

BRAF Genetic Testing to Select Melanoma or Glioma Patients for Targeted Therapy

Effective: August 1, 2018

Next Review: August 2019

Last Review: July 2018

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

BRAF and MEK inhibitors are drugs that were originally designed to target a variant in the BRAF gene found in some advanced melanoma tumors. This BRAF-variant kinase is believed to be actively involved in oncogenic proliferation, and specific inhibition of the kinase has been shown to slow tumor growth and may improve patient survival.

MEDICAL POLICY CRITERIA

- I. Testing for *BRAF* V600 variants in tumor tissue to select melanoma patients for treatment with Food and Drug Administration (FDA)-approved BRAF or MEK inhibitors may be considered **medically necessary** for any of the following:
 - A. Metastatic (stage IV) melanoma, or
 - B. Unresectable melanoma, or
 - C. Resected stage III melanoma
- II. Testing for *BRAF* V600 variants for all other patients with melanoma is considered **investigational**.
- III. Testing for *BRAF* V600 variants in patients with glioma is considered **investigational**.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

It is critical that the list of information below is submitted for review to determine if the policy criteria are met. If any of these items are not submitted, it could impact our review and decision outcome.

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variants being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing?
6. Medical records related to this genetic test
 - o History and physical exam
 - o Conventional testing and outcomes
 - o Conservative treatment provided, if any

CROSS REFERENCES

1. [Genetic Testing for Inherited Susceptibility to Colon Cancer](#), Genetic Testing, Policy No. 06
2. [KRAS, NRAS, and BRAF Variant Analysis and MicroRNA Expression Testing for Colorectal Cancer](#), Genetic Testing, Policy No. 13
3. [Genetic and Molecular Diagnostic Testing](#), Genetic Testing, Policy No. 20
4. [Molecular Analysis for Targeted Therapy of Non-Small Cell Lung Cancer \(NSCLC\)](#), Genetic Testing, Policy No. 56

BACKGROUND

MELANOMA

Overall incidence rates for melanoma have been increasing for at least 30 years; in 2015, more than 70,000 new cases will be diagnosed.^[1] In advanced (Stage 4) melanoma, the disease has spread beyond the original area of skin and nearby lymph nodes. Although only a small proportion of cases are Stage 4 at diagnosis, prognosis is poor, with a five-year survival of only 15-20%. For several decades since its approval in 1975, cytotoxic chemotherapy with dacarbazine was considered the standard systemic therapy but has low response rates of only 15 to 25% and median response durations of five to six months. Less than 5% of responses are complete.^[2] Temozolomide has similar efficacy with a greater ability to penetrate the central nervous system. Recently immunotherapy with ipilimumab or with checkpoint inhibitors such as pembrolizumab and nivolumab has demonstrated superior efficacy to chemotherapy^[3-7] regardless of *BRAF* status and is now recommended as one potential first-line treatment of metastatic or unresectable melanoma by the National Comprehensive Cancer Network (NCCN).^[8]

Variants in the *BRAF* kinase gene are common in tumors of patients with advanced melanoma and result in constitutive activation of a key signaling pathway that is associated with oncogenic proliferation. In general, 50 to 70% of melanoma tumors harbor a *BRAF* variant and of these, 80% are positive for *BRAF* V600E and 16% are positive for *BRAF* V600K.^[9] Thus, approximately 45-60% of advanced melanoma patients might respond to a BRAF inhibitor targeted to this variant kinase.

Two BRAF inhibitors and two mitogen-activated extracellular signal-regulated kinase (MEK) inhibitors have been developed for use in patients with advanced melanoma. Vemurafenib (trade name Zelboraf®, also known as PLX4032 and RO5185426) was co-developed under an agreement between Roche (Genentech) and Plexxikon. Vemurafenib was developed using a fragment-based, structure-guided approach that allowed the synthesis of a compound with high potency to inhibit the BRAF V600E variant kinase and significantly lower potency to inhibit most of many other kinases tested.^[10] Preclinical studies demonstrated that vemurafenib selectively blocked the RAF/MEK/ERK pathway in *BRAF*-variant cells^[11-13] and caused regression of *BRAF*-variant human melanoma xenografts in murine models.^[10] Paradoxically, preclinical studies also showed that melanoma tumors with the *BRAF* wild-type gene sequence could respond to variant BRAF-specific inhibitors with accelerated growth,^[11-13] suggesting that it might be harmful to administer BRAF inhibitors to patients with BRAF wild-type melanoma tumors. Potentiated growth in BRAF wild-type tumors has not yet been confirmed in melanoma patients as the supportive clinical trials were enrichment trials, enrolling only those patients with tumors positive for the *BRAF* V600E variant.

Dabrafenib (trade name Tafinlar®, also known as GSK2118436 or SB-590885) is a BRAF inhibitor developed by GlaxoSmithKline, now Novartis.^[14,15] Dabrafenib inhibits several kinases, including variant forms of BRAF, with greatest activity against the V600E BRAF variant. In vitro and in vivo studies demonstrated dabrafenib's ability to inhibit growth of *BRAF* V600 variant-positive melanoma cells.^[16]

Trametinib (trade name Mekinist™) is an inhibitor of MEK1 and MEK2 developed by GlaxoSmithKline. MEK kinases regulate extracellular signal-related kinase (ERK), which promotes cellular proliferation. *BRAF* V600E and V600K variants result in constitutive activation of MEK1 and MEK2.^[17] Trametinib inhibits growth of BRAF V600 variant-positive melanoma cells in vitro and in vivo.^[18]

Cobimetinib, formally GDC-0973/XL518 (trade name Cotellic®) was developed by Genentech^[19] and Exelixis^[20]. It is a MEK inhibitor indicated for the treatment of patients with unresectable or metastatic melanoma with a *BRAF* V600E or V600K variant, in combination with vemurafenib. Cobimetinib is not indicated for treatment of patients with wild-type *BRAF* melanoma.

Nivolumab (OPDIVO®), developed by Bristol-Myers Squibb, is not a BRAF or MEK inhibitor, but instead inhibits the PD-1 protein on cells.^[21] PD-1 blocks the body's immune system from attacking melanoma tumors. Nivolumab is intended for patients who have been previously treated with ipilimumab and, for melanoma patients whose tumors express a *BRAF* V600 variant, for use after treatment with ipilimumab and a BRAF inhibitor.

GLIOMA

More than 79,000 new cases of primary malignant and nonmalignant brain and other central nervous system tumors are expected to be diagnosed in the United States in 2017, the majority of which are gliomas.^[22] Gliomas encompass a heterogeneous group of tumors and classification of gliomas has changed over time. In 2016, World Health Organization (WHO) published an update of its classification of gliomas based on both histopathologic appearance and molecular parameters.^[23] The classification ranges from grade I to IV corresponding to the degree of malignancy (aggressiveness) with WHO grade I being least aggressive and grade IV being most aggressive.

Low-grade gliomas were historically those classified as WHO grade I or II and include pilocytic astrocytoma, diffuse astrocytoma, and oligodendroglioma. Surgical resection of the tumor is generally performed, along with additional radiation and chemotherapy following surgery except in the case of pilocytic astrocytoma. The optimal timing of additional therapies is unclear. Many patients will recur following initial treatment with a clinical course similar to high-grade glioma. High-grade gliomas (WHO grade III/IV) include anaplastic gliomas and glioblastoma. Maximal surgical resection is the initial treatment followed by combined adjuvant chemoradiotherapy. Temozolomide, an oral alkylating agent, is considered standard systemic chemotherapy for malignant gliomas. The prognosis for patients with high-grade gliomas is poor: the one-year survival in U.S. patients with anaplastic astrocytoma is about 63% and with glioblastoma is about 38%.^[24]

There is a high frequency of *BRAF* V600E variants in several types of gliomas. For example, *BRAF* V600E variants have been found in approximately 5% to 10% of pediatric diffusely infiltrating gliomas, 10% to 15% of pilocytic astrocytoma, 20% of ganglioglioma, and more than 50% of pleomorphic xanthoastrocytoma.^[25-30] However, it may be rare in adult glioblastoma.^[31] There is considerable interest in targeted therapies that inhibit the MAPK pathway, particularly in patients with high-grade glioma and low-grade gliomas whose tumors are in locations that prevent full resection. Evidence from early phase trials in patients with *BRAF* variant-positive melanoma with brain metastases suggest some efficacy for brain tumor response with vemurafenib and dabrafenib,^[32,33] indicating that these agents might be potential therapies for primary brain tumors.

REGULATORY STATUS

The FDA Centers for Devices and Radiological Health (CDRH), for Biologics Evaluation and Research (CBER), and for Drug Evaluation and Research (CDER) developed a draft guidance on in vitro companion diagnostic devices, released on July 14, 2011,^[34] to address the “emergence of new technologies that can distinguish subsets of populations that respond differently to treatment.” As stated, the FDA encourages the development of treatments that depend on the use of companion diagnostic devices “when an appropriate scientific rationale supports such an approach.” In such cases, the FDA intends to review the safety and effectiveness of the companion diagnostic test as used with the therapeutic treatment that depends on its use. The rationale for co-review and approval is the desire to avoid exposing patients to preventable treatment risk.

- **Vemurafenib**

Vemurafenib and a Class III companion diagnostic test, the cobas® 4800 *BRAF* V600 Mutation Test, were co-approved by the FDA in August 2011.^[35] The test is approved as an aid in selecting melanoma patients whose tumors carry the *BRAF* V600 variant for treatment with vemurafenib.^[36] Vemurafenib is indicated for the treatment of patients with unresectable or metastatic melanoma with a *BRAF* V600 variant. The vemurafenib full prescribing information states that confirmation of a *BRAF* V600 variant using an FDA-approved test is required for selection of patients appropriate for therapy.^[37]

- **Dabrafenib**

Dabrafenib was originally FDA-approved in May 2013 for the treatment of patients with unresectable or metastatic melanoma with *BRAF* V600E variant, as detected by an FDA-approved test.^[16] A 2018 updated approval indicates that it may be used in combination

with trametinib for adjuvant treatment of patients with resected stage III melanoma with *BRAF* V600E or V600K variants. Dabrafenib is specifically not indicated for the treatment of patients with wild-type *BRAF* melanoma.

- **Trametinib**

Trametinib was originally FDA-approved in May 2013 for the treatment of patients with unresectable or metastatic melanoma with *BRAF* V600E or V600K variants, as detected by an FDA-approved test.^[18] A 2018 update indicates that it may be used in combination with dabrafenib for adjuvant treatment of patients with resected stage III melanoma with *BRAF* V600E or V600K variants. Trametinib is specifically not indicated for the treatment of patients previously treated with *BRAF* inhibitor therapy.^[18]

- **Nivolumab**

Nivolumab was originally FDA-approved December 2014 for the treatment of unresectable or metastatic melanoma.^[38] Nivolumab is intended for patients who have been previously treated with ipilimumab and, for melanoma patients whose tumors express an activating *BRAF* V600 variant, for use after treatment with ipilimumab and a *BRAF* inhibitor. Nivolumab may also be used in combination with ipilimumab in patients without a *BRAF* V600 variant.

