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Review

Clinical Cancer Research

# Developments in the Space of New MAPK Pathway Inhibitors for BRAF-Mutant Melanoma

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## Abstract

The characterization of the MAPK signaling pathway has led to the development of multiple promising targeted therapy options for a subset of patients with metastatic melanoma. The combination of BRAF and MEK inhibitors represents an FDA-approved standard of care in patients with metastatic and resected BRAF-mutated melanoma. There are currently three FDA-approved BRAF/MEK inhibitor combinations for the treatment of patients with BRAF-mutated melanoma. Although there have been significant advances in the field of targeted therapy, further exploration of new targets within the MAPK pathway will strengthen therapeutic options for patients. Important clinical and translational research focuses on mechanisms of resistance, predictive biomarkers, and challenging patient populations such as those with brain metastases or resected melanoma.

## Introduction

Treatment options for patients with melanoma have expanded dramatically over the past decade. Mutations in MAPK signaling augment cell growth and proliferation in melanoma and other solid tumors (1, 2). Both clinical and translational research focuses on exploration of the MAPK signaling pathway to detect predictors of resistance and response. Simultaneously targeting more than one mediator of the pathway, such as the inhibition of BRAF and MEK, has become the foundation of therapeutic development. There are currently three combinations of BRAF/MEK inhibitors FDA approved for patients with $BRAF^{V600E/K}$-mutated metastatic melanoma and one combination approved in the adjuvant, stage III, setting. Additionally, there are new targets in the MAPK pathway in development.

The clinical benefit of targeted therapies in metastatic melanoma is not durable in the great majority of patients due to several mechanisms of resistance that have been described (3, 4). Clinical trials attempting to overcome resistance are focused on optimal dosing and alternative scheduling of BRAF/MEK inhibition, exploring the safety and efficacy of three and four drug combinatorial regimens, and determining optimal combination or sequencing with immunotherapy and/or other immune modulating therapies. Combined with translational efforts there has been an expansion of therapeutic options for patients with mutations in the MAPK pathway.

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## MAPK Pathway Inhibition in Melanoma

The MAPK pathway is primarily responsible for responses to growth signals within cells. Aberrations of various steps along this pathway occur with increased activity of receptor tyrosine kinases (RTK), RAS, or RAF and result in constitutive activation of MEK and ERK (1, 5). This leads to uncontrolled cellular proliferation seen in melanoma and a number of other malignancies. *BRAF* is mutated in up to 7% of all malignancies and 40% to 50% of melanomas (6, 7). Activation of the BRAF kinase leads to interaction of BRAF and MEK, which subsequently results in phosphorylation of MEK and ERK. Although BRAF inhibitors predictably inhibit MEK/ERK signaling in cells harboring *BRAF* mutations, they paradoxically activate MEK/ERK signaling in cells harboring *RAS* mutations by promoting BRAF-CRAF heterodimers and homodimers. When this occurs, CRAF remains constitutively activated, which leads to MEK/ERK activation (8–10).

The most common *BRAF* mutation, accounting for 70% to 88% of all *BRAF* mutations, is a substitution of glutamic acid for valine at amino acid 600 (V600E; ref. 7, 11). Other mutations in *BRAF* occur less frequently and include V600K, V600R, V600M, non-V600 alterations, and fusions. The three distinct classes of *BRAF* mutations predict response to BRAF inhibitors (Table 1; ref. 12). Class I (V600 mutations) signal as RAS-independent monomers and respond well to first-generation BRAF inhibitors (vemurafenib, dabrafenib, encorafenib) as well as combined BRAF/MEK inhibitor therapy. Class II (non-V600 mutations) function independently of upstream RTK and RAS but signal as activated dimers and are less activating than V600 mutations. These mutations do not respond to first-generation BRAF inhibitors but may respond to paradoxical blocking BRAF inhibitors (e.g., PLX8394), as well as downstream inhibition of MEK or ERK (13). Finally, class III mutations (N581, D594) have no kinase activity, however facilitate RAS binding and CRAF activation (14). As class II and III mutants represent <5% of all BRAF mutations in melanoma, there has been little clinical development of MEK, ERK, and newer BRAF inhibitors, however the effectiveness of these agents in patients with any solid tumor malignancy and one of these mutations is an area of active investigation.

