Corporate Medical Policy

Genetic Testing and Genetic Expression Profiling in Patients with Cutaneous Melanoma AHS-M2029

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Description of Procedure or Service

Cutaneous melanoma is a common and serious form of skin cancer. Diagnosis of this type of cancer often involves visual examination of the skin by a dermatologist; however, due to the various presentations of nodules, it can be difficult to properly recognize a melanoma case. Although several visual systems have been developed to assist in diagnosis (such as the ABCDE system for signature features), a biopsy is often performed to diagnose a case (Swetter, 2020). Genetic testing (particularly gene expression panels) has been proposed to assist in diagnosing these cases without a biopsy (DermTech, 2020a).

Another application of genetic testing in cutaneous melanoma is for "targeted testing". Certain genetic mutations demonstrate better response to certain treatments, and it is therefore useful to identify these mutations so proper treatment can be delivered.

BRAF (V-raf murine sarcoma viral oncogene homolog B1) is a serine-threonine protein kinase involved in cell survival, proliferation, and differentiation (H. Davies et al., 2002; Tatsuno et al., 2016). The most common missense mutation of *BRAF* (mainly V600E) contributes to the incidence of various cancers, including melanoma (Flaherty et al., 2012). Up to half of cutaneous melanoma cases harbor a mutation in *BRAF*. Two other common mutations found in cutaneous melanoma cases are *NRAS* (up to 20% of cases) and *KIT* (up to 15% of cases); both mutations may have useful targeted therapies available for patients (Sosman, 2020).

Related Policies
KRAS, NRAS, and BRAF Mutation Analysis In Colorectal Cancer AHS- M2026
Testing for Targeted Therapy of Non-Small-Cell Lung Cancer AHS- M2030
Molecular Panel Testing of Cancers for Diagnosis, Prognosis, and Identification of Targeted Therapy AHS-M2109

******Note: This Medical Policy is complex and technical. For questions concerning the technical language and/or specific clinical indications for its use, please consult your physician.***

Policy

BCBSNC will provide coverage for genetic testing and genetic expression profiling in patients with cutaneous melanoma when it is determined the medical criteria or reimbursement guidelines below are met.

Benefits Application

This medical policy relates only to the services or supplies described herein. Please refer to the Member's Benefit Booklet for availability of benefits. Member's benefits may vary according to benefit design; therefore member benefit language should be reviewed before applying the terms of this medical policy.

Genetic Testing and Genetic Expression Profiling in Patients with Cutaneous Melanoma AHS-M2029

When Genetic Testing and Genetic Expression Profiling in Patients with Cutaneous Melanoma is covered

Testing of tumor tissue for BRAF V600, KIT, and NRAS mutation analysis is considered **medically necessary** for individuals with stage III or stage IV melanoma, prior to initiation of molecular-targeted treatment.

When Genetic Testing and Genetic Expression Profiling in Patients with Cutaneous Melanoma is not covered

Genetic expression profiling testing (eg. DecisionDx Melanoma, DermTechPLA) for cutaneous melanoma is considered **investigational**.

Testing for BRAF V600, KIT, NRAS and other mutations in other forms or stages of melanoma* (Please see note below) is considered **investigational**.

BRAF, KIT, and NRAS testing of the primary cutaneous melanoma is considered **investigational** unless required to guide systemic therapy.

*Note: For testing of 5 or more genes for an affected individual with cutaneous melanoma, please refer to AHS-M2109 Molecular Panel Testing of Cancers for Diagnosis, Prognosis, and Identification of Targeted Therapy.

Policy Guidelines

Melanoma is particularly lethal and aggressive, with the ability to metastasize to any organ (Leong et al., 2011). Cutaneous tumors as little as 1 mm in thickness are capable of lymph node metastasis (Stage III) which results in a significant decrease in the 5-year survival rate from 90% to 56% (Yee et al., 2005). Spread beyond the lymph nodes (Stage IV) results in an even more dramatic decrease in 5-year survival to 15%. (Grossmann, Grossmann, & Wallander, 2012)

BRAF, KIT, PIK3CA and *NRAS* mutations are commonly seen in melanoma cases (Alrabadi et al., 2019; Lokhandwala et al., 2019) with an estimated 50% of melanomas exhibiting the *BRAF* V600E mutation (Burjanivova et al., 2019). BRAF is a member of the RAF family of protein serine/threonine kinases (ARAF, BRAF, CRAF) that is activated by Ras proteins during intracellular signaling cascades. Mutations in *BRAF* appear to be the most common genetic alteration in melanoma (Hocker & Tsao, 2007) and occur more frequently in melanoma than lung, colon, and ovarian carcinoma (Grossmann et al., 2012). More than 30 mutations of the *BRAF* gene associated with human cancers have been identified (Siroy et al., 2015). In 90% of the cases, thymine is substituted with adenine at nucleotide 1799. This leads to valine (V) being substituted for by glutamate (E) at codon 600 (now referred to as V600E) in the kinase domain (Tan et al., 2008). Importantly, *BRAF* activating mutations occur in up to 80% of benign nevi or moles and, therefore, cannot be used to distinguish benign from malignant melanocytic lesions (Grossmann et al., 2012; Poynter et al., 2006).

Table 1. Frequency of melanoma subtypes with activating genetic alterations in BRAF and KIT (Grossmann et al., 2012)

Aberration	Cutaneous −CSD	Cutaneous + CSD	ALM	MM
BRAFa mutation	53%	8–11%	10%	15%
KIT mutation; amplification	0	17%	11–38%; 19–27%	6–19%; 20–33%

A variety of methods are utilized for *BRAF* and *KIT* mutational analysis testing in melanoma, which has resulted in no standardized procedures for testing. Due to the fact that numerous techniques are available and updated methods continue to be released, labs have been reluctant to switch *BRAF* platforms to accommodate one specific drug for one disease (Grossmann et al., 2012). Current *BRAF* genetic testing methods include *BRAF* V600E by real-time PCR, *BRAF* (V600E) mutation only by Sanger sequencing, *BRAF* full gene sequence analysis, and *BRAF* next generation sequencing (CMGL, 2014).

However, a recent study found good overall compliance of labs with the College of American Pathologists (CAP) (Cree, 2014) and National Comprehensive Cancer Network (NCCN) guidelines for molecular diagnosis of tumors (Volmar, Idowu, Souers, & Nakhleh, 2015) despite not using the specific FDA-approved test.

Table 2. Molecular Testing Adherence to NCCN Guidelines (taken from (Volmar et al., 2015))

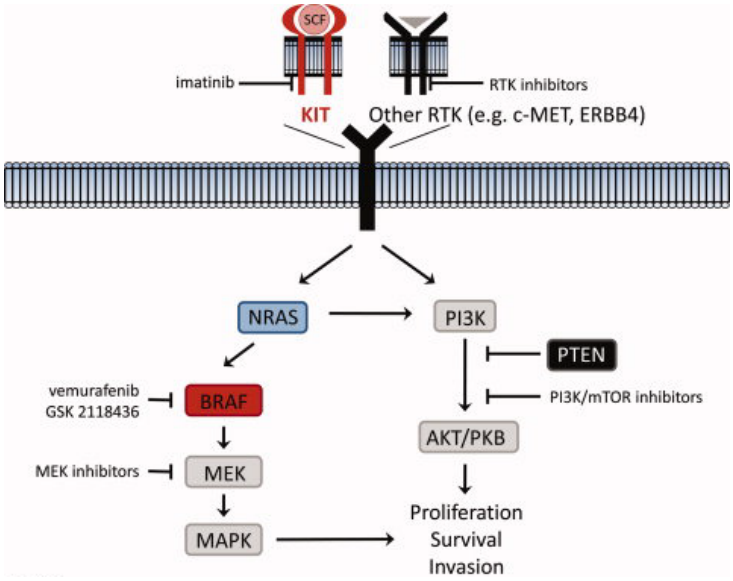
	All Institutions Percentiles					
	n	**10th**	**25th**	**Median**	**75th**	
Retrospective study (lung, colorectal, melanoma)						
Percentage of tests that strictly meet the guideline	26	32.6	64.7	70.9	82.7	
Percentage of tests that at least loosely meet the guideline	26	57.4	90.7	95.1	98.9	
Prospective study (all case type)						
Percentage of tests that strictly meet the guideline	23	20.0	31.4	53.3	66.7	
Percentage of tests that at least loosely meet the guideline	23	75.0	87.0	94.3	100.0	

Genetic testing is important to determine the most efficient treatment method for a melanoma patient. Despite the high frequency in nevi, the role of *BRAF* mutations in oncogenesis is well established (Davies & Gershenwald, 2011) and has been confirmed in clinical trials (Flaherty et al., 2010). The remarkable efficacy of *BRAF* inhibitors led to the accelerated approval of vemurafenib for unresectable and metastatic

melanoma. Importantly, *BRAF* mutation testing is warranted for determining therapeutic eligibility as selective *BRAF* inhibitors pose significant risk of cutaneous squamous cell carcinoma and have the potential to increase disease progression in *BRAF* wild type (mutation negative) tumors (Grossmann et al., 2012).