- **Cobimetinib**

Cobimetinib was FDA-approved November 2015 for the treatment of unresectable or metastatic melanoma with a *BRAF* V600E or V600K variant, in combination with vemurafenib, as detected by an FDA-approved test. Cobimetinib is not indicated for treatment of patients with wild-type *BRAF* melanoma.^[39]

The companion diagnostic test co-approved for both dabrafenib and trametinib is the THxID™ *BRAF* Kit manufactured by bioMérieux. The kit is intended “as an aid in selecting melanoma patients whose tumors carry the *BRAF* V600E mutation for treatment with dabrafenib and as an aid in selecting melanoma patients whose tumors carry the *BRAF* V600E or V600K mutation for treatment with trametinib.”^[36]

In 2014, the FDA granted accelerated approval of trametinib and dabrafenib as a combination therapy for the treatment of patients with unresectable or metastatic melanoma with *BRAF* V600E or V600K variants, as detected by an FDA-approved test.^[40] Approval of the combination therapy was based on the demonstration of durable objective responses in a multicenter, open-label, randomized (1:1:1), active-controlled, dose-ranging trial enrolling 162 patients with histologically confirmed Stage IIIC or IV melanoma determined to be *BRAF* V600E or V600K. No more than one prior chemotherapy regimen and/or interleukin-2 were permitted. Patients with prior exposure to *BRAF* inhibitors or MEK inhibitors were ineligible.

In November 2015, cobimetinib was approved by the U.S. Food and Drug Administration (FDA) for the treatment of patients with unresectable or metastatic melanoma with *BRAF* V600E or V600K variant, in combination with vemurafenib. Additionally, in 2011, ipilimumab (Yervoy®) was approved by the FDA for the treatment of patients with unresectable or metastatic melanoma.^[41] For the first time, a survival advantage was demonstrated in previously treated patients: median survival on ipilimumab of 10 months versus 6.4 months on control medication. However, side effects of ipilimumab can include severe and fatal immune-

mediated adverse reactions, especially in patients who are already immune-compromised. Ipilimumab's clinical study did not test metastatic melanoma patients' tumors for BRAF status; therefore, it's not know what, if any, clinical relevance BRAF status has with respect to ipilimumab.

NOTE: Currently only vemurafenib, dabrafenib, cobimetinib and trametinib are FDA-approved specifically for the treatment of advanced *BRAF*-variant melanoma. There are no FDA-approved targeted therapies for *BRAF* V600 variant-positive glioma.

EVIDENCE SUMMARY

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, which refers to how the results of the diagnostic test will be used to change management of the patient, and whether these changes in management lead to clinically important improvements in health outcomes.

This policy was originally created in 2011 based on a Special Report by the BlueCross BlueShield Association Technology Evaluation Center (TEC).^[42]

UNRESECTABLE OR METASTATIC MELANOMA

The purpose of testing for BRAF pathogenic variants in individuals with unresectable or metastatic melanoma is to inform a decision whether to treat with BRAF and/or MEK inhibitors versus other standard treatments for metastatic melanoma. At the time of the early trials of targeted therapy for metastatic melanoma, cytotoxic chemotherapy (e.g., dacarbazine, temozolomide) was widely used to treat metastatic melanoma although it was never demonstrated to improve survival. However, chemotherapy is now generally used only in second- or third-line settings or not at all. Current standard treatment for patients with metastatic melanoma includes immunotherapy, which is effective in patients with and without BRAF V600 variants. Patients whose tumors contain a BRAF V600 pathogenic variant may receive a BRAF inhibitor and/or a MEK inhibitor instead of or following immunotherapy. There are no randomized controlled trials (RCTs) directly comparing BRAF and MEK inhibitors with immunotherapy and no prospective data on optimal sequencing of BRAF and MEK inhibitors and immunotherapy for patients with a BRAF V600 pathogenic variant.

Analytic Validity

The analytic validity of a genetic test is its ability to accurately and reliably measure the genotype (or analyte) of interest in the clinical laboratory, and in specimens representative of the population of interest.^[43] Submission to the Office of In Vitro Diagnostics of the FDA for marketing clearance or approval of a diagnostic test requires an extensive demonstration of the analytic validity of the test. Data for cleared or approved tests are summarized in the kit insert (prepared by the manufacturer) and in the Summary of Safety and Effectiveness of the test (prepared by the FDA and publicly available).

Cobas® 4800 BRAF V600 Mutation Test

The cobas® 4800 BRAF V600 Mutation Test is a real-time polymerase chain reaction (PCR) test intended for the qualitative detection of the BRAF V600E variant specifically in DNA that has been extracted from formalin-fixed, paraffin-embedded (FFPE) human melanoma tissue. Correlation of cobas 4800 BRAF V600 Mutation Test results to Sanger sequencing was tested in the phase III trial of vemurafenib^[44] on 596 consecutive patients, 449 of which were evaluable. The percent agreement of the BRAF V600 Mutation Test with Sanger sequencing is shown in the first line of Table 1 when only V600E results were counted as positive. The cobas 4800 BRAF V600 Mutation Test detected 27 V600 variants (primarily V600K) that were not V600E by Sanger sequencing. Limited evidence suggests that patients with V600K variant tumors may also respond to vemurafenib.

Tumor specimens from the patients enrolled in the phase II trial^[45] were also sequenced by Sanger sequencing; specimens that were invalid by Sanger, or that were identified as V600K variant or as V600 wild type by Sanger, were re-sequenced by the more sensitive 454 pyrosequencing method to resolve differences. Correlation to 454 pyrosequencing was 100% if V600K-positive samples were counted as true positives (see Table 1).

Tumor specimens from 55 patients enrolled in a phase I clinical trial of vemurafenib were subjected to cobas 4800 BRAF V600 Mutation Test and to Sanger sequencing. The limit of detection was 5% variant allele for cobas 4800 BRAF V600 Mutation Test and 20% for Sanger sequencing. The cobas 4800 BRAF Mutation Test is highly predictive for V600E; however, it also detects other BRAFV600 variants (V600K 65.8% agreement with Sanger sequencing, V600D, V600E2, and V600R not determined) with less sensitivity. Data presented on study 3 is in Table 1.^[46]

Halait (2012) analyzed the analytical performance of cobas 4800 BRAF V600 Mutation Test and Sanger sequencing in 219 melanoma specimens.^[47] A greater than 96% correct call rate was obtained across all specimen types with 5% variant sequences. The cobas 4800 BRAF V600 Mutation Test and Sanger sequencing correlation results for V600E are presented in Table 1. After discrepant analysis with 454 sequencing, the positive percent agreement increased to 100%, the negative percent agreement increased to 93%, and the overall percent agreement increased to 96%.

A similar study by Anderson (2012) used screening specimens from phase II and phase III trials of vemurafenib.^[48] Of 477 available specimens, 433 had both a valid cobas result and valid Sanger sequencing. Correlation results were similar to those obtained by Halait (2012) and are shown in Table 1.^[47] Of 42 discordant results (cobas variant-positive/Sanger V600E-negative), 17 (40%) were V600E-positive and 24 (57%) were V600K-positive by 454 pyrosequencing; one sample with a V600D variant on Sanger sequencing was wild-type by 454 pyrosequencing. Reproducibility was assessed across three sites. Correct interpretations were made for all wild-type specimens and for specimens with more than 5% variant allele, the limit of detection of the cobas test.

Regulatory documents contain additional data detailing the evaluation of analytic sensitivity and specificity, cross reactivity, interference, reproducibility, repeatability, and additional studies of test robustness. In general, correlation with sequencing and extensive analytic validation data support that the test is a sensitive, specific, and robust assay for the detection of the V600E variant in FFPE melanoma specimens. Patients with V600K variants will also be identified as positive, although it is not clear that all patients with V600K variants will be positive. There is very limited evidence that patients with V600K variants may respond to

vemurafenib. Infrequently, patients with V600E2 and V600D variants may also be detected. Additionally, the method is available as a kit and is partially automated, which should result in wide access and rapid turnaround time relative to the reference standard of sequencing.

Table 1. Correlation of Cobas 4800 BRAF V600 Mutation Test results with Sanger sequencing.

Definition of Positive	Positive % Agreement	Negative % Agreement	Overall % Agreement
<i>Phase III trial</i> ^[44]			
Only V600E	97.3	84.6	90.9
All V600	87.7	95.4	90.6
V600E + V600K	92.7	95.2	91.1
<i>Phase II trial</i> ^[49]			
Only V600E	92.4		
V600E + V600K	100		
<i>Phase I trial</i> ^[46]			
Only V600E	97.3		
<i>Analytical Performance Trial</i> ^[47,48]			
Only V600E	96	82	88
Only V600E	96.4	80	88.5

THxID™ BRAF Kit

The THxID™ BRAF kit is a real-time PCR test intended for the qualitative detection of BRAF V600E and V600K variants in DNA samples extracted from formalin-fixed paraffin-embedded (FFPE) human melanoma tissue.^[50] Two oligonucleotide probes labeled with different fluorescent dyes (one for internal controls and the other for variant sequence alleles) were measured at characteristic wavelengths and compared by an autoanalyzer. Results were reported as either “mutation(s) detected” or “mutation(s) not detected” (or “invalid,” which requires troubleshooting and a repeat of the test). The threshold of detection, defined as the smallest proportion of mutated alleles for which the assay yields a positive result in 95% of tests, is 5% for V600E and V600K variants.

Correlation of the THxID BRAF assay with Sanger sequencing was tested in 898 consecutive clinical trial samples. Forty-three samples (5%) were invalid or quantity not sufficient. Excluding these samples, there were 35 discordant cases (4%). The THxID BRAF kit detected as V600E variant-positive two samples determined by Sanger sequencing to be V600D variant-positive.

Immunohistochemical Analysis

Immunohistochemical (IHC) analysis is potentially a cheaper, more efficient alternative to DNA-based testing, particularly in laboratories without access to DNA-based testing. A BRAF V600E monoclonal antibody (VE1) has been developed. Anwar (2016) reported a systematic review and meta-analysis of 21 studies (total n=1,687 patients) comparing VE1 IHC to DNA-based methods for identification of BRAF V600E variants in melanoma tissue specimens.^[51] The studies used varying cutoffs for identifying VE1 IHC as positive and there was high heterogeneity observed between studies ($I^2=95\%$, $p<0.001$). The pooled sensitivity and specificity of VE1 IHC for BRAF V600E detection were 96% (95% confidence interval [CI] 94% to 98%) and 100% (95% CI 97% to 100%), respectively. The area under the receiver operating characteristic curve (AUROC) was 0.99 (95% CI 0.98 to 1.00). Subsequent studies similarly report high concordance of VE1 IHC with DNA-based testing.^[52,53]

Section Summary: Analytic Validity

The analytic validity of BRAF genetic tests that are FDA-approved has been described in FDA documents. Detection of BRAF V600E by immunohistochemistry has been shown to have very high concordance with DNA-based testing.

Clinical Validity and Utility

The clinical validity of a genetic test is its ability to accurately and reliably predict the clinically defined disorder or phenotype of interest; the clinical utility of a genetic test is the evidence of improved measurable clinical outcomes and its usefulness and added value to patient management and decision making compared with current management without genetic testing.^[45]

When a treatment is developed for a specific biological target that characterizes only some patients with a particular disease, and a test is co-developed to identify diseased patients with that target, clinical validity and clinical utility studies are no longer separate and sequential. Rather, the clinical studies of treatment benefit, which use the test to select patients, provide evidence of both clinical validity and clinical utility.

