or metastatic melanoma were systemic chemotherapy (dacarbazine, fotemustine, or temozolomide), and immunotherapy with high-dose interleukin-2 (HD IL-2). None of these treatments had any impact on median overall survival (OS) and only few patients survived for more than 1 year [1]. However, since 2011, immune checkpoint inhibitor

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antibodies, including ipilimumab, nivolumab, and pembrolizumab, and targeted agents, such as vemurafenib, dabrafenib, encorafenib, trametinib, cobimetinib, and binimetinib, have dramatically improved survival rates and have been approved for the treatment of patients with advanced melanoma [2]. The immune checkpoint inhibitors have been approved for patients irrespective of mutational status, while the targeted therapies (BRAF inhibitors and MEK inhibitors) are specifically intended for the nearly 50% of patients with melanomas that harbor BRAF V600 mutations. The increasing number of treatment options, especially for patients with BRAF-mutant melanoma, has led to new questions, still unanswered, regarding the best treatment as first line, the best sequence of treatment, the superiority of a combined approach versus a sequential approach, and also the duration of treatment. Many ongoing clinical trials combining MAPK pathway inhibition with cytotoxic T lymphocyte-associated antigen (CTLA)-4 and programmed cell death (PD)-1/programmed death ligand (PD-L)-1 blockade in patients with metastatic melanoma are assessing whether there is a benefit of the combination over the monotherapy, while others are prospectively evaluating the best treatment sequence.

Immunotherapy

Novel immunotherapies, especially anti-CTLA-4 and anti-PD-1 checkpoint inhibitors, have resulted in dramatic improvements in survival in patients, not only with advanced melanoma, but also with other cancers, including non-small cell lung cancer (NSCLC), bladder cancer, kidney cancer, and head and neck cancers. CTLA-4 and PD-1 are essential negative regulators of T cell immune responses. Inhibition of these targets results in increased activation of the immune system. CTLA-4, a CD28 homolog expressed on the surface of T lymphocytes, is a protein receptor that, functioning as an immune checkpoint, downregulates immune responses. The inhibition of CD8 T cells by CTLA-4 is mediated by two co-stimulatory molecules CD80 and CD86. PD-1 is a member of the B7/CD28 family of co-stimulatory receptors. It regulates T cell activation through binding to its ligands, PD-L1 and PD-L2. Both CTLA-4 and PD-1 bindings have similar negative effects on T cell activity; however, the timing of downregulation, the responsible signaling mechanisms, and the anatomic locations of immune inhibition by these two immune checkpoints differ. Unlike CTLA-4, which is confined to T cells, PD-1 is more broadly expressed on activated T cells, B cells, and myeloid cells. While CTLA-4 functions during the priming phase of T cell activation, PD-1 functions during the effector phase, predominantly within peripheral tissues [3].

Anti-CTLA-4

Ipilimumab was the first drug shown to extend survival among patients with advanced melanoma. Ipilimumab is a fully human, monoclonal antibody that overcomes CTLA-4-mediated T cell suppression to enhance the immune response against tumors. Preclinical and clinical studies of patients with advanced melanoma have shown that ipilimumab promotes antitumor activity as monotherapy and in combination with treatments such as chemotherapy, radiotherapy, vaccines, cytokines, and other checkpoint inhibitors. Ipilimumab is a feasible treatment option in patients with metastatic melanoma, regardless of BRAF and NRAS mutational status. Data from a large cohort of patients support clinical trial evidence that ipilimumab can induce durable disease control and long-term survival [4]. In a phase III study of ipilimumab in previously treated patients with metastatic melanoma (study MDX010-020), 676 pretreated patients received ipilimumab with a peptide vaccine (gp100) or placebo or gp100 plus placebo [5]. The OS was significantly longer with ipilimumab, both alone or in combination with the vaccine (10.1 vs.6.4 months). In addition, in a pooled analysis from 12 ipilimumab clinical studies that included 1860 patients, an OS plateau started at approximately 3 years with a 22% 3-year survival rate [6]. We now have data at 10 years from 4846 patients, including those from an expanded access program (EAP) which showed that 21% of patients are alive at 3 years, and the majority of these remain alive after 10 years. More recently, it was demonstrated that higher-doses of ipilimumab (10 mg/kg) showed an advantage in terms of OS (15.7 vs.11.5 months), with more frequent immune-mediated toxicity. Moreover, the higher-dose ipilimumab also resulted in improved OS in patients with BRAF mutations (HR 0.65, 95% CI 0.44–0.96) and in M1c patients with brain metastases (HR 0.71, 95% CI 0.49–1.04) [7]. Ipilimumab is generally well tolerated but can be associated with different kinds of side effects, due to the immune system activation by CTLA-4 blockade. Collectively, the spectrum of side effects is described as immune-related adverse events (irAEs). irAEs most commonly affected the skin (rash/vitiligo/pruritis), the liver (hepatitis/rise in liver enzymes), the bowel (diarrhea/colitis), and the endocrine system (hypophysitis, thyroiditis, adrenal insufficiency). More rarely, uveitis, conjunctivitis, neuropathy, myopathy, and nephritis have been known to occur. Retrospective analysis suggests that patients who experience irAEs may be more likely to benefit from anti-CTLA-4 therapy. Data from phase I and II trials with tremelimumab, another anti-CTLA-4 antibody, showed an estimated 5-year survival rate of 20% (95% CI, 13–26%) [8]. However, tremelimumab did not demonstrate a significant survival advantage compared to standard of care chemotherapy in the first-line treatment of patients with metastatic melanoma in a phase III trial [9].