Historically, systemic therapy for metastatic melanoma provided very low response rates and little to no benefit in overall survival (Atkins, Kunkel, Sznol, & Rosenberg, 2000; Tsao, Atkins, & Sober, 2004). Recently, the immune-boosting anti-CTLA-4 antibody ipilimumab (Hodi et al., 2010) and testing and development of small molecule kinase (KIT and BRAF) inhibitors have yielded improvements in long-term survival (M. A. Davies & Gershenwald, 2011; Ribas & Flaherty, 2011; Woodman & Davies, 2010).

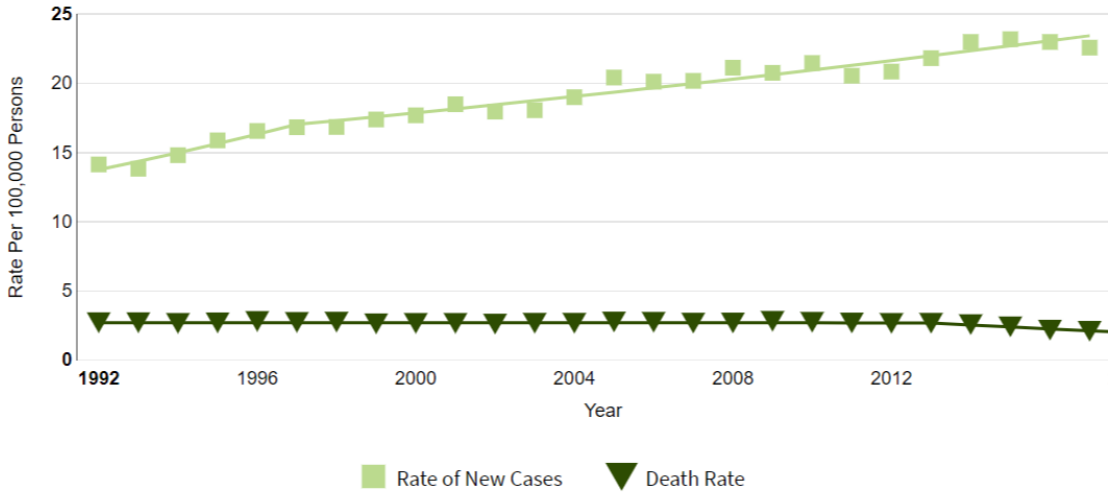
Figure 1: Imatinib and RTK Inhibitor Pathways for Melanoma Treatment (image taken from (Grossmann et al., 2012))



The incidence of melanoma in the United States appears to be increasing rapidly over the past few decades (NCI, 2020). The lifetime risk for the general population of developing melanoma is 1 in 55 (NCI, 2020) and that risk has increased approximately 2% annually since 1960 (Rashid & Zager, 2015). The American Cancer Society now reports that 2.6% of Caucasian Americans, 0.1% of African Americans and 0.6% of Hispanics will develop melanoma in America each year; approximately 100,350 Americans in total will be diagnosed with melanoma in 2020 (ACS, 2020).

In 2017, the number of new cases of melanoma of the skin per 100,000 people was 22.7; this accounted for 5.6% of all new cancer cases. Further, the number of melanoma patient deaths in 2017 was 2.3 per 100,000 individuals, accounting for 1.1% of all cancer deaths in United States (NCI, 2020).

Figure 2. New Cases and Deaths of Melanoma of the Skin per 100,000 Persons (taken from (NCI, 2020))



Clinical Validity

Burjanivova et al. (2019); Corean, George, Patel, and Li (2019); McEvoy et al. (2018) analyzed a total of 113 samples from patients with malignant melanoma; the authors state, "The aims of our study were to detect *BRAF* V600E mutations within circulating cell-free DNA in plasma ("liquid biopsy") by a droplet digital PCR (ddPCR) method." A *BRAF* V600E mutation was identified in 37/113 samples, showing that this method is "highly sensitive" in the detection of *BRAF* V600E mutations and may be used for both mutation detection and treatment monitoring (Burjanivova et al., 2019). A second team of researchers also used ddPCR to identify melanoma mutations including *BRAF*, *NRAS*, and *TERT*; results were compared to both Sanger sequencing and pyrosequencing methods (McEvoy et al., 2018). Overall, ddPCR was found to be more sensitive in detecting mutations than the aforementioned testing methods with an increased sensitivity "more apparent among tumors with <50% tumor cellularity" (McEvoy et al., 2018).

O'Brien et al. (2017) analyzed samples to determine if *BRAF* mutation identification by immunohistochemistry was a suitable alternative to PCR; 132 patients were included in this study, and the anti-*BRAF* V600E VE1 clone antibody was used for immunohistochemistry detection. A sensitivity of 86.1% and specificity of 96.9% was shown with the anti-*BRAF* V600E VE1 clone antibody; "The concordance rate between PCR and immunohistochemical BRAF status was 95.1% (116/122) (O'Brien et al., 2017)." As both methods were in high agreement, immunohistochemistry may be a viable alternative to PCR for *BRAF* mutation testing.

Corean et al. (2019) utilized several different techniques on metastatic melanoma samples, including bone marrow morphology, histology, immunophenotyping, molecular genetic testing and *BRAF* V600E immunohistochemistry. *BRAF* immunohistochemistry was detected in two patients, and molecular testing confirmed these results; researchers then stated that "*BRAF* V600E immunohistochemistry is useful as a surrogate marker of molecular results," once again highlighting the fact that immunohistochemistry may be a viable alternative for *BRAF* mutation testing (Corean et al., 2019).

Available *BRAF* tests and analytical sensitivities:

- "The *BRAF* V600E by real-time PCR test uses a TaqMan® Mutation Detection Assay to detect the V600E mutation in exon 15 of *BRAF* in tumor (somatic) cells. The sensitivity of the TaqMan assay is ~0.1% mutant DNA in a wild-type background. Poor DNA quality, insufficient DNA quantity or the presence of PCR inhibitors can result in uninterpretable or (rarely) inaccurate results.

- The *BRAF* (V600E) mutation only by Sanger sequencing uses a DNA-based PCR-sequencing assay to detect the V600E in exon 15 of *BRAF*. The limit of detection for Sanger sequencing is >20% mutant DNA in a wild-type background.
- The *BRAF* full gene sequence analysis test uses a DNA-based PCR-sequencing assay to detect point mutations in the coding sequence and intron/exon boundaries of the *BRAF* gene. The sensitivity of DNA sequencing is over 99% for the detection of nucleotide base changes, small deletions and insertions in the regions analyzed. Rare variants at primer binding sites may lead to erroneous results. The limit of detection for Sanger sequencing is >20% mutant DNA in a wild-type background (CMGL, 2014)."
- The *BRAF* NGS with TruSeq had sensitivity of over 99% (Froyen et al., 2016).