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**Table 1.** Classification of BRAF mutations

| BRAF class | BRAF mutation | Kinase activity | Potential targets |
|---|---|---|---|
| Class I | V600 | RAS independent | BRAFi or BRAFi/MEKi combination |
| Class II | Non-V600 | RAS and RTK independent | MEKi, ERKi, or paradoxical blocking BRAFi |
| Class III | N581, D594 | No kinase activity, CRAF activation | MEKi + RTKi |

Abbreviations: BRAFi, BRAF inhibitor; ERKi, ERK inhibitor; MEKi, MEK inhibitor; RTKi, RTK inhibitor.

The majority of clinical trials to date have focused on patients with $BRAF^{V600E}$ and $BRAF^{V600K}$ mutations and the safety, efficacy, and responses of BRAF inhibitors in combination with MEK inhibitors has been in patients with tumors that harbor these mutations. Interestingly, the V600E and V600K *BRAF* mutations are subtypes with distinct clinical phenotypes, mutational load profiles, and responses to therapy (15). In fact, it has been known for over many years that the ratio of *BRAF* mutations (V600E:V600K) in melanoma patients varies by region. For example, patients from warmer climates with higher UV exposure (e.g., Australia, Houston, Texas) have a higher rate of V600K mutations than patients from cooler climates with lower UV exposure areas. Additionally, V600K-mutant melanoma patients are more likely to involve chronic sun damage areas of the skin than those with V600E mutations. These features likely result from the fact that V600K mutations require two nucleotide substitutions (GTG to AAG) versus the one nucleotide substitution (GTG to GAG) for V600E mutations. Furthermore, the most common substitutions in *BRAF V600K* are C to T transitions, a classic UV signature mutation, and not surprisingly, patients with V600K mutations have a higher mutational load than those with V600E mutations. This likely explains the recent report that patients with *BRAFV600K* mutations have higher response rates to immune checkpoint inhibitor therapy. Finally, *BRAF V600K* mutations tend to be associated with less activation of the ERK pathway, which may explain the lower responses to targeted therapy and higher responses to immunotherapy.

Mutations in *RAS* oncogene subtypes (K-, H-, N) are seen in up to a quarter of patients with melanoma, are typically mutually exclusive of $BRAF^{V600}$ mutations, and are seen in all subtypes of patients of melanoma except uveal. *NRAS* mutations represent the great majority of *RAS* mutations in patients with cutaneous melanoma and are associated with a poor prognosis and more aggressive clinical course than patients without *NRAS* mutations (e.g., *BRAF*-mutant or *BRAF*/*NRAS* wild-type (WT) patients; refs. 16–18). Initial studies suggested that patients with NRAS-mutant, versus non-NRAS-mutant, melanoma may have better outcomes with immunotherapy, however, this has not been corroborated in other datasets. Targeted therapy has also been studied in *NRAS*-mutated melanoma, however, inhibiting BRAF can paradoxically activate MEK-ERK signaling. Therefore, the focus of targeted therapies for patients with *NRAS* mutations has been MEK and, more recently, ERK inhibitors. Importantly, *RAS* mutations and specifically *NRAS* mutations can active alternative signaling pathways, such as the PI3K pathway, which likely limits the effectiveness of single-agent MAPK pathway inhibition. A convergent point of both MAPK and PI3K pathways is cell-cycle regulation. A number of groups have demonstrated synergy of dual MEK plus cyclin-dependent kinase 4/6 (CDK4/6) inhibition, although the clinical efforts to combine these types of agents (described below) has proven tricky, as toxicity has limited the ability to give these inhibitors at doses with a predicted efficacious exposure level.

## BRAF plus MEK Inhibition: Old and New Developments

In 2002, Davies and colleagues described *BRAF* mutations in up to 66% of patients with melanoma (19). This resulted in a surge of research dedicated to the development of BRAF inhibitors for the treatment of patients with *BRAF*-mutated melanoma. Multiple pivotal phase III trials showed improved overall survival (OS), progression-free survival (PFS), and overall response rate (ORR) in patients who received BRAF inhibitor monotherapy versus chemotherapy. In a short period of time, the combination of BRAF and MEK inhibitors were tested and demonstrated to be effective treatments for patients with tumors harboring $BRAF^{V600E/K}$ mutations (20, 21). The initial studies included vemurafenib plus cobimetinib and dabrafenib plus trametinib (20), COMBI-d (dabrafenib plus trametinib vs. dabrafenib plus placebo), COMBI-v (dabrafenib plus trametinib vs. vemurafenib; refs. 22, 23), and coBRIM (vemurafenib plus cobimetinib vs. vemurafenib; refs. 24, 25). These studies consistently demonstrated response rates of approximately 70% and median PFS of 12 months and paved the way for further development of targeted combinations in the field of *BRAF*-mutated melanoma and other malignancies (24, 26). Most recently, the combination of encorafenib plus binimetinib was FDA approved based on the results from the COLUMBUS study (encorafenib plus binimetinib vs. vemurafenib), which showed superior ORR and PFS of the combination.