Anti-PD-1/PD-L1 Agents

The anti-PD-1 agents, nivolumab and pembrolizumab, have demonstrated improved survival and less toxicity compared with ipilimumab. For this reason, they have become the new standard first-line checkpoint inhibitor treatment. In a phase I trial, nivolumab had a 48% objective response rate (ORR) and 32% OS rate at 4 years [10]. In the phase III Checkmate 066 trial, nivolumab demonstrated superior OS, with 72.9% of patients in the nivolumab arm and 42.1% in the dacarbazine arm alive at 1 year. All the patients enrolled in this trial were BRAF wild-type [11]. An update reported a 3-year OS of 51.2% with nivolumab versus 21.6% with dacarbazine [12]. In this trial, the incidence of grade 3/4 treatment-related adverse events was 11.7% in the nivolumab arm and 17.6% in the chemotherapy arm. In the Checkmate 067 trial, nivolumab demonstrated substantially improved ORR, progression-free survival (PFS), and OS compared with ipilimumab as first-line treatment, even in patients with BRAF mutations (31.5% of patients). Moreover, in the BRAF wild-type group, OS appears to be similar in patients treated with the combination nivolumab plus ipilimumab and nivolumab alone (2-year OS 61% and 57%, respectively), while in the BRAF-mutated group, the combination is like to be more effective than monotherapy with nivolumab (2-year OS of 71% and 62%, respectively) [13••]. It appears likely that BRAF V600 mutations in melanoma cells may affect the tumor microenvironment and consequently the response to immunotherapy [14] (Fig. 1). In the phase I trial KEYNOTE-001 of pembrolizumab, there was a 5-year OS rate of 34% in patients with previously treated and treatment-naive advanced melanoma and 41% in treatment-naive patients only [15]. In the phase II trial (KEYNOTE-002), pembrolizumab at two different dosages (2 and 10 mg/kg every 3 weeks) were compared to investigator choice chemotherapy in patients pretreated with ipilimumab, and both doses improved PFS and ORR [16]. Grade 3/4 adverse events occurred in 11% of patients in the pembrolizumab 2 mg/kg group, 14% in the pembrolizumab 10 mg/kg group, and 26% in the chemotherapy group [17]. Median OS was 13.4 months with pembrolizumab 2 mg/kg and 14.7 months with pembrolizumab 10 mg/kg and 11.0 months with chemotherapy, but this improvement was not statistically significant in the final analysis. Two-year survival rates were 36% and 38% versus 30%. PFS, ORR, and duration of response (DOR) were all improved with pembrolizumab versus chemotherapy. In a phase III trial, pembrolizumab compared to ipilimumab (KEYNOTE-006 trial) showed 12-month OS rates of 74.1%, 68.4%, and 58.2%, for pembrolizumab every 2 weeks, for pembrolizumab every 3 weeks, and for ipilimumab, respectively. Response rates were also superior with pembrolizumab versus ipilimumab. Treatment-related grade 3–5 adverse events were less frequent with pembrolizumab (13.3% and 10.1%) than with ipilimumab (19.9%) [18•]. Several observations suggest that BRAF inhibitor therapy may affect negatively the subsequent response to immunotherapy, maybe due to a selection of a more aggressive disease. In the KEYNOTE-002 study, all patients with BRAF-mutant melanoma were previously treated with a BRAF inhibitor. Patients with BRAF wild-type melanoma had better PFS and ORR than patients with BRAF-mutant melanoma [19]. In a single-center retrospective analysis, patients with BRAF-mutant melanoma treated with pembrolizumab, who were previously treated with a BRAF inhibitor if mutated, had a significantly lower median PFS than patients with BRAF wild type [20].