Colombino et al. (2020) compared *BRAF* mutation testing performed with conventional nucleotide sequencing approaches (Sanger sequencing and pyrosequencing) with real-time polymerase chain reaction (RT-PCR) or next-generation sequencing (NGS) assays to assess the levels of concordance between these various techniques. 319 tissue samples were analyzed and initially screened with conventional approaches. The initial screen found pathogenic *BRAF* mutations in 144 (45.1%) cases. RT-PCR (Idylla™ *BRAF* mutation assay) detected 11 (16.2%) and 3 (4.8%) additional *BRAF* mutations after Sanger sequencing and pyrosequencing, respectively. NGS detected one additional *BRAF*-mutated case (2.1%) among 48 wild-type cases previously tested with pyrosequencing and RT-PCR. According to the data, RT-PCR is more accurate than both Sanger sequencing and pyrosequencing in detecting *BRAF* mutations. Overall, RT-PCR had a good concordance with the other tests; 60/61 (98.4%) RT-PCR tests confirmed the presence of the same *BRAF* mutation identified by the sequencing assay. "Real-time PCR is a rapid method which achieves the same maximum level of sensitivity of NGS (up to 98%), without requiring particular skills" (Colombino et al., 2020). Although NGS can provide a detailed evaluation with a high diagnostic sensitivity, the interpretation of sequencing data may be complex, requires a high level of expertise, and makes it difficult to apply in the clinical practice. "Sanger-based direct sequencing achieves the highest specificity (100%) and can detect all sequence mutations in *BRAF* exons, but it presents the lowest diagnostic sensitivity (80–85%). Pyrosequencing is a simple-to-perform method and provides a good level of sensitivity (92–95%), but it does not achieve a complete mutation coverage specificity (up to 90%) (Colombino et al., 2020)." According to the author, "[RT-PCR and NGS] improved the diagnostic accuracy of *BRAF* testing via the detection of additional *BRAF* mutations in a subset of false-negative cases previously tested with Sanger sequencing or pyrosequencing. In attendance of further confirmations in larger prospectively designed studies, the use of two sensitive molecular methods may ensure the highest level of diagnostic accuracy (Colombino et al., 2020)."

Clinical Utility

BRAF analysis is an accepted medical practice for patients with unresectable, metastatic stage IV melanoma (NCCN, 2018). However, recent randomized controlled trials have indicated the benefits of expanding *BRAF* analysis. A study published in The Lancet Oncology by Amaria et al. (2018) compared standard of care in patients with high-risk, surgically resectable melanoma (stage III or IV) to similar patients receiving a regimen of a neoadjuvant plus adjuvant dabrafenib and trametinib. All patients had to be of confirmed *BRAF* V600E or *BRAF* V600K status to participate in either the control or experimental groups. In the follow-up (median of 18.6 months), 10/14 (or 71%) of patients in the experimental group remained event-free (i.e. alive without disease progression), whereas 0/7 (0%) of the control group receiving standard of care remained event-free. The authors conclude that the "Neoadjuvant plus adjuvant dabrafenib and trametinib significantly improved event-free survival versus standard of care in patients with high-risk, surgically resectable, clinical stage III-IV melanoma (Amaria et al., 2018)."

Another study published by Zippel et al. (2017) researched the use of perioperative *BRAF* inhibitors on patients with stage III melanoma. All patients had to be confirmed *BRAF* V600E to participate in the study. Of the thirteen patients, twelve "patients showed a marked clinical responsiveness to medical treatment, enabling a macroscopically successful resection in all cases"; moreover, "at a median follow up of 20

months, 10 patients remain free of disease" (Zippel et al., 2017). One patient died prior to surgery in this study. The authors conclude that the "Perioperative treatment with *BRAF* inhibiting agents in *BRAF*V600E mutated Stage III melanoma patients facilitates surgical resection and affords satisfactory disease free (sic) survival (Zippel et al., 2017)."

Gene Expression Profiling for Diagnosis and Prognosis

Currently, patients presenting with a suspicious pigmented lesion undergo excisional biopsy which is then subjected to histopathologic examination by a pathologist (NCCN, 2020c). The majority of melanocytic neoplasms can be accurately classified by this approach; however, in some cases confidently differentiating benign melanocytic nevi from malignant melanoma can be extremely difficult or impossible despite additions to histopathologic assessment, such as the evaluation of Breslow depth (Chiaravalloti, Jinna, Kerr, Whalen, & Grant-Kels, 2018; Lee & Lian, 2018). In these cases, even diagnoses from expert pathologists can be discordant (Elmore et al., 2017; Farmer, Gonin, & Hanna, 1996; Gerami et al., 2014) and subject to diagnostic drift (Bush, Hunt, & Fraga, 2015). A number of diagnostic and prognostic genetic tests for melanoma have been developed as ancillary tests to assist in this differentiation and resultant risk stratification (Lee & Lian, 2018), including microRNAs as biomarkers to distinguish between melanomas and nevi (Torres et al., 2019). In particular, gene expression profiling is growing in popularity for the diagnosis and prognosis of cutaneous melanoma. Molecular tests based on gene expression profiling of cutaneous melanoma are commercially available. They include 23-GEP MyPath Melanoma (Myriad, 2020), 2-GEP Pigmented Lesion Assay (DermTech, 2020a), and 31-GEP Decision-Dx Melanoma (Castle_Biosciences, 2020). Under clinical development is a clinicopathological and gene expression profile (CP-GEP) model by SkylineDx (SkylineDx, 2020).

Clinical Validity and Utility of Gene Expression Profiling

Gerami, Cook, Wilkinson, et al. (2015) developed a 28-gene signature for the identification of high-risk cutaneous melanoma tumors; this test accurately predicted metastasis risk in a multicenter cohort of primary cutaneous melanoma tumors by identifying genes that were upregulated in metastatic melanoma but not in primary melanoma. Metastatic risk was predicted with high accuracy in development (ROC = 0.93) and validation (ROC = 0.91) cohorts of primary cutaneous melanoma tumor tissue. The sensitivity was 100% and specificity of 78%; Kaplan–Meier analysis indicated that the 5-year disease-free survival (DFS) rates in the development set were 100% and 38% for predicted classes 1 and 2 cases, respectively ($P < 0.0001$) (Gerami, Cook, Wilkinson, et al., 2015). A second study by Gerami, Cook, et al. (2015b) found that the gene expression profile was a more accurate predictor than sentinel lymph node biopsy independently and also improved prognostication in combination with sentinel lymph node biopsy. A multi-center study (Zager et al., 2018) validated the prognostic accuracy in an independent cohort of cutaneous melanoma patients and found that the gene expression profile was a significant predictor of recurrence free survival and distant metastasis free survival in univariate analysis (hazard ratio [HR] = 5.4 and 6.6, respectively, $P < 0.001$ for each); this study also provided additional independent prognostic information to traditional staging which helps to estimate an individual's risk for cancer recurrence. A prospective evaluation of the gene expression profile's performance in 322 patients enrolled in two clinical trials found that patient outcomes from the combined prospective cohort supports the gene expression profile's ability to stratify early-stage cutaneous melanoma patients into two groups with significantly different metastatic risk; further, it was determined that survival outcomes in this real-world cohort are consistent with previously published analyses with retrospective specimens and that gene expression profile testing complements current clinicopathologic features and increases identification of high-risk patients (Hsueh et al., 2017).

myPath Melanoma (Myriad Genetics)

A 23-gene expression profile and algorithm that assigns various weights and thresholds of expression for each gene was developed to differentiate benign melanocytic nevi from malignant melanoma; this gene expression profile and algorithm was determined to have a sensitivity of 89% and specificity of 93% (Clarke et al., 2015). Further, three experienced dermatopathologists validated this method against an independent histopathologic evaluation; a sensitivity of 91.5% and a specificity of 92.5% was determined (Clarke, Flake,

et al., 2017). This gene expression profile was developed into a commercial test known as myPath Melanoma; this test generates a single positive or negative score where a negative number indicates a benign lesion and a positive number indicates melanoma with a reported sensitivity of 90-94% and specificity of 91-96% (Ko et al., 2017). MyPath Melanoma correlates closely with long-term clinical outcomes by adding valuable adjunctive information to aid in the diagnosis of melanoma. An examination of the utility of this test (Cockerell et al., 2017) found that the results of this gene expression signature have a significant clinical impact with 71.4% (55/77) of cases changing from pretest recommendations to actual treatment. The majority of changes were consistent with the test result. There was an 80.5% (33/41) reduction in the number of biopsy site re-excisions performed for cases with a benign test result. However, when more challenging samples were included (Minca et al., 2016) with 39 histopathologically unequivocal lesions (15 malignant, 24 benign) and 78 challenging lesions interpreted by expert consensus (27 favor malignant, 30 favor benign, and 21 ambiguous), myPath Melanoma had a lower sensitivity and specificity than fluorescence in situ hybridization (FISH) (FISH: 69% sensitivity, 91% specificity; myPath; 55% sensitivity, 88% specificity). In the unequivocal group, FISH and myPath score showed 97% and 83% agreement with the histopathologic diagnosis, respectively, with 93% and 62% sensitivity, 100% and 95% specificity, and 80% inter-test agreement. In the challenging group, FISH and the myPath score showed 70% and 64% agreement with the histopathologic interpretation, respectively, with 70% inter-test agreement. The myPath Melanoma testing method may have limited sensitivity in cases of desmoplastic melanoma, a rare fibrosing variant of melanoma (Clarke, Pimentel, Zalaznick, Wang, & Busam, 2017). The exclusion of melanocytic neoplasms that did not have a triple concordant diagnosis, and the lowered sensitivity and specificity when these sample types were included may significantly limit the applicability of this test in the most challenging diagnostic circumstances (Lee & Lian, 2018).

