

Medical Policy



Title: **Microarray-Based Gene Expression Testing for Cancers of Unknown Primary**

Professional

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DESCRIPTION

Cancers of unknown primary (CUP) represent 3% of all cancer cases in the U.S. A detailed history and physical, as well as radiologic and histologic testing, can identify some but not all primary sources of secondary tumor. It is suggested that identifying a likely primary source with microarray-based gene expression testing and directing treatment accordingly may improve health outcomes.

Background

Cancers of Unknown Primary

Cancers of unknown primary (CUP), or occult primary malignancies, are tumors that have metastasized from an unknown primary source; they make up approximately 3% of all cancer cases in the U.S. Identifying the primary origin of a tumor can dictate cancer-specific treatment, expected outcome, and prognosis. (1)

Most cancers of unknown primary are adenocarcinomas or undifferentiated tumors; less commonly, they may be squamous carcinomas, melanoma, soft tissue sarcoma, or neuroendocrine tumors. Osteo- and chondrosarcomas rarely produce cancers of unknown primary. The most common primary sites of cancers of unknown primary are lung and pancreas, followed by colon and stomach, then breast, ovary, prostate, and solid-organ carcinomas of the kidney, thyroid, and liver. Conventional methods used to aid in the identification of the origin of a CUP include a thorough history and physical examination; computed tomography (CT) scans of the chest, abdomen, and pelvis; routine laboratory studies; and targeted evaluation of specific signs and symptoms. (2)

Biopsy of a CUP with detailed pathology evaluation may include immunohistochemical (IHC) analysis of the tumor. IHC identifies different antigens present on different types of tumors and can usually distinguish an epithelial tumor (i.e., carcinoma) from a melanoma or sarcoma. Detailed cytokeratin panels often allow further classification of a carcinoma; however, tumors of different origins may show overlapping cytokeratin expression. The results of IHC may provide a narrow differential of possible sources of a tumor's origin, but not necessarily a definitive answer.

The current success rate of the diagnostic workup of a CUP is 20–30%, including consideration of clinical, radiologic, and extensive histopathologic methods. (3) Recent advances in the understanding of gene expression in normal and malignant cells have led researchers to explore molecular classification as a way to improve the identification of the site of origin of a cancer of unknown primary.

Molecular Classification of Cancers

The molecular classification of cancers is based on the premise that, despite different degrees of loss of differentiation, tumors retain sufficient gene expression “signatures” as to their cell of origin, even after metastasis. Theoretically, it is possible to build a gene expression database spanning many different tumor types to compare to the expression profile of very poorly differentiated tumors or a cancer of unknown primary to aid in the identification of the tumor type and organ of origin. The feasibility of using molecular classification schemes with gene expression profiling (GEP) to classify these tumors of uncertain origin has been demonstrated in several studies. (4-7)

Ramaswamy et al., using microarray gene expression analysis of more than 16,000 genes, showed 78% classification accuracy of 14 common tumor types. (5) Su et al.,

using large-scale RNA profiling with microarrays, accurately predicted the anatomical site of tumor origin for 90% of 175 carcinomas. (6) Bloom et al. combined multiple tumor microarray databases, creating a large collection of tumors, including 21 types, resulting in a molecular classification scheme that reached 85% accuracy. (8) Although microarray technology enables large numbers of genes to be evaluated at the same time, it is complex and time-consuming and is limited in its use as mostly a research tool. (4) In addition, since formalin fixation can degrade RNA, fresh/frozen tissue is preferred for better accuracy with microarray technology; however, formalin-fixed is the standard for pathology material in current practice. (9)

One such microarray technology is the Pathwork® test (Response Genetics Inc., Los