Combination Anti-CTLA-4 and Anti-PD-1

Combined PD-1 plus CTLA-4 inhibition resulted in better survival outcomes than single-agent monotherapy, with the exception of OS in first-line therapy for which single-agent PD-1 inhibition had similar results as combined PD-1 plus CTLA-4 inhibition [2]. In a phase 1 trial, 2-year and 3-year OS of 79% and 63% were reported, respectively [21]. In the phase II CheckMate-069 study, patients treated with ipilimumab plus nivolumab had an ORR of 52% versus 10% with ipilimumab alone among patients with BRAF-mutant tumors. Similar results were also observed in patients with BRAF wild-type tumors [22]. At a median follow-up of 24.5 months, 2-year OS was 63.8% with nivolumab plus ipilimumab and 53.6% with ipilimumab alone [23]. However, grade 3–4 treatment-related adverse events were reported in 54% of patients receiving combination therapy compared with 24% of patients receiving ipilimumab. In the CheckMate-067 phase III trial, nivolumab alone or nivolumab plus ipilimumab were compared with ipilimumab alone in 945 previously untreated patients with metastatic melanoma [24]. At a 9-month median follow-up, median PFS was higher with the combination treatment versus ipilimumab alone (11.5 vs. 2.9 months) and with nivolumab monotherapy versus ipilimumab alone (6.9 vs. 2.9 months). Treatment-related grade 3/4 adverse events were observed in 59% of patients in the nivolumab plus ipilimumab group, in 22% of the nivolumab group, and 28% of the ipilimumab group. At the last follow-up, 4-year OS rate was 53% in the combination group and 46% in the nivolumab group, as compared with 30% in the ipilimumab group [13••]. Although the trial was not designed to compare nivolumab plus ipilimumab versus nivolumab alone, these results show the combination to be superior to nivolumab monotherapy in patients with tumors harboring BRAF mutations. The 3-year and 4-year survival results for nivolumab plus ipilimumab in patients with BRAF-mutant metastatic melanoma were 68% and 62%, respectively. In the KEYNOTE-029, the combination of pembrolizumab with ipilimumab has shown a clear benefit, with 61% ORR, an