Pigmented Lesion Assay (DermTech)

DermTech has developed a pre-diagnostic quantitative polymerase chain reaction (qPCR)-based pigmented lesion assay (PLA) that measures the expression of two genes in the stratum corneum to assist with diagnostic or prognostic information for potential melanoma cases (Varedi et al., 2019). DermTech's PLA identifies malignant changes on a genomic level that cannot be detected with the human eye; this assay can be used to support clinicians in their decision to biopsy suspicious nevi. This test has the potential to increase the number of early melanomas biopsied and reduce the number of benign lesions biopsied, thereby improving patient outcomes (Ferris et al., 2017). A recent study has given this pigmented lesion assay a sensitivity of 91-96%, a specificity of 69-91%, and a negative predictive value of approximately 99% (Ferris, Rigel, et al., 2019).

To help support clinicians in their decision to biopsy, this noninvasive 2-gene expression assay of the *LINC00518* and *PRAME* genes has been developed for use on adhesive patch biopsies. Skin sampling via an adhesive patch allows for DNA, RNA, skin tissue and microbiome samples to be safely obtained and transported cost effectively by mail at room temperature; skin cells, T-cells, dendritic cells, melanocytes and other types of cells can be analyzed by this method (Yao, Moy, Allen, & Jansen, 2017). The use of an adhesive patch also allows 100% of the lesion to be sampled, compared to less than 1-2% of surgical biopsies (DermTech, 2020b). Further, this technique is a much more cost-effective option. Hornberger and Siegel (2018) report that PLA testing could save approximately $447 per lesion compared to traditional biopsies.

Additional researchers have determined the utility of this pigmented lesion assay for *LINC00518/PRAME* expression; using this assay, dermatologists improved their mean biopsy sensitivity from 95.0% to 98.6% ($P = .01$) and improved their specificity from 32.1% to 56.9% ($P < .001$) (Gerami et al., 2017). This result may increase the number of early melanomas biopsied and reduce the number of benign lesions biopsied, thereby improving patient outcomes and reducing health care costs (Ferris et al., 2017). An application study of 381 patients found that the estimated real-world sensitivity of the DermTech was 95% and specificity was 91% (Ferris et al., 2018); overall, 93% of PLA results positive for both *LINC00518* and *PRAME* were diagnosed histopathologically as melanoma. Further, this study was also used to identify if the real-world clinical use of the DermTech PLA could change physician behavior and reduce the overall number of biopsies performed. The PLA identified 51 PLA(+) test results, and 100% of these pigmented skin lesions were biopsied (37% were melanomas). Further, "Nearly all (99%) of 330 PLA(-) test results

were clinically managed with surveillance. None of the three follow-up biopsies performed in the following 3-6 months, were diagnosed as melanoma histopathologically (Ferris et al., 2018)." The PLA test altered clinical management of pigmented lesions and shows high clinical performance.

The PLA test has also been validated against driver mutations in melanoma, including *BRAF*, *NRAS*, and *TERT*. Ferris, Moy, et al. (2019) studied the mutation frequency of these genes using samples obtained from the PLA technique (e.g. adhesive patch) in both histopathologically confirmed melanomas (n = 30) and non-melanoma controls (n = 73). "The frequency of these hotspot mutations in samples of early melanoma was 77%, which is higher than the 14% found in nonmelanoma samples ($P < 0.0001$). *TERT* promoter mutations were the most prevalent mutation type in PLA-positive melanomas; 82% of PLA-negative lesions had no mutations, and 97% of histopathologically confirmed melanomas were PLA and/or mutation positive (Ferris, Moy, et al., 2019)." 86% of the non-melanomas within this validation cohort contained no mutations. The authors next analyzed 519 real-world PLA samples for the same mutations. Similar to the previous validation cohort, 88% of this larger cohort also contained no mutations., indicating that the PLA test can rule out lesions with few mutational risk factors for melanoma.

Another study by Ferris, Rigel, et al. (2019) followed up with patients who were given PLA(-) results for a year to determine the utility of this pigmented lesion assay; no lesions biopsied in the twelve month period were given a histopathologic diagnosis of melanoma, highlighting the accuracy of this technique. Further, Brouha et al. (2020) completed a large United States registry study which included the assessment of 3,148 suspicious pigmented skin lesions. All skin lesions were analyzed by PLA and were considered PLA(+) if *LINC* and/or *PRAME* was identified. All PLA(+) samples (9.48%) were surgically biopsied and analyzed. The PLA was found to have a negative predictive value >99% and reduced cost as well as biopsies by 90%; further, "97.53% of PLA(+) lesions were surgically biopsied, while 99.94% of PLA(-) cases were clinically monitored and not biopsied" (Brouha et al., 2020).

A recent report by Robinson and Jansen outlined a proof-of-concept pilot program of remote physician-guided self-sampling (i.e. telehealth administration of the adhesive patch) during the Illinois stay-at-home order of the COVID-19 pandemic. The authors also surveyed skin self-examination (SSE) anxiety as well. Two cohorts were used in this pilot, an experimental group (n=7), and a randomly selected physician-sampled control case group (n=10). The authors report that SSE-induced anxiety has increased during the COVID-19 pandemic. It should be noted that surveys were administered to much larger groups than those administering self-sampling tests. 258 surveys about SSE anxiety were conducted prior to the COVID-19 pandemic, and 211 surveys during the COVID-19 pandemic. The authors state, "Guided self-sampling led to molecular risk factor analyses in 7/7 (100%) of cases compared to 9/10 (90%) randomly selected physician-sampled control cases... Adhesive patch self-sampling under remote physician guidance is a viable specimen collection option (Robinson & Jansen, 2020)."

DecisionDx Melanoma (Castle Biosciences)

After a melanoma case has been identified, several management approaches may be considered. However, best melanoma management practices are constantly evolving. A common technique to assess the spread of a tumor, such as melanoma, is a sentinel lymph node biopsy (SLNB). This procedure is used to evaluate whether the cancer has spread beyond the original tumor site and into the lymphatic system. The lymphatic or lymph system transports fluid known as lymph throughout the body; this fluid contains white blood cells which help to fight infections. The lymphatic system also aids in ridding the body of other waste and toxins. The SLNB technique essentially helps the physician to stage the tumor. However, melanoma-related lymph node spread is very complex and is associated with many factors, including age, location, thickness, ulceration, gender and regression (Ribero et al., 2017).

The DecisionDx-Melanoma test is a gene expression profile (GEP) test that measures the expression of 31 different genes in a tumor tissue sample. This test was designed for cutaneous melanoma patients undergoing or considering SLNB and can help to identify the risk of cancer recurrence or metastasis in stage I-III melanoma (CastleBiosciences, 2020). DecisionDx-Melanoma may help physicians guide treatment options, including whether to perform a SLNB in eligible patients, and what type of follow up treatment is necessary (CastleBiosciences, 2020). After GEP analysis, the DecisionDx-Melanoma provides

a score of either Class 1 (low risk) or Class 2 (high risk), as well as A or B scores with "A" reflecting a better outcome and "B" reflecting a worse outcome; this allows physicians to receive final scores of Class 1A, 1B, 2A, and 2B (Gastman, Zager, et al., 2019).

The DecisionDx-Melanoma is performed on tumor tissue biopsies that have been preserved as formalin-fixed, paraffin-embedded (FFPE) samples (CastleBiosciences, 2020). Researchers have highlighted that the identification of tissue-based prognostic markers in melanoma has been a challenging obstacle for researchers, as "One major limitation is that most primary melanomas are preserved as formalin-fixed, paraffin-embedded (FFPE) samples rather than fresh-frozen tissues because of the small size of the specimens (Weiss, Hanniford, Hernando, & Osman, 2015)." High-quality genetic material is more challenging to extract from FFPE samples, and messenger RNA is inconsistent in FFPE samples (Weiss et al., 2015).

