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Medical Policy

Subject:	Gene Expression Profiling of Melanomas		
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Description/Scope

This document addresses gene expression profiling to assist in determining the diagnosis, risk stratification and clinical management of cutaneous and uveal (ocular) melanoma.

Position Statement

Investigational and Not Medically Necessary:

I. Gene expression profiling of suspected or established cutaneous melanoma is considered **investigational and not medically necessary.**

II. Gene expression profiling of suspected or established uveal melanoma is considered **investigational and not medically necessary.**

Rationale

Gene Expression Profiling of Cutaneous Melanoma

myPath® Melanoma

The myPath Melanoma (Myriad Genetic Laboratories, Inc., Salt Lake City, UT) gene expression profiling test is intended for use as an adjunct to histopathology when the distinction between a benign nevus and a malignant melanoma is uncertain based on histopathology alone. The myPath Melanoma test measures 23 genes involved in cell differentiation, cell signaling, and immune response signaling. The 23 genes included in the myPath Melanoma test are PRAME (a single gene involved in cell differentiation), a group of genes involved in multiple cell signaling pathways (S100A7, S100A8, S100A9, S100A12 and PI3), another group involved in tumor immune response signaling (CCL5, CD38, CXCL10, CXCL9, IRF1, LCP2, PTPRC and SELL) and 9 housekeeping genes that are measured to normalize RNA expression for analysis (CLTC, MRFAPI, PPP2CA, PSMA1, RPL13A, RPL8, RPS29, SLC25A3, and TXNLI).

A retrospective study of the myPath Melanoma aimed to quantify the impact of this test on diagnosis and treatment of cutaneous melanoma. Diagnostically challenging melanocytic lesions encountered during routine dermatopathology check-ups were submitted for gene expression testing using the myPath Melanoma test and were assigned a resulting melanoma diagnostic score (MDS). Data from 1695 eligible individuals were evaluated for inclusion in the 'diagnostically challenging' subset (n=218). Dermatopathologists who submitted cases were asked to complete a pre-test survey documenting diagnosis, level of diagnostic confidence, and their recommendations for treatment. After the MDS was assigned, a post-test survey was completed. Changes in dermatopathologists' survey

responses were analyzed. Definitive diagnoses were increased by 56.6% for cases that were initially indeterminate and changes in treatment recommendations occurred in 49.1% of cases. Treatment recommendations were changed to align with the test result in 76.6% of diagnostically challenging cases (Cockerell, 2016). Based on this study's findings, a prospective analysis without the risk of bias and including clinically meaningful outcomes may be warranted.

In another, similarly-designed study evaluating the myPath Melanoma test, diagnostically challenging melanocytic neoplasms were submitted by dermatopathologists for gene expression testing as part of a prospective evaluation assessing the impact of the test on diagnostic and medical management decision-making. The diagnostically challenging samples were submitted along with pretest surveys which assessed physicians' initial diagnosis and treatment-plan recommendations. After testing with the myPath Melanoma, the dermatopathologists' actual treatment was analyzed for changes from the pre-test, baseline recommendations. In 71.4% (55 out of 77) of cases, there was a change from pretest recommendations to actual treatment, 75.0% (39 out of 52) of changes were aligned with the results of myPath testing. There was an 80.5% (33 out of 41) reduction in the number of biopsy site re-excisions performed for cases with a benign test result (Cockerell, 2017). A larger, randomized, prospective and blinded clinical trial in individuals with diagnostically challenging melanocytic neoplasms, comparing diagnoses and health outcomes of those evaluated with histopathology alone and those with myPath Melanoma as an adjunct to histopathology, may be warranted.

A set of 1400 melanocytic lesions were selected from samples prospectively submitted for gene expression analysis. The primary objective of this study was to independently assess the ability of myPath Melanoma to differentiate melanoma from benign nevi. Samples were evaluated by independent histopathologic testing read by experienced dermatopathologists. Diagnostic concordance among the three dermatopathologists was required for inclusion in the final analysis. The sensitivity and specificity of the myPath Melanoma MDS in differentiating benign and malignant lesions was calculated to assess the association between the MDS and the histopathologic diagnosis. Within this cohort, 349 samples (24.9%) received a malignant score, 823 (58.8%) received a benign score, and 228 (16.3%) received an indeterminate score. Among the 860 cases with triple concordance, that did not receive an indeterminate score, 204 (23.7%) received a malignant diagnosis, and 656 (76.3%) received a benign diagnosis. The myPath gene expression signature differentiated benign nevi from malignant melanoma with a sensitivity of 91.5% and a specificity of 92.5% (Clarke, 2017a). This manufacturer-sponsored study warrants further investigation in the setting of a randomized controlled trial with long-term, clinically meaningful outcomes to determine the impact of myPath Melanoma on treatment decisions and health outcomes.

DermTech Pigmented Lesion Assay (PLA)

DermTech Inc. 's (La Jolla, CA) PLA is a gene expression profiling test designed to detect atypical pigmented lesions (or moles) at high risk for melanoma from examination of samples obtained via an adhesive patch, to avoid potentially unnecessary surgical procedures necessitated by histopathology, the gold standard in definitive diagnosis. DermTech's PLA's design is unique in that it uses an adhesive patch to obtain an RNA sample for analysis, thus sparing individuals from a more invasive biopsy of a potentially benign nevi. PLA measures expression of LINC00518 and PRAME, both of which are known to play a role in oncogenesis and are over expressed in cutaneous melanoma. This test has not been validated in the use of analyzing pigmented lesions on the palms of hands, soles of feet, fingernails, bleeding or ulcerated lesions or mucous membranes.

In 2016, a retrospective analysis of 555 adhesive patch samples of pigmented lesions (157 training and 398 validation samples) from enrolled individuals, who were 18 years or older and had a clinically suspicious pigmented lesion at least 4 mm in diameter, was conducted. The only study exclusions were use of topical steroids within the past 30 days, obvious or suspected nodular melanoma and generalized skin disorders not related to melanoma. Each sample collection included analysis of RNA from the adhesive patch, followed by a conventional surgical biopsy of the same pigmented lesion of suspicion. Results of the samples were compared with standard histopathologic assessment in lesions where there was a consensus in diagnosis amongst three dermatopathologists. Of the 398 validation samples, 87 melanomas and 311 benign nevi, LINC00518 and/or PRAME expression correctly differentiated the samples with as sensitivity of 91% and a specificity of 69%. The three experts had discordant diagnoses on 11% of the samples, as a result, they were excluded from analysis. This manufacturer-sponsored, retrospective analysis of a large number of samples evaluated by three dermatologists, does not provide evidence that the PLA test improves clinical outcomes beyond the gold standard of histopathology testing to differentiate between benign and malignant pigmented lesions (Gerami, 2017).

In 2017, a study was conducted by Ferris and colleagues to determine the impact of the PLA on dermatologists' decision to biopsy pigmented skin lesions. A total of 45 board-certified dermatologists were asked to evaluate 60 web-based images of clinically atypical pigmented lesions, collected using the PLA adhesive patch, to recommend whether the lesions should be biopsied (2700 decisions analyzed). Dermatologists were given relevant health history information (sex, race, and age; personal history of melanoma; first-degree relative with melanoma; history of atypical nevi, basal cell cancer, or squamous cell cancer; more than 5 severe sunburns before 20 years of age; use of tanning beds; UV-A or UV-B treatment; 1 to 10, 11 to 50, or 51 or more moles; Fitzpatrick skin type; location of the lesion; presence of a new lesion; pain or itching; diameter greater than 6 mm; actual diameter 1 to 2 mm; evolving lesion; ulceration, weeping, or oozing; border irregularity; a pigmented lesion very different from surrounding pigmented lesions; and patient concern). Web-based images were viewed twice by each dermatologist, first without the PLA information and then with the PLA information. Overall, introduction of the PLA led to 581 fewer decisions to biopsy benign lesions. The study reported that dermatologists improved their mean biopsy sensitivity from 95% to 98.6% ($p=0.1$) and specificity increased from 32.1% to 56.9% ($p<0. 001$), the latter of which was the primary outcome of this study. For benign lesions, the mean physician confidence score was 3.0, on a Likert scale (1 to 5; 5 being most confident), without using the PLA and 3.2 using the PLA ($p<0.001$). For malignant lesions, the mean physician confidence score was 3.6, on a Likert scale, without using the PLA and 4.3 using the PLA ($p<0.001$). While the increased specificity is statistically significant and encouraging, further study in the setting of a randomized, blinded trial is warranted.

In 2017, multi-center, retrospective chart review of 12-month follow-up data was conducted on 734 pigmented lesions previously categorized as benign using the PLA test. Of the 734 lesions, 98.2% (n=721) were monitored without further intervention, whereas 1.8% (n=13) were biopsied for histopathologic review. None of lesions biopsied were determined to be melanoma. The test's utility further was studied in a registry (n=1575) including 62 participating providers, which demonstrated that 99.9% of PLA-negative lesions were clinically monitored, thereby avoiding a surgical procedure, and 96.5% of all PLA-positive lesions were appropriately biopsied. This study concludes that the PLA demonstrates an encouraging negative predictive value and may aid in decision making, whereby lessening unnecessary surgical procedures in benign nevi. Further study of relevant clinical outcomes in a randomized, prospective cohort comparing PLA to histopathology review, the gold standard for melanoma diagnosis, is warranted (Ferris, 2019).

DecisionDx-Melanoma

The DecisionDx-Melanoma test (Castle Bioscience, Inc., Friendswood, TX) is a multigene expression assay designed to predict metastasis in individuals with stage I or stage II cutaneous melanoma who have no sign of disease beyond the original tumor. The laboratory test is a signature of 31 genes, 28 discriminating genes and 3 control genes, that classifies tumors as class 1 (low risk of metastasis) or class 2 (high risk of metastasis), using reverse transcription polymerase chain reaction (RT-PCR) on formalin-fixed paraffin-embedded (FFPE) primary tumor tissue specimens obtained from either biopsy or excision of a cutaneous melanoma.

There is wide variability in metastatic rates within and across Tumor-Node-Metastasis (TNM) stage groupings in individuals with cutaneous melanoma. The DecisionDx-Melanoma test is purported to predict the risk of tumor metastasis in confirmed melanoma independent of currently used metrics of risk assessment such as Breslow thickness, ulceration status (present or absent), dermal mitotic rate, microstellitosis (present or absent), American Joint Committee on Cancer (AJCC) stage, and sentinel lymph node biopsy status. It is suggested this information would add to a comprehensive baseline evaluation and determination of the initial surveillance and treatment of an individual with high-risk stage I or II disease.

The clinical validity of the DecisionDx-Melanoma test was evaluated in a prospective, multicenter study of class 1 cutaneous melanoma tumors which analyzed microarray expression data to identify a prognostic 28-gene signature to predict risk of metastasis (Gerami, 2015b). Based on modeling analysis of cohorts of primary cutaneous melanoma tumor tissue and Kaplan-Meier analysis, the study reported the 5-year disease-free survival (DFS) rates in the development set were 100% and 38% for predicted classes 1 and 2 tumors, respectively ($p<0.0001$). DFS rates for the validation set were 97% and 31% for predicted classes 1 and 2 tumors, respectively ($p<0.0001$). The investigators suggested their preliminary analysis indicates the 28-gene signature is an independent predictor of metastasis risk in the studied cohort of cutaneous melanoma tumors.

Gerami and colleagues (2015a) assessed the prognostic accuracy of gene expression profiling for molecular staging of cutaneous melanoma in a multicenter cohort study of 217 individuals undergoing sentinel lymph node biopsy (SLNB). The prognostic accuracy of each test was determined using Kaplan-Meier and Cox regression analysis of disease-free, distant metastasis-free, and overall survival. For individuals with a negative SLNB and a class 2 gene expression profile signature (that is, a high risk outcome), Kaplan-Meier 5-year disease-free, distant metastasis-free, and overall survival rates were 35%, 49%, and 59%, respectively; however, there was no statistical difference in disease-free survival, or overall survival rates for individuals with class 2 gene expression profile signature and a negative SLNB result and individuals with a class 2 gene expression profile score and a positive SLNB result. A limitation of the study is the lack of data obtained from a randomized sample of cases, which the authors conclude as resulting "in a higher rate of distant metastasis than commonly observed or reported in the SLNB-negative group." Additional study is needed in a randomized sample of individuals to determine how gene expression profiling combined with SLNB would contribute to the accurate staging and treatment planning of individuals with cutaneous melanoma.

Berger and colleagues (2016) performed a retrospective chart review of 156 individuals with cutaneous melanoma who were consecutively tested with the DecisionDx-Melanoma gene expression profile assay at three dermatology and three surgical oncology practices between May 2013 and December 2015. The primary purpose of the study was to evaluate clinical management plans before and after gene expression profile testing, including frequency of physical examinations (initial work-up and follow-up), frequency and modality of imaging (chest x-ray, computed tomography [CT], positron emission tomography CT [PET-CT], magnetic resonance imaging [MRI], or

ultrasound), SNLB procedure recommendations and results (if performed), use and frequency of routine blood work, and referral patterns to surgical and medical oncologists. The clinical characteristics of the cohort's tumors by AJCC staging were Stage I (n=66, 42%), Stage II (n=74, 47%), Stage III (n=13, 8%), and Unknown (n=3, 2%). Overall, 95 (61%) were classified as class 1 and 61 (39%) were class 2 by gene expression profile testing. The majority of individuals were male (62%), had a median Breslow thickness of 2.0 mm, and were 63 years old (median age). The majority of tumors were located on the extremities and had superficial spreading and nodular growth patterns. Of the 156 cases, 100 (64%) of individuals received care in surgical and oncology practices and 56 (36%) were seen in dermatology practices. Individuals categorized as class 2 by the 31-gene expression profile test were managed by surgical oncology (51% vs. 18%, p<0.001 [Fisher's exact test]). A total of 82 (53%) individuals had a documented change in management, with the majority of class 2 (n=47 of 61, 77%) undergoing surveillance changes compared to 35 (37%) class 1 (p<0.0001 [Fisher's exact test]). When stratifying results according to low-risk and high-risk AJCC stage, gene expression profile testing confirmed a low-risk, class 1 tumor for 56% of Stage I and IIA individuals, resulting in no change in management. However, 13 of 18 early stage individuals who were identified as high-risk class 2 by gene expression profile testing had more intense management, primarily in the form of more frequent imaging requested by the surgical oncologist. Overall, the majority (77 of 82, 94%) of individuals had a change in intensity of surveillance and/or referral pattern as a result of the gene expression profile classification; these changes were concordant with the risk indicated by the test result (p<0.0001 [Fisher's exact test]), with increased management intensity for class 2 and reduced management intensity for class 1. Limitations of this study include the retrospective design and lack of follow-up data collection in the study cohort, which limits drawing conclusions on the impact of gene expression profile testing to alter clinical practice management and improve outcomes for individuals with cutaneous melanoma.

Similarly, Ferris and colleagues (2017) conducted a study that included 205 participants, retrospectively enrolled, with previously reported stage I and II melanoma in addition to sufficient clinical data to obtain 5-year survival outcomes. The primary goal of the study was the comparison of the DecisionDx-Melanoma-based classification with the AJCC online prediction tool as an independent predictor of recurrence-free survival (RFS), distant metastasis-free survival (DMFS, defined as a distant metastasis detected beyond the regional basin), or overall survival (OS). The secondary aim was evaluation of the utility of gene expression profiling combined with AJCC predictions for enhancing identification of melanomas at high-risk of metastasis. The final cohort was comprised of 109 stage I and 96 stage II melanoma cases. Risk classification of DMFS and OS were determined to be significant based on a cox univariate analysis (hazard ratio [HR] range 3.2-9.4; p=0.001) for both tools. Overall, 43 (21%) cases had discordant DecisionDx and AJCC classification; 11 out of 13 (85%) deaths in that group were predicted as high risk by DecisionDx but low risk by AJCC. This study suggests that the DecisionDx test, when used in combination with AJCC, may improve identification of stage I and II cutaneous melanoma with a high-risk of recurrence or metastatic disease. The retrospective nature precludes the ability to determine if use of the combined tools would alter disease management and subsequently improve clinically meaningful outcomes.

More recently, a manufacturer-sponsored, 5-year, multi-center, prospective study of the DecisionDx-Melanoma test was published. At interim analysis (median 1.5 years), a total 322 participants had completed at least one follow-up visit and were evaluable. Individuals were enrolled in one of two on-going prospective studies, EXPAND and INTEGRATE. Eligibility for study inclusion included a diagnosis of cutaneous melanoma, age ≥ 16 years and a successful DecisionDx-test result. Overall 282 (88%) of 322 cases had stage I/II disease and 237 (74%) had a SLNB. A total of 248 (77%) had class-1 molecular profiles. At this interim analysis, the difference in primary outcomes between class 1 vs. class 2 profiles were as follows: 97 vs. 77% RFS, 99 vs. 89% DMFS, and 99 vs. 92% OS (p<0.0001 for each). In multi-variate analysis, Breslow thickness, mitotic rate, and DecisionDx-test result

significantly predicted recurrence (p<0.01), whereas tumor thickness was the only significant predictor of DMFS and OS at 1.5 years. While these results are encouraging, the questions remains as to whether or not use of DecisionDx will improve clinically meaningful outcomes beyond current risk-stratification techniques (Hsueh, 2017).

A retrospective, multi-center study analyzed primary melanoma tumors to determine the DecisionDx's prognostic accuracy. A total of 523 individuals with biopsy-confirmed stage I–III cutaneous melanoma and at least 5 years of follow-up (unless there was an earlier documented recurrence or metastatic event) were enrolled. The DecisionDx was used to classify individuals as Class 1 (low risk) and Class 2 (high risk) and this classification was correlated to clinical outcome and assessed along with AJCC staging criteria. The primary endpoints of interest were RFS and DMFS. At 5 years from baseline diagnosis, RFS rates for Class 1 tumors was 88% and Class 2 tumors was 52%. DMFS rates were 93% for Class 1 and 60% for Class 2 (p<0.001). The authors concluded that the DecisionDx is a significant predictor of RFS (HR=5.4; p<0.001) and DMFS (HR=6.6; p<0.001). DecisionDx classification was also significantly associated with secondary outcomes, including Breslow thickness, ulceration, mitotic rate, and SLN status (p<0.001, for each). The retrospective, open-label, case-series design of this industry-sponsored study, are limitations that preclude definitive determination of the DecisionDx's prognostic validity (Zager, 2018).

The DecisionDx was evaluated in a Spanish-Caucasian prospective cohort of 86 individuals with resected pathologic AJCC stages IB and II primary cutaneous melanoma. Median follow-up time was 26 months. AJCC stages IB and IIA were considered Class 1 (low-risk) and IIB and IIC as Class 2 (high-risk). Within a median time-frame of 12 months, 7 (8.1%) relapses occurred in the Class 2 group (overall, 21. 2% of the Class 2 group). Of the 7 relapses, 5 were also classified as high-risk by AJCC stage, 2 were classified as low-risk. Both univariate analysis (HR=28.4; 95% CI, 3.5-3682.9) and multivariate analysis (HR=18.8; 95% CI, 1.81-2549.7) showed the DecisionDx test to be an independent predictor of relapse and metastatic disease. Authors caution that the low incidence of events may compromise the external validity of the study findings, and the very large confidence intervals associated with the reported HRs raise additional concern regarding the precision of the estimates generated by this study's analysis (Podlipnik, 2019).

A prospective cohort of 159 individuals age 26-88 (median age 59 years) who were diagnosed with cutaneous melanoma underwent SNB and adjunctive testing with DecisionDx. Melanoma cases were classified as low-risk Class 1 (n=117) or high-risk Class 2 (n=42). The primary outcomes of interest were RFS and DMFS. The median, overall follow-up was 42 months. Breslow thickness, ulceration, SNB positivity, and AJCC stage were significantly associated with DecisionDx class (p=0.009, p=0.0001, p<0.0001, p<0.0001, p=0.011, and p<0.0001, respectively). Recurrence rate was 5% for Class 1 status and 55% for Class 2. Distant metastatic rate was 1% for Class 1 status and 36% for Class 2. The median time to recurrence in this cohort was 13.3 months and 90% of recurrences occurred by 30.1 months. Out of the 10 SNB-positive, Class 2 patients, 9 experienced a recurrence. By multivariate analysis, only SNB result and GEP class were independently, statistically associated with both RFS (p=0.008 and 0.0001, respectively) and DMFS (p=0.019 and 0.001, respectively). The authors conclude that the combination of DecisionDx testing and SNB may improve our ability to determine prognosis in individuals with primary cutaneous melanoma, but how this would alter disease management and health outcomes remains unclear (Keller, 2019).

In summary, there is insufficient evidence to evaluate the clinical validity and clinical utility of the myPath Melanoma, PLA or DecisionDx-Melanoma test. Additional study is required to further validate if gene expression profile testing of suspicious pigmented lesions will alter clinically meaningful outcomes beyond histopathology, and if classifying cutaneous melanoma by gene expression results will accurately identify individuals with more

aggressive disease to the extent that treatment plans change and health outcomes improve in the surveillance and treatment of high-risk cutaneous melanoma.

The National Comprehensive Cancer Network® (NCCN) Clinical Practice Guideline (CPG) in Oncology® recommendations for the clinical staging and workup of cutaneous melanoma (V2. 2021) (principles of pathology), states:

> The use of gene expression profiling (GEP) testing according to specific AJCC-8 melanoma stage (before or after sentinel lymph node biopsy [SLNB] requires further prospective investigation in large, contemporary data sets of unselected patients. Prognostic GEP testing to differentiate melanomas at low versus high risk for metastasis should not replace pathologic staging procedures. Moreover, since there is a low probability of metastasis in stage 1 melanoma and higher proportion of false-positive results, GEP testing should not guide clinical decision-making in this subgroup.
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> Gene expression profiling for melanoma could be an enormously valuable contribution to understanding the biology of the disease. However, the difficulty of embracing gene expression profiling as an independent predictor or outcome is illustrated by inconsistency of results across studies aimed at defining the most predictive gene sets for melanoma (including Gerami, 2015b; Nsengimana, 2015)…While there is interest in newer prognostic molecular techniques such as gene expression profiling to differentiate melanomas at low- versus high-risk for metastasis, routine (baseline) genetic testing of primary cutaneous melanomas (before or following sentinel lymph node biopsy [SLNB]) is not recommended outside of a clinical study.

Gene Expression Profiling of Uveal Melanoma

Uveal melanoma, also referred to as ocular or choroidal melanoma, while relatively rare, is the most common primary ocular malignancy in adults and has a high incidence of metastases to the liver. Even with successful treatment of the primary tumor, up to 50% of individuals will subsequently develop systemic metastases, with liver involvement in up to 90% of these individuals. Metastatic liver disease remains the most common cause of tumor-related mortality in choroidal malignant melanoma, even with aggressive systemic treatments, with a median survival rate of 2 to 7 months and a 1-year survival rate of less than 10%.

In the management of uveal melanoma, clinicopathologic features and tumor genetics are used to predict prognosis, including the risk of metastatic disease. The results of two large case series have shown that tumor size has consistently been demonstrated to be of prognostic significance, in terms of the subsequent risk of metastasis and death from uveal melanoma (Diener-West, 2005; Shields, 2009). Approximately one-half of uveal melanoma tumors will metastasize at some point prior to diagnosis of the primary eye tumor, and at the time of diagnosis of the primary eye tumor, metastatic disease (micrometastases) will only be detectable in about 3% of individuals. Recent estimates of tumor doubling time have suggested that as a result of these micrometastases, clinicians may be able to identify individuals who are at higher risk for uveal melanoma through molecular signatures unique to their specific ocular tumor or those tumors with a known tendency to metastasize (Singh, 2004).

Large-scale genetic alterations, such as the presence of only one copy of chromosome 3 (monosomy 3), have been reported in uveal melanoma and associated with metastatic disease. A type of genetic test called gene expression

profiling has been proposed as a tool to identify those individuals having a high risk of developing metastasis from primary uveal melanoma. Gene expression profiling for uveal melanoma suggests that tumors can be sorted into two classes with different characteristics and prognosis: class 1 tumors are thought to be at lower risk for metastasis, while class 2 tumors may be at high risk.

An early study by Worley and colleagues (2007) reported that the sensitivity and specificity for a molecular classifier using two microarray gene expression profiling platforms (84.6% and 92.9%, respectively) was superior when compared to monosomy 3 detected by an array comparative genomic hybridization (aCGH) (58.3% and 85.7%, respectively) and fluorescence in situ hybridization (FISH) (50.0% and 72.7%, respectively). The investigators concluded that "molecular classification based on gene expression profiling of the primary tumor was superior to monosomy 3 and clinicopathologic prognostic factors for predicting metastasis in uveal melanoma." This study, however, is limited in application by inconsistencies in the reported data.

DecisionDx-UM

The DecisionDx-UM test (Castle Biosciences Inc., Friendswood, TX) is a commercially marketed gene expression profiling test intended for use in assessing metastatic risk in individuals with uveal melanoma. It consists of a 15-gene PCR-based assay that stratifies individuals with uveal melanoma into two classes based on the molecular signature of tumor tissue. The peer-reviewed literature related to the DecisionDx-UM test consists of studies describing the derivation of the gene expression profile and analytical and clinical validation of the technology (Onken, 2010a; Onken, 2010b; Onken, 2012).

Onken and colleagues (2004) presented a derivation of class 1 and class 2 molecular signatures and explored their relationship with known prognostic factors and survival. Tumor samples were taken from 25 enucleated eyes of individuals with uveal melanoma. Gene expression was examined using high-density oligonucleotide arrays. An analysis showed that gene expression profiling of uveal melanoma tumors tends to yield two classes of molecular signatures, class 1 (14 of 25, 56%) and class 2 (11 of 25, 44%) tumors. A 3-gene set (*PHLDA1*, *FZD6*, *ENPP2*) that predicted tumor class with no errors ($p=3.5 \times 10^{-5}$) was obtained from the analysis of the top 26 discriminating genes; however, none of these three genes are included in the current DecisionDx-UM gene list (Onken, 2010a). The investigators compared tumor class with clinical and pathological features (that is, cytology rank, participant age and sex, tumor diameter and thickness, presence of local invasion, ciliary body involvement, and pigmentation rank) associated with metastasis in uveal melanoma. Advanced age, a known risk factor for metastasis, correlated significantly with tumor class, as did cytology rank. Survival analysis was performed on an additional 25 participants. Kaplan-Meier analysis showed that the 92-month survival probability for class 1 participants was 95%, compared with 31% in class 2 participants ($p=0.01$). Of the total individuals analyzed (n=50), 1 subject in the class 1 group died, compared with 8 subjects in the class 2 group. The investigators ranked tumors from lowest to highest proportion of epithelioid cells, an indicator of tumor severity, and found that tumor class corresponded significantly with cytology rank ($p<0.0001$). Cytogenetic analysis of a small subset (n=10) of samples indicated that 4 of 5 (80%) class 2 tumors exhibited monosomy 3, and no class 1 tumors exhibited monosomy 3. The investigators concluded that molecular classification may better detect high-risk individuals than chromosomal analysis (monosomy 3) testing, but stated that this finding should be confirmed using a larger data set. A limitation of this study is the use of enucleated specimens in the analysis of molecular yield. Uveal melanoma tumors that are treated with enucleation are typically larger in size and exhibit extraocular tumor extension, and currently represent a subpopulation of only 10% of all uveal melanoma due to the current use of eye-sparing treatment modalities (Onken, 2006a). According to one of the developers of the DecisionDx-UM test, it is inappropriate to generalize the

results of molecular testing developed from the study of larger tumors to smaller tumors without confirming that molecular testing accurately predicts metastasis in smaller primary tumors as well.

> Most tests reported in the literature to date do not provide adequate scientific and statistical validation to be used outside of an ethically supervised investigational environment…Well-controlled prospective studies are necessary to identify the most accurate, widely accessible, and affordable tests for routine clinical use (Harbour, 2009).

Onken and colleagues (2010b) conducted a prospective and technical validation study (n=609) describing the derivation of the DecisionDx-UM test utilizing the PCR-based 15-gene assay comprising 12 discriminating genes and 3 endogenous control genes from previously published data sets (Onken, 2004; Onken, 2006a; Onken, 2006b). Technical performance of the assay was assessed in tumor samples, including 553 fine needle aspiration biopsy and 56 enucleation specimens from the authors' laboratory (n=188) and 11 collaborating sites (n=421). According to the study protocol, sample failure rate due to incorrect specimen handling was low, occurring in 32 of 609 (5.3%) of samples ($p<0.0001$). Preliminary data suggested the potential for increased sensitivity of gene expression profiling compared with cytologic diagnosis, as the assay failed in only 1 of 51, or 2% of samples with insufficient material for cytological diagnosis; however, point estimates of overall test accuracy (for example, sensitivity, specificity, or both) were not provided. In a subset of 172 individuals with uveal melanoma, the relationship between tumor class and metastasis was studied with available clinical data and a median follow-up time of 16 months. Within this group, the assay was reported to correctly identify individuals who went on to develop metastatic disease. Kaplan-Meier analysis showed approximately 24% class 2 individuals with uveal melanoma surviving at 48 months and close to 100% survival in the class 1 group, although more specific data was not provided. This study evaluates primarily fine-needle aspiration biopsy (FNAB) specimens (553 of 609, or 90.8%) rather than enucleation specimens (Onken, 2004); however, the data reported on the relationship between tumor class and metastasis are limited and median follow-up time was reported as a relatively short duration (16 months).

The prognostic performance of the 15-gene assay was subsequently validated by Onken and colleagues (2012) in a prospective, multicenter study involving 459 cases of posterior uveal melanoma. The Collaborative Ocular Oncology Group (COOG), comprised of 12 ocular oncology centers in North America, assigned samples obtained directly from individuals (usually at the time of treatment: FNAB, n=359; post-enucleation FNAB, n=92; and local tumor resection, n=8 cases) to prognostic subgroups: class 1 (low metastatic risk) and class 2 (high metastatic risk). After treatment of the primary tumor, participants were monitored for metastatic disease with a liver function panel every 6 months and liver imaging once per year or anytime the liver function panel was abnormal or there were symptoms suspicious for metastasis. The first 260 samples were also analyzed for chromosome 3 status (monosomy 3) using a single nucleotide polymorphism assay. Net reclassification improvement analysis was performed to compare the prognostic accuracy of the 15-gene assay with the 7th edition clinical TNM classification and chromosome 3 status. The 15-gene assay successfully classified 446 of 459 cases (97.2%). The 15-gene assay was class 1 in 276 cases (61.9%) and class 2 in 170 cases (38. 1%) at the median follow-up of 17.4 months (mean, 18.0 months). Metastasis was detected in 3 class 1 cases (1.1%) and 44 class 2 cases (25.9%). Although there was an association between the 15-gene assay class 2 and monosomy 3 ($p<0.0001$), 54 of 260 tumors (20.8%) were discordant for the 15-gene assay and chromosome 3 status, among which the 15-gene assay demonstrated superior prognostic accuracy ($p=0.0001$). The 15-gene assay class had a stronger independent association with metastasis than any other prognostic factor ($p<0.0001$). Chromosome 3 status did not contribute additional prognostic information that was independent of the 15-gene assay ($p=0.2$). At 3-year follow-up, the net reclassification improvement of the 15-gene assay over TNM classification was 0.43 ($p=0.001$) and 0.38 ($p=0.004$) over

chromosome 3 status. A total of 34 deaths occurred, 28 (82.4%) which were due to metastatic disease. Another 19 individuals developed metastases but were still alive at the time of the last follow-up evaluation. There was a strong association observed between the 15-gene assay class 2 and other adverse prognostic factors, including increased age of the individual, ciliary body involvement, larger tumor diameter and thickness, epithelioid cell type, and monosomy 3. The investigators stated the 15-gene assay was more strongly associated with metastasis than the other adverse prognostic factors and more accurate as a prognostic marker than monosomy 3 status for clinical validation and utility in stratifying individuals for entry into clinical trials of adjuvant therapy. The study, however, did not measure or report how classifying the tumors into subgroups altered the clinical treatment plans and improved health outcomes for these study participants with uveal melanoma.

Augsburger and colleagues (2015) performed a prospective single institution longitudinal study (in conjunction with a multicenter validation study) to determine the frequency of discordant gene expression profile classification of posterior uveal melanoma. FNAB aspirates of 80 clinically diagnosed primary choroidal and ciliochoroidal melanomas were obtained from two tumor sites prior to or at the time of initial ocular tumor treatment. Two different machine learning algorithms were calibrated against 30 uveal melanomas of known prognostic category and were used to translate results for each individual specimen into a prognostic gene expression profile class: 1) class 1: low risk of future emergence of distant metastasis; or, 2) class 2: relatively high risk of short-term emergence of distant metastases. A discordant result was defined as a "gene expression profile class assignment of different sign for the tumor cells obtained from the two sites or a failed gene expression profile test. . . for the tumor cells obtained from one but not both tumor sites." The results indicated that single-site FNAB for gene expression profile testing and prognostic classification was associated with a substantial probability of misclassification of a class 2 tumor as a class 1 tumor or an inconclusive class assignment because of a low confidence result plus a small risk of a failed gene expression profile test in 9 of 80 cases (11.3%; 95% confidence interval [CI], 9.0% to 13.6%). If cases with a "low confidence" gene expression profile class assignment for one or both aspirates and the 2 cases with a failed test on one aspirate were also classified as "discordant," as many as 13 cases (16.3%) by weighted algorithm and 15 cases (18.8%) by machine algorithm could have been classified as discordant. The authors concluded that gene expression profile testing is likely to result in the correct prognostic classification of the tumor about 85% of the time in smaller tumors.

Correa and Augsburger (2016) performed a prospective, single-institution interventional case series of 299 individuals to evaluate the clinical features, cytopathology, and gene expression profile of posterior uveal melanoma tumor cells sampled by FNAB at the time of or shortly prior to initial treatment. The melanoma tumor cells were classified by gene expression profile testing as class 1 in 211 cases (70.6%) and class 2 in 88 cases (29.4%). With use of a univariate prognostic model with Kaplan-Meier event rate curves and univariate Cox proportional hazard modeling, the investigators reported that gene expression profiling class was the strongest prognostic factor for metastatic death in this case series; however, it was noted that in the analysis, the largest linear basal diameter of the tumors (LBD), tumor thickness, and intraocular tumor location also proved to be significant individual prognostic factors. Clinical utility of these prognostic factors remains to be established.

Two additional retrospective observational studies have attempted to validate whether any clinicopathologic factors provide independent prognostic information that may enhance the accuracy of gene expression profile classifications (Walter, 2016), and what associations exist between gene expression profile classification (class 1 or class 2), clinicopathologic features, mutation status and patient outcomes in individuals with uveal melanoma (Decatur, 2016). The study by Walter and colleagues (2016) was similar in methodology to the previously discussed Onken study (2012). The primary cohort included 339 individuals and a validation cohort of 241

individuals. Both cohorts included 132 individuals that were in the Onken (2012) study. The validation cohort was used to test a prediction model using the gene expression profile plus pretreatment largest basal diameter (LBD). Cox proportional hazards analysis was used in the primary cohort to examine gene expression profile classification and other clinicopathologic variables including age, sex, tumor thickness, LBD, ciliary body involvement, and pathologic cell type. Gene expression profile class 2 was determined to be the most significant predictor of metastases and mortality in uveal melanoma. Tumor diameter was also an independent predictor of outcomes when using a 12 mm LBD cutoff value. The authors concluded that class 2 uveal melanoma had a better prognosis when the LBD was less than 12 mm at the time of treatment. In follow-up the authors are planning "a prospective, multicenter study to validate these findings and to determine the optimal use of LBD in guiding primary tumor treatment, clinical trial inclusion criteria, and systemic adjuvant therapy." Decatur and colleagues (2016) retrospectively studied a small group (n=81) with uveal melanoma treated with enucleation by a single ocular oncologist between 1998 and 2014. Tumor samples were used for gene expression profile testing, and were classified as class 1 in 35 (43%), class 2 in 42 (52%), and unknown in 4 (5%) individuals. Tumors with *BAP1* mutations were associated with poor prognostic factors, and *EIF1AX* and *SF3B1* mutations were associated with good prognostic factors. Gene expression profile class 2 was strongly associated with *BAP1* variants (r=0.70; p<0.001). On Cox proportional hazards analysis, GEP class 2 was the strongest predictor of metastases and melanoma mortality.

Plasseraud and colleagues (2016) evaluated the clinical validity and utility of the DecisionDx-UM test in individuals (n=70) enrolled in the industry-sponsored, observational CLEAR study (NCT02376920), a cohort registry of data from four participating centers across the United States that was designed to assess information on how physicians use the DecisionDx-UM results to manage treatment plans with regards to surveillance regimens and treatment referral patterns for uveal melanoma. Surveillance regimens were not prespecified but independently determined by each participating physician utilizing the DecisionDx-UM test result and documented as part of the registry data. None of the individuals in the registry had technical failures with DecisionDx-UM testing. The intensity of surveillance was categorized based on data collection methods used in a retrospective case study and cross-sectional survey of physician practice patterns in the management of uveal melanoma in Medicare beneficiaries (Aaberg, 2014). High-intensity surveillance was defined as clinical visits every 3-6 months and liver function tests and/or liver imaging/systemic evaluation (for example, CT, ultrasound, or MRI) every 3-6 months. Low-intensity surveillance was characterized by annual imaging and/or liver function tests. A total of 70 enrolled individuals with documented class 1 (low-risk) tumors (n=37 [53%]) and class 2 (high risk) tumors (33 [47%]) were included in the analysis. Of those with class 1 tumors, 30 (81%) were class 1A and 7 (19%) were class 1B. At a median follow-up of 2.4 years, 12 (36%) class 2 individuals experienced metastasis compared to 2 (5%) class 1 (p=0.002, Fisher's exact test). The median time to metastasis for class 2 was 1.4 years and time to death was 2.7 years. At 3 years, 100% of class 1 were metastasis-free compared to 63% (95% CI, 43%-83%) of class 2 (log rank test, p=0.003). The majority of metastases were localized in the liver (8 of 12 individuals, of which 1 person had liver/lung metastasis), but metastases were also found in the bone (n=3) and lungs/brain (n=1). Of the class 2 metastatic tumors (n=12), 9 were treated with enucleation, 2 with plaque radiotherapy, and 1 with transpupillary thermotherapy. A total of 30 of 37 class 1 were treated with low-intensity follow-up while all 33 class 2 were managed with high-intensity follow-up. Two individuals with intermediate risk class 1B results received high-intensity surveillance and 4 of 37 (11%) class 1 were referred to medical oncology. Six of 33 (18%) class 2 were referred to medical oncology and 8 (24%) class 2 were referred to adjuvant clinical trials. Four of the class 2 went on to receive systemic adjuvant therapy, of which 3 received combination chemotherapy within a clinical trial, and 1 received IVIG immunotherapy. No one in class 1 was referred to a clinical trial or had systemic adjuvant therapy. The results suggest that class 2 is managed by medical oncology (with imaging and liver function tests) and offered

clinical trial participation significantly more often than class 1 (Fisher's exact test for intensity of surveillance, p<0.0001; for medical oncology/clinical trial referral, p=0.04). The authors suggest that for class 2, with higher-intensity surveillance, the results are consistent with the "goal of potentially identifying metastases earlier, thus permitting intervention, while the patient is asymptomatic and likely amenable to treatment(s). Conversely, unnecessary surveillance can potentially be avoided for patients in whom extraocular recurrence of disease is unlikely." And that "the decision to enroll class 2 patients in clinical trials is directly related to the level of evidence for metastatic propensity that has been reported for the [DecisionDx-UM] test." The CLEAR registry data collection is ongoing, with an estimated final collection date of October 2020 for the primary outcome measure (that is, individuals followed for up to 10 years with measurement for metastatic event performed at 6 month intervals).

In 2020, 5-year results of the CLEAR multicenter clinical trial were published by Aaberg and colleagues. In total, 89 subjects were prospectively enrolled and evaluated every 6 months for a medium follow-up (for event-free participants) of 4.9 years. Out of the 49 individuals categorized as class 1 (low-risk), 10 (20%) underwent high-intensity management, whereas all 40 class 2 (high-risk) individuals underwent high-intensity management (p<0.0001). At the time of censorship, a total of 23 subjects with class 2 tumors and 5 subjects with class 1 tumors experienced metastasis (p<0.0001). Class 1 and 2 metastasis-free, 5-year melanoma-specific survival rates were 94% (87-100%) and 63%, respectively (49-82%; p=0.0007). Although authors report that class 2 tumor classification was the only independent predictor of metastasis and was associated with increased risk of metastasis and mortality, the data used to calculate relative risk was pooled from a meta-analysis of the current trial along with several previous small trials (some unpublished), due to the current study's sample size being prohibitively small in the calculation. Additional long-term, larger trials comparing clinical outcomes of individuals managed with 15-GEP testing to standard of care are warranted to define the value of adding this relatively new testing technique to clinical practice.

In 2020, the results of the prospective, multicenter CLEAR II clinical trial were published by Schefler and colleagues. Based on prior study, apriori analysis estimated at least 58 participants were needed for enrollment to show a minimum of difference of 40% in disease management (defined as statistical difference [alpha=0.05] in the frequency of intervention) between class 1 and class 2 GEP stratification. Between 2018 and 2019, 138 evaluable participants were recruited and enrolled who were between 18 and 90 years of age and had 15-GEP testing performed as a routine an aspect of routine clinical care during radiation or enucleation. Results of the GEP classified 93 (63%) study participants with class I tumors and 45 (33%) with class 2 tumors. Based on the results of the GEP test, providers were significantly more like to refer study participants to medical oncology, and regular follow-up for chest-imagining, abdominal imaging or liver function testing. Although the study's primary outcomes were met, a change in practice management, the impact of this change on clinical outcomes warrants investigation to further elucidate the role 15-GEP testing may have in the uveal melanoma clinical setting.

In summary, it has been suggested that use of gene expression profile testing of primary uveal melanoma to identify individuals at high risk of metastatic disease (class 2) could select those who would benefit from adjuvant treatment to reduce the risk of metastasis and more frequent screening for the earliest development of metastatic disease. It is also purported that low risk stratification can obviate potentially unnecessary medical care, including surveillance testing and imaging. Although gene expression profiling of uveal melanoma has been shown to be an independent predictor of risk of metastasis, in the absence of effective adjuvant or systemic therapy, it is uncertain how risk stratification based upon this type of testing would improve health outcomes. There is a lack of published data from well-designed, prospective studies of sufficient sample size and follow-up that support the clinical utility of gene

expression profile testing of uveal melanoma, including evidence that either low-risk stratification results in less unnecessary care without leading to harm, including missed or delayed metastatic disease identification, or that high-risk stratification results in improved health outcomes as compared to usual care.

The current NCCN CPG for uveal melanoma (V2. 2021) states:

> Biopsy of the primary tumor may provide prognostic information that can help inform frequency of follow-up and may be needed for eligibility for clinical trials. If biopsy is performed, molecular/chromosomal testing for prognostication is preferred over cytology alone. The risk/benefits of biopsy for prognostic analysis should be carefully considered and discussed.

A 2015 guideline of the United Kingdom Uveal Melanoma Guideline Development Working Group (Nathan, 2015) makes two recommendations for molecular testing for uveal melanoma, stating:

- Consider collecting molecular genetic and/or cytogenetic data for research and prognostication purposes where tumour material is available and where patient consent has been obtained as part of an ethically approved research programme.
- Use of multifactorial prognostication models incorporating clinical, histological, immunohistochemical and genetic tumour features – should be considered (Grade D).

The local treatment of uveal melanoma is well-established. Preservation of the eye, when attempted, is considered conservative treatment. Other conservative treatments include brachytherapy and proton beam radiotherapy. As reported in the randomized trial data from the Collaborative Ocular Melanoma Study (COMS) (Hawkins, 2011), there is no statistical difference in risk of metastasis between enucleation and plaque radiotherapy, or of brachytherapy prior to enucleation for large tumors; both strategies offer the same prognosis in terms of survival rates and risk of metastasis. Despite the established treatment protocols for primary uveal melanoma, no decrease has been observed in the mortality rate of this tumor. The 5-year survival rate has not changed over the last 3 decades (81.6%), suggesting that life expectancy is independent of successful local eye treatment (Pereira, 2013).

Background/Overview

Cutaneous Melanoma

Cutaneous melanoma occurs in all parts of the skin, including the soles of feet, on the palms of the hand, in between toes and fingers, and underneath the finger and toe nails. The four main categories of cutaneous melanoma described by the Melanoma Research Foundation (2016) include superficial spreading melanoma (SSM), nodular melanoma, acral lentiginous melanoma (ALM) (also called subungual melanoma), and, lentigo maligna melanoma (LMM).

According to the National Cancer Institute (NCI, 2021), melanoma accounts for less than 5% of skin cancer cases but is the major cause of skin cancer deaths with the incidence rising over the past 4 decades. In 2021, it is estimated that 106, 110 new cases of melanoma will be diagnosed and 7180 individuals may die of the condition in the United States. Elderly men are at highest risk; however, melanoma is the most common cancer in young adults aged 25 to 29 years and the second most common cancer in those between the ages of 15 to 29 years of age.

Uveal Melanoma

According to the National Cancer Institute (NCI, 2021), melanoma of the uveal tract (iris, ciliary body, and choroid) is the most common primary intraocular (eye) malignancy in adults. Metastatic uveal melanoma is a rare aggressive malignancy with limited effective treatment options. The mean age-adjusted incidence of uveal melanoma in the United States is approximately 4.3 new cases per million population. The age-adjusted incidence of this cancer has remained stable since the early 1970s. Uveal melanoma is diagnosed mostly at older ages, with a progressively rising age-specific incidence rate that peaks near the age of 70. Of the three types, iris melanomas have the best prognosis, whereas those of the ciliary body have the poorest. Most uveal tract melanomas originate in the choroid. The ciliary body is less commonly a site of origin, and the iris is the least common. The comparatively low incidence of iris melanomas has been attributed to the characteristic features of these tumors, that is, they tend to be small, slow growing, and relatively dormant in comparison with their posterior counterparts. Iris melanomas rarely metastasize. Melanomas of the posterior uveal tract are cytologically more malignant, detected later, and metastasize more frequently than iris melanomas. Extrascleral extension usually confers a poor prognosis. In addition, regular screening tests for the development of liver metastases, including measurement of liver function tests, liver ultrasound, computed tomography scan, or magnetic resonance imaging, have not shown evidence of any effect on health outcomes (Augsburger, 2009).

Gene expression profiling assays are being investigated as a tool to assist in the risk stratification and clinical management of individuals with uveal (ocular) melanoma. The DecisionDx-UM test is a proprietary, multi-gene expression profiling assay intended for use in assessing metastatic risk in individuals with uveal melanoma. The DecisionDx-UM assay requires a single biopsy specimen. For individuals with the confirmed diagnosis of uveal melanoma, the tumor specimen can be obtained with a fine needle aspiration biopsy at the time of enucleation or at a later date from the FFPE slides that are made from the enucleated globe.

According to Castle Biosciences Inc., the DecisionDx-UM test results are used for the following:

- To develop specific monitoring or surveillance plans, including a more frequent monitoring with advanced imaging procedures for those individuals identified as having a high risk of developing metastasis;
- For individuals at a low risk of developing metastasis, a less intensive surveillance plan may balance the risks of radiation exposure associated with less frequent imaging;
- To initiate referral to a medical oncologist for treatment planning which may include adjuvant treatment; and
- To improve life-planning.

The cancer-testis antigen PRAME (preferentially expressed antigen in melanoma) has been identified as a potential biomarker for increased metastatic risk in Class 1 uveal melanoma tumors. Some investigators believe this may have important implications for precision management in uveal melanoma and may aid in stratification of risk for clinical trials. It is theorized that cancers expressing PRAME may be more susceptible to immunotherapy and therefore identifying PRAME could enhance prognostic accuracy by identifying Class 1 tumors with intermediate metastatic risk (Fields, 2016a). The DecisionDx®-PRAME test (Castle Bioscience, Inc., Friendswood, TX) has been developed for this purpose. Castle Biosciences is offering PRAME testing as an optional add-on test to the DecisionDx-UM test; the newly developed DecisionDx-PRAME testing is only relevant in the context of the DecisionDx-UM results. The myPath Melanoma 23-gene assay and DermTech's PLA also include PRAME testing.

Regulatory Approval

Laboratories performing gene expression profiling tests are regulated under the Clinical Laboratory Improvement Amendments (CLIA) Act of 1988. Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the CLIA Act. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U. S. Food and Drug Administration (FDA) does not require regulatory review of these tests.

Definitions

Breslow thickness: Maximal thickness of a primary cutaneous melanoma measured in tissue sections from the top of the epidermal granular layer, or from the ulcer base (if the tumor is ulcerated), to the bottom of the tumor; metastatic rates correlate closely with tumor thickness.

Cytology: The study of the formation and function of cells.

Deoxyribonucleic acid (DNA): The hereditary material in humans and almost all other organisms. Genetic material contained in nearly every cell in a person's body has the same DNA.

Gene expression profile/profiling (GEP): The individual pattern of expression of a panel of genes that is regarded as a "signature" for that tissue; a major determinant of the biology of both normal and malignant cells.

Histology: The study of the microscopic structure of tissue and cells.

Tumor node metastasis (TNM) system: One of the most widely used cancer staging systems accepted by the American Joint Committee on Cancer (AJCC). The TNM system is based on the size and/or extent (reach) of the primary tumor (T), the amount of spread to nearby lymph nodes (N), and the presence of metastasis (M) or secondary tumors formed by the spread of cancer cells to other parts of the body. A number is added to each letter to indicate the size and/or extent of the primary tumor and the degree of cancer spread.

Coding

The following codes for treatments and procedures applicable to this document are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement policy. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

When services are Investigational and Not Medically Necessary:
When the code describes a procedure indicated in the Position Statement section as investigational and not medically necessary.

CPT	
81401	Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat) [when specified as the following, e.g., DecisionDx-PRAME]:

	• *LINC00518 (long intergenic non-protein coding RNA 518)* (eg, melanoma), expression analysis • *PRAME (preferentially expressed antigen in melanoma)* (eg, melanoma), expression analysis
81529	Oncology (cutaneous melanoma), mRNA, gene expression profiling by real-time RT-PCR of 31 genes (28 content and 3 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as recurrence risk, including likelihood of sentinel lymph node metastasis DecisionDx® Melanoma, Castle Biosciences, Inc
81552	Oncology (uveal melanoma), mRNA, gene expression profiling by real-time RT-PCR of 15 genes (12 content and 3 housekeeping), utilizing fine needle aspirate or formalin-fixed paraffin-embedded tissue, algorithm reported as risk of metastasis DecisionDx®-UM test, Castle Biosciences, Inc
81599	Unlisted multianalyte assay with algorithmic analysis [when specified as uveal or cutaneous melanoma gene expression tests]
84999	Unlisted chemistry procedure [when specified as uveal or cutaneous melanoma gene expression tests]
0089U	Oncology (melanoma), gene expression profiling by RTqPCR, *PRAME* and *LINC00518*, superficial collection using adhesive patch(es) Pigmented Lesion Assay (PLA), DermTech
0090U	Oncology (cutaneous melanoma), mRNA gene expression profiling by RT-PCR of 23 genes (14 content and 9 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a categorical result (ie, benign, intermediate, malignant) myPath® Melanoma, Castle Biosciences, Inc
0314U	Oncology (cutaneous melanoma), mRNA gene expression profiling by RT-PCR of 35 genes (32 content and 3 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a categorical result (ie, benign, intermediate, malignant) DecisionDx® DiffDx™- Melanoma, Castle Biosciences, Inc, Castle Biosciences, Inc

ICD-10 Diagnosis

C43. 0-C43. 9	Malignant melanoma of skin
C69. 30-C69. 32	Malignant neoplasm of choroid
C69. 40-C69. 42	Malignant neoplasm of ciliary body
Z85. 820	Personal history of malignant melanoma of skin

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DecisionDx-Melanoma
DecisionDx-UM

The use of specific product names is illustrative only. It is not intended to be a recommendation of one product over another, and is not intended to represent a complete listing of all products available.

Document History

Status	Date	Action
	04/01/2022	Updated Coding section with 04/01/2022 CPT changes; added 0314U.
	12/29/2021	Updated Coding section with 01/01/2022 CPT descriptor change for 0090U.
Reviewed	08/12/2021	Medical Policy & Technology Assessment Committee (MPTAC) review. Updated Rationale, Background, References and Websites sections.
	12/16/2020	Updated Coding section with 01/01/2021 CPT changes; added 81529.
Reviewed	08/13/2020	MPTAC review. Updated Rationale, Background, References and Websites for Additional Information.
	12/31/2019	Updated Coding section with 01/01/2020 CPT changes; added 81552 replacing 0081U deleted 12/31/2019.
Revised	08/22/2019	MPTAC review. Expanded Scope to include diagnosis of melanoma and INV&NMN statement to include suspicion of melanoma. Updated Rationale,

		Background/Overview and References section. Updated Coding section, added 0089U, 0090U; added 10/01/19 CPT change revising descriptor for 0081U.
Reviewed	03/21/2019	MPTAC review.
Reviewed	03/20/2019	Hematology/Oncology Subcommittee review. Updated References section.
	12/27/2018	Updated Coding section with 01/01/2019 CPT changes; added 0081U.
	09/20/2018	Updated Coding section to clarify wording, removed reference to diagnostic test.
Reviewed	05/03/2018	MPTAC review.
Reviewed	05/02/2018	Hematology/Oncology Subcommittee review. Updated Rationale, Background, References and Websites for Additional Information.
	12/27/2017	The document header wording updated from "Current Effective Date" to "Publish Date." Updated Coding section with 01/01/2018 CPT changes; added Tier 2 code 81401, genes *LINC00518*, *PRAME*.
Reviewed	05/04/2017	MPTAC review.
Reviewed	05/03/2017	Hematology/Oncology Subcommittee review. Updated formatting in Position Statement section. Updated Rationale, Background, References, Websites for Additional Information, and Index sections.
Reviewed	05/05/2016	MPTAC review.
Reviewed	05/04/2016	Hematology/Oncology Subcommittee review. Updated Rationale, Background, References, and Websites for Additional Information sections. Removed ICD-9 codes from Coding section.
Revised	05/07/2015	MPTAC review.
Revised	05/06/2015	Hematology/Oncology Subcommittee review. Revised scope of document including Subject, Description, Position Statement, Rationale, Background, Coding, References, Websites for Additional Information and Index sections, adding an investigational and not medically statement for gene expression profiling of cutaneous melanoma.
Reviewed	11/13/2014	MPTAC review.
Reviewed	11/12/2014	Hematology/Oncology Subcommittee review. Updated Rationale, References, and Websites for Additional Information sections.
Reviewed	11/14/2013	MPTAC review.
Reviewed	11/13/2013	Hematology/Oncology Subcommittee review. Format changes to Coding. Updated Rationale, Background, References, Websites for Additional Information, and Index sections.
Reviewed	05/09/2013	MPTAC review.
Reviewed	05/08/2012	Hematology/Oncology Subcommittee review. Updated Rationale, Background, References, Websites for Additional Information and Index.
	01/01/2013	Updated Coding section with 01/01/2013 CPT changes.
Reviewed	05/10/2012	MPTAC review.
Reviewed	05/09/2012	Hematology/Oncology Subcommittee review. Updated Websites for Additional Information.
New	02/16/2012	MPTAC review. Initial document development.