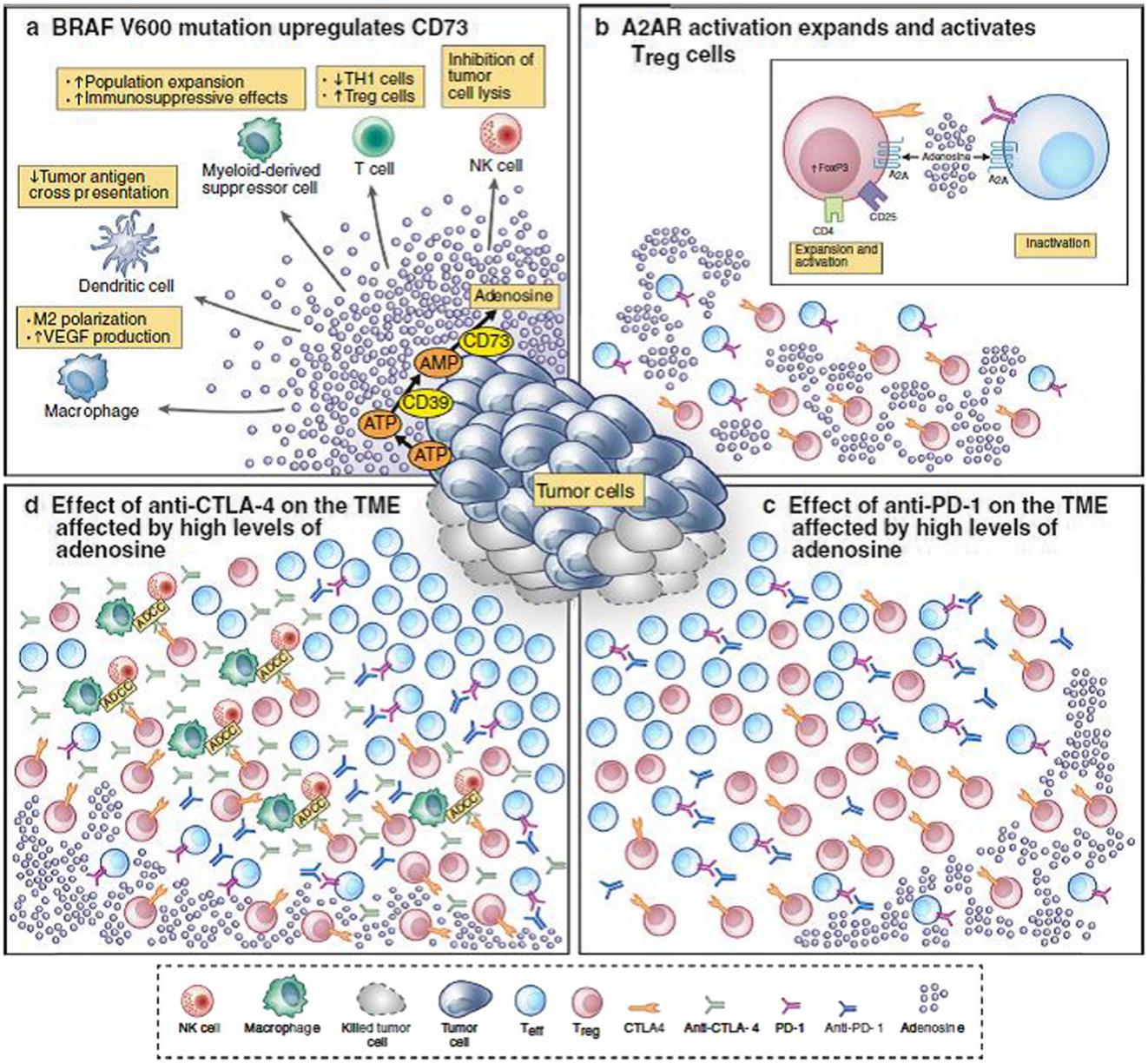

Fig. 1 Hypothetical model about how BRAFV600 mutation in melanoma cells could affect the tumor microenvironment and response to ipilimumab and combination of ipilimumab and nivolumab [14]. Reproduced from Ascierto PA, McArthur GA. Checkpoint inhibitors in melanoma and early phase development in solid tumors: what's the future? J Transl Med. 2017 Aug 8;15(1):173, which is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), courtesy of the authors

estimated 69% 1-year PFS and an estimated 89% 1-year OS; furthermore, the combination showed a manageable toxicity profile, with grade 3/4 treatment-related adverse events occurring in 45% of patients [25].

Brain metastases are a common clinical problem in patients with melanoma. Three trials evaluated the combinations of checkpoint inhibitors and BRAF/MEK inhibitors in patients with melanoma and brain metastases. In COMBI-MB, 58% of patients responded intracranially to the BRAF inhibitor dabrafenib plus the MEK inhibitor trametinib [26•]. In the phase II study (CheckMate 204), patients received ipilimumab (2 mg/kg) plus nivolumab (1 mg/kg) every 3 weeks for up to 4 weeks followed by nivolumab (3 mg/kg) every 2 weeks. At a median follow-up of 14 months, the rate of intracranial clinical benefit was 57%; these results suggest that the use of this combination is safe and effective for treatment-naive metastatic melanoma patients with brain metastases [27•]. In the phase II ABC trial, the response rate was higher when nivolumab plus ipilimumab was given upfront. In patients with no prior BRAF or MEK inhibitor therapy, the intracranial response rate was 50%, compared to 16% for patients with previous targeted treatment [28••]. These data suggest that

first-line treatment for patients with asymptomatic brain metastases should be combination ipilimumab and nivolumab rather than combined BRAF and MEK inhibition.