The prognostic utility of this test has been measured by Keller et al. (2019); a total of 159 patients participated in this study and were followed up with, on average, 44.9 months after initial testing. Gene expression profiling results helped to categorize patients into two groups: low-risk patients were placed in Class 1, and high-risk patients in Class 2; 117 patients were placed in Class 1 and 42 patients in Class 2 (Keller et al., 2019). Results showed that this gene expression profiling test had great prognostic abilities. "Gender, age, Breslow thickness, ulceration, SNB positivity, and AJCC stage were significantly associated with GEP classification ($P < 0.05$ for all). Recurrence and distant metastasis rates were 5% and 1% for Class 1 patients compared with 55% and 36% for Class 2 patients. Sensitivities of Class 2 and SNB for recurrence were 79% and 34%, respectively (Keller et al., 2019)."

Podlipnik et al. (2019) also studied the prognostic utility of this test in a similar way: patients were categorized into Class 1 (low risk) and Class 2 (high risk) based on the DecisionDx-Melanoma gene expression profile test results. Results showed that this testing method could correctly identify patients in accordance with the American Joint Committee on Cancer (AJCC) staging system for prognostic purposes. "We believe that gene expression profile in combination with the AJCC staging system could well improve the detection of patients who need intensive surveillance and optimize follow-up strategies (Podlipnik et al., 2019)."

Berman et al. (2019) gathered an expert panel of nine dermatologists/dermatologic surgeons/ dermatopathologists and completed 29 clinical scenarios in which gene expression tests could be used appropriately; several gene expression profiling (GEP) tests were used including a 2-GEP assay, 23-GEP assay and 31-GEP assay. "The 2-GEP assay for melanoma diagnosis received 1 B-strength and 6 C-strength recommendations. The 23-GEP diagnostic test received 1 A-strength, 3 B-strength, and 4 C-strength recommendations. The 31-GEP prognostic assay received 1 A-strength, 7 B-strength, and 6 C-strength recommendations"; these recommendations show that the 31-GEP assay received the highest recommendations by this expert panel (Berman et al., 2019).

Another study reported that the 31-GEP was validated in almost 1600 patients "as an independent predictor of risk of recurrence, distant metastasis and death in Stage I-III melanoma and can guide SLNB decisions in patient subgroups, as demonstrated in 1421 patients"; further, an appropriate 31-GEP testing population was identified and concluded that it is best used on patients with cutaneous melanoma tumors greater than or equal to 0.3 mm thick (Marks et al., 2019). However, this study reports several conflicts of interest that are important to note as multiple authors are employees at Castle Biosciences, Inc., and one author is a consultant and speaker for the company (Marks et al., 2019).

Dubin, Dinehart, and Farberg (2019) also published an article that reviewed seven studies aiming to validate 31-GEP testing for cutaneous melanoma patients; the authors found "the 31-GEP test to be particularly useful for patients with invasive melanoma or older patients with T1/T2 melanomas. For patients with invasive melanoma, the results of the molecular test may help guide the frequency of skin examinations and utilization of SLNB or imaging following diagnosis." However, conclusions stated that differences were identified between the author's findings and official published guidelines which "may be attributed to chronological issues, as many of the studies were not yet published when the aforementioned organizations conducted their reviews"; the authors also recognized that "There was also difficulty in applying the

National Comprehensive Cancer Network criteria to this prognostic test, as their guidelines were intended for evaluation of predictive markers. Nevertheless, based upon the most current data available, integration of the 31-GEP test into clinical practice may be warranted in certain clinical situations (Dubin et al., 2019)." Once again, relevant conflicts of interest for this study include that two authors serve on the advisory board of Castle Biosciences (Dubin et al., 2019).

Greenhaw et al. (2020) completed a meta-analysis of the DecisionDx-Melanoma 31-GEP prognostic test in a total of 1,479 patients. This meta-analysis included participants from three different studies. The patient analysis showed that the five-year recurrence and distant metastasis-free survival rate for Class 1A patients was 91.4% and 94.1% respectively and were 43.6% and 55.5% for Class 2B patients. The 31-GEP was then used to estimate the likelihood of recurrence and distance metastasis. The GEP test exhibited a sensitivity of 76% for each endpoint, showing consistency and accuracy for the identification of at increased risk of metastasis (Greenhaw et al., 2020).

Gastman, Zager, et al. (2019) used the commercially available DecisionDx-Melanoma to classify the recurrence risk of 157 cutaneous melanoma tumors as low-risk Class 1 or high-risk Class 2; a total of 110 of these patients had a SLNB. Results showed that the 31-GEP was able to identify 74% of patients who developed distant metastases; further, 88% of patients who were categorized as Class 2 died from the disease over a 5-year period (Gastman, Zager, et al., 2019).

Gastman, Gerami, et al. (2019) used data from three previous studies with GEP-results, totaling 690 participants. Analyses were performed and showed that 70% of patients with SLNB negative results (SLN-negative) were categorized as Class 2 and exhibited metastasis. Both the DecisionDx-Melanoma 31-GEP class 2B and SLN positivity were shown to be independent predictors of cancer recurrence in patients with T1 tumors (Gastman, Gerami, et al., 2019).

Zager et al. (2018) evaluated the DecisionDx-Melanoma 31-GEP test's prognostic accuracy in a multi-center study of 523 patients with cutaneous melanoma; all participants were classified as Class 1 (low risk) or Class 2 (high risk). The molecular classification from the GEP test was correlated to the clinical outcome of each patient, as well as the AJCC staging criteria. The authors note that "The 5-year RFS [recurrence free survival] rates for Class 1 and Class 2 were 88% and 52%, respectively, and DMFS [distant metastasis-free survival] rates were 93% versus 60%, respectively ($P < 0.001$). The GEP was a significant predictor of RFS and DMFS in univariate analysis (hazard ratio [HR] = 5.4 and 6.6, respectively, $P < 0.001$ for each), along with Breslow thickness, ulceration, mitotic rate, and sentinel lymph node (SLN) status ($P < 0.001$ for each) (Zager et al., 2018)." This study showed that the GEP was able to assist with prognostic information to estimate cancer recurrence.

Hsueh et al. (2017) completed a multi-registry study to analyze the survival estimate of a group of 322 cutaneous melanoma patients with the 31-GEP test. The median follow-up time for event-free patients was 1.5 years. The authors note that "1.5-year RFS, DMFS, and OS [overall survival] rates were 97 vs. 77%, 99 vs. 89%, and 99 vs. 92% for Class 1 vs. Class 2, respectively ($p < 0.0001$ for each)" (Hsueh et al., 2017). These results support the idea that the 31-gene GEP can accurately classify cutaneous melanoma patients into two groups based on significantly different metastatic risk.

Gerami, Cook, et al. (2015a) assessed the prognostic accuracy of the 31-GEP compared to SLNB tests in a multicenter cohort of 217 individuals. End point analyses include disease-free, distant metastasis-free, and overall survivals. Results showed that the "GEP outcome was a more significant and better predictor of each end point in univariate and multivariate regression analysis, compared with SLNB ($P < .0001$ for all)"; further, the combination of both GEP and SLNB improved prognostication (Gerami, Cook, et al., 2015a). Finally, for patients who received a high-risk GEP result and a negative SLNB, Kaplan-Meier 5-year disease-free was 35%, distant metastasis-free was 49% and overall survival was 54%. This study showed that the 31-GEP could accurately predict metastatic risk in patients undergoing SLNB.

