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REVIEW

Mechanisms of resistance to BRAF and MEK inhibitors and clinical update of US Food and Drug Administration-approved targeted therapy in advanced melanoma

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Abstract: Approximately 50% of melanomas harbor an activating *BRAF* mutation. Combined BRAF and MEK inhibitors such as dabrafenib and trametinib, vemurafenib and cobimetinib, and encorafenib and binimetinib are US Food and Drug Administration (FDA)-approved to treat patients with $BRAF^{V600}$-mutated advanced melanoma. Both genetic and epigenetic alterations play a major role in resistance to BRAF inhibitors by reactivation of the MAPK and/or the PI3K–Akt pathways. The role of BRAF inhibitors in modulating the immunomicroenvironment and perhaps enhancing the efficacy of checkpoint inhibitors is gaining interest. This article provides a comprehensive review of mechanisms of resistance to BRAF and MEK inhibitors in melanoma and summarizes landmark trials that led to the FDA approval of BRAF and MEK inhibitors in metastatic melanoma.

Keywords: malignant melanoma, targeted therapy, BRAF inhibitor, MEK inhibitor, resistance

Introduction

Nearly half of patients with metastatic melanomas harbor a valine–glutamine substitution in codon 600 of the serine/threonine kinase BRAF ($BRAF^{V600}$ mutation).[1] These melanomas have all the features of oncogene addiction to the *BRAF*-mutated gene (Figure 1).[2] Vemurafenib, previously known as PLX4032 or RG7204, dabrafenib, known as GSK2118436, and encorafenib are BRAF inhibitors (BRAFi) approved by the US Food and Drug Administration (FDA) in combination with MEK inhibitors to treat patients with $BRAF^{V600E/K}$-mutated metastatic melanomas.[3,4] BRAFi result in high response rates; however, responses are short-lived, with a median time to progression of 5.1–8.8 months.[3,5–7] The addition of an MEK inhibitor to a BRAFi extends the median duration of response from 5.6 months to 9.5 months.[8,9] Genetic and/or epigenetic changes in melanoma cells (Figure 2) via reactivation of the MAPK pathway and to a lesser extent the PI3K–Akt pathway play a crucial role in acquired resistance to BRAFi[10–14] and contribute extensively to tumor heterogeneity.[15] There is an intense effort to better understand mechanisms of resistance to BRAFi and develop new agents that target areas of resistance.[16–19] Moreover, the role of BRAFi in enhancing immunoresponses and boosting the efficacy of checkpoint inhibitors is an area of extensive research. This article provides a comprehensive review of mechanisms of resistance and summarizes landmark trials that led to the approval of BRAF and MEK inhibitors in metastatic melanoma. We will briefly discuss how BRAFi could modulate the tumor microenvironment and enhance immunoresponses.

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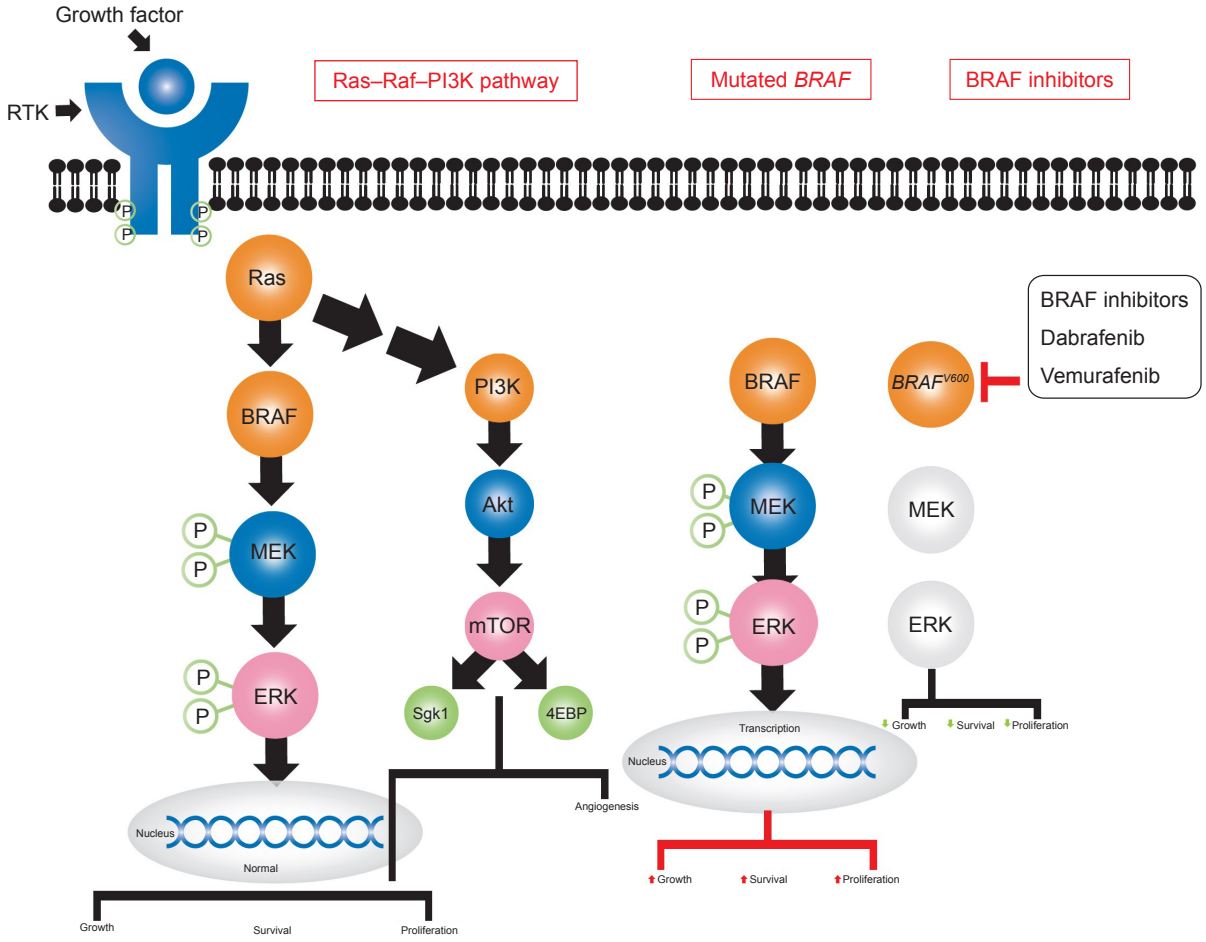