The differences between the three approved combinations lies in the adverse effects and schedule of dosing (Table 2; ref. 27). The combination of vemurafenib and cobimetinib is given orally, on an empty stomach and a total of 11 pills are taken daily at full doses (four pills of vemurafenib twice daily and three pills of cobimetinib daily); of note, cobimetinib is taken for 3 weeks followed by 1 week off of cobimetinib whereas vemurafenib is given continuously. The most common toxicities in the trials were diarrhea, nausea, vomiting, rash, fatigue, arthralgia, photosensitivity, and increased liver function tests. Dabrafenib and trametinib are also oral and taken on an empty stomach with a total of five pills every day. Compared with monotherapy, the combination caused pyrexia, chills, fatigue, headache, nausea, diarrhea, arthralgia, rash, and hypertension. MEK inhibitor toxicities occurred at a higher frequency with the combination including peripheral edema, decrease in cardiac ejection fraction, and acneiform dermatitis. Finally, the combination of encorafenib and binimetinib requires 12 pills daily, however can be taken with or without food. The most common AEs reported include nausea, vomiting, diarrhea, fatigue, increased creatinine phosphokinase, and headache. Importantly, the most characteristic and troublesome toxicities with vemurafenib/cobimetinib (photosensitivity)

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**Table 2.** Toxicity comparison between three FDA-approved BRAFi + MEKi combinations

| Combination | Most common toxicities | Less common toxicities | Dose schedule |
|---|---|---|---|
| Vemurafenib + cobimetinib | Rash, diarrhea, nausea, arthralgia, fatigue, photosensitivity, pyrexia, vomiting, serous retinopathy, alopecia, and hyperkeratosis. | cuSCC, keratoacanthoma, and Bowen disease. | Orally, with or without food. Vemurafenib is twice daily every day. Cobimetinib is once daily on days 1 to 21. |
| Dabrafenib + trametinib | Pyrexia, nausea, diarrhea, chills, fatigue, headache, and vomiting. | Rash, palmer-plantar erythrodysesthesia, photosensitivity, skin papillomas, cuSCC, keratoacanthomas, and hyperkeratosis. | Orally, on an empty stomach. Dabrafenib is twice daily. Trametinib is once daily. |
| Encorafenib + binimetinib | Diarrhea, constipation, vomiting, abdominal pain, asymptomatic CPK increase, and blurred vision. | Pruritis, hyperkeratosis, rash, keratosis pilaris, palmoplantar keratoderma, palmer-plantar erythrodysesthesia, dry skin, skin papilloma, maculopapular rash, sunburn, alopecia, photosensitivity, arthralgia, myalgia, extremity pain, decreased appetite, musculoskeletal pain, and decreased weight. | Orally, with or without food. Encorafenib is once daily, and binimetinib is twice daily. |

NOTE: Vemurafenib and combimetinib typically cause more skin toxicities. Dabrafenib and trametinib have more fevers. Encorafenib and binimetinib have more gastrointestinal toxicities.

Abbreviations: BRAFi, BRAF inhibitor; CPK, creatine phosphokinase; cuSCC, cutaneous squamous cell carcinoma; MEKi, MEK inhibitor.

and dabrafenib/trametinib (febrile syndrome) were not regularly seen in clinical trials. The full spectrum of toxicity of encorafenib and binimetinib remains to be seen, given its recent FDA approval and commercial availability.