Litchman, Prado, Teplitz, and Rigel (2020) published a systematic review and meta-analysis of GEP for primary cutaneous melanoma prognosis. The authors included 29 articles within the systematic review. Even though nine unique gene signatures were reported, the authors decided to focus on the 31-gene GEP

test by Castle Biosciences (and the six studies on the prognostic validity) due to the heterogeneity between the gene signatures and the lack of reported studies for some of the other tests. They note "significant heterogeneity between studies". They report a pooled hazard ratio (HR) for recurrence-free survival (RFS) of 7.22 (95% CI, 4.75 – 10.98). Likewise, the pooled HR for distant metastasis-free survival (DMFS) was 6.62 (95% CI, 4.91-8.91), and the pooled HR for overall survival was 7.06 (95% CI, 4.44-11.22). The authors state, "A high-risk GEP result may appropriately influence a clinician to refer a patient for this [SLNB] procedure. Current NCCN guidelines suggest that patients with a 5% risk of positive SLNB should undergo SLNB. Although GEP testing may help stratify patient risk for SLNB positivity, GEP is not currently recommended to replace SLNB as evidenced by the results of this review… In conclusion, the findings of this review have clinical implications for patients with melanoma to better assess their prognosis leading to more effective management of their disease. The results of this study may be useful when deciding to offer GEP testing to primary cutaneous melanoma patients (Litchman et al., 2020)."

Mirsky, Prado, Svoboda, Glazer, and Rigel (2018) studied the impact of the 31-GEP test on management decisions made by physician assistants (PA) and nurse practitioners (NP). A total of 164 PAs and NPs attending a national dermatology conference completed an online survey on the potential impact of the 31-GEP test. The authors note that "In the majority of cases, a lower risk 31-GEP test result led to a statistically significant decrease in the proportion of PA/NPs who would recommend SLNBx [sentinel lymph node biopsy, SLNB], imaging, or quarterly follow-up. Conversely, a higher risk 31-GEP result significantly altered management toward increased intensity (more recommendations for SLNBx, imaging, or quarterly follow-up) in all cases (Mirsky et al., 2018)." However, these are hypothetical management scenarios.

Schuitevoerder et al. (2018) completed a retrospective review of 91 patients seen between September 2015 and August 2016 to determine the impact of the GEP results on patients with clinically node negative cutaneous melanoma, as determined after SLNB. Of 91 patients, 38 were identified as stage I, 42 were identified as stage II, 10 were identified as stage III, and 1 was identified as stage IV. GEP results were found to be significantly associated with the management of both stage I and stage II patients; a difference was not found in the follow-up in stage III of IV results (Schuitevoerder et al., 2018). Further, a Class 2 GEP result led to more aggressive disease management.

Svoboda, Glazer, Farberg, and Rigel (2018) researched the factors that cause a clinician to utilize the 31-GEP. A survey was completed by 181 dermatologists attending a national conference. A majority of clinicians stated that they would use the 31-GEP test if the tumor had a Breslow thickness $\leq$0.5mm. Further, the presence of ulceration also showed a statistically significant increase in potential 31-GEP use. Finally, "A negative SLN was only associated with a statistically significant increase in the percentage of clinicians who would recommend the test for the thinnest (0.26 mm) tumors (22% to 34%, P=0.033) (Svoboda et al., 2018)." The authors note that ulceration was the most important factor in this group of dermatologists to influence the use of the 31-GEP.

Dillon et al. (2018) studied the impact of the 31-GEP on clinical management of melanoma patients. Pre- and post-test recommendations were assessed before and after 31-GEP results were provided to physicians at 16 dermatology, surgical, or medical oncology centers. A total of 247 melanoma samples were included in this study. Results showed that after 31-GEP results were obtained, post-test management plans changed for 49% of cases (36% class I and 85% class II cases). "GEP class was a significant factor for change in care during the study ($p<0.001$), with Class 1 accounting for 91% (39 of 43) of cases with decreased management intensity, and Class 2 accounting for 72% (49 of 68) of cases with increases (Dillon et al., 2018)." These results showed that the 31-GEP did affect the clinical management of cutaneous melanoma cases.

Berger et al. (2016) studied the clinical impact of the 31-GEP test on 156 cutaneous melanoma patients. A total of 42% of the participants were identified as stage I, 47% were identified as stage II, and 8% were identified as stage III. The 31-GEP classified 61% of participants as Class 1 and 39% of participants as Class 2. After 31-GEP results were received, 53% of patients experienced changes in disease management, and "The majority (77/82, 94%) of these changes were concordant with the risk indicated by the test result ($p < 0.0001$ by Fisher's exact test), with increased management intensity for Class 2 patients and reduced

management intensity for Class 1 patients (Berger et al., 2016).” These results show that the 31-GEP did have a clinical impact on cutaneous melanoma disease management.

A follow up study (Cook et al., 2018) on the analytic validity of DecisionDx found inter assay concordance of 99% and inter instrument concordance of 95% with a technical success of 98%, demonstrating that DecisionDx-Melanoma demonstrates strong reproducibility between experiments and has high technical reliability on clinical samples. It may be a useful diagnostic and prognostic adjunct in the workup of thin to intermediate thickness melanomas, especially in counseling patients who are candidates for sentinel lymph node biopsy. However, this assay has not been tested on the full spectrum of histologic subtypes of melanoma, and it is unclear how these results should be integrated into current staging criteria (Lee & Lian, 2018).

State and Federal Regulations, as applicable

A search of the FDA database on 11/12/2020 using the term “*BRAF*” yielded 6 results and a search using the term “melanoma” yielded 9 results. Additional tests may be considered laboratory developed tests (LDTs); developed, validated and performed by individual laboratories. LDTs are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA’88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

The FDA-approvals for all of the BRAF-targeted therapies include the requirement that BRAF mutation testing be performed by an FDA-approved test.

On August 17, 2011 the U.S. Food and Drug Administration (FDA) announced the approval of Zelboraf (vemurafenib) for unresectable or metastatic melanoma with oncogenic BRAF mutation (V600E). The Cobas® 4800 BRAF V600 Mutation Test was approved as the companion diagnostic for vemurafenib (Bollag et al., 2012).

Dabrafenib was FDA-approved in May 2013 for the treatment of patients with unresectable or metastatic melanoma with *BRAF* V600E mutation, as detected by an FDA-approved test. Dabrafenib is specifically not indicated for the treatment of patients with wild-type *BRAF* melanoma (Tafinlar (dabrafenib), Jan 2014).

Trametinib was FDA-approved in May 2013 for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E or V600K mutations, as detected by an FDA-approved test. Trametinib is specifically not indicated for the treatment of patients previously treated with BRAF inhibitor therapy (GlaxoSmithKline. Mekinist Aug 2014).

The companion diagnostic test coapproved 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” (Genentech, Inc. Zelboraf® March, 2014).

The FDA approved the use of the Oncomine Dx target test NGS panel for somatic or germline variants, which includes the BRAF V600E mutation for consideration with dabrafenib therapy as one of the gene variants (Life Technologies Corporation, approved in June 2017).

The FDA approved the FoundationOne CDx NGS panel in November 2017, which does include both the V600E and V600K mutation for possible dabrafenib or vemurafenib therapy. (Foundation Medicine, Inc.).

The FDA approved the use of Therascreen BRAF V600E RGQ PCR Kit in April 2020, a real time PCR test that detects *BRAF V600E* mutations. The Therascreen BRAF V600E RGQ PCR Kit is for use on the Rotor-Gene Q MDx (US) instrument.

Guidelines and Recommendations

The National Comprehensive Cancer Network (NCCN, 2019, 2020c)

The NCCN Melanoma Panel strongly recommends testing for and reporting the presence or absence of *BRAF* and *KIT* gene mutations that may impact treatment options in patients with metastatic melanoma (stage IV patients); this testing is only recommended for patients with advanced disease for whom molecular targeted therapies could be beneficial (NCCN, 2020c). In addition, "Mutational analysis for *BRAF* or multigene testing of the primary lesion is not recommended for patients with cutaneous melanoma who are without evidence of disease (NED), unless required to guide adjuvant or other systemic therapy or consideration of clinical trials (NCCN, 2020c)." The panel also states, "GEP to differentiate melanomas at low versus high risk for metastasis may provide information on individual risk of recurrence. However, the current available prognostic molecular techniques should not replace pathological staging procedures, and the use of GEP testing according to specific melanoma stage (before or after SLNB) requires further prospective investigation (NCCN, 2020c)." Finally, the NCCN declared that "*BRAF* mutation testing is recommended for patients with stage III at high risk for recurrence for whom future *BRAF*-directed therapy may be an option (NCCN, 2020c)."