Nivolumab

Larkin (2015a) published results systematic review and meta-analysis to evaluate the efficacy and safety of nivolumab in patients with wild-type BRAF and variant BRAF metastatic melanoma.^[54] The analysis included four trials: three phase I studies and one phase III trial known as CheckMate 037. Four hundred and forty patients from these trials with unresectable stage III or stage IV melanoma who had been tested for BRAF variants were included in this review. Of a total of 440 patients, 334 were BRAF wild-type and 106 were positive for BRAF V600 variant. With the exception of prior BRAF inhibitor therapy, the demographics were well balanced between the two cohorts. In patients evaluable for response, the objective response rates were 34.6% (95% confidence interval [CI] 28.3 to 41.3) for the 217 patients with wild-type BRAF status and 29.7% (95% CI, 19.7 to 41.5) for the 74 with variant BRAF status. The objective response rates did not seem to be affected by prior BRAF inhibitor therapy, prior ipilimumab therapy, or PD-1 ligand 1 (PD-L1) status of the tumor. The median duration of objective response was 14.8 months (95% CI 11.1 to 24.0 months) for wild-type BRAF and 11.2 months (95% CI 7.3 to 22.9 months) for variant BRAF. Median time to objective response was 2.2 months in both patient groups. The incidence of treatment-related adverse events of any grade was 68.3% in the wild-type BRAF group and 58.5% in the variant BRAF group, with grade 3 or 4 adverse events (AEs) in 11.7% and 2.8% of patients, respectively. Treatment-related AEs of any grade that occurred in at least 5% of patients in either group were fatigue, pruritus, rash, and diarrhea.

The overall survival (OS) in the CheckMate 037 trial, which compared outcomes with nivolumab treatment to those with chemotherapy, was reported by Larkin (2017).^[55] The patients were stratified by BRAF status, PD-L1 expression, and prior cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) therapy response, and 272 patients were randomized to nivolumab and 133 were randomized to the investigator's choice of chemotherapy. Treatment continued until patients had disease progression or unacceptable toxicity, and there was approximately two years of follow-up. The nivolumab group had a higher frequency of brain metastasis (20% vs. 14% in the chemotherapy group) and increased lactate dehydrogenase levels (52% vs. 38% in the chemotherapy group) at baseline, and more patients in the

chemotherapy group received anti-PD-1 agents after therapy assignment (41% vs. 11% in the nivolumab group). Although overall response rate and median duration of response were higher in the nivolumab group than in the chemotherapy group (27% vs. 10% and 32 months vs. 13 months, respectively), there were no significant differences in OS or progression-free survival (PFS) between groups.

Larkin (2015b) published results from a randomized, double-blind, phase III trial, called CheckMate067, that included 945 previously untreated patients with unresectable stage III or IV melanoma and compared nivolumab alone, nivolumab plus ipilimumab, or ipilimumab alone.^[56] PFS and OS were coprimary end points of the trial. The median PFS was 11.5 months (95% CI 8.9 to 16.7) with nivolumab plus ipilimumab, as compared with 2.9 months (95% CI 2.8 to 3.4) with ipilimumab (hazard ratio [HR] for death or disease progression 0.42, 99.5% CI 0.31 to 0.57, $p < 0.001$), and 6.9 months (95% CI 4.3 to 9.5) with nivolumab (HR for the comparison with ipilimumab 0.57, 99.5% CI 0.43 to 0.76, $p < 0.001$). In patients with tumors positive for the PD-L1, the median PFS was 14.0 months in the nivolumab-plus-ipilimumab group and in the nivolumab group, but in patients with PD-L1-negative tumors, PFS was longer with the combination therapy than with nivolumab alone (11.2 months [95% CI 8.0 to not reached] vs. 5.3 months [95% CI 2.8 to 7.1]). Treatment-related AEs of grade 3 or 4 occurred in 16.3% of the patients in the nivolumab group, 55.0% of those in the nivolumab-plus-ipilimumab group, and 27.3% of those in the ipilimumab group. The health-related quality of life (HRQoL) results from this study were reported by Shadendorf (2017), which showed no significant differences between the groups.^[57]

Hazarika (2017) reported on a trial of nivolumab for patients with unresectable or metastatic melanoma following progression on ipilimumab, and, if BRAF V600 variant-positive, a BRAF inhibitor, which led to accelerated FDA approval of nivolumab for these indications.^[58] This open-label trial showed a clinically meaningful objective response rate in 120 patients treated with 3 mg/kg intravenously every two weeks, who had at least six months of follow-up. The response rate of 31.7% (95% CI 23.5 to 43.8) was determined by a blinded independent review committee using the Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1. There were 13 patients that had a response duration of six months or more.

An international, double-blinded trial reported by Beaver (2017) supported the FDA approval of nivolumab as a first-line treatment for patients with unresectable or metastatic melanoma with wild-type BRAF V600.^[59] The trial randomized 418 patients to either nivolumab (3 mg/kg intravenously every two weeks) or dacarbazine (1,000 mg/m² intravenously every three weeks). OS was significantly higher in the nivolumab group compared with the dacarbazine group (HR 0.42, 95% CI 0.30 to 0.60, $p < 0.0001$), as was PFS (HR 0.43, 95% CI 0.34 to 0.56, $p < 0.0001$). The most common AEs in the nivolumab group were fatigue, diarrhea, constipation, nausea, rash, pruritus, and musculoskeletal pain. The authors stated that although nivolumab had a more favorable risk-benefit profile than dacarbazine, it was not clear that treatment beyond disease progression with nivolumab led to overall clinical benefit.

Vemurafenib

The primary evidence of clinical validity and utility for the cobas® 4800 BRAF V600 Mutation Test is provided by the phase III clinical trial of vemurafenib. This comparative trial, known as BRIM-3, randomly assigned 675 patients to either vemurafenib (960 mg twice daily orally) or dacarbazine (1,000 mg/m² body surface area by intravenously every three weeks) to compare the rates of overall or PFS for the two medications.^[45] All enrolled patients had unresectable,

previously untreated Stage IIIC or IV melanoma with no active central nervous system (CNS) metastases. Melanoma specimens from all patients tested positive for the BRAF V600E variant on the cobas 4800 BRAF V600 Mutation Test. Included were 19 patients with the BRAF V600K variant and one with a BRAF V600D variant. Final OS results from BRIM-3 were reported by Chapman (2017).^[60] Eighty-four (25%) of the 338 dacarbazine patients crossed over to vemurafenib, and overall 173 (51%) of the 338 patients in the dacarbazine group and 175 of the 337 patients (52%) in the vemurafenib group received subsequent anticancer therapies, most commonly ipilimumab. Median OS without censoring at crossover was 13.6 months (95% CI 12.0 to 15.4) in vemurafenib vs 10.3 months (95% CI 9.1 to 12.8 months) in dacarbazine (HR 0.81, 95% CI 0.68 to 0.96, $p=0.01$).

Tumor assessments including computed tomography (CT) were performed at baseline, at weeks 6 and 12, and every 9 weeks thereafter. Tumor responses were determined by the investigators according to RECIST v.1.1. Primary endpoints were the rate of OS and PFS. An interim analysis was planned at 98 deaths and a final analysis at 196 deaths; the published report is the interim analysis, reporting 118 deaths. The median survival had not been reached. AEs in the vemurafenib group included grade 2 or 3 photosensitivity skin reactions in 12% of patients and cutaneous squamous cell carcinoma in 18% of patients. The Data and Safety Monitoring Board determined that both co-primary endpoints had met prespecified criteria for statistical significance and recommended that patients in the dacarbazine group be allowed to cross over and receive vemurafenib. The results of this trial comprised the data supporting the efficacy and safety of vemurafenib for submission to the FDA and established the safety and effectiveness of the cobas 4800 BRAF V600 Mutation Test, resulting in co-approval of drug and companion test.

A phase II trial known as BRIM-2 enrolled patients at 13 centers who had failed at least one previous treatment for metastatic melanoma.^[49] All patients were selected with the cobas 4800 BRAF V600 Mutation Test; 122 cases had BRAF V600E–positive melanoma, and 10 cases were positive for BRAF V600K. The target overall response rate (primary outcome) was 30%, with a lower boundary of the 95% CI of at least 20%. At a median follow-up of 10 months, this target was met with an overall response rate of 53% by independent review committee (95% CI 44 to 62%). At 10 months, 27% of patients were still on treatment; the majority of discontinuations were due to disease progression. The most common AEs of any grade were arthralgias (58%), skin rash (52%), and photosensitivity (52%). The most common grade 3 AEs were squamous cell carcinomas; these were seen in about 25% of patients, tended to occur in the first two months of treatment, and were managed with local excision. There were very few grade 4 AEs.

Puzanov (2015) reported a long-term follow-up phase I clinical trial to assess disease progression and clinical management of vemurafenib monotherapy in BRAF V600E melanoma patients.^[61] Patients received vemurafenib 240-1120 mg (dose escalation cohort) or 960 mg (extension cohort) orally twice daily. Clinical response was evaluated every eight weeks by RECIST. Patients with progressive disease amenable to local therapy (surgery or radiotherapy) were allowed to continue vemurafenib after progression. Forty-eight patients (escalation cohort, $n=16$; extension cohort, $n=32$) received therapeutic doses of vemurafenib (≥ 240 mg twice daily). Forty-four patients had progressive disease by the time of this analysis and four remained progression free (follow-up time 1.2 to 56.1 months). Median OS was 14 months (range 1.2 to 56.1); three- and four-year melanoma-specific survival rate in the extension cohort was 26% and 19%, respectively. Median OS was 26.0 months (range 7.7 to 56.1) among 20 patients who continued vemurafenib after local therapy. Median treatment

duration beyond initial disease progression was 3.8 months (range 1.1 to 26.6). In the extension cohort, six and five patients were alive after three and four years, respectively, on vemurafenib monotherapy.

The two-year results of a multicenter, open-label safety study of vemurafenib in 3219 patients with BRAF V600 variant-positive metastatic melanoma were reported by Blank (2017).^[62] All patients had previously treated or untreated metastatic melanoma, and received 960 mg of vemurafenib twice a day. The median follow-up was 32.2 months, and 3079 (96%) of patients had discontinued treatment, mainly due to disease progression. The most common AEs related to treatment were arthralgia (37%), alopecia (25%), and hyperkeratosis (23%). Squamous cell carcinoma of the skin (8%) and keratoacanthoma (8%) were the most common grade 3/4 AEs.

Flaherty (2010) published results from an RCT where the goal was to first determine the maximum dose in a dose-escalation phase, then determine the objective response rate and monitor toxicity.^[63] This trial used a PCR assay that was likely a prototype of the final test; only a brief description of the assay was provided in the publication. In the dose-escalation phase, five patients with metastatic melanoma tumors who did not have the BRAF V600E variant received 240 mg or more vemurafenib twice daily (final recommended dose is 960 mg twice daily); of these, none responded. In the extension phase of the trial, 26 of 32 patients with the BRAF V600E variant responded (81%: 24 partial, two complete responses).

Dabrafenib

One phase III randomized controlled trial on dabrafenib for melanoma has been published.^[64] The main objective of this RCT was to study the efficacy of dabrafenib vs. standard dacarbazine treatment in patients selected to have BRAF V600E variant-positive metastatic melanoma. Two-hundred-fifty patients were randomized 3:1 to receive oral dabrafenib 150 mg twice daily versus intravenous dacarbazine 1,000 mg/m² every 3 weeks. The primary outcome was PFS and secondary outcomes were overall survival, objective response rates, and adverse events.

Median PFS for the dabrafenib and dacarbazine groups was 5.1 months and 2.7 months, respectively. OS did not differ significantly between groups; 11% of patients in the dabrafenib group died compared with 14% in the dacarbazine group (hazard ratio [HR] 0.61-30, 95% CI 0.25 to 1.48). However, 28 patients (44%) in the dacarbazine arm crossed over at disease progression to receive dabrafenib. The objective response rate, defined as complete plus partial responses was higher in the dabrafenib group (50%, 95% CI 42.4 to 57.1%) compared with the dacarbazine group (6%, 95% CI 1.8 to 15.5%). Treatment-related AEs grade 2 or higher occurred in 53% of patients who received dabrafenib and in 44% of patients who received dacarbazine. Grade 3-4 AEs were uncommon in both groups. The most common serious AEs were cutaneous squamous cell carcinoma (7% vs. none in controls); serious non-infectious, febrile drug reactions (3% grade 3 pyrexia vs. none in controls); and severe hyperglycemia (>250-500 mg/dL), requiring medical management in non-diabetic or change in management of diabetic patients (6% vs. none in controls). The results demonstrate that targeting dabrafenib against BRAF V600E variant-positive melanoma results in a benefit in PFS. Patients were allowed to cross over at the time of progression, and the effect of dabrafenib on OS was favorable but not statistically significant.