Finally, in the adjuvant setting, the results of COMBI-AD lead to the approval of dabrafenib and trametinib for patients with resected *BRAF*-mutated melanoma (28). In this double blind, placebo-controlled, phase III trial, patients with resected stage III melanoma with $BRAF^{V600E/K}$ mutations were assigned to received dabrafenib and trametinib versus matched placebos. The 3-year rate of relapse-free survival and OS in the combination group was superior compared with the placebo group. Furthermore, these patients had an improved rate of distant metastasis-free survival and freedom from relapse. The combination of dabrafenib and trametinib, however, in the adjuvant setting appears to have significant toxicity with 97% of patients reporting at least one adverse effect. Twenty-six percent of patients in the targeted therapy arm required discontinuation of the drugs whereas 38% required dose reduction and 66% required dose interruption. Despite the toxicities in the adjuvant setting, data show that quality of life is not negatively impacted (29).

### Other MAPK targets and new combinations

Studies targeting *NRAS* mutations in patients with metastatic melanoma have had limited success and there are currently no RAS inhibitor therapies approved. Binimetinib compared with chemotherapy in patients with *NRAS*-mutated melanoma (part of the NEMO study) showed a favorable response rate and PFS, however there was no difference in overall survival observed (30). Building upon this single-agent data and based on the previously discussed preclinical data showing that CDK4/6 inhibition combined with MEK inhibition was more efficacious, two clinical trials were launched with an aim to define the clinical efficacy of dual inhibitor therapy in patients with *NRAS*-mutated melanoma. One of these studies (NCT01781572) combined binimetinib with the CDK4/6 inhibitor ribociclib, which is FDA approved for the treatment of breast cancer. Response rates were slightly better than seen with single-agent MEK inhibitors (ranging from 25% to 40%), however toxicity was the limiting factor preventing more rapid clinical development of this approach. In a second study (NCT02065063), the combination of the MEK inhibitor trametinib and the CDK4/6 inhibitor palbociclib was studied in patients with solid tumor malignancies, with a focus on treated patients with aberrancies of the MAPK pathway (e.g., mutant or amplified *KRAS, NRAS, BRAF*) and/or cell cycle (e.g., *CDK4* amplification, cyclin D amplification or mutation, or loss of *CDKN2A*). Unfortunately, this combination was toxic, with maximum tolerated doses of the combination less than that of the individual agents. The combination was not particularly active (responses seen in <10% of patients), likely due to inadequate exposure levels and/or due to an enrollment strategy that resulted in a paucity of patients with *NRAS*-mutant melanoma treated.

Pan-RAF and ERK inhibitors represent additional therapeutic opportunities in the MAPK pathway. The mechanism of pan-RAF inhibitors suggests that they would not have the same paradoxical activation of MAPK as more specific RAF inhibitors. Sorafenib is a multikinase inhibitor, blocking CRAF, BRAF (WT and mutant), VEGFR1/2, FLT1, PDGFR, and KIT. Multiple trials with sorafenib monotherapy failed to show efficacy, regardless of the presence of a BRAF mutation (31, 32). Another pan-RAF inhibitor, RAF-265, had a disappointing response rate and significant toxicities (33, 34). Results from ongoing phase I clinical trials with TAK580, BGB-283, and PLX8394 will provide insight into potential efficacy in patients with *BRAF* or *NRAS* mutations. ERK activation inhibits RAF, creating an ideal negative feedback mechanism to target. A number of ERK inhibitors are currently in development (CDC-0994, ulixertinib, SCH772984, MK-8353; refs. 35–38). There may be opportunities to combine these with BRAF/MEK inhibitors or as monotherapy in patients with BRAF mutations.

Perhaps most promising is the combination of immunotherapy with BRAF/MEK inhibitors for patients with BRAF mutations. The theoretical concept is to combine the rapid response with BRAF/MEK inhibitors and the durability of response with immunotherapy. However, there is also a rationale beyond the ethereal to justify such a combination. Specifically, serial biopsy studies in patients with BRAF-mutant melanoma treated with BRAF-targeted therapy (performed at baseline and early on therapy) show that BRAF-targeted therapy is associated with increased tumor antigen expression, upregulation of antigen presentation machinery, and enhanced $CD8^+$ T-cell tumor infiltration (39–43) Also, BRAF and MEK inhibitors are associated, *in vitro*, leading to increased melanoma differentiation antigen expression and