Figure 1 MAPK–PI3K–Akt pathway and *BRAF*^{V600} mutation in melanoma.
Notes: MAPK pathway in normal cells (left), where growth factors bound to RTK result in phosphorylation of Ras kinase, which further activates downstream kinases (Raf–MEK–ERK and PI3K–Akt–mTOR) and regulates the activities of several transcription factors responsible for cell growth, survival, and proliferation. *BRAF*^{V600} mutations in melanoma lead to constitutive activation of the MAPK pathway, which leads to uncontrolled cell survival, growth, and proliferation in malignant melanoma (right) that might be reversed, at least temporarily, by treatment with BRAF inhibitors.

Genetic causes of resistance to BRAFi in melanoma

MAPK-pathway activation is a fundamental step in several intracellular processes, including cell growth and differentiation. Physiological upstream negative feedback prevents persistent MAPK-pathway activation in normal cells, but this is lost in melanoma cells that harbor the *BRAF*^{V600} mutations, leading to constitutive activation of the MAPK pathway.[11]

Whole-exome sequencing of serial melanoma biopsies obtained at baseline and upon progression reveal a spectrum of genetic alterations in ~51%–58% of patients with *BRAF*^{V600}-mutated metastatic melanoma who receive vemurafenib or dabrafenib (Table 1). These genetic alterations (Figure 2) mainly result in reactivation of MAPK and to lesser extent activation of PI3K–Akt pathways.[10,11,15,16,20–26]

Secondary mutations in *NRAS* or *MAP2K* suggest acquired resistance mechanisms that maintain dependence on the MAPK pathway.[27] Mutations in *RAS* (25%) and *BRAF*^{V600} (22%) are mutually exclusive, representing the most frequently detected genetic alterations leading to resistance.[15,24,25,28] Resistance to BRAFi or combined BRAF and MEK inhibitors is also associated to a lesser extent with activation of the PI3K–Akt pathways (Table 1).[29] Preclinical data have shown that PI3K-pathway activation via loss of PTEN prevents apoptosis of melanoma cells treated with BRAFi or activation of Akt can contribute to BRAFi resistance.[29,30] Patients with PTEN loss have shorter progression-free survival (PFS) on dabrafenib.[31] The presence of PI3K-pathway alterations does not necessarily preclude clinical response.[9,20,32]

Epigenetic or transcriptomic changes

Epigenetic or transcriptome-based changes were speculated to be the likely drivers of resistance to BRAFi among