Targeted Therapy

The MAPK pathway, which features the sequence RAS, RAF, MEK, and ERK, is a most important intracellular signaling involved in regulating cellular proliferation and the survival of melanoma cells [29]. In melanoma, BRAF is the most frequently mutated gene of the MAPK signaling cascade. BRAF mutation induces hyperactivation of the MAPK pathway, leading to cell division and survival signaling. There are several different mutations, involving BRAF or NRAS, and the most commonly observed in BRAF mutation are V600E (80–90% of the total), and V600 K, V600D, and non-V600 mutations, accounting for the remaining 10–20%. Mutations in BRAF have been reported in nearly 50% of melanomas and mutations in NRAS in 15–25% [30]. These mutations have become targets for new therapeutic strategies in patients with melanoma. For patients with BRAF-mutant melanomas, two BRAF inhibitors, vemurafenib and dabrafenib, as monotherapy, showed improved PFS and OS and an ORR of around 50% [31, 32]. The safety profiles were manageable, with arthralgia, rash, and photosensitivity more common with vemurafenib, and hyperkeratosis, arthralgia, and pyrexia more frequent with dabrafenib. However, 50% of patients who are treated with BRAF inhibitors have disease progression after nearly 6 months of therapy. Several mechanisms mediating resistance to BRAF inhibitors through MAPK reactivation have been described, including the upregulation of bypass pathways mediated by COT (Osaka thyroid kinase), development of new NRAS or MEK mutations, and dimerization or variant splicing of mutant BRAF V600. New therapeutic strategies are needed to address these resistance mechanisms [33–36]. Combining BRAF inhibitors with MEK inhibitors is a way to overcome MAPK-dependent resistance mechanisms [37]. Moreover, the combination reduces cutaneous toxicities, such as squamous cell carcinoma, determined by the paradoxical activation of the MAPK pathway during BRAF inhibitor monotherapy. The superiority of combination BRAF plus MEK inhibition to BRAF monotherapy was confirmed in by several phase III trials with median PFS of 10–14 months and median OS of around 24 months [2]. To date, three regimens (vemurafenib plus cobimetinib, dabrafenib plus trametinib, and encorafenib plus binimetinib) have been approved for the treatment of patients with BRAF V600-mutant melanoma.

Vemurafenib and Cobimetinib

A total of 129 patients with advanced BRAF V600-mutant melanoma who were either BRAF inhibitor-naïve or had recently progressed on vemurafenib were treated with vemurafenib plus cobimetinib in a phase Ib trial BRIM 7 [38]. The maximum tolerated dose was established as vemurafenib 960 mg twice daily in combination with cobimetinib 60 mg for 21 days on and 7 days off. The ORR rate was 87% in BRAF inhibitor-naïve patients and 15% in the group who had recently progressed on vemurafenib. In an update of this study, median OS was 28.5 months in the first group and 8.4 months in the second group [39]. In the latest update, 5-year OS rates reached a plateau at 39.2% in BRAF inhibitor-naïve patients [40]. In the phase III coBRIM trial, vemurafenib in combination with cobimetinib was compared with vemurafenib alone. The median PFS was 9.9 months in patients receiving combined therapy compared with 6.2 months in patients who received vemurafenib alone (hazard ratio [HR] = 0.51, $p < 0.001$), with an improved ORR (68% with combination vs. 45% with monotherapy) [41]. The most frequent adverse events were diarrhea, nausea, vomiting, photosensitivity, elevated aminotransferase levels, increased CPK, and central serous retinopathy, slightly more frequent with the combination (65% vs. 59%). At a median follow-up of 14.2 months, median PFS was 12.3 months for cobimetinib and vemurafenib versus 7.2 months for vemurafenib in monotherapy [42]. At a median follow-up of 18.6 months, median OS among patients receiving vemurafenib plus cobimetinib was 22.5 months versus 17.4 months in patients receiving vemurafenib alone [43•].