Trametinib

The clinical efficacy and safety of trametinib was assessed in the phase III, open-label METRIC trial.^[65] Patients with stage IV or unresectable stage IIIC cutaneous melanoma were randomized 2:1 to receive trametinib 2 mg orally once daily (n = 214) or chemotherapy (n = 108), either dacarbazine 1,000 mg/m² IV every three weeks or paclitaxel 175 mg/m² IV every three weeks at investigator discretion. Most patients (67%) were previously untreated. The primary efficacy endpoint was PFS; secondary endpoints included OS, overall response rate, and safety. Tumor assessments were performed at baseline and at weeks 6, 12, 21, and 30, and then every 12 weeks.

Median PFS was 4.8 months (95% CI 4.3 to 4.9) in the trametinib arm and 1.5 months (95% CI 1.4 to 2.7) in the chemotherapy arm, a statistically significant difference. Although median overall survival had not been reached at the time of the report publication, 6-month survival was statistically longer in the trametinib group than in the chemotherapy group (p=0.01); 51 of 108 patients (47%) in the chemotherapy group crossed over at disease progression to receive trametinib. In the trametinib and chemotherapy groups, AEs led to dose interruption in 35% and 22% of patients, respectively, and to dose reduction in 27% and 10% of patients, respectively. Decreased ejection fraction or ventricular dysfunction was observed in 14 patients (7%) in the trametinib group; two patients had grade 3 cardiac events that led to permanent drug discontinuation. Twelve percent of the trametinib group and 3% of the chemotherapy group experienced grade 3 hypertension. Nine percent of patients in the trametinib group experienced ocular events (mostly grade 1 or 2), most commonly blurred vision (4%). The most common AEs in the trametinib group were rash, diarrhea, peripheral edema, and fatigue; rash was grade 3 or 4 in 16 patients (8%). Cutaneous squamous cell carcinoma was not observed during treatment.

Tumor tissue was evaluated for BRAF variants at a central site using a clinical trial assay. Retrospective THxID BRAF analysis was conducted on tumor samples from 289 patients (196 [92%] in the trametinib arm and 93 [86%] in the chemotherapy arm). Reanalysis of PFS in patients who were V600E or V600K-positive by the THxID BRAF kit showed a treatment effect that was almost identical to the overall result by central assay. Additional analysis for discordant results assuming a worst case scenario as above yielded a hazard ratio of 0.48 (95% CI 0.35 to 0.63).^[50]

Combination BRAF and MEK Inhibition

Dabrafenib and Trametinib

Long (2016) reported the OS and clinical characteristics of BRAF inhibitor-naïve, long-term responders and survivors treated with dabrafenib plus trametinib in a phase I and II trial of 78 patients with BRAF V600 variant-positive (V600E or V600K) metastatic melanoma.^[66] In one group, 24 BRAF inhibitor-naïve patients received dabrafenib 150 mg twice daily plus trametinib 2 mg once daily (the 150/2 group). In group two, 54 patients were randomly assigned to each of three treatment groups: dabrafenib monotherapy, dabrafenib plus trametinib 1 mg once daily, and dabrafenib plus trametinib 2 mg once daily (the 150/2 group). For patients on the combination therapy (n = 78), the PFS at 1, 2, and 3 years was 44%, 22%, and 18%, respectively, for group one (n = 24) and 41%, 25%, and 21%, respectively, for group two (n = 54). Median OS was 27.4 months in group one and 25 months in group two. OS at 1, 2, and 3 years was 72%, 60%, and 47%, respectively, for group one and 80%, 51%, and 38%, respectively, for group two. The median OS for BRAF inhibitor-naïve variant-positive patients who received dabrafenib plus trametinib (150/2) in the randomized phase II part of this study

was more than two years, and the two- and three-year survival rates were 51% and 38%, respectively.

Menzies (2015) assessed the features associated with efficacy and long-term survival in BRAF variant-positive metastatic melanoma patients treated with BRAF inhibitor monotherapy (dabrafenib [n = 70]; or vemurafenib [n = 41]) or combined dabrafenib and trametinib (n = 31).^[67] One hundred and nineteen patients (84%) had the V600E variant, whereas 23 patients (16%) had either V600K or V600R. The median follow-up was 15.7 months (range 0.6 to 60.5 months). Patients treated with monotherapy were grouped together for analysis. The two-, three-, and four-year OS rates were 43%, 24%, and 24%, respectively. Factors associated with longer PFS and OS were female sex and a normal pretreatment serum lactate dehydrogenase level. The *BRAF* V600E genotype was independently associated with longer PFS (HR 0.51, p=0.006) but not OS. One of the limitations of this study is the heterogeneous patient population in the monotherapy group; the type of monotherapy provided was not accounted for in the analysis.

A similar study by Schadendorf (2017) examined factors associated with clinical outcomes for dabrafenib and trametinib combination therapy in a pooled analysis of phase III trials.^[68] They found that baseline lactate dehydrogenase level and the number of organ sites were significantly associated with PFS and OS. Individuals with normal LDH, baseline sum of lesion diameters of less than 66 mm, and less than three organ sites (n=183 [33% of 563]) had the most favorable prognosis, with 42% demonstrating three-year PFS.

Johnson (2015) published results from an open-label phase I/II trial to assess the safety and efficacy of dabrafenib and trametinib in patients who had received prior BRAF inhibitor treatment.^[69] Seventy-one patients were enrolled in the study and treated with combination therapy after disease progression with BRAF inhibitor treatment administered before study enrollment (part B; n = 26) or after cross-over at progression with dabrafenib monotherapy (part C, n = 45). In parts B and C, confirmed objective response rates (ORR) were 15% (95% CI 4% to 35%) and 13% (95% CI 5% to 27%), respectively; an additional 50% and 44% experienced stable disease \geq 8 weeks, respectively. The median PFS was 3.6 months (95% CI 2 to 4), and median overall survival was 11.8 months (95% CI 8 to 25) from cross-over. Patients who previously received dabrafenib \geq 6 months had superior outcomes with the combination compared with those treated < 6 months; median PFS was 3.9 (95% CI 3 to 7) versus 1.8 months (95% CI 2 to 4, HR 0.49, p=0.02), and ORR was 26% (95% CI 10% to 48%) versus 0% (95% CI 0% to 15%).

A study by Schreuer (2017) also evaluated dabrafenib plus trametinib in a small, single-arm, open-label study with 25 pretreated patients. In this case, patients had previously experienced disease progression on BRAF inhibitors with or without MEK inhibitor use.^[70] After patients were off treatment for 12 weeks or more, they began dabrafenib and trametinib therapy. The primary endpoint was overall response rate, as determined using RECIST v.1.1., on two occasions, at least 28 days after the first recorded response. Eight patients had a partial response, and 10 patients appeared to have stable disease during this period. Grade 3 AEs occurred in two patients (pyrexia and panniculitis), and there were no grade 4 or 5 AEs.

Robert (2015) published results from an open-label phase III clinical trial to examine overall survival in patients with metastatic melanoma.^[71] There were 704 patients with a BRAF V600 variant that received either a combination of dabrafenib (150 mg twice daily) and trametinib (2 mg once daily) or vemurafenib (960 mg twice daily) orally as first-line therapy. At the

preplanned interim overall survival analysis, which was performed after 77% of the total number of expected events occurred, the OS rate at 12 months was 72% (95% CI 67 to 77) in the combination-therapy group and 65% (95% CI 59 to 70) in the vemurafenib group. The study was stopped in July 2014 because the prespecified interim stopping boundary had been crossed. Median PFS was 11.4 months in the combination-therapy group and 7.3 months in the vemurafenib group (HR 0.56, 95% CI 0.46 to 0.69, $p < 0.001$). The objective response rate was 64% in the combination-therapy group and 51% in the vemurafenib group ($p < 0.001$). Rates of severe AEs and study-drug discontinuations were similar in the two groups. Cutaneous squamous-cell carcinoma and keratoacanthoma occurred in 1% of patients in the combination-therapy group and 18% of those in the vemurafenib group.

Schadendorf (2015) reported results from a double-blind randomized phase III COMBI-d trial that investigated the combination of dabrafenib and trametinib versus dabrafenib monotherapy in patients with BRAF V600E/K-variant metastatic melanoma.^[72] COMBI-d showed significantly prolonged PFS for the combination. Health-related quality of life was evaluated by questionnaire at baseline, during study treatment, at progression, and post progression to assess various dimensions (global health/quality of life, functional status, and symptom impact). Questionnaire completion rates were $>95\%$ at baseline, $>85\%$ to week 40 and $>70\%$ at disease progression. Baseline scores across both arms were comparable for all dimensions. Global health dimension scores were significantly better at weeks 8, 16 and 24 for patients receiving the combination during treatment and at progression. The majority of functional dimension scores (physical, social, role, emotional and cognitive functioning) trended in favor of the combination. Pain scores were significantly improved and clinically meaningful (6- to 13-point difference) for patients receiving the combination for all follow-up assessments compared to those receiving dabrafenib monotherapy. For other symptom dimensions (nausea and vomiting, diarrhea, dyspnea, and constipation), scores trended in favor of dabrafenib monotherapy. A three-year survival and safety analysis from this study was reported by Long (2017). The PFS at three years was higher in the combination group (22%) than in the monotherapy group (12%), as was OS (44% vs. 32%, respectively).^[73]

Long (2015) published results from a double-blind phase III industry sponsored study at 113 sites in 14 countries.^[74] The 423 enrolled participants were previously untreated patients with BRAF V600E or V600K variant-positive unresectable tumors and were randomly assigned to receive: 1) dabrafenib and trametinib ($n = 211$) or 2) dabrafenib only ($n = 212$). Overall survival was 74% at one year and 51% at two years in the dabrafenib and trametinib group versus 68% and 42%, respectively, in the dabrafenib only group. Based on 301 events, median PFS was 11.0 months (95% CI 8.0 to 13.9) in the dabrafenib and trametinib group and 8.8 months (5.9 to 9.3) in the dabrafenib only group (HR 0.67, 95% CI 0.53 to 0.84, $p = 0.0004$, unadjusted for multiple testing). Treatment-related AEs occurred in 181 (87%) of 209 patients in the dabrafenib and trametinib group and 189 (90%) of 211 patients in the dabrafenib only group; the most common were pyrexia (108 patients, 52%) in the dabrafenib and trametinib group, and hyperkeratosis (70 patients, 33%) in the dabrafenib only group. Grade 3 or 4 AEs occurred in 67 (32%) patients in the dabrafenib and trametinib group and 66 (31%) patients in the dabrafenib only group.