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reactivity to antigen-specific T lymphocytes without causing significant immunosuppression (40). Targeting BRAF and MEK leads to a decrease in immunosuppressive proteins such as IL6 and IL8 and an increase in PD-1, PD1-L1, and TIM-3 (41) and an inhibition of tumor-associated fibroblasts, which results in inhibition of IL-1a and IL-1B transcription (44). Also, CCL2 is decreased in the setting of BRAF inhibition, which may result in decreased $CCR2^{+}$ tumor-infiltrating lymphocytes (TIL). Finally, a number of preclinical murine models of melanoma have demonstrated synergy of BRAF-targeted therapy with immune checkpoint therapy (45–47). Together, these data support the synergistic effect of combining targeted therapy with immune checkpoint inhibitors, adoptive cell therapy, or anticancer cytokine therapies such as IL2 or IFN-α2b. The early data from phases I and II trials of BRAF plus MEK plus anti-PD-1/PD-L1 therapy demonstrate high response rates, but not necessarily higher than that of dual BRAF/MEK inhibitor therapy. Randomized phase III trials (NCT02130466/NCT03149029, NCT02902029/NCT02908672/NCT01656642, NCT02967692) will determine if the preliminary efficacy of these combinations is superior to standard therapy and whether this approach leads to more durable responses (48–51).

### Resistance mechanisms

Despite the significant advances in developmental therapeutics focused on MAPK pathway inhibition, resistance, both acquired and intrinsic, remains a major obstruction to the durable success of these therapies. Despite the initial rapid response rates with BRAF/MEK inhibitor combinations in BRAF-mutated malignancy, acquired resistance typically develops within the first two years of therapy (52). Intrinsic resistance is unresponsiveness to therapy from the outset. This phenomenon is rare, occurring in <10% of BRAF-mutated melanomas and may be linked with PTEN and mitogen-activated protein kinase kinase 1 (MAP2K1; ref. 53). Acquired resistance is more common has been extensively described and occurs through various mechanisms (54–60). Patients who fail to respond to BRAF monotherapy also fail to respond to MEK inhibitors, suggesting cross-resistant and heterogenous mechanisms (54, 61–63). In fact, the genetic analyses of samples from patients, pretreatment and progressing on BRAF inhibitor therapy, demonstrate separate resistance mechanisms within tumors and between tumors in the same patient (64). Similar data confirmed these findings in 100 patients with disease progression on BRAF inhibitor therapy (65). Identified mutations included *NRAS, KRAS, BRAF* splice variants, $BRAF^{V600E/K}$ amplifications, *MAP2K1* and *MAP2K2*, and non-MAPK pathway alterations. Resistance mechanisms did not correlate with clinical outcome. Patients in whom MAPK signaling is restored may have improved outcomes, suggesting activity of BRAF inhibitors beyond progression (64, 66).

The majority of the time, however, resistance occurs through reactivation of the MAPK pathway (3, 65, 67). Growth factors are upregulated, leading to pathway reactivation through SRC-family kinases signaling. Alternatively, activation of alternate oncogenic signaling pathways, such as NRAS, which signals through CRAF, can also lead to resistance. In fact, activation of CRAF may lead to hyperactivation of MEK and ERK (8–10). Similarly, alternative splicing of BRAF may also contribute to driving resistance. Nevertheless, a proportion of BRAF inhibitor-resistant melanomas do not display MAPK reactivation, typically through PI3K/AKT pathway activation through RTK activity or genetic changes, such as tumor suppressor gene functional loss (e.g., PTEN) or mutation or activation of pathway mediators (e.g., AKT3; ref. 60).

Given the multiple pathways that mediate resistance, results of second-line trials in BRAF/MEK-resistant patients will provide insight into future directions in this field. One potential solution is to target the PI3K pathway, including targeting PI3K and mTOR. Another possible approach includes intermittent dosing of BRAF/MEK inhibitors, which has shown benefit in a mouse model (68, 69). Pan-RAF inhibitors, which also inhibit SFKs, have been studied and show promise in preclinical models (70). Finally, ERK inhibition is associated with responses in 15% to 20% of patients with BRAFV600E/K-mutant melanoma previously treated with and progressed on BRAF-targeted therapy (37, 38). Of note, 25% to 40% of patients have unidentified mechanisms of resistance, again emphasizing, the complexity of resistance to targeted therapies (64).

A variety of mutations appear to arise within the context of acquired resistance, and reignite MAPK signaling (or parallel signaling networks) despite the presence of BRAF inhibition, and include mutations in NRAS, PI3K/AKT pathway members, and amplification and alternative splicing of BRAF (57–59). Unraveling intrinsic resistance has been a greater challenge, although preexisting mutations in PTEN and MAP2K1 appear to correlate with shorter responses (60, 61). These mutations, however, do not preclude therapeutic responses.