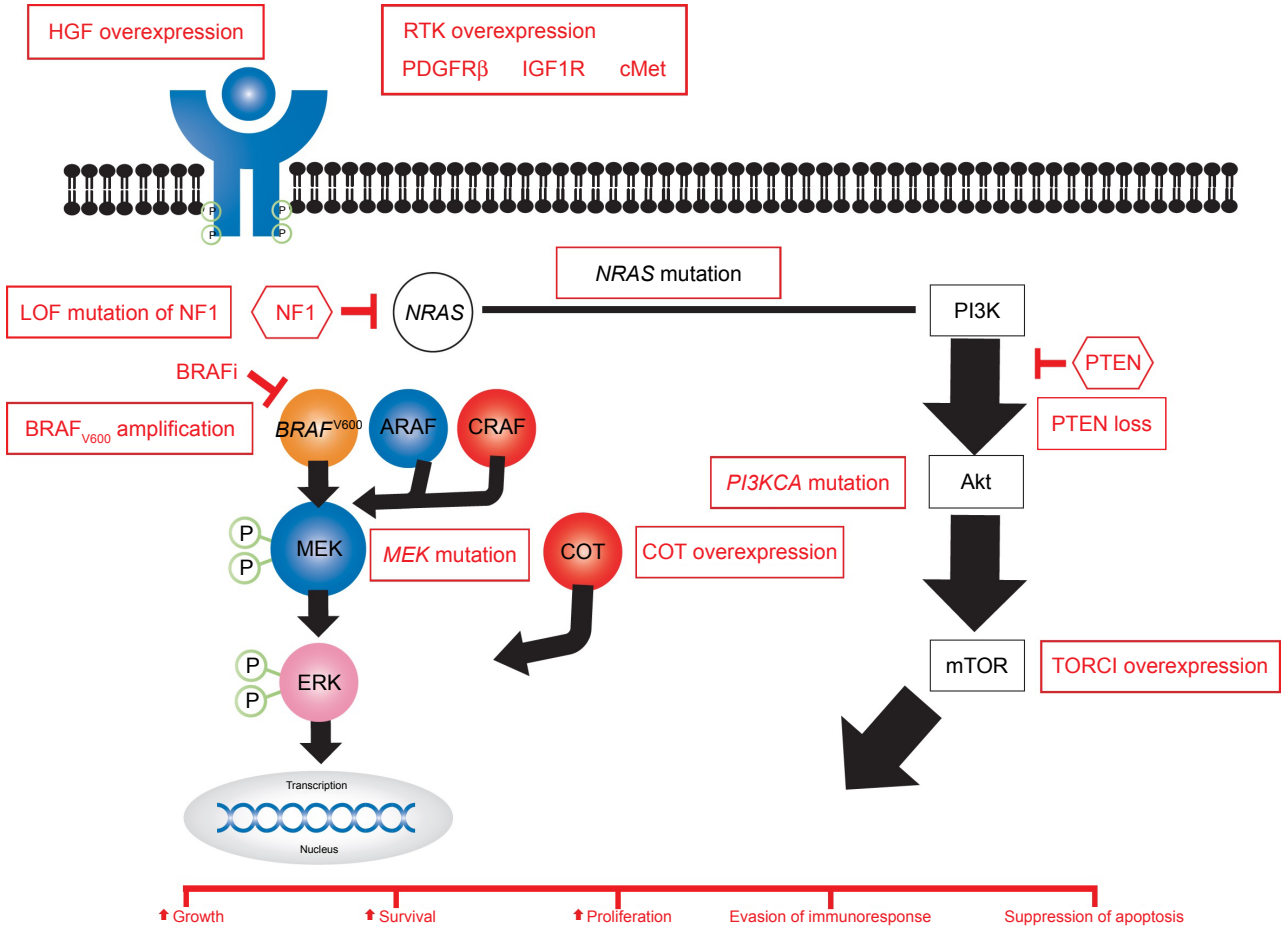

Figure 2 Genetic and epigenetic causes of resistance to BRAF inhibitors in melanoma.
Notes: Mechanisms of resistance to BRAF inhibitors in metastatic melanoma. Genetic changes leading to resistance to BRAF inhibitors include *NRAS* mutation, BRAF amplification, *MEK* mutations, NF1 mutations Akt amplification (genetic or epigenetic), and loss of PTEN (genetic or epigenetic), while epigenetic changes include Akt amplification, loss of PTEN, overexpression of HGF, RTK, PDGFRβ, and IGF1R.

39%–42% of melanomas that progressed on BRAFi and lacked any identifiable genetic abnormality to explain such resistance (Table 2).[15,42,43]

BRAFi and the immune system

Preclinical data suggest that *BRAF*^{V600E} mutation contributes to immunoescape and that both BRAFi and MEKi have beneficial effects on antitumor immunity and the tumor microenvironment as a whole, which is mediated by different mechanisms.[50] Treatment with BRAFi and MEK inhibitors may modulate the immunomicroenvironment.[15,49,50] Increased expression of melanoma antigens (MART, TYRP1, TYRP2, and Gp100), CD8$^+$ T-cell infiltrates, and markers of T-cell cytotoxicity (perforin, granzyme B) and decreased levels of immunosuppressive cytokines occur during BRAFi therapy.[51] However, immunoresponses may be limited, due to increased markers of T-cell exhaustion, such TIM3, PD1, and the immunosuppressive ligand PDL1.[52] In melanoma cell lines, BRAFi/MEKi increase the rates of PD1$^+$ melanoma cells that may sustain tumor relapse.[48] These findings are intriguing, as immunocheckpoint blockade may be critical if combined with BRAF, in enhancing antitumor immunity and augmenting therapeutic responses.[48,52] This proimmunotherapy microenvironment is lost upon melanoma progression on BRAFi. Several studies have revealed a decrease in melanoma antigen expression, an increase in T-cell exhaustion, and a decrease in CD8$^+$ T-cell infiltrates in melanoma tumor specimens obtained at time of progression on BRAFi.[15,51,52]

The number of infiltrating macrophages and levels of macrophage-produced factors (such as growth factors, cytokines, chemokines, extracellular matrix, and proteinases) correlates inversely with patient outcomes in melanoma.[53–60] Infiltrating macrophages contribute to cancer resistance to chemotherapy, radiotherapy, and immunotherapy.[61–64] BRAFi paradoxically activate the MAPK pathway in macrophages to produce VEGF, which directly activates the MAPK pathway and stimulates cell growth in both macrophages

Table 1 Genetic causes of resistance to BRAF inhibitors (BRAFi) in melanoma

Study	Mechanisms of resistance	Comment
van Allen et al[20]	*NRAS* mutation 17.8% *MAP2K1* mutation 15.6% BRAF amplification 8.9% *MAP2K2* mutation 8.8% Mutations in the PI3K pathway (*PIK3CA*, *PTEN*, *PIK3R1*) MITF amplification *HOXD8* nonsense gene mutation	Genetic alterations observed in 23 of 45 patients (51%) *NRAS* mutations occurred exclusively in patients on therapy for more than 12 weeks (P=0.04): Q61 (n=7) and T58 (n=1) loci Nonsense mutation in *HOXD8* (n=1) and missense mutations in *RAC1* (n=3) correlated with early resistance *MAP2K1*^{P124S} and *MAP2K1*^{P124L} detected in pretreatment tumors correlated with rapid progression to BRAFi, while *MAP2K1*^{G276W} and *MAP2K1*^{F53Y} correlated with clinical response to BRAFi
Shi et al[33]	*BRAF*$^{V600E/K}$ amplification	
Jakob et al[34] Nazarian et al[23]	*NRAS* mutation	
Trunzer et al[9] Wagle et al[35]	*KRAS* mutation	
Trunzer et al[9] Wagle et al[35]	*MAP2K1*/*MAP2K2* mutations	
Whittaker et al[36]	*MAP2K1*, *PIK3CA*, *AKT1*, *AKT3*	
Whittaker et al[36]	LOF events in *PIK3R2*, *DUSP4*, *CDKN2A*, *PTEN*, *NF1*	
Montagut et al[37] Paraiso et al[38]	Reactivation of phosphorylated ERK	
Gray-Schopfer et al[39] Shi et al[25,33] Paraiso et al[29]	LOF of PTEN[a] *AKT* mutation or amplification	
Hodis et al[40] Krauthammer et al[41]	*RAC1*^{P29S} gain-of-function oncogene mutation	*RAC1*^{P29S} mutation in pretreatment biopsies of patients with metastatic melanoma was associated with early disease progression in the setting of BRAFi
Johnson et al[26]	*NRAS* mutation 17% *KRAS* mutation 2% *BRAF*-splice variants 16% BRAF amplification 13% *MAP2K1*/*MAP2K2* mutations 7% Non-MAPK-pathway alterations 11%	Marked heterogeneity was observed within tumors and patients *NRAS* mutations, *BRAF* splice variants, and *MAP2K1*/*MAP2K2* mutations usually occurred in mutually exclusive fashion with each other, whereas *BRAF*$^{V600E/K}$ amplification overlapped with *NRAS* mutations, non-MAPK alterations, and a *MEK2* mutation Non-MAPK pathway alterations largely occurred in the PI3K–Akt pathway but also included *MITF* amplification, and overexpression of PDGFR/IGF1R. *CDKN2A* deletion and *DUSP4* loss occurred in three samples Mutational complexity increases over time on BRAFi with earlier progression sample had fewer resistance mechanisms compared to the later sample (mean 0.42 vs 0.83, P=0.054) NRAS mutations correlated with vemurafenib use (P=0.045) and intracranial metastases (P=0.036), whereas *MAP2K1*/*MAP2K2* mutations correlated with hepatic progression (P=0.011) Median survival after disease progression was 6.9 months, and responses to subsequent BRAF and MEK inhibition were uncommon (2 of 15; 13%)
Poulikakos et al[22]	Alternate splicing of *BRAF*	