An open-label Phase I/II trial examined the pharmacokinetics, safety, and efficacy of dabrafenib plus trametinib combination therapy in 247 patients with metastatic (stage IV) melanoma and BRAF V600E or V600K variants.^[75] Maximum tolerated combination dosing was not reached. One dose-limiting toxic effect, recurrent neutrophilic panniculitis, occurred in 24 patients who received the highest dose level (dabrafenib 150 mg twice daily plus trametinib

2 mg daily), and this was the recommended dose for efficacy testing. Median PFS, the primary efficacy endpoint, was 9.4 months in the combination therapy group (n=54) and 5.8 months in the dabrafenib (150 mg twice daily) monotherapy group (n=54, HR 0.39, 95% CI 0.25 to 0.62, $p<0.001$). Complete or partial response occurred in 76% of patients in the combination therapy group and 54% of the monotherapy group ($p=0.03$). Median duration of response was 10.5 (95% CI 7.4 to 14.9) months and 5.6 months (95% CI 4.5 to 7.4), respectively. Cutaneous squamous cell carcinoma occurred in 7% of the combination therapy group and 19% of the monotherapy group ($p=0.09$). Fever was more common in the combination therapy group (71% vs. 26% monotherapy, $p<0.001$).

Vemurafenib and Cobimetinib

A multicenter, double-blinded, phase III RCT, known as coBRIM, evaluated the combination of the BRAF inhibitor vemurafenib and the MEK inhibitor cobimetinib in 495 patients with previously untreated, BRAF V600 variant-positive, unresectable or metastatic melanoma.^[76] All patients received vemurafenib 960 mg orally twice daily on days 1 to 28 and were randomized in a 1:1 ratio to also receive cobimetinib 60 mg once daily on days 1 to 21 or cobimetinib placebo. The primary outcome was PFS. Analyses were done on the intention-to-treat population. Median follow-up was 14 months. PFS was significantly increased with vemurafenib and cobimetinib compared to vemurafenib and placebo (median PFS 12.3 months vs 7.2 months, HR 0.58, 95% CI 0.46 to 0.72, $p<0.001$). Median OS was 22 months for vemurafenib and cobimetinib versus 17 months for vemurafenib and placebo (HR 0.70, 95% CI 0.55 to 0.90, $p=0.005$). Serious AEs were reported in 92 (37%) patients in the vemurafenib and cobimetinib group and 69 (28%) patients in the vemurafenib and placebo group. The most common serious AEs in the vemurafenib and cobimetinib group were pyrexia and dehydration. The most common grade 3 to 4 AEs occurring more frequently in the vemurafenib and cobimetinib group were γ -glutamyl transferase increase, blood creatine phosphokinase increase, and alanine transaminase.

Dréno (2017) published a report on toxicities in the coBRIM study, after a median follow-up of 18.5 months.^[77] Nearly all of the 493 patients that received treatment experienced an AE. The majority of AEs occurred during the first treatment cycle. The frequency of serious AEs (grade 3 and above) was higher in the combination therapy group than the monotherapy group (75% and 61%, respectively). Common AEs, including rash, diarrhea, photosensitivity, pyrexia, and serous retinopathy decreased in incidence over time. A study by de la Cruz-Merino (2017) focused on patients in this trial who had serous retinopathy.^[78] There was a total of 86 serous retinopathy events in 70 patients, with the vast majority reported in the combination therapy group (79 vs. 7 events in the monotherapy group). Most retinopathy events were managed by observation and did not require discontinuation or dose modification of cobimetinib.

Larkin (2015) published results from a phase III trial that evaluated the combination of vemurafenib and cobimetinib in 495 patients with previously untreated, unresectable, locally advanced or metastatic, BRAF V600 variant-positive melanoma.^[79] Patients were randomly assigned to received vemurafenib and cobimetinib (combination group) or vemurafenib and placebo (control group). The median PFS was 9.9 months in the combination group and 6.2 months in the control group (HR for death or disease progression 0.51, 95% CI 0.39 to 0.68, $p<0.001$). The rate of complete or partial response in the combination group was 68%, as compared with 45% in the control group ($p<0.001$), including rates of complete response of 10% in the combination group and 4% in the control group. PFS, as assessed by independent

review, was similar to investigator-assessed PFS. Interim analyses of OS showed 9-month survival rates of 81% (95% CI 75 to 87) in the combination group and 73% (95% CI 65 to 80) in the control group. Vemurafenib and cobimetinib was associated with a nonsignificantly higher incidence of AEs of grade 3 or higher, as compared with vemurafenib and placebo (65% vs. 59%), and there was no significant difference in the rate of study-drug discontinuation. The number of secondary cutaneous cancers decreased with the combination therapy.

Ribas (2014) published results from a phase Ib clinical trial to assess the safety and efficacy of combined BRAF inhibition with vemurafenib and MEK inhibition with cobimetinib in patients with advanced BRAF V600 variant-positive melanoma.^[80] The primary endpoint was safety of the drug combination and to identify dose-limiting toxic effects and the maximum tolerated dose. One hundred and twenty-nine patients were included who had either recently progressed on vemurafenib or never received a BRAF inhibitor. Patients received vemurafenib twice a day continuously and cobimetinib once a day for either 14 days on and 14 days off (14/14), 21 days on and 7 days off (21/7), or continuously (28/0).

Across all dosing regimens, the most common AEs were diarrhea (83 patients, 64%), non-acneiform rash (77 patients, 60%), liver enzyme abnormalities (64 patients, 50%), fatigue (62 patients, 48%), nausea (58 patients, 45%), and photosensitivity (52 patients, 40%). Most AEs were mild-to-moderate in severity. The most common grade 3 or 4 AEs were cutaneous squamous-cell carcinoma (12 patients, 9%; all grade 3), raised amounts of alkaline phosphatase (11 patients, 9%), and anaemia (nine patients, 7%). Confirmed objective responses were recorded in 10 (15%) of 66 patients who had recently progressed on vemurafenib, with a median PFS of 2.8 months (95% CI 2.6 to 3.4). Confirmed objective responses were noted in 55 (87%) of 63 patients who had never received a BRAF inhibitor, including six (10%) who had a complete response; median PFS was 13.7 months (95% CI 10.1 to 17.5).

Encorafenib and Binimetinib

Dummer (2018) reported on results of a phase III COLUMBUS RCT comparing encorafenib, a BRAF inhibitor, alone or in combination with the MEK inhibitor binimetinib, with vemurafenib in patients who had advanced BRAF V600-variant unresectable or metastatic melanoma.^[81] The COLUMBUS trial was conducted in 162 hospitals in 28 countries between 2013 and 2015; patients were randomized (1:1:1) to oral encorafenib 450 mg once daily plus oral binimetinib 45 mg twice daily (n=192), oral encorafenib 300 mg once daily (n=194), or oral vemurafenib 960 mg twice daily (n=191). The primary outcome was PFS for encorafenib plus binimetinib vs vemurafenib. Analyses were done on the intention-to-treat population. Median follow-up was 17 months. PFS was significantly increased with encorafenib plus binimetinib compared with vemurafenib (median PFS was 14.9 months vs 7.3 months in the vemurafenib group; HR 0.54, 95% CI 0.41 to 0.71, p<0.001). The OS was not reported. The most common grade 3 or 4 AEs were increased γ -glutamyltransferase (9%), increased creatine phosphokinase (7%), and hypertension (6%) in the encorafenib plus binimetinib group; palmoplantar erythrodysesthesia syndrome (14%), myalgia (10%), and arthralgia (9%) in the encorafenib group; and arthralgia (6%) in the vemurafenib group.

BRAF and MEK inhibition vs. Immunotherapy

For patients who are BRAF V600 variant-positive unresectable or metastatic melanoma, NCCN has suggested that both immunotherapy and BRAF/MEK inhibition are appropriate first-

line therapies. There are no RCTs directly comparing BRAF and MEK inhibitors with immunotherapy. Network meta-analyses providing indirect comparisons are discussed below.

Amdahl (2016) reported a network meta-analysis of RCTs to compare dabrafenib plus trametinib in previously untreated patients versus other first-line treatments that were approved by Health Canada as of February 2015 (dabrafenib, vemurafenib, trametinib, ipilimumab, dacarbazine) for submission to Canadian reimbursement authorities.^[82] Seven studies (total n=2,834 patients) were included. Bayesian network meta-analyses were performed to estimate hazard ratios for PFS and OS. The combination of dabrafenib and trametinib was associated with prolonged PFS and OS compared to all other first-line therapies included in analysis. For PFS, the HRs favoring dabrafenib and trametinib were: 0.23 (95% credible interval [CrI] 0.18 to 0.29) versus dacarbazine; 0.32 (95% CrI 0.24 to 0.42) versus ipilimumab plus dacarbazine; 0.52 (95% CrI 0.32 to 0.83) versus trametinib; 0.57 (95% CrI 0.48 to 0.69) versus vemurafenib; and 0.59 (95% CrI 0.50 to 0.71) versus dabrafenib. For OS, the hazard ratios were: 0.41 (0.29 to 0.56) versus dacarbazine; 0.52 (95% CrI 0.38 to 0.71) versus ipilimumab plus dacarbazine; 0.68 (0.47 to 0.95) versus trametinib; 0.69 (95% CrI 0.57 to 0.84) versus vemurafenib; and 0.72 (95% CrI 0.60 to 0.85) versus dabrafenib. Nivolumab, pembrolizumab, and cobimetinib were not approved in Canada at the time the analysis was conducted.

Devji (2017) performed a network meta-analysis comparing first-line treatments and including RCTs in treatment-naïve patients in which at least one intervention was a BRAF and MEK inhibitor or an immune checkpoint inhibitor.^[83] Fifteen RCTs (total n=6,662 patients) were included. Treatments were combined into drug class: targeted therapy (BRAF and/or MEK inhibitor), immunotherapy (CTLA-4, PD-1, and/or granulocyte macrophage colony-stimulating factor [GM-CSF]), chemotherapy, and combinations of these treatments. Bayesian network meta-analyses were performed to calculate hazard ratios for OS and PFS and ORs for overall response rate. The risk of bias for the included studies was low. BRAF plus MEK inhibition and PD-1 were both individually associated with improved OS compared with all other treatments except CTLA-4/GM-CSF; there was no significant difference in OS between BRAF plus MEK inhibition and PD-1 (HR 1.02; 95% CrI 0.72 to 1.45). The network meta-analysis showed a significant advantage of BRAF plus MEK inhibition compared with all other treatment strategies for PFS and overall response rate. Chemotherapy and PD-1 therapy had the lowest risk of serious AEs.

Pasquali (2017) also compared immune checkpoint inhibitors and BRAF targeted therapies in a network meta-analysis including 12 RCTs (total n = 6207 patients) reporting on anti-PD1 antibodies, antiCTLA-4 antibodies, BRAF inhibitors, and MEK inhibitors.^[84] BRAF plus MEK inhibition was associated with longer PFS compared to BRAF inhibition alone and immunotherapy (BRAF plus MEK vs. anti-CTLA-4: HR 0.22, 95% CI 0.12 to 0.41, BRAF vs. MEK vs. anti-PD-1 antibodies: HR 0.38, 95% CI 0.20 to 0.72; BRAF plus MEK vs. BRAF alone: HR 0.56, 95% CI 0.44 to 0.70). Anti-PD-1 monoclonal antibodies were estimated to be the least toxic while the combination of anti-CTLA-4 and anti-PD-1 monoclonal antibodies were associated with the most toxicity.

Section Summary: Clinical Validity and Clinical Utility

RCTs of BRAF and MET inhibitor therapy in patients selected on the basis of BRAF V600 variant testing have shown improvements in OS and PFS. Single-agent BRAF inhibitor treatment with vemurafenib and dabrafenib compared with chemotherapy shows superior outcomes for response and PFS. Combination BRAF and MEK inhibitor treatment with

vemurafenib plus cobimetinib or dabrafenib plus trametinib shows superior OS when compared with either vemurafenib or dabrafenib alone. There are no RCTs directly comparing BRAF and MEK inhibitor therapy with immunotherapy as first-line treatment for patients with BRAF pathogenic variants. Network meta-analyses including indirect comparisons suggest that BRAF and MEK combination therapy might prolong PFS but with higher toxicity compared to immunotherapy.