Dabrafenib and Trametinib

Patients treated with dabrafenib 150 mg once daily combined with trametinib 2 mg daily had 9.4 months PFS versus 5.8 months in patients receiving dabrafenib alone in a phase I/II trial [44]. One-year OS rate was 79% versus 70%, but 80% of patients in the monotherapy arm crossed over to the combination group at disease progression. In an updated report, median OS was 25 months with the combination with a 3-year OS rate of 38% [45]. The most frequent toxicities with combination therapy were pyrexia and chills, and the incidence of cutaneous squamous cell carcinoma was dramatically reduced. Subsequently, combined dabrafenib and trametinib was compared with dabrafenib or vemurafenib alone in two randomized phase III trials, COMBI-d and COMBI-v. In the COMBI-d trial, median PFS in the dabrafenib plus cobimetinib arm was 9.3 months versus 8.8 months with dabrafenib alone. ORR was 67% in the combination group and 51% in the monotherapy group. Regarding the safety profile, pyrexia was more frequent in the combination arm (51% vs. 28%) while cutaneous squamous cell carcinoma incidence was higher with monotherapy (2% vs. 9%). After longer follow-up, median PFS was 11.0 months with the combination versus 8.8 months with monotherapy, OS was

25.1 months versus 18.7 months [46]. Three-year OS was 44% for the combination arm versus 32% for the monotherapy [47]. The best outcomes with the combination were observed in patients with normal lactate dehydrogenase (LDH) and less than three disease sites. In the COMBI-v trial, patients treated with dabrafenib plus trametinib had a median PFS of 11.4 months, compared to 7.3 months with vemurafenib monotherapy [48]. Median OS for the combination had not been reached, while it was 17.2 months for monotherapy. The trial was closed because of the evident benefit with the combination, and patients treated with monotherapy were switched to combination treatment. Three-year landmark data of a pooled analysis (from the phase III trials COMBI-d and COMBI-v) showed durable responses, with 23% of patients remaining progression-free and 44% remaining alive at 3 years. In addition, some prognostic factors, such as baseline LDH level and number of organ sites, were strongly associated with a better outcome [49••].

Encorafenib and Binimetinib

In a phase Ib/II trial, 55 patients received different doses of encorafenib (400, 450, or 600 mg once daily) plus binimetinib 45 mg twice daily. ORR was 74.5%, with a median PFS of 11.3 months in patients naïve to BRAF inhibitor therapy [50]. In a phase III trial, median PFS was 14.9 months in the encorafenib plus binimetinib group and 7.3 months in the vemurafenib group. Median DOR was 16.2, 14.8, and 8.4 months with the combination, encorafenib alone, and vemurafenib alone, respectively. ORR was 63% in patients treated with the combination, 51% in patients treated with encorafenib, and 40% in patients treated with vemurafenib. OS was 33.6 months in the combination group compared with 23.5 months in the encorafenib group and 16.9 months in the vemurafenib group [51•]. Encorafenib plus binimetinib seems to improve OS by around 10 months compared with the other BRAF/MEK inhibitor combinations. Treatment with this combination was also associated with a better tolerability profile, with less events requiring dose interruption or modification and fewer treatment discontinuations compared with encorafenib or vemurafenib alone.

Combination Targeted Therapy and Immunotherapy

Despite the improved OS and ORR with BRAF/MEK combinations, most patients develop mechanisms of acquired resistance and about 15% have primary resistance with no response to treatment [52]. The combination of BRAF/MEK inhibitors with immunotherapy may overcome the limited durability of responses and potentially could increase response rates and long-term survival compared with immunotherapy or targeted therapy alone. In a phase I study, vemurafenib plus ipilimumab showed severe hepatic toxicity and the trial was stopped for this reason [53]. However, the combination of vemurafenib with the anti-PD-L1 agent atezolizumab and the triple combination atezolizumab plus vemurafenib and cobimetinib showed good tolerability and encouraging clinical activity, with a response rate of 85.3% (6 complete, 23 partial) and a median DOR of 20.9 months [54]. The combination of dabrafenib and ipilimumab was well-tolerated; however, the addition of trametinib in a triple combination was associated with gastrointestinal events, with two of seven patients developing grade 3 colitis complicated by perforation [55]. A clinical benefit of the combination atezolizumab plus cobimetinib was observed both in BRAF-mutated and BRAF wild-type patients [56]. A total of 26 patients treated with the triple combination of dabrafenib, trametinib, and the anti-PD-L1 antibody durvalumab (MEDI4736) had ORR of 69% [57]. The treatment was well tolerated. In the randomized phase II part of the KEYNOTE-022 trial, 60 patients were treated with pembrolizumab plus dabrafenib and trametinib, and 60 patients with placebo plus dabrafenib and trametinib. At a median follow-up of 9.6 months, median PFS was 16.0 months with pembrolizumab plus dabrafenib and trametinib compared to 10.3 months with placebo plus dabrafenib and trametinib [58]. Median DOR was 18.7 months with the triple combination compared with 12.5 with dabrafenib plus trametinib. However, the pembrolizumab-containing arm showed higher toxicity, and a higher percentage of patients who discontinued the study due to treatment-related adverse events (40% vs. 20%). In the phase III COMBI-I trial, dabrafenib plus trametinib is being evaluated in combination with and without the PD-1 inhibitor, spartalizumab (PDR001). Preliminary results indicate that the triple combination has promising clinical activity (80% unconfirmed ORR) [59]. No new safety signals were observed. The combination of BRAF/MEK inhibitors with anti-PD-1/PD-L1 agents appears safe and effective for patients with unresectable or metastatic melanoma. However, to date, the data are not conclusive regarding a significant improvement in response and survival rates. For this reason, we must await the results of ongoing and future trials.