Regarding GEP testing, the NCCN recognizes this test as a potential method to detect melanocytic neoplasms following histopathology. In regards to prognostic testing, the NCCN states that "Commercially available GEP tests are marketed as being able to classify cutaneous melanoma into separate categories based on risk of metastasis. However, it remains unclear whether these tests provide clinically actionable prognostic information when used in addition to or in comparison with known clinicopathologic factors or multivariate nomograms that incorporate patient sex, age, tumor location and thickness, ulceration, mitotic rate, lymphovascular invasion, microsatellites, and SLNB status. Furthermore, the impact of these tests on treatment outcomes or follow-up schedules has not been established." The NCCN continues by stating that even though several studies have been published highlighting the prognostic capabilities of the 31-GEP test for class 2 assignments, "It remains unclear whether this GEP profile is reliably predictive of outcome across the risk spectrum of melanoma (NCCN, 2020c)." Further, the NCCN also stated that most of the studies measuring the prognostic capability of the 31-GEP test are retrospective, and that prospective validation studies are required "to more accurately define the clinical utility of molecular testing prior to widespread implementation of GEP for prognostication of cutaneous melanoma, and in particular to determine its role in guiding surveillance imaging, SLNB, and adjuvant treatment decisions. Existing and emerging GEP platforms and other prognostic techniques should also be compared with optimized contemporary multivariable phenotypic models (ie, the AJCC 8th edition melanoma risk calculator/prognostic tool in development) (NCCN, 2020c)."

Furthermore, NCCN guidelines indicate that "If a patient's risk of a positive sentinel lymph node (SNL) is <5%, NCCN does not recommend SLNB. This would indicate clinical stage 1A, T1a melanoma with Breslow depth of 0.8 mm without ulceration, or other adverse features, unless there is significant uncertainty about the adequacy of microstaging (positive deep margins). If a patient's risk of a positive SLNB is 5%-10%, NCCN recommends discussing and considering SLNB. This would include clinical stage 1B, T1b melanoma (Breslow depth <0.8 mm with ulceration of 0.8-1 mm with or without ulceration), or T1a lesions with Breslow depth <0.8 mm and with other adverse features (eg, very high mitotic index $\geq 2/mm^2$ [particularly in the setting of young age], lymphovascular invasion, combination of these factors) (NCCN, 2020a)."

The NCCN does not recommend *BRAF* or NGS testing for "resected stage I-II cutaneous melanoma unless it will inform clinical trial participation". However, *BRAF* mutation testing is recommended for patients with stage III at "high risk for recurrence for whom future *BRAF*-directed therapy may be an option". The NCCN also notes that if *BRAF* single-gene testing was the "initial test" performed, and is negative, clinicians should strongly consider larger NGS panels to identify other potential genetic targets" such as *KIT*. (NCCN, 2020c)

The NCCN also observes *NRAS* as a relevant mutation for melanoma, noting that *NRAS* mutations are present in "approximately 15% of melanomas with chronic and intermittent sun exposure, acral surfaces, and mucosal surfaces". Due to the low probability of an overlapping targetable mutations (such as *BRAF*

or *KIT*), the NCCN remarks that "the presence of an [sic] *NRAS* mutation may identify patients who will not benefit from additional molecular testing" (NCCN, 2020c).

The NCCN notes that repeat molecular testing "upon recurrence or metastases is likely to be of low yield". Further, use of mutation burden to "guide treatment decisions" remains "investigational" (NCCN, 2020c).

In 2019, the NCCN listed "Pre-diagnostic technologies to inform decision about whether to biopsy", such as DermTech PLA, as one of the emerging molecular technologies; however, they then went on to state, "The NCCN Guidelines for Cutaneous Melanoma focus on the management of cutaneous melanoma following pathology diagnosis. As such, emerging molecular technologies for pre-diagnostic purposes (eg, noninvasive genomic adhesive patch testing), are not within the guidelines' purview (NCCN, 2019)." The 2020 NCCN guidelines again do not include a review of pre-diagnostic testing (NCCN, 2020b).

The American Academy of Dermatology (AAD) (Swetter et al., 2019)

The AAD recently published guidelines for the care and management of primary cutaneous melanoma. Regarding skin biopsies, the AAD states that while many different molecular and imaging techniques have been developed, "skin biopsy remains the first step to establish a definitive diagnosis of CM [cutaneous melanoma]"; further, the guidelines also state that "Newer noninvasive techniques (eg, reflectance confocal microscopy [RCM], as well as electrical impedance spectroscopy, gene expression analysis, optical coherence tomography, and others [see the section Emerging Diagnostic Technologies]) can also be considered as these become more readily available (Swetter et al., 2019)."

The AAD also notes that these guidelines highlight several gaps in research including "the clinical utility and prognostic significance of various biomarkers and molecular tests; optimal clinical situations in which to pursue multigene somatic and germline mutational analysis; and the value of ancillary molecular tests in comparison with well-established clinicopathologic predictors of outcome (Swetter et al., 2019)." it is then noted that "Efforts to standardize the histopathologic diagnosis and categorization of melanocytic neoplasms are under way to reduce the significant interobserver variability among pathologists. Ongoing advances in genomic medicine may make many of the aforementioned issues obsolete before the next AAD melanoma CPG is issued (Swetter et al., 2019)."

In regards to patients with a family history of invasive cutaneous melanoma (at least 3 affected members on 1 side of the family), "Cancer risk counseling by a qualified genetic counselor is recommended" (Swetter et al., 2019).

Finally, the AAD states that "There is insufficient evidence to recommend routine molecular profiling assessment for baseline prognostication. Evidence is lacking that molecular classification should be used to alter patient management outside of current guidelines (eg. NCCN and AAD). The criteria for and the utility of prognostic molecular testing, including GEP, in aiding clinical decision making (eg. SLNB eligibility, surveillance intensity, and/or therapeutic choice) needs to be evaluated in the context of clinical study or trial (Swetter et al., 2019)."

European Society for Medical Oncology (ESMO, 2019)

The ESMO has stated that "Mutation testing for actionable mutations is mandatory in patients with resectable or unresectable stage III or stage IV [I, A], and is highly recommended in high-risk resected disease stage IIC but not for stage I or stage IIA-IIB. *BRAF* testing is mandatory [I, A]. If the tumour is *BRAF* wild type (WT) at the V600 locus (Class I *BRAF* mutant) sequencing the loci of the other known minor *BRAF* mutations (Class II and Class III *BRAF* mutant) to confirm WT status and testing for *NRAS* and c-kit mutations are recommended [II, C] (ESMO, 2019)."

American Joint Committee on Cancer (AJCC) (Gershenwald et al., 2017)

The AJCC did not include any mention of molecular testing in the most recent 8th edition guidance on melanoma staging (Gershenwald et al., 2017).

U.S. Preventive Services Task Force (USPSTF) (Wernli et al., 2016)

The USPSTF examined the utility of visual skin examination for the prevention of melanoma and found that "Only limited evidence was identified for skin cancer screening, particularly regarding potential benefit of skin cancer screening on melanoma mortality (Wernli et al., 2016)." The use of molecular tests in screening for melanoma is not mentioned.

American Society of Clinical Oncology (ASCO) and the Society of Surgical Oncology (SSO) (Wong et al., 2018)

The ASCO and SSO have formed an expert panel and published guidelines for SLNB in melanoma. This guideline includes the following recommendations:

- "Routine SLN biopsy is not recommended for patients with thin melanomas that are T1a (nonulcerated lesions < 0.8 mm in Breslow thickness). SLN biopsy may be considered for thin melanomas that are T1b (0.8 to 1.0 mm Breslow thickness or < 0.8 mm Breslow thickness with ulceration) after a thorough discussion with the patient of the potential benefits and risk of harms associated with the procedure.

- SLN biopsy is recommended for patients with intermediate-thickness melanomas (T2 or T3; Breslow thickness of > 1.0 to 4.0 mm).

- SLN biopsy may be recommended for patients with thick melanomas (T4; > 4.0 mm in Breslow thickness), after a discussion of the potential benefits and risks of harm.

- In the case of a positive SLN biopsy, CLND [completion lymph node dissection] or careful observation are options for patients with low-risk micrometastatic disease, with due consideration of clinicopathological factors. For higher-risk patients, careful observation may be considered only after a thorough discussion with patients about the potential risks and benefits of foregoing CLND. Important qualifying statements outlining relevant clinicopathological factors and details of the reference patient populations are included within the guideline (Wong et al., 2018)."