RESECTED STAGE III MELANOMA

The purpose of testing for BRAF pathogenic variants in individuals with resected stage III melanoma is to inform a decision whether to use adjuvant treatment with BRAF and/or MEK tyrosine kinase inhibitors after surgical resection. Observation, as well as treatment with nivolumab or ipilimumab, are also options for resected, stage III melanoma. There are no RCTs directly comparing BRAF and MEK inhibitors with immunotherapy.

Long (2017) reported on results of COMBI-AD, a phase III RCT comparing adjuvant combination therapy using dabrafenib plus trametinib with placebo in 870 patients who had stage III melanoma with BRAF V600E or V600K variants.^[85] In 2013 and 2014 when patients were being enrolled in COMBI-AD, observation was the standard of care after resection of stage III melanoma in most countries. With a median follow-up of 2.8 years, the three-year rate of relapse-free survival was 58% in the combination group and 39% in the placebo group (HR 0.47, 95% CI 0.39 to 0.58, $p < 0.001$). The OS rates at three years were 86% and 77%, respectively (HR 0.57, 95% CI 0.42 to 0.79, $p < 0.001$).

Maio (2018) reported on results of BRIM8, a phase III RCT comparing adjuvant vemurafenib monotherapy with placebo in 498 patients who had stage IIC, IIIA, IIIB, or IIIC BRAF V600 variant-positive melanoma.^[86] Patients with stage IIC, IIIA, or IIIB disease were enrolled in cohort 1 ($n=314$), and patients with stage IIIC disease were enrolled in cohort 2 ($n=184$). As stated previously, during enrollment, observation was standard care for stage III melanoma. A hierarchical testing strategy was prespecified for the primary outcome (disease-free survival) based on the assumption that observing a biologic effect in higher risk disease (i.e., cohort 2) would suggest a treatment effect across the continuum of melanoma given the effect is already established in metastatic melanoma. In the hierarchical strategy, only a p -value of 0.05 or less in cohort 2 would allow for results in cohort 1 to be considered significant. The median trial follow-up was 34 months (interquartile range 26 to 42 months) in cohort 2 and 31 months (interquartile range, 26 to 41 months) in cohort 1. In cohort 2, median disease-free survival was 23 months (95% CI 19 to 27 months) in the vemurafenib group and 15 months (95% CI, 11 to 36 months) in the placebo group (HR 0.80, 95% CI 0.54 to 1.18, $p=0.26$). In cohort 1, median disease-free survival was not reached (95% CI not estimable) in the vemurafenib group and 37 months (95% CI 21 to not estimable) in the placebo group (HR 0.54, 95% CI 0.37 to 0.78); however, this result cannot be considered statistically significant because of the prespecified hierarchical testing strategy.

Section Summary: Clinical Validity and Clinical Utility

RCTs of BRAF and MET inhibitor therapy in stage III melanoma patients selected by BRAF V600 variant testing have shown reductions in recurrence risk. One well-conducted RCT of combination BRAF and MEK inhibitor treatment with dabrafenib plus trametinib has shown superiority for recurrence risk and OS in BRAF variant-positive, stage III patients compared with placebo. Single-agent BRAF inhibitor treatment using vemurafenib compared with placebo showed numeric benefit for disease-free survival in patients with stage IIC, IIIA, or IIIB BRAF

V600 variant–positive melanoma but this result must be considered exploratory given the lack of statistically significant benefit in stage IIIC disease and the hierarchical statistical testing strategy. There are no RCTs directly comparing BRAF and MEK inhibitor therapy with immunotherapy as an adjuvant treatment for stage III patients with BRAF pathogenic variants.

GLIOMA

The purpose of testing for BRAF pathogenic variants in individuals with glioma is to inform a decision whether to treat with BRAF and/or MEK inhibitors versus other standard treatments for glioma. Standard treatment for patients with glioma includes surgical resection followed by radiotherapy and/or chemotherapy with temozolomide.

Analytical Validity

Currently there is no standard method for testing BRAF status in neuropathology. DNA-based tests for melanomas and immunohistochemistry are used. The analytic validity of these methods is described in the previous section.

Clinical Validity and Clinical Utility

Sorafenib

Sorafenib is a multikinase inhibitor with potent in vitro activity against both wild-type BRAF and V600E variant, as well as vascular endothelial growth factor receptors (VEGFR), platelet-derived growth factor receptors, and c-kit. Several phase II single-arm prospective studies have investigated the use of sorafenib in newly diagnosed and recurrent, adult and pediatric, low- and high-grade gliomas in various combinations with other treatments, but results have not shown sorafenib to be effective. Most studies did not report BRAF V600 variant status. Table 4 describes prospective studies of sorafenib in glioma.

Table 4. Prospective Studies of Sorafenib in Patients With Glioma

Author (Year)	Populations	N	Treatment(s)	Results (95% CI), mo	
				Median PFS	Median OS
Karajannis (2014) ^[87]	Children with recurrent or progressive low-grade astrocytomas	11 overall; 5 positive for constitutive <i>BRAF</i> activation (<i>KIAA-BRAF</i> fusion or <i>BRAF</i> -activating variant including <i>BRAF</i> V600E)	Sorafenib bid at 200 mg/m ² per dose in continuous 28-d cycles	2.8 (2.1 to 31.0) ^a	
Hottinger (2014) ^[88]	Adults with newly diagnosed high-grade glioma	17; <i>BRAF</i> status not reported	60-Gy RT plus TMZ 75 mg/m ² per day and sorafenib 200 mg qd, 200 mg bid, or 400 mg bid	7.9 (5.4 to 14.6)	17.8 (14.7 to 25.6)
Galanis (2013) ^[89]	Adults with recurrent GBM	54; <i>BRAF</i> status not reported	Bevacizumab 5 mg/kg per 2 wk plus sorafenib 200 mg qd or bid	Six-month 20.4%	5.6 (4.7 to 8.2)
Zustovich (2013) ^[90]	Adults with recurrent GBM	53; <i>BRAF</i> status not reported	TMZ 40 mg/m ² per day plus	3.2 (1.8 to 4.8)	7.4 (5.6 to 9)

Author (Year)	Populations	N	Treatment(s)	Results (95% CI), mo	
				Median PFS	Median OS
			sorafenib 400 mg bid		
Den (2013) ^[91]	High-grade glioma (primary or recurrent) with at least 2 wk RT	18; <i>BRAF</i> status not reported	Sorafenib 200-400 mg bid plus: <ul style="list-style-type: none"> • Primary disease, TMZ 75 mg/m² per day and 60-Gy RT • Recurrent disease, 35 Gy in 10 fractions 		18 (6 to undefined)
Peereboom (2013) ^[92]	Adults with recurrent or progressive GBM	56; <i>BRAF</i> status not reported	Erlotinib 150 mg qd plus sorafenib 400 mg bid	2.5 (1.8 to 3.7)	5.7 (4.5 to 7.9)
Lee (2012) ^[93]	Adults with recurrent GBM or gliosarcoma	18; <i>BRAF</i> status not reported	Sorafenib 800 mg qd plus temsirolimus 25 mg/wk	8 wk (5 to 9) ^a	

bid: twice daily; GBM: glioblastoma multiforme; Gy: gray; OS: overall survival; PFS: progression-free survival; qd: every day; RT: radiotherapy; TMZ: temozolomide.

^a Study terminated early.

Vemurafenib, Dabrafenib, and Trametinib

Several case reports and small case series have suggested clinical benefit with vemurafenib, dabrafenib, and trametinib in patients with glioma and BRAF V600 pathogenic variants.

Hyman (2015) published results of a multicenter phase II “basket” study of vemurafenib in BRAF V600 variant-positive nonmelanoma cancers.^[94] A total of 122 patients with BRAF V600 pathogenic variants were enrolled, including eight patients with gliomas. Response was assessed by site investigators using RECIST criteria. Of the eight glioma patients, two died before the one-month evaluation; four had stable disease at 12, 6, 4, and 3 months and two had progressive disease at two and seven months, all respectively.

Section Summary: Clinical Validity and Clinical Utility

Studies of sorafenib in patients with newly diagnosed and recurrent gliomas combined with various other treatments have not shown benefit, although most did not report BRAF V600 status. Evaluation of the BRAF and MEK inhibitors vemurafenib, dabrafenib, and trametinib in patients with gliomas has been limited to one phase II “basket” study (including eight patients with glioma), case reports, and small case series. Several early phase studies are ongoing.

PRACTICE GUIDELINE SUMMARY

NATIONAL COMPREHENSIVE CANCER NETWORK (NCCN)

NCCN guidelines for melanoma, version 2.2018, recommend that BRAF variant status testing should be performed using an FDA-approved test or by a facility approved by CLIA.^[8] Combination dabrafenib-trametinib or vemurafenib-cobimetinib therapies both have a category 1 recommendation as preferred regimens for advanced or metastatic melanoma. Vemurafenib

and dabrafenib also have category 1 recommendations for advanced or metastatic melanoma. National Comprehensive Cancer Network also recommends dabrafenib plus trametinib combination therapy as an option for patients with stage III melanoma who have a BRAF V600-activating variant and sentinel lymph node metastasis greater than 1 mm (category 1).

National Comprehensive Cancer Network guidelines for central nervous system cancers, version 1.2018 indicate the following on the use of BRAF molecular markers to guide treatment decisions for primary brain cancers: “BRAF V600E tumors may respond to BRAF inhibitors such as vemurafenib, but comprehensive clinical trials are still ongoing.”^[95]

SUMMARY

There is enough research to show that *BRAF* V600 variant testing can improve health outcomes for some melanoma patients by helping them to select targeted treatment. In addition, clinical guidelines based on research recommend treatment with these BRAF inhibitors in patients with a V600 *BRAF* variant. Therefore, *BRAF* V600 variant testing that uses a test approved by the U.S. Food and Drug Administration (FDA) may be considered medically necessary to select melanoma patients for treatment with FDA-approved BRAF inhibitors, when policy criteria are met. Testing for *BRAF* V600 variants for all other patients with melanoma is considered investigational.

There is not enough research to show that genetic testing for targeted treatment with BRAF or MEK inhibitors can improve survival and other health outcomes for patients with glioma. In addition, there are no clinical guidelines based on research that recommend such testing. Therefore, testing for *BRAF* V600 variants for patients with glioma is considered investigational.