Note: [a]Both genetic and epigenetic changes may result in LOF of PTEN.
Abbreviation: LOF, loss of function.

and melanoma cells.[10,65–67] The TNFα produced by activated macrophages may also contribute to melanoma resistance to BRAFi.[68] BRAFi may transition the macrophage from being a passenger to a driver of melanoma progression, and hence agents that target infiltrating macrophages may overcome resistance to BRAFi.[10,59]

Potential ways to overcome resistance to BRAF inhibitors

BRAFi cause increased expression of melanocyte differentiation antigens, increased recognition by antigen-specific T cells, increased CD8$^+$ T-cell infiltration in the melanoma microenvironment, decreased expression of immunoinhibitory cytokines, decreased myeloid-derived suppressor cells, and increased T-cell-exhaustion markers (eg, TIM3, PD1, and PDL1). Reactivation of the MAPK pathway causes suppression of melanoma antigens and reemergence of an immunosuppressive tumor microenvironment. Subsequent MAPK-pathway inhibition by an MEK inhibitor has restored melanoma antigen expression and promoted infiltration of CD8$^+$ T cells.[48,69,70] Adding MEK inhibitors to BRAFi can overcome resistance to BRAFi and enhance

Table 2 Epigenetic or transcriptomic causes of resistance to BRAF inhibitors (BRAFi) in melanoma

Study	Mechanisms of resistance	Comment
Johannessen et al[21]	Overexpression of MAP3K8 (also called COT)	COT overexpression drives resistance to BRAFi through MAPK-pathway reactivation COT activates ERK primarily through MEK-dependent mechanisms that do not require Raf signaling
Wily Hugo et al[15]	Overexpression of cMet Underexpression of *LEF1* and *YAP1*-signature enrichment	Melanoma acquires MAPKi resistance with highly dynamic and recurrent nongenomic alterations and coevolving intratumoral immunity
Paraiso et al[29]	Underexpression of BIM via PTEN loss	Loss of PTEN contributes to intrinsic BRAFi resistance via suppression of BIM-mediated apoptosis
Poulikakos et al[22]	Expression of *BRAF*^{V600E}-splicing variants	Expression of a BRAF splicing variant leads to structural change in BRAF and the ability of BRAFi to bind to it
Straussman et al[19]	Stromal secretion of HGF	Proteomic analysis showed that stromal cell secretion of HGF resulted in activation of the HGF receptor Met, reactivation of the MAPK and PI3K–Akt signaling pathways, and immediate resistance to Raf inhibition in melanoma
Wily Hugo et al[15]	Underexpression of *CTLA4* Underexpression of antigen presentation genes (*B2M*, *HLA-A*, *HLA-B*, and *TAP1*) Underexpression of Wnt-signaling genes (*LEF1*, *FZD6*, *WNT11*, and *WNT10A*) Underexpression of RTK genes (*AXL*, *EGFR*, *ALK*, *NTRK2*, and *FGFR2*)	Transcriptomic underexpression accounted for the majority of highly recurrent LOF gene-based events in genes considered vital for active immunosurveillance in melanoma Gene- and signature-based transcriptomic alterations in acquired MAPKi-resistant melanoma highly recurrent
Sanchez-Laorden et al[44]	cMet and IL8 overexpression	cMet and IL8 overexpressed in 44% and 40% of resistant tumors, respectively
Villanueva et al[45] Nazarian et al[23]	Overexpression of PDGFRβ or IGF1R	
Shi et al[25] Lidsky et al[46]	Overexpression of wild-type *NRAS* or *KRAS*	
Villanueva et al[45] Nazarian et al[23]	RTK dysregulation	
van Allen et al[20] Garraway et al[47]	*MITF* amplification	*MITF* amplification associated with resistance to MAPK inhibition; this gene encodes a master lineage transcription factor that governs melanocyte development and is also an amplified oncogene within the melanocyte lineage

Abbreviation: LOF, loss of function.

immunosurveillance. In a mouse model of *BRAF*^{V600E} melanoma, improved antitumor activity, in vivo cytotoxic activity, and intratumoral cytokine secretion have been reported after combining adoptive T-cell transfer with BRAFi.[71] As such, BRAF-targeted therapy may be critical in augmenting responses to immunocheckpoint blockade in patients with metastatic melanoma. The optimal treatment sequence remains to be elucidated.[76]

Preclinical studies have shown a decreased in $CD8^+$ T cells in the tumor microenvironment upon progression to targeted therapy. Indeed, patients with disease progression on targeted therapy have lower response rates to immunocheckpoint blockade in retrospective studies.[72–75] Therefore, theoretically we should not treat *BRAF*-mutated melanoma patients to progression with targeted therapy before starting immunocheckpoint blockade. Instead, we should consider adding it soon after initiation of targeted therapy.[51,77] The critical question of therapy sequencing is being tested in a randomized Phase III trial (NCT02224781).