## Unmet Needs

### Brain metastases

Approximately 43% of patients with metastatic melanoma have clinically or radiologically detected brain metastases and up to 75% have brain metastases detected on autopsy (71). The majority of clinical trials with targeted therapies for the treatment of patients with metastatic melanoma excluded patients with brain metastases or retrospectively studied this cohort, however a number of prospective studies have more recently been performed. There is evidence that molecularly targeted therapies can effectively penetrate the blood–brain barrier (BBB) and lead to improved intracranial responses in this patient population (72–74). Vemurafenib monotherapy showed intracranial responses in 16% of patients with symptomatic brain metastases who had prior central nervous system (CNS)-directed therapy (75). In the BREAK-MB trial, dabrafenib monotherapy showed intracranial clinical activity in 39% of patients without previous local therapy and 31% in patients who had previous CNS-directed therapy (76). COMBI-MB was the first trial dedicated to assessing response to combination BRAF/MEK inhibitors in patients with BRAF-mutated brain metastases (77). In this study, patients receiving dabrafenib and trametinib were enrolled in one of four cohorts depending on their type of BRAF mutation, previous treatments, and symptoms. In BRAFV600E-mutated patients with asymptomatic, untreated brain metastases, 58% [95% confidence interval (CI), 46–69] achieved intracranial response. Fifty-six percent (95% CI, 30–80) of patients with $BRAF^{V600E}$, asymptomatic yet previously treated metastases had intracranial responses. Patients with non-BRAFV600E (D/K/R) with or without prior therapy were also included. This cohort had a 44% (95% CI, 20–70) intracranial response rate. Finally, 59% (95% CI, 33–82) of patients with symptomatic metastases with or

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without prior treatment and any BRAF mutation had intracranial responses. Although the median duration of response in all patients was relatively short, the results of this study definitively demonstrate clinical benefit in patients with BRAF-mutated brain metastases.

The reason for differential efficacy in intracranial and extracranial metastases is not specifically known, but there are a few possibilities that may provide a rationale for a new wave of trials for patients with brain metastasis. In melanoma specifically, brain metastases may have significantly higher activation-specific protein markers in the PI3K/AKT pathway compared with matched extracranial metastases (78, 79). Subsequently, whole-exome sequencing in 86 matched brain metastases, primary tumors, and normal tissue (not melanoma specific) showed genetic alterations in brain metastases in 53% of cases, which were not detected in the matched primary tumor (80). Confirming earlier findings, distal extracranial and regional lymph node metastases were highly divergent from brain metastases harboring alterations in PI3K/AKT/mTOR, CKD, HER2/EGFR. These results argue for an individualized and genomically targeted treatment approach for patients with brain metastases. Specifically, there is a focus on improving intracranial responses to therapies targeting the MAPK pathway by increasing the BBB penetration further with intermittent scheduling of targeted therapy, pulsed high-dose therapy, and combination of therapies (targeted, immune checkpoint inhibitors, surgery, or radiotherapy; refs. 73, 80, 81). There are also ongoing trials addressing other targets in the MAPK pathway. For example, Brastianos and colleagues currently have a clinical trial in patients whose brain metastases harbor CDKN2A mutations (NCT02896335). Additional genomically guided trials for patients with brain metastases are in the pipeline.

**Adjuvant**

Treatment of adjuvant melanoma in patients with MAPK aberrations also remains an area requiring improvement. The FDA approval of adjuvant dabrafenib and trametinib was a major breakthrough; however, many centers continue to give adjuvant immunotherapy despite the data showing superior recurrence free survival and distant metastasis-free survival. In the absence of randomized data, clinical bias favors treating patients with immune checkpoint inhibitors for several reasons. The adverse effects of adjuvant targeted therapy results in dose reductions, dose interruptions, and early discontinuation, not to mention a major decrease in the quality of life in patients receiving this therapy (27). Immunotherapy, conversely, appeals to patients, offering them an advertised "durable" benefit. Finally, there is a concern about the high-likelihood of recurrence in setting of BRAF/MEK discontinuation, which is valid in the metastatic setting and less likely to occur in the adjuvant setting. Ultimately, targeted therapy and immune checkpoint inhibitor sequencing needs to be reexamined now that both BRAF/MEK inhibitors and anti-PD-1 are available for this patient population.