REFERENCES

1. American Cancer Society (ACS). Cancer Facts and Figures. [cited 7/3/2018]; Available from: <http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2015/index>
2. Gogas, HJ, Kirkwood, JM, Sondak, VK. Chemotherapy for metastatic melanoma: time for a change? *Cancer*. 2007 Feb 1;109(3):455-64. PMID: 17200963
3. Ribas, A, Puzanov, I, Dummer, R, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. *The Lancet Oncology*. 2015 Aug;16(8):908-18. PMID: 26115796
4. Maio, M, Grob, JJ, Aamdal, S, et al. Five-year survival rates for treatment-naive patients with advanced melanoma who received ipilimumab plus dacarbazine in a phase III trial. *J Clin Oncol*. 2015 Apr 01;33(10):1191-6. PMID: 25713437
5. Robert, C, Long, GV, Brady, B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med*. 2015 Jan 22;372(4):320-30. PMID: 25399552
6. Robert, C, Thomas, L, Bondarenko, I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med*. 2011 Jun 30;364(26):2517-26. PMID: 21639810
7. Weber, JS, D'Angelo, SP, Minor, D, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate

- 037): a randomised, controlled, open-label, phase 3 trial. *The Lancet Oncology*. 2015 Apr;16(4):375-84. PMID: 25795410
8. National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology™. Melanoma v.2.2018. [cited 7/3/2018]; Available from: http://www.nccn.org/professionals/physician_gls/pdf/melanoma.pdf
 9. Vultur, A, Villanueva, J, Herlyn, M. Targeting BRAF in advanced melanoma: a first step toward manageable disease. *Clin Cancer Res*. 2011 Apr 1;17(7):1658-63. PMID: 21447722
 10. Bollag, G, Hirth, P, Tsai, J, et al. Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. *Nature*. 2010 Sep 30;467(7315):596-9. PMID: 20823850
 11. Sondergaard, JN, Nazarian, R, Wang, Q, et al. Differential sensitivity of melanoma cell lines with BRAFV600E mutation to the specific Raf inhibitor PLX4032. *J Transl Med*. 2010;8:39. PMID: 20406486
 12. Joseph, EW, Pratilas, CA, Poulikakos, PI, et al. The RAF inhibitor PLX4032 inhibits ERK signaling and tumor cell proliferation in a V600E BRAF-selective manner. *Proc Natl Acad Sci U S A*. 2010 Aug 17;107(33):14903-8. PMID: 20668238
 13. Yang, H, Higgins, B, Kolinsky, K, et al. RG7204 (PLX4032), a selective BRAFV600E inhibitor, displays potent antitumor activity in preclinical melanoma models. *Cancer Res*. 2010 Jul 1;70(13):5518-27. PMID: 20551065
 14. King, AJ, Patrick, DR, Batorsky, RS, et al. Demonstration of a genetic therapeutic index for tumors expressing oncogenic BRAF by the kinase inhibitor SB-590885. *Cancer Res*. 2006 Dec 1;66(23):11100-5. PMID: 17145850
 15. Takle, AK, Brown, MJ, Davies, S, et al. The identification of potent and selective imidazole-based inhibitors of B-Raf kinase. *Bioorg Med Chem Lett*. 2006 Jan 15;16(2):378-81. PMID: 16260133
 16. Novartis. Tafinlar (dabrafenib) capsules prescribing information. June 2017. [cited 7/3/2018]; Available from: <https://www.pharma.us.novartis.com/sites/www.pharma.us.novartis.com/files/tafinlar.pdf>
 17. Rubinstein, JC, Sznol, M, Pavlick, AC, et al. Incidence of the V600K mutation among melanoma patients with BRAF mutations, and potential therapeutic response to the specific BRAF inhibitor PLX4032. *J Transl Med*. 2010;8:67. PMID: 20630094
 18. Novartis. Mekinist (trametinib) tablets prescribing information, June 2017. [cited 7/3/2018]; Available from: <https://www.pharma.us.novartis.com/sites/www.pharma.us.novartis.com/files/mekinist.pdf>
 19. Genentech: Cobimetinib press release. [cited 7/3/2018]; Available from: <http://www.gene.com/media/press-releases/14611/2015-11-10/fda-approves-genentechs-cotellic-cobimet>
 20. Exelixis: Cobimetinib. [cited 7/3/2018]; Available from: <https://www.exelixis.com/our-medicines/>
 21. Bristol-Myers Squibb: Nivolumab. [cited 7/3/2018]; Available from: <http://www.opdivohcp.com/metastatic-melanoma/efficacy/clinical-trial-results>
 22. Central Brain Tumor Registry of the United States. 2016 CBTRUS Fact Sheet. [cited 7/3/2018]; Available from: <http://www.cbtrus.org/factsheet/factsheet.html>
 23. Louis, DN, Perry, A, Reifenberger, G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta neuropathologica*. 2016 Jun;131(6):803-20. PMID: 27157931

24. Chien, LN, Gittleman, H, Ostrom, QT, et al. Comparative Brain and Central Nervous System Tumor Incidence and Survival between the United States and Taiwan Based on Population-Based Registry. *Front Public Health*. 2016;4(151). PMID:
25. Dougherty, MJ, Santi, M, Brose, MS, et al. Activating mutations in BRAF characterize a spectrum of pediatric low-grade gliomas. *Neuro-oncology*. 2010 Jul;12(7):621-30. PMID: 20156809
26. Schindler, G, Capper, D, Meyer, J, et al. Analysis of BRAF V600E mutation in 1,320 nervous system tumors reveals high mutation frequencies in pleomorphic xanthoastrocytoma, ganglioglioma and extra-cerebellar pilocytic astrocytoma. *Acta neuropathologica*. 2011 Mar;121(3):397-405. PMID: 21274720
27. Myung, JK, Cho, H, Park, CK, Kim, SK, Lee, SH, Park, SH. Analysis of the BRAF(V600E) Mutation in Central Nervous System Tumors. *Translational oncology*. 2012 Dec;5(6):430-6. PMID: 23323158
28. Zhang, J, Wu, G, Miller, CP, et al. Whole-genome sequencing identifies genetic alterations in pediatric low-grade gliomas. *Nature genetics*. 2013 Jun;45(6):602-12. PMID: 23583981
29. Horbinski, C, Nikiforova, MN, Hagenkord, JM, Hamilton, RL, Pollack, IF. Interplay among BRAF, p16, p53, and MIB1 in pediatric low-grade gliomas. *Neuro-oncology*. 2012 Jun;14(6):777-89. PMID: 22492957
30. Forshev, T, Tatevossian, RG, Lawson, AR, et al. Activation of the ERK/MAPK pathway: a signature genetic defect in posterior fossa pilocytic astrocytomas. *The Journal of pathology*. 2009 Jun;218(2):172-81. PMID: 19373855
31. Behling, F, Barrantes-Freer, A, Skardelly, M, et al. Frequency of BRAF V600E mutations in 969 central nervous system neoplasms. *Diagnostic pathology*. 2016 Jun 27;11(1):55. PMID: 27350555
32. Dummer, R, Goldinger, SM, Turttschi, CP, et al. Vemurafenib in patients with BRAF(V600) mutation-positive melanoma with symptomatic brain metastases: final results of an open-label pilot study. *Eur J Cancer*. 2014 Feb;50(3):611-21. PMID: 24295639
33. Long, GV, Trefzer, U, Davies, MA, et al. Dabrafenib in patients with Val600Glu or Val600Lys BRAF-mutant melanoma metastatic to the brain (BREAK-MB): a multicentre, open-label, phase 2 trial. *The Lancet Oncology*. 2012 Nov;13(11):1087-95. PMID: 23051966
34. Food and Drug Administration (FDA). Draft guidance for industry and food and drug administration staff: in vitro companion diagnostic devices. August 2014. [cited 7/3/2018]; Available from: <http://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm262327.pdf>
35. Kim, G, McKee, AE, Ning, YM, et al. FDA approval summary: vemurafenib for treatment of unresectable or metastatic melanoma with the BRAFV600E mutation. *Clin Cancer Res*. 2014;20:4994-5000. PMID: 25096067
36. Food and Drug Administration (FDA). Companion diagnostic devices: in vitro and imaging tools. [cited 7/3/2018]; Available from: <http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm>
37. Genentech, Inc. Zelboraf® (vemurafenib) tablet prescribing information. [cited 7/3/2018]; Available from: <http://www.zelboraf.com>

38. Raedler, LA. Opdivo (Nivolumab): Second PD-1 Inhibitor Receives FDA Approval for Unresectable or Metastatic Melanoma. *American health & drug benefits*. 2015 Mar;8(Spec Feature):180-3. PMID: 26629287
39. U.S. Food and Drug Administration (FDA): Cobimetinib. [cited 7/3/2018]; Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2015/206192Orig1s000Approv.pdf
40. NIH National Cancer Institute; FDA Approval for Dabrafenib;. [cited 7/3/2018]; Available from: <https://www.cancer.gov/about-cancer/treatment/drugs/fda-dabrafenib>
41. Bristol-Myers Squibb. Press Release. FDA Approves Yervoy (ipilimumab) for the Treatment of Patients with Newly Diagnosed or Previously-Treated Unresectable or Metastatic Melanoma, the Deadliest Form of Skin Cancer. [cited 7/3/2018]; Available from: <https://news.bms.com/press-release/rd-news/fda-approves-yervoy-ipilimumab-treatment-patients-newly-diagnosed-or-previous>
42. TEC Assessment "Special Report: Companion Diagnostics - Example of *BRAF* Gene Mutation Testing to Select Patients with Melanoma for Treatment with BRAF Kinase Inhibitors." BlueCross BlueShield Association Technology Evaluation Center, Vol. 26, Tab 7.
43. Teutsch, SM, Bradley, LA, Palomaki, GE, et al. The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Initiative: methods of the EGAPP Working Group. *Genet Med*. 2009 Jan;11(1):3-14. PMID: 18813139
44. Chapman, PB, Hauschild, A, Robert, C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med*. 2011 Jun 30;364(26):2507-16. PMID: 21639808
45. Ribas, A, Kim, KB, Schuchter, LM, et al. BRIM-2: An open-label, multicenter phase II study of vemurafenib in previously treated patients with BRAF V600E mutation-positive metastatic melanoma. *J Clin Oncol*. 2011;29(Suppl):Abstract 8509. PMID: No PMID Entry
46. Luke, JJ, Hodi, FS. Vemurafenib and BRAF inhibition: a new class of treatment for metastatic melanoma. *Clin Cancer Res*. 2012 Jan 1;18(1):9-14. PMID: 22083257
47. Halait, H, Demartin, K, Shah, S, et al. Analytical performance of a real-time PCR-based assay for V600 mutations in the BRAF gene, used as the companion diagnostic test for the novel BRAF inhibitor vemurafenib in metastatic melanoma. *Diagnostic molecular pathology : the American journal of surgical pathology, part B*. 2012 Mar;21(1):1-8. PMID: 22306669
48. Anderson, S, Bloom, KJ, Vallera, DU, et al. Multisite analytic performance studies of a real-time polymerase chain reaction assay for the detection of BRAF V600E mutations in formalin-fixed, paraffin-embedded tissue specimens of malignant melanoma. *Archives of pathology & laboratory medicine*. 2012 Nov;136(11):1385-91. PMID: 22332713
49. Sosman, JA, Kim, KB, Schuchter, L, et al. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N Engl J Med*. 2012 Feb 23;366(8):707-14. PMID: 22356324
50. Food and Drug Administration (FDA). THxID™-BRAF kit for use on the ABI 7500 Fast Dx Real-Time PCR Instrument - P120014. BioMérieux labeling, May 2013. [cited 7/3/2018]; Available from: https://www.accessdata.fda.gov/cdrh_docs/pdf12/P120014c.pdf
51. Anwar, MA, Murad, F, Dawson, E, Abd Elmageed, ZY, Tsumagari, K, Kandil, E. Immunohistochemistry as a reliable method for detection of BRAF-V600E mutation in