Sequencing Target Therapy/Immunotherapy

MAPK inhibitors can provide early and high rates of response but these are generally short-lived and overall clinical benefit is often limited due to the emergence of drug-resistance clones. In contrast, immune checkpoint inhibitors have a slower onset of action but can provide more durable responses and potentially longer-term disease control in a considerable percentage of patients. However, many others do not benefit

from immunotherapy at all, and others relapse after an initial response. Patients with metastatic BRAF-mutant melanoma have both therapeutic options available, and choosing the best first-line treatment remains an unanswered question to date. Combination BRAF/MEK inhibitors are often chosen as first-line therapy in patients with bulky and/or symptomatic disease. In all other patients who do not require rapid symptom control, up-front treatment with immune checkpoint inhibitors is often favored, because of the potential long-term survival. First-line treatment with targeted therapy may select more aggressive melanomas clones leading to a rapidly progressive disease [60]. Moreover, many patients were unable to complete ipilimumab treatment after progression with MAPK inhibitors [61]. That was true with anti-BRAF monotherapy but is less so with the BRAF/MEK combination. There are some factors associated with rapid progression such as elevated LDH levels, ECOG ≥ 1, and the presence of brain metastases. The presence of two of these risk factors at baseline is linked to a higher probability of rapid disease progression. There is evidence that pre-treatment with a BRAF inhibitor may reduce subsequent response to immunotherapy [62]. In the phase III trial KEYNOTE-006, patients with BRAF wild-type and BRAF-mutant melanoma were randomized to first-line pembrolizumab or ipilimumab. Among patients with BRAF-mutant melanoma, those who were not previously treated with a BRAF inhibitor had longer PFS than patients who received a previous BRAF inhibitor (median 7.0 vs. 2.8 months) [19]. Similar observations have also been made for nivolumab [63]. However, this negative impact could be a reflection of baseline tumor biology rather than an impact of the BRAF inhibitor therapy itself. In the other hand, objective responses are still ongoing in patients treated with immunotherapy after disease progression on BRAF inhibitor therapy [64]. Other data suggest that the use of first-line immunotherapy does not appear to negatively impact the prognosis for patients receiving subsequent BRAF inhibitor treatment [65, 66]. Additional data suggests that prior immunotherapy might also worsen the safety profile of subsequent BRAF-targeted therapy [67]. This may be due to some alteration in tumor biology, or selection for a more aggressive disease, or even a persistent immune activation. Overall, these data suggest that immunotherapy may be the best first choice in patients with BRAF-mutant melanoma that is non-symptomatic and without a high tumor burden. However, this approach is based on retrospective data and may not be fully accepted. Several clinical trials are ongoing or planned to answer the question of which treatment to use first or which treatment sequence is preferable (Table 1). The best sequence of treatment is also being evaluated in the neoadjuvant setting.

Challenges and Future Directions

Continuous combination of MAPK inhibitors and anti-PD1/PDL-1 agents improve outcomes of BRAFV600 mutated melanoma patients, but with a high frequency of grade 3/4 treatment-related adverse events. Moreover, resistance mechanisms in combination therapy are similar to the monotherapy. Because of this, other strategies to avoid resistance emergence are also being studied, including the use of sequential and intermittent schedules. The IMPemBra study recently showed that combination of pembrolizumab plus intermittent

Table 1 Ongoing clinical trials evaluating combination treatment or in sequence with targeted therapy and immunotherapy in advanced-metastatic BRAF mutated melanoma