There is also growing interest in modifying our current approach of identifying and targeting driver mutations to one that focuses more on targeting elements of resistance in the tumor microenvironment.[10] The presence of macrophages in the tumor microenvironment plays a critical role in melanoma resistance and predicts early relapse to targeted therapy, as aforementioned. Targeting macrophages alone in vivo can inhibit melanoma growth and increase the efficacy of BRAFi, which provides a rationale for combining BRAFi with therapies that target macrophages (NCT01826448, NCT03101254).[10]

Activation of the PI3K–Akt pathways contributes to melanoma resistance to targeted therapy. Therefore, approaches aiming simultaneously to inhibit both the MAPK and PI3K–Akt pathways have been proposed in melanoma.[78] Preclinical

data have demonstrated the superior antitumor activity of a combination of MAPK and PI3K–Akt–mTOR pathway inhibitors in *BRAF*^{V600E}-mutant cell lines.[29,45,79] Melanoma cells resistant to BRAFi have an MEK-independent survival driver that can be blocked by inhibitors of the PI3K–Akt–mTOR pathway.[80] Upon progression, the addition of an Akt or mTOR inhibitor to continued therapy with vemurafenib or switching to a combination of an MEK inhibitor plus an Akt or an mTOR inhibitor may provide additional inhibitory activity.[27]

NRAS mutations and *BRAF* amplifications may still prove responsive to subsequent MEK-inhibitor-based regimens, although the existing clinical data suggest that patients who progress following single-agent Raf inhibition are less likely to benefit from MEK inhibitors.[81] Complete NRAS extinction is difficult to achieve pharmacologically, due to redundant feedbacks and the likely induction of toxicity in patients, and thus *NRAS*-mutant melanoma remains without effective therapy.[82] Combined MEK and CDK4 inhibition has revealed synergistic antitumor effects in a human *NRAS*-mutant melanoma xenograft model, providing a rationale for combining a CDK4 inhibitor with MEKi to achieve therapeutic synergy.[83] Vemurafenib-resistant cell lines with acquired *NRAS*^{Q61K} mutation exhibit some sensitivity to sequential treatment with an MEK inhibitor and combinations of drugs inhibiting both Akt and MAPK pathways. This may be due to possible cross talk between mutated *NRAS* and the Akt pathway.[27] Therapeutically, these findings imply that multiple pathways may need to be targeted simultaneously if not limited by toxicity or sequentially as part of an intermittent-dosing schedule.[84]

Update on FDA-approved targeted therapy in metastatic melanoma

Historically, the prognosis of metastatic melanoma has been poor, with 5-year survival ~6% and median overall survival (OS) of 7.5 months.[85,86] Dacarbazine was the mainstay of treatment for metastatic melanoma until 2011, with an overall response rate of 7%–12% and median OS of 5.6–7.8 months.[96–98] BRAF-targeted therapy and immunotherapy have transformed the landscape of melanoma treatment drastically.[99–101] Vemurafenib and dabrafenib have shown significant improvement in response rates, PFS, and OS in *BRAF*$^{V600E/K}$-mutated metastatic melanoma.[87,88]

In the BRIM-3 randomized Phase III trial, patients (n=675) with stage IIIC unresectable or stage IV *BRAF*^{V600E}-mutated melanoma with no prior therapy were randomized 1:1 to vemurafenib (960 mg orally twice daily) or dacarbazine (1,000 mg/m^2 intravenously every 3 weeks; Table 3).[87] Median OS and PFS were 13.6 and 5.3 months in the vemurafenib arm compared to 9.7 (HR 0.81) and 1.6 months in the dacarbazine arm, respectively. Vemurafenib reduced the risk of death by 63% (HR 0.37) and risk of progression by 74% (HR 0.26). Normal baseline LDH, Eastern Cooperative Oncology Group performance status score 0, and stage M1a/b melanoma predicted long-term response to vemurafenib. Adverse events seen more frequently in vemurafenib included skin rash, arthralgia, alopecia, fatigue, and photosensitivity reactions. Squamous cell carcinoma of the skin or keratoacanthoma occurred in 18% of patients on vemurafenib. Based on the results of the BRIM-3 trial, the FDA approved vemurafenib on August 17, 2011 for the treatment of patients with unresectable or metastatic *BRAF*^{V600E}-mutated melanoma.

BREAK-3 was a multicenter open label Phase III randomized trial where patients (n=250) with unresectable stage III or stage IV *BRAF*^{V600E}-mutated melanoma were randomized 3:1 to dabrafenib (150 mg orally twice daily) or dacarbazine (1,000 mg/m^2 intravenously every 3 weeks; Table 3).[88] Median OS and PFS were 20 and 6.9 months for dabrafenib compared with 15.6 (HR 0.61) and 2.7 (HR 0.3) months for dacarbazine, respectively.[88] Common adverse events seen with dabrafenib included fever, fatigue, headache, arthralgia, cutaneous squamous cell carcinoma and keratoacanthoma. Based on the results of the BREAK-3 trial, the FDA approved dabrafenib on May 29, 2013 for the treatment of patients with unresectable or metastatic *BRAF*^{V600E}-mutated melanoma.