- melanoma: a systematic review and meta-analysis of current published literature. *The Journal of surgical research*. 2016 Jun 15;203(2):407-15. PMID: 27363650
52. O'Brien, O, Lyons, T, Murphy, S, Feeley, L, Power, D, Heffron, C. BRAF V600 mutation detection in melanoma: a comparison of two laboratory testing methods. *Journal of clinical pathology*. 2017 Apr 19. PMID: 28424234
 53. Huang, WK, Kuo, TT, Wu, CE, et al. A comparison of immunohistochemical and molecular methods used for analyzing the BRAF V600E gene mutation in malignant melanoma in Taiwan. *Asia-Pacific journal of clinical oncology*. 2016 Dec;12(4):403-8. PMID: 27488807
 54. Larkin, J, Lao, CD, Urba, WJ, et al. Efficacy and Safety of Nivolumab in Patients With BRAF V600 Mutant and BRAF Wild-Type Advanced Melanoma: A Pooled Analysis of 4 Clinical Trials. *JAMA oncology*. 2015;1:433-40. PMID: 26181250
 55. Larkin, J, Minor, D, D'Angelo, S, et al. Overall Survival in Patients With Advanced Melanoma Who Received Nivolumab Versus Investigator's Choice Chemotherapy in CheckMate 037: A Randomized, Controlled, Open-Label Phase III Trial. *J Clin Oncol*. 2017 Jul 03;JCO2016718023. PMID: 28671856
 56. Larkin, J, Chiarion-Sileni, V, Gonzalez, R, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med*. 2015 Jul 2;373(1):23-34. PMID: 26027431
 57. Schadendorf, D, Larkin, J, Wolchok, J, et al. Health-related quality of life results from the phase III CheckMate 067 study. *Eur J Cancer*. 2017 Sep;82:80-91. PMID: 28651159
 58. Hazarika, M, Chuk, MK, Theoret, MR, et al. U.S. FDA Approval Summary: Nivolumab for Treatment of Unresectable or Metastatic Melanoma Following Progression on Ipilimumab. *Clin Cancer Res*. 2017 Jul 15;23(14):3484-8. PMID: 28087644
 59. Beaver, JA, Theoret, MR, Mushti, S, et al. FDA Approval of Nivolumab for the First-Line Treatment of Patients with BRAFV600 Wild-Type Unresectable or Metastatic Melanoma. *Clin Cancer Res*. 2017 Jul 15;23(14):3479-83. PMID: 28073844
 60. Chapman, PB, Robert, C, Larkin, J, et al. Vemurafenib in patients with BRAFV600 mutation-positive metastatic melanoma: final overall survival results of the randomized BRIM-3 study. *Annals of oncology : official journal of the European Society for Medical Oncology*. 2017 Oct 1;28(10):2581-7. PMID: 28961848
 61. Puzanov, I, Amaravadi, RK, McArthur, GA, et al. Long-term outcome in BRAF(V600E) melanoma patients treated with vemurafenib: Patterns of disease progression and clinical management of limited progression. *Eur J Cancer*. 2015 Jul;51(11):1435-43. PMID: 25980594
 62. Blank, CU, Larkin, J, Arance, AM, et al. Open-label, multicentre safety study of vemurafenib in 3219 patients with BRAFV600 mutation-positive metastatic melanoma: 2-year follow-up data and long-term responders' analysis. *Eur J Cancer*. 2017 Jul;79:176-84. PMID: 28501764
 63. Flaherty, KT, Puzanov, I, Kim, KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med*. 2010 Aug 26;363(9):809-19. PMID: 20818844
 64. Hauschild, A, Grob, JJ, Demidov, LV, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *Lancet*. 2012 Jul 28;380(9839):358-65. PMID: 22735384
 65. Flaherty, KT, Robert, C, Hersey, P, et al. Improved survival with MEK inhibition in BRAF-mutated melanoma. *N Engl J Med*. 2012 Jul 12;367(2):107-14. PMID: 22663011

66. Long, GV, Weber, JS, Infante, JR, et al. Overall Survival and Durable Responses in Patients With BRAF V600-Mutant Metastatic Melanoma Receiving Dabrafenib Combined With Trametinib. *J Clin Oncol*. 2016;34:871-8. PMID: 26811525
67. Menzies, AM, Wilmott, JS, Drummond, M, et al. Clinicopathologic features associated with efficacy and long-term survival in metastatic melanoma patients treated with BRAF or combined BRAF and MEK inhibitors. *Cancer*. 2015 Nov 01;121(21):3826-35. PMID: 26218930
68. Schadendorf, D, Long, GV, Stroiakovski, D, et al. Three-year pooled analysis of factors associated with clinical outcomes across dabrafenib and trametinib combination therapy phase 3 randomised trials. *Eur J Cancer*. 2017 Sep;82:45-55. PMID: 28648698
69. Johnson, AS, Crandall, H, Dahlman, K, Kelley, MC. Preliminary results from a prospective trial of preoperative combined BRAF and MEK-targeted therapy in advanced BRAF mutation-positive melanoma. *Journal of the American College of Surgeons*. 2015 Apr;220(4):581-93 e1. PMID: 25797743
70. Schreuer, M, Jansen, Y, Planken, S, et al. Combination of dabrafenib plus trametinib for BRAF and MEK inhibitor pretreated patients with advanced BRAFV600-mutant melanoma: an open-label, single arm, dual-centre, phase 2 clinical trial. *The Lancet Oncology*. 2017 Apr;18(4):464-72. PMID: 28268064
71. Robert, C, Karaszewska, B, Schachter, J, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. *N Engl J Med*. 2015 Jan 01;372(1):30-9. PMID: 25399551
72. Schadendorf, D, Amonkar, MM, Stroyakovskiy, D, et al. Health-related quality of life impact in a randomised phase III study of the combination of dabrafenib and trametinib versus dabrafenib monotherapy in patients with BRAF V600 metastatic melanoma. *Eur J Cancer*. 2015 May;51(7):833-40. PMID: 25794603
73. Long, GV, Flaherty, KT, Stroyakovskiy, D, et al. Dabrafenib plus trametinib versus dabrafenib monotherapy in patients with metastatic BRAF V600E/K-mutant melanoma: long-term survival and safety analysis of a phase 3 study. *Annals of oncology : official journal of the European Society for Medical Oncology*. 2017 May 05. PMID: 28475671
74. Long, GV, Stroyakovskiy, D, Gogas, H, et al. Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma: a multicentre, double-blind, phase 3 randomised controlled trial. *Lancet*. 2015 Aug 1;386(9992):444-51. PMID: 26037941
75. Flaherty, KT, Infante, JR, Daud, A, et al. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *N Engl J Med*. 2012 Nov;367(18):1694-703. PMID: 23020132
76. Ascierto, PA, McArthur, GA, Dreno, B, et al. Cobimetinib combined with vemurafenib in advanced BRAF(V600)-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. *The Lancet Oncology*. 2016 Sep;17(9):1248-60. PMID: 27480103
77. Dreno, B, Ribas, A, Larkin, J, et al. Incidence, course, and management of toxicities associated with cobimetinib in combination with vemurafenib in the coBRIM study. *Annals of oncology : official journal of the European Society for Medical Oncology*. 2017 May 01;28(5):1137-44. PMID: 28444112
78. de la Cruz-Merino, L, Di Guardo, L, Grob, JJ, et al. Clinical features of serous retinopathy observed with cobimetinib in patients with BRAF-mutated melanoma treated in the randomized coBRIM study. *J Transl Med*. 2017 Jun 24;15(1):146. PMID: 28646893

79. Larkin, J, Ascierto, PA, Dreno, B, et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. *N Engl J Med*. 2014 Nov 13;371(20):1867-76. PMID: 25265494
80. Ribas, A, Gonzalez, R, Pavlick, A, et al. Combination of vemurafenib and cobimetinib in patients with advanced BRAF(V600)-mutated melanoma: a phase 1b study. *The Lancet Oncology*. 2014 Aug;15(9):954-65. PMID: 25037139
81. Dummer, R, Ascierto, PA, Gogas, HJ, et al. Encorafenib plus binimetinib versus vemurafenib or encorafenib in patients with BRAF-mutant melanoma (COLUMBUS): a multicentre, open-label, randomised phase 3 trial. *The Lancet Oncology*. 2018 May;19(5):603-15. PMID: 29573941
82. Amdahl, J, Chen, L, Delea, TE. Network Meta-analysis of Progression-Free Survival and Overall Survival in First-Line Treatment of BRAF Mutation-Positive Metastatic Melanoma. *Oncology and therapy*. 2016;4(2):239-56. PMID: 28261653
83. Devji, T, Levine, O, Neupane, B, Beyene, J, Xie, F. Systemic Therapy for Previously Untreated Advanced BRAF-Mutated Melanoma: A Systematic Review and Network Meta-Analysis of Randomized Clinical Trials. *JAMA oncology*. 2017 Mar 01;3(3):366-73. PMID: 27787543
84. Pasquali, S, Chiarion-Sileni, V, Rossi, CR, Mocellin, S. Immune checkpoint inhibitors and targeted therapies for metastatic melanoma: A network meta-analysis. *Cancer treatment reviews*. 2017 Mar;54:34-42. PMID: 28189914
85. Long, GV, Hauschild, A, Santinami, M, et al. Adjuvant Dabrafenib plus Trametinib in Stage III BRAF-Mutated Melanoma. *N Engl J Med*. 2017 Nov 9;377(19):1813-23. PMID: 28891408
86. Maio, M, Lewis, K, Demidov, L, et al. Adjuvant vemurafenib in resected, BRAF(V600) mutation-positive melanoma (BRIM8): a randomised, double-blind, placebo-controlled, multicentre, phase 3 trial. *The Lancet Oncology*. 2018 Apr;19(4):510-20. PMID: 29477665
87. Karajannis, MA, Legault, G, Fisher, MJ, et al. Phase II study of sorafenib in children with recurrent or progressive low-grade astrocytomas. *Neuro-oncology*. 2014 Oct;16(10):1408-16. PMID: 24803676
88. Hottinger, AF, Aissa, AB, Espeli, V, et al. Phase I study of sorafenib combined with radiation therapy and temozolomide as first-line treatment of high-grade glioma. *British journal of cancer*. 2014 May 27;110(11):2655-61. PMID: 24786603
89. Galanis, E, Anderson, SK, Lafky, JM, et al. Phase II study of bevacizumab in combination with sorafenib in recurrent glioblastoma (N0776): a north central cancer treatment group trial. *Clin Cancer Res*. 2013 Sep 01;19(17):4816-23. PMID: 23833308
90. Zustovich, F, Landi, L, Lombardi, G, et al. Sorafenib plus daily low-dose temozolomide for relapsed glioblastoma: a phase II study. *Anticancer research*. 2013 Aug;33(8):3487-94. PMID: 23898124
91. Den, RB, Kamrava, M, Sheng, Z, et al. A phase I study of the combination of sorafenib with temozolomide and radiation therapy for the treatment of primary and recurrent high-grade gliomas. *International journal of radiation oncology, biology, physics*. 2013 Feb 01;85(2):321-8. PMID: 22687197
92. Peereboom, DM, Ahluwalia, MS, Ye, X, et al. NABTT 0502: a phase II and pharmacokinetic study of erlotinib and sorafenib for patients with progressive or recurrent glioblastoma multiforme. *Neuro-oncology*. 2013 Apr;15(4):490-6. PMID: 23328813

93. Lee, EQ, Kuhn, J, Lamborn, KR, et al. Phase I/II study of sorafenib in combination with temsirolimus for recurrent glioblastoma or gliosarcoma: North American Brain Tumor Consortium study 05-02. *Neuro-oncology*. 2012 Dec;14(12):1511-8. PMID: 23099651
94. Hyman, DM, Puzanov, I, Subbiah, V, et al. Vemurafenib in Multiple Nonmelanoma Cancers with BRAF V600 Mutations. *N Engl J Med*. 2015 Aug 20;373(8):726-36. PMID: 26287849
95. National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology™. Central Nervous System Cancers v.1.2018. [cited 7/3/2018]; Available from: https://www.nccn.org/professionals/physician_gls/pdf/cns_blocks.pdf
96. BlueCross BlueShield Association Medical Policy Reference "BRAF Gene Mutation Testing To Select Melanoma Patients for BRAF Inhibitor Targeted Therapy." Policy No. 2.04.77

CODES

Codes	Number	Description
CPT	81210	BRAF (B-Raf proto-oncogene, serine/threonine kinase) (e.g., colon cancer, melanoma), gene analysis, V600 variant(s)
HCPCS	None	

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