Trial	Phase	Study design	Primary endpoint
KEYNOTE- 022 NCT02130466	II Randomized	• Dabrafenib/trametinib + pembrolizumab • Dabrafenib/trametinib + placebo	mPFS
TRILOGY NCT02908672	III randomized	• Vemurafenib/cobimetinib + atezolizumab • Vemurafenib/cobimetinib + placebo	mPFS
COMBI-I NCT02967692	III Randomized	• Dabrafenib/trametinib + spartalizumab • Dabrafenib/trametinib + placebo	mPFS
ECOG NCT02224781	III Randomized comparative	• Dabrafenib/trametinib ➔ ipilimumab/nivolumab at PD • Ipilimumab/nivolumab ➔dabrafenib/trametinib at PD	OS
SECOMBIT NCT02631447	II Randomized not comparative	• Encorafenib/binimetinib ➔ ipilimumab/nivolumab at PD • Ipilimumab/nivolumab ➔ encorafenib/binimetinib at PD • Encorafenib/binimetinib run-in (8 weeks)➔ ipilimumab/nivolumab ➔ encorafenib/ binimetinib at PD	OS
ImmunoCobiVem NCT02902029	II Randomized not comparative	• Run-in vemurafenib/cobimetinib (3 months) ➔ vemurafenib/cobimetinib ➔ atezolizumab at PD • Run-in vemurafenib/cobimetinib (3 months) ➔ atezolizumab ➔ vemurafenib/cobimetinib at PD	Time to PFS
EORTC 1612 NCT03235245	III Randomized comparative	• Ipilimumab/nivolumab ➔ IC at PD • Encorafenib/binimetinib run-in (12 weeks)➔ ipilimumab/nivolumab ➔ encorafenib/ binimetinib at PD	mPFS

dabrafenib and trametinib is safe and effective [68]. In the ongoing phase II COMBAT trial, patients are randomized to the combination of dabrafenib and trametinib after 8 weeks of either as monotherapy; in the ongoing SWOG S1320 study, patients receive dabrafenib plus trametinib for 8 weeks and are then randomized to dabrafenib plus trametinib as continuous or intermittent dosing.

Increasing evidence exists for the role of immunosuppressive adenosine in promoting tumor growth, invasion, and proliferation. Suppression of adenosine signaling by inhibiting adenosine receptors or adenosine-generating enzymes (CD73) on melanoma cells represents a novel therapeutic target. Furthermore, in melanoma patients with BRAF mutation, CD73 is overexpressed. Administration of anti-BRAF, with or without anti-MEK agents, led to a decrease of CD73 expression, and in a mouse model, a significant therapeutic suppression of tumor initiation and metastasis was observed following the combination of BRAF, MEK, and adenosine A2a receptor inhibitors [69]. Increased CDK4 activity occurs in the majority of melanomas and the combination of CDK4/6 inhibition with BRAF and MEK inhibition seems to be a promising strategy [70]. In a Phase 1b study, the triple combination of encorafenib, binimetinib, and the CDK 4/6 inhibitor ribociclib showed a confirmed ORR of 52.4%, with a median PFS of 9.0 months. Ten patients (23.8%) discontinued treatment due to an adverse event, of which four had increased transaminases [71]. Combination of CDK4 inhibitors and BRAF and MEK inhibitors may be a strategy for overcoming resistence [70]. However, the increase of toxicity with an increase of the discontinuation rate of drug assumption compared to BRAF/MEK inhibition alone may limit the effect of the triple inhibition. Further investigations are warranted. A phase I–II study with PD-0332991 (an inhibitor of cyclin-dependent kinase 8) added to vemurafenib in patients with metastatic melanoma and BRAF mutation is ongoing (OPTIMAL trial).

Conclusion

In recent years, the treatment of patients with metastatic melanoma has dramatically changed and several options are now available, especially for patients with the BRAF mutation. Immunotherapy (anti-PD-1/PDL-1) and targeted therapy (anti-BRAF and anti-MEK) became the new standard of treatment. These advances have substantially improved survival rates. However, many questions still remain unanswered, such as what is the best first- and second-line treatment and the best treatment sequence. New combinations of drugs, targeted therapy combined with immunotherapy and sequencing approaches, are now underway in many ongoing clinical trials. Moreover, a personalized approach based on the identification of biomarkers could help to better treatment choices.

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Compliance with Ethical Standards

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

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