Preclinical data have revealed that resistance to BRAFi occurs largely at the level of MEK, and hence increased interest in MEK inhibitors has emerged. Trametinib is a small molecule that selectively inhibits MEK1 and MEK2.[102] In Phase I and II trials, trametinib caused tumor regression and stabilization of disease in patients with *BRAF*$^{V600E/K}$ mutation.[103,104] In the METRIC trial, patients (n=322) with stage IIIC or stage IV *BRAF*$^{V600E/K}$-mutated melanoma were randomly assigned 2:1 to receive trametinib (2 mg orally daily) or chemotherapy (dacarbazine or paclitaxel; Table 3).[105] Median PFS was 4.9 months in the trametinib arm and 1.6 months in the chemotherapy arm ($HR_{progression}$ 0.45; HR_{death} 0.54). OS at 6 months was 81% in the trametinib arm compared to 67% in the chemotherapy arm. Rash, edema, diarrhea, fatigue, and dermatitis acneiform were commonly encountered with trametinib. Cardiomyopathy was observed in 7% of subjects receiving trametinib, with serious cardiac events leading to discontinuation of the drug seen in three patients. Retinal vein occlusion and central serous retinopathy were encountered with trametinib.[105] Based on the

Table 3 Clinical trials of BRAF inhibitors and BRAF + MEK inhibitors in metastatic melanoma

Trial	PEP	Treatment arms (number of patients)	OS (months/ rate)	PFS (months)	ORR	TTR (months)	DOR (months)	Most common AEs
BRIM-3[87]	OS + PFS	Vem (338) Dac (337)	13.6 9.7	5.3 1.6	48% 5%	1.45 2.7	5.49 NA	Cutaneous lesions Arthralgia Fatigue
BREAK III[88]	PFS	Dab (187) Dac (63)	20 15.6	6.9 2.7	50% 6%	1.5 NR	5.5 NA	Cutaneous lesions Fever Fatigue Headache Arthralgia
METRIC[105]	PFS	Tr (214) Dac/Pac (108)	15.6 11.3	4.9 1.6	19% 5%	NR NR	5.6 NA	Rash Edema Diarrhea Fatigue Dermatitis
COMBI-V[5]	OS	Dab + Tr (352) Vem (352)	NR 17.2	11.4 7.3	64% 51%	NR NR	13.8 7.5	Fever Nausea Diarrhea Chills Arthralgia Rash Alopecia Diarrhea Nausea
COMBI-D[86]	PFS	Dab + Tr (211) Dab + Pl (212)	44% (at 3 years) 32% (at 3 years)	22% (at 3 years) 12% (at 3 years)	68% 55%	NR NR	12 10.6	Fever, chills Diarrhea Vomiting Edema Hyperkeratosis Alopecia SCC/KA Skin papilloma
coBRIM[95]	PFS	Vem + Cob (247) Vem + Pl (248)	81% (at 9 months) 73% (at 9 months)	9.9 6.2	68% 45%	NR NR	NR 7.3	Diarrhea Nausea Vomiting Rash Arthralgia Fever Fatigue Alopecia Arthralgia Diarrhea Hyperkeratosis Cutaneous SCC/KA
COLUMBUS[107]	PFS	Enc + Bin (192) Enc (194) Vem (191)	33.6 47% (at 3 years) 23.5 16.9 32% (at 3 years)	14.9 9.6 7.3	64% 52% 41%	NR NR NR	18.6 15.2 12.3	Increased γGT, CPK, and hypertension Hand–foot syndrome, myalgia, and arthralgia

Abbreviations: AE, adverse events; Bin, binimetinib; Cob, cobimetinib; Dab, dabrafenib; Dac, dacarbazine; DOR, duration of response; Enc, encorafenib; KA, keratoacanthoma; NA, not applicable; NR, not reported; ORR, overall response rate; OS, overall survival; Pac, paclitaxel; PEP, primary end point; PFS, progression-free survival; Pl, placebo; RFS, relapse-free survival; SCC, squamous cell carcinoma; Tr, trametinib; TTR, time to response; Vem, vemurafenib.

results of the METRIC trial, the FDA approved trametinib on May 29, 2013 for the treatment of patients with unresectable or metastatic *BRAF*$^{V600E/K}$-mutated melanoma.

As discussed earlier, MAPK-pathway activation plays an important role in melanoma resistance to BRAFi, which limit median PFS to 6–8 months after BRAFi therapy alone.[18,20,25,87,88] Moreover, paradoxical activation of the MAPK pathway in other normal BRAF wild-type cells, such as keratinocytes, has been associated with development of secondary cutaneous squamous cell carcinoma and

keratoacanthomas.[90–94] Preclinical and clinical data suggest that inhibition of both MEK and mutant *BRAF* kinases may result in greater initial tumor response, prevent MAPK-driven acquired resistance, and decrease the incidence and severity of toxicities, such as secondary skin tumors, owing to the paradoxical activation of the MAPK pathway seen in BRAFi monotherapy.[18,20,25,89,95]