Scottish Intercollegiate Guidelines Network (SIGN, 2017)

The SIGN has given the following recommendations:

- "Serine/threonine-protein kinase B-Raf (*BRAF*) status should be requested in all patients with advanced disease and recorded on the pathology report
- All patients with advanced melanoma should be tested for mutations in *BRAF* and have their management discussed at a specialist MDT in order to determine the optimal management strategy taking into account patient fitness, co-morbidity, disease burden and overall aim of treatment (SIGN, 2017)."

American Society of Clinical Oncology (ASCO) (Seth et al., 2020)

The ASCO expert panel recommends needle biopsy as the preferred diagnostic approach to clinically detected lymphadenopathy in any patient with known or suspected metastatic melanoma in regional nodes. Excisional biopsy, a more invasive approach, is not routinely required for diagnosis or characterization of melanoma. The panel recommends that *BRAF* mutation testing should be performed at time of diagnosis, but clinicians are not required to wait for the results of that testing if the decision has been made to initiate immunotherapy. If prior testing resulted in a false negative, the panel states that re-testing RAF status upon

progression could be considered, but it would not be of value as the panel is not aware of data that supports re-testing. Regarding immunohistochemistry, the panel states "Immunohistochemistry for the *BRAF* V600E mutation has the most rapid turnaround time; however, less common mutations may be found with other methodologies such as *BRAF* gene sequencing" (Seth et al., 2020).

European Dermatology Forum (EDF), the European Association of DermatoOncology (EADO), and the European Organization of Research and Treatment of Cancer (EORTC) (Garbe et al., 2020)

A panel of experts from EDF, EADO, and EORTC recommend that *BRAF* mutation testing is required "in patients with distant metastasis or non-resectable regional metastasis to identify those who are eligible to receive treatment with combined *BRAF* and MEK inhibitors, and in resected high-risk stage III melanoma patients in the adjuvant setting (Garbe et al., 2020)." Mutational analysis should be performed on metastatic tissue, either distance or regional, or on the primary tumor if metastatic tissue is not feasible. There may be a discrepancy rate in the *BRAF* status between the primary versus metastatic melanoma lesions. *BRAF* mutation testing of the primary tumor is not recommended in a patient with cutaneous melanoma who has no evidence of the disease. Regarding next generation sequencing, the panel claims that it may help in identifying genetic alterations, but data is still limited for its use in a standardized diagnostic setting.
The guideline also commented on *NRAS*, stating that *NRAS* mutations are present in 15-20% of melanoma cases and are almost always mutually exclusive with *BRAF* mutations. The guideline remarked that *NRAS* status may inform clinicians regarding the *BRAF* status, although *NRAS*-based treatments are still under investigation.
Finally, the guideline commented on c-*KIT*, recommending that acral and mucosal melanomas should initially be tested for *BRAF* and *NRAS* mutations; if both genes were found to be wild-type, the sample should be tested for c-*KIT* (Garbe et al., 2020).

Melanoma Prevention Working Group (MPWG) (Grossman et al., 2020)

This Working Group published a guideline regarding prognostic gene expression profile [GEP] testing for cutaneous melanoma. Although the Group is "optimistic" about the future use of prognostic GEP testing to "improve risk stratification and enhance clinical decision-making", it states that "more evidence is needed to support GEP testing to inform recommendations regarding SLNB [sentinel lymph node biopsy], intensity of follow-up or imaging surveillance, and postoperative adjuvant therapy." Overall, the Group remarks that "there are insufficient data to support routine use of currently available prognostic GEP tests to inform management of patients with CM [cutaneous melanoma" (Grossman et al., 2020).

Expert Panel Consensus-Based Modified Delphi Process Assessment (Berman et al., 2019)

An expert panel gathered to develop consensus-based guidelines on the appropriate use criteria for the integration of diagnostic and prognostic GEP assays into the management of cutaneous malignant melanoma. These guidelines include recommendations for the 2-GEP test, the 23-GEP test, and the 31-GEP test. Regarding the 31-GEP test, a total of 14 recommendations were considered. Each recommendation is shown below:

"One of 14 received an A-strength recommendation:

- Use of the 31-GEP test to aid in the management of patients who are SLNBx negative

Seven of the recommendations received a B-strength recommendation:

- Integration of 31-GEP results into the decision to adjust follow-up frequency
- Integration of 31-GEP results into the decision to order adjunctive imaging studies
- Integration of 31-GEP results into management of patients with T1a tumors with Breslow depth <0.8 mm and other adverse prognostic factors

- Integration of 31-GEP results into management of patients with T1 or T2 tumors who are sentinel lymph node biopsy (SLNBx) eligible
- Integration of 31-GEP results into management of patients with T1b tumors
- Integration of 31-GEP results into management of patients with T2 tumors
- Integration of 31-GEP results into management of patients with a low-risk category based on traditional AJCC factors

Six received a C-strength recommendation:

- Integration of 31-GEP results into the assessment of prognosis and management options for patients with T1a tumors with a positive deep margin
- Integration of 31-GEP results into the assessment of prognosis and management options for patients with T1b tumors with a positive deep margin
- Integration of 31-GEP results to for risk-stratification of patients in clinical trials
- Use of 31-GEP results as a criterion for eligibility for a chemotherapy regimen
- T4 disease as a contraindication for use of the 31-GEP test
- Melanoma in situ as a contraindication for use of the 31-GEP test (Berman et al., 2019)"

Billing/Coding/Physician Documentation Information

This policy may apply to the following codes. Inclusion of a code in this section does not guarantee that it will be reimbursed. For further information on reimbursement guidelines, please see Administrative Policies on the Blue Cross Blue Shield of North Carolina web site at www.bcbsnc.com. They are listed in the Category Search on the Medical Policy search page.

Applicable service codes: 0089U, 0090U 81210, 81272, 81273, 81311, 81529

BCBSNC may request medical records for determination of medical necessity. When medical records are requested, letters of support and/or explanation are often useful, but are not sufficient documentation unless all specific information needed to make a medical necessity determination is included.

Scientific Background and Reference Sources

For Policy Titled: BRAF Genetic Testing in Patients with Melanoma

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For Policy Titled: Genetic Testing and Genetic Expression Profiling in Patients with Cutaneous Melanoma

Specialty Matched Consultant Advisory Panel review 3/2020

Medical Director review 3/2020

Medical Director review 1/2021

Specialty Matched Consultant Advisory Panel review 3/2021

Medical Director review 3/2021

Policy Implementation/Update Information

For Policy Titled: BRAF Genetic Testing in Patients with Melanoma

1/1/2019 New policy developed. BCBSNC will provide coverage for BRAF genetic testing in patients with melanoma when it is determined to be medically necessary and criteria are met. Medical Director review 1/1/2019. Policy noticed 1/1/2019 for effective date 4/1/2019. (lpr)

11/12/19 Wording in the Policy, When Covered, and/or Not Covered section(s) changed from Medical Necessity to Reimbursement language, where needed. (hb)

For Policy Titled: Genetic Testing and Genetic Expression Profiling in Patients with Cutaneous Melanoma

2/11/20 Reviewed by Avalon Q4 2019 CAB. Under "When Not Covered" section added statement: Genetic expression profiling testing for cutaneous melanoma is considered investigational. Added Note: For testing of 5 or more genes for an affected individual with cutaneous melanoma, please refer to AHS-M2109 Molecular Panel Testing of Cancers for Diagnosis, Prognosis, and Identification of Targeted Therapy. Under Billing/Coding section: Deleted CPT codes 81445, 81450, 81455 and added PLA codes: 0089U and 0090U. **Policy Title changed from: BRAF Genetic Testing in Patients with Melanoma to: Genetic Testing and Genetic Expression Profiling in Patients with Cutaneous Melanoma**. Medical Director review 1/2020. (lpr)

3/31/20 Specialty Matched Consultant Advisory Panel review 3/18/2020. No change to policy statement. (lpr)

2/9/21 Reviewed by Avalon Q4 2020 CAB. Under "When Covered' section: added NRAS and consolidated statements 1. and 2. into one statement addressing both stage III and IV melanoma. References and Description section updated. Extensive revisions/updates to Policy Guidelines section. Added related policies section. Added CPT codes 81311, 81529 to Billing/Coding section. Medical Director review 1/2021. (lpr)

4/6/21 Specialty Matched Consultant Advisory Panel review 3/17/2021. No change to policy statement. (lpr)