**Biomarkers**

An important unmet need in the field is the development of tissue and blood-based biomarkers that will (i) improve frontline treatment selection, (ii) facilitate serial monitoring for determination of response/progression in stage IV and no evidence of disease/disease recurrence in stage I to III, (iii) determine mechanisms of resistance, (iv) aide in the detection of minimal residual disease (MRD). Circulating tumor DNA (ctDNA) is one such biomarker, which may provide clues as to who will benefit from adjuvant targeted therapy. In the AVAST-M adjuvant trial of bevacizumab versus placebo, droplet digital PCR (ddPCR) detected BRAF and NRAS mutations in the baseline plasma of 161 patients with high-risk, pretreated, stage II and stage III patients with melanoma (82). ctDNA ($\geq$1 copy of mutant ctDNA) was detected in 11% of BRAF-mutant patient samples. Patients with detectable ctDNA had decreased disease-free interval and distant metastasis-free intervals versus those patients with undetectable ctDNA. Additionally, the 5-year OS rate for patients with detectable ctDNA (BRAF and NRAS) was 33% (95% CI, 14–55%) versus 65% (95% CI, 56–72%) for those with undetectable ctDNA. The study clearly demonstrates that ctDNA can predict for relapse and survival in high-risk resected melanoma and it will be critical to determine if patients with detection at baseline are those most likely to benefit from adjuvant BRAF-targeted therapy (83–85).

In metastatic patients, residual ctDNA after starting treatment predicts earlier progression of disease and conversion of positive to negative ctDNA indicates a favorable response to treatment (86). Additionally, immune and cell-cycle gene signatures may predict outcomes in patients with *BRAF*$^{V600}$-mutated melanoma (87–89). Recently, an exploratory analysis compared genomic features of baseline tumors in patients who had a complete response versus those who had rapid progression on treatment with BRAF $\pm$ MEK inhibitors (89). Specifically, MITF and TP53 alterations were expressed more frequently in patients with rapid progression whereas NF1 alterations were expressed more frequently in patients with complete responses. RNA profiling showed of the same population focused on immune response-related genes. Results from the analysis showed tumors with an immune profile including signatures of CD8$^+$ effector T cells, cytolytic T cells, antigen-presenting cells, and natural killer cells were associated with a complete response to therapy whereas those with keratin signature (keratin and kallikrein gene expression) were associated with rapid progression of disease. In another analysis, patients on the COMBI-v study receiving dabrafenib and trametinib, PD-L1 and CD8$^+$ expression were analyzed and results showed patients had clinical benefit regardless of immune phenotype (90). Also, Eskiocak and colleagues (91) identified SOX10 addiction as a clue to predicting sensitivity to BRAF/MEK inhibition. In an exploratory analysis, Corcoran and colleagues (92) showed that suppression of TORC1 activity in patients receiving BRAF/MEK inhibition predicts induction of cell death. Therefore, in resistant BRAF-mutated melanomas, TORC1 activity is maintained after treatment with BRAF/MEK inhibitors. Additionally, paired biopsies in patients pre- and on-treatment with BRAF/MEK inhibition showed P-S6 (measuring TORC1 activity) suppression predicted improved PFS. As noted previously, Pires da Silva and colleagues (15) explored predictors of response in patients with BRAF$^{V600E}$ versus BRAF$^{V600K}$, noting that higher mutation burden (TMB) in patients with BRAF$^{V600K}$. Therefore, TMB may be a marker of low response rates to targeted therapy and may justify treatment upfront with immune checkpoint inhibitors. The impact of other secondary mutations as biomarkers, such as PTEN/AKT or CDKN2A, has not yet been explored fully.

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## Conclusions

Despite major advances in the treatment of patients with melanoma who harbor mutations in the MAPK signaling pathway, there are still many unanswered questions. Efforts focus on the remaining critical questions including overcoming mechanisms of resistance, new combinations that would allow for higher and/or intermittent dosing, effectiveness in patients with brain metastases, and predictive biomarkers. Ultimately, a combination of clinical trials and aggressive translational and correlative research will move the field of targeted therapeutics forward.

## Disclosure of Potential Conflicts of Interest

J.V. Cohen is a consultant/advisory board member for Sanofi-Genzyme. R.J. Sullivan is a consultant/advisory board member for Array, Merck, Novartis, Amgen, Replimmune, Compugen, and Genentech. No other potential conflicts of interest were disclosed.

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