In the COMBI-D double-blinded Phase III randomized control trial, patients (n=423) with unresectable stage IIIC or stage IV *BRAF*$^{V600E/K}$-mutated melanoma were randomized 1:1 to receive dabrafenib plus trametinib or dabrafenib plus placebo (Table 3).[86] The 3-year landmark analysis of COMBI-D provided evidence that long-term benefit and tolerability are achievable with combination dabrafenib and trametinib in patients with previously untreated *BRAF*$^{V600E/K}$-mutant metastatic melanoma. Three-year PFS and OS rates were 22% and 44% with dabrafenib plus trametinib vs 12% (HR 0.71) and 32% (HR 0.75) with monotherapy, respectively.[86] These results corroborated the Phase III BRF113220 trial demonstrating OS of 38%.[8] The highest 3-year OS of 62% and PFS of 38% was observed in patients with normal baseline LDH level and fewer than three organ-site metastases compared to 45% and 16% in monotherapy.[8] Patients with LDH above the upper limit of normal had 3-year OS of 25% vs 14%.[86] Common adverse events in the combination vs monotherapy arm included fever (59% vs 33%), chills (32% vs 17%), diarrhea (31% vs 17%), vomiting (26% vs 15%), and peripheral edema (22% vs 9%). Hyperkeratosis, alopecia, cutaneous squamous cell carcinoma, keratoacanthoma, basal cell carcinoma, and skin papilloma were more common in the dabrafenib arm. Extended 5-year follow-up data of the Phase II BRF113220 trial revealed increased OS in patients who received dabrafenib and trametinib with normal baseline LDH (5 years, 45%) and normal LDH with fewer than three organ sites with metastasis (5 years, 51%).[89]

The COMBI-V open label, multicenter, Phase III trial randomly assigned patients (n=704) with metastatic *BRAF*^{V600}-mutated melanoma 1:1 to receive either a combination of dabrafenib (150 mg orally twice daily) and trametinib (2 mg orally once daily) or vemurafenib (960 mg orally twice daily) as first-line therapy (Table 3).[5] Median PFS and OS were 7.3 months and 17.2 months, respectively, for patients in the vemurafenib group compared with 11.4 months (HR 0.56, 95% CI, 0.46–0.69; $P<0.001$) and had not been reached for patients in the combination therapy group. The most common reasons for either drug discontinuation or dose modification were pyrexia and decreased left-ventricle ejection fraction in the combination therapy group and arthralgia in the vemurafenib group. Pyrexia was more frequent in the combination therapy group than the vemurafenib group (53% vs 21%). Skin toxicity effects were more frequent in the vemurafenib group, as a result of paradoxical reactivation of the MAPK pathway in BRAF wild-type keratinocytes, compared to the combination group, in particular rash (43% vs 22%), photosensitivity reaction (22% vs 4%), hand–foot syndrome (25% vs 4%), skin papilloma (23% vs 2%), squamous cell carcinoma and keratoacanthoma (18% vs 1%), and hyperkeratosis (25% vs 4%).[5]

coBRIM was a multinational trial that randomly assigned 495 patients with previously untreated unresectable locally advanced or metastatic *BRAF*^{V600}-mutation-positive melanoma to vemurafenib and cobimetinib in the combination group vs vemurafenib and placebo in the control group (Table 3).[95] Median PFS was 9.9 months in the combination group compared with 6.2 months in the control group (HR 0.51, $P<0.001$), and 68% of patients in the combination group had an objective response compared with 45% in the control group ($P<0.001$). The rate of complete response was also significantly higher in the combination group compared with the control group (10% vs 4%). Central serous retinopathy, gastrointestinal events, photosensitivity, and elevated aminotransferase and creatinine levels were seen with high frequency in the combination group. More than 50% of such events were grade 1 or 2. Equivalent rates of grade 3 events were noted in both study groups. CK elevation is a known class effect of MEK blockade and was noted be to the most common grade 4 event (4%) in the combination group. It is notable that the majority of events related to CK elevation were grade 1 or 2. Keratoacanthoma and cutaneous squamous cell carcinoma were less common in the combination group. Six deaths were attributed to adverse events in the combination group compared with three deaths in controls.[5]

COMBI-AD enrolled patients (n=870) with stage IIIA/B/C *BRAF*-mutated melanoma after complete surgical resection.[106] Patients were randomized to receive a combination of dabrafenib (150 mg orally twice daily) and trametinib (2 mg orally once daily) or two placebos in a double-blind manner (Table 3). After a median follow-up of 2.8 years, the recurrence rate was 37% in the combination therapy group and 57% in the placebo group. The risk of relapse was 53% lower in the combination therapy group compared with the placebo group ($HR_{relapse}$ 0.47). The mortality rate was 14% in the combination-therapy group and 22% in the placebo group. OS at 3 years was 86% in the combination therapy

group and 77% in the placebo group.[106] The most common adverse reactions in the combination therapy group were fever, fatigue, and nausea, and 26% of patients in this group had severe adverse effects leading to permanent discontinuation of the drugs.[106]

The COLUMBUS trial enrolled patients with stage IIIB, IIIC, or IV unresectable or metastatic *BRAF*$^{V600E/K}$-mutated melanoma who were treatment-naïve or had progressed on or after previous first-line immunotherapy.[107] Patients were randomized 1:1:1 to receive oral encorafenib (450 mg orally once daily) plus binimetinib (45 mg orally twice daily), oral encorafenib alone (300 mg once daily), or oral vemurafenib alone (960 mg orally twice daily; Table 3). Median PFS was 14.9 months in the encorafenib plus binimetinib group and 7.3 months in the vemurafenib group (HR 0.54, 95% CI 0.41–0.71; two-sided $P<0.0001$). The most common grade 3–4 adverse events seen in the encorafenib plus binimetinib group were increased γGT (9%), increased CPK (7%), and hypertension (6%). There were no treatment-related deaths, except for one in the combination group, which was considered by the investigator to be possibly related to treatment.[107]

BRAF inhibitors in management of metastatic melanoma to brain

Up to 60% of patients with metastatic melanoma develop brain metastasis.[108] OS for metastatic brain melanoma (MBM) is 4–6 months.[108] Despite the recent advances in management of metastatic melanoma with either immunotherapy or BRAFi (among those with BRAF^{V600}-mutated melanoma), MBM remains a therapeutic challenge and an area of unmet need. From the 6,000 patients who were enrolled in the pivotal studies that led to the approval of targeted or immunotherapy in metastatic melanoma, none included patients with previously untreated MBM.[87,88,108]

Prior use of cytotoxic chemotherapy or temozolomide, a second-generation oral alkylating agent, showed poor activity against MBM with intracranial response of <10%.[108,109] A fotemustine Phase III randomized study that included 43 patients with MBM showed a brain response rate of only 5.9% compared with no response in the dacarbazine arm.[110] There are a growing number of trials that have shown activity of BRAFi therapy in patients with MBM (Table 4). One involved the use of dabrafenib with stable or tapering doses of steroids in 172 patients with *BRAF*$^{V600E/K}$-mutated MBM. Intracranial response rates were 39.2% in treatment-naïve patients and 30.8% in patients with prior central nervous system radiation or surgery. OS >8 months was observed in both groups.[108,111] An intracranial response rate of 37% with median OS of 5.3 months was detected in a pilot trial involving the use of vemurafenib in 24 patients with unresectable previously treated symptomatic MBM.[112] A larger Phase II trial of vemurafenib showed an intracranial disease control rate and OS of 18% and 8.9 months in previously untreated patients with MBM compared with 20% and 9.6 months in previously treated patients with MBM, respectively.[113]

Treatment with BRAFi achieves a rapid response in the majority of patients with *BRAF*^{V600}-mutated melanoma; however, resistance to BRAFi is almost inevitable, as discussed earlier.[108] In a cohort of patients treated with vemurafenib, it was noted that 59% of those who developed brain metastases

Table 4 Clinical trials of BRAF inhibitors in metastatic melanoma to brain

Trial	Phase	Drug	Patients	ICRR	Median OS
Long et al[111]	II	Dab	Treatment naïve *BRAF*^{V600E} (n=74)	39.2%	33.1 weeks
			BRAF^{V600K} (n=15)	6.7%	16.3 weeks
			Previously treated *BRAF*^{V600E} (n=65)	30.8%	31.4 weeks
			BRAF^{V600K} (n=18)	22.2%	21.9 weeks
Dummer et al[112]	II	Vem	*BRAF*-mutation-positive previously treated unresectable MBM (n=24)	42%	5.3 months
Mcarthur et al[113]	II	Vem	Treatment naïve MBM (n=90)	18%	8.9 months
			Previously treated (n=56)	NR	9.6 months
Davies et al[116]	II	Dab + Tr	*BRAF*-mutant asymptomatic MBM without previous local treatment (n=76)	58%	10.8 months
			BRAF-mutant asymptomatic MBM with previous local therapy (n=16)	56%	24.3 months
			BRAF-mutant symptomatic MBM with or without previous local therapy (n=16)	44%	13 months
			BRAF-mutant symptomatic MBM with or without previous local therapy (n=17)	59%	11.5 months

Abbreviations: Dab, Dabrafenib; ICRR, intracranial response rate; OS, overall survival; Tr, trametinib; Vem, vemurafenib.

while receiving vemurafenib had controlled extracranial disease.[108,114]

To overcome secondary resistance to BRAFi, combinations of BRAFi with other treatment modalities are being studied. Though melanoma is generally considered a radioresistant tumor, small retrospective case series suggest an increased response by a combination of vemurafenib and radiation therapy, which is consistent with preclinical data suggesting that vemurafenib has a radiosensitizing effect.[108,115] Dabrafenib plus trametinib in 125 patients after median follow-up of 8.5 months showed intracranial response in 44 (58%) patients with asymptomatic *BRAF*-positive brain metastases without previous treatment, nine (56%) with asymptomatic *BRAF*-positive MBM with previous treatment, seven (44%) with asymptomatic *BRAF*-positive MBM with or without previous treatment, and 10 (59%) with symptomatic *BRAF*-positive MBM with or without previous treatment.[116]

Conclusion

Approximately 50% of melanomas harbor an activating *BRAF* mutation. Combined BRAF–MEK inhibitor therapy is the standard of care for $BRAF^{V600}$-mutant advanced melanoma. The three FDA-approved combination (BRAFi + MEKi) therapies in melanoma are vemurafenib plus cobimetinib, dabrafenib plus trametinib, and encorafenib plus binimetinib. Developing grade 3 or 4 adverse events to one combination does not preclude the use of other combinations, as each has its own unique adverse events that might be different than the others. Combination therapy is also effective in MBM; however, responses are short lived.

Common genetic alterations that lead to BRAFi resistance include: *NRAS* mutation, *MAP2K1* and *MAP2K2* mutations, *BRAF* amplification, and mutations in the PI3K pathway. Approaches aiming simultaneously to inhibit both the MAPK and PI3K–Akt pathways have been proposed. The role of epigenetic alterations in the emergence of BRAFi resistance is being recognized. Finally, the relationship between BRAFi and the immune system is of great importance.

BRAFi result in increased expression of melanocyte-differentiation antigens, increased recognition by antigen-specific T cells, increased $CD8^+$ T-cell infiltration in the melanoma microenvironment, decreased expression of immunoinhibitory cytokines, decreased myeloid-derived suppressor cells, and increased T-cell-exhaustion markers (eg, TIM3, PD1, PDL1). This proimmunotherapy microenvironment is lost upon melanoma progression on BRAFi, and hence immunocheckpoint blockade may be critical if combined with BRAFi in enhancing antitumor immunity and augmenting therapeutic responses. The optimal treatment sequence remains to be elucidated in melanoma. Targeting elements of resistance in the melanoma microenvironment, such as macrophages, can inhibit melanoma growth and increase the efficacy of BRAFi.

Disclosure

The authors report no conflicts of interest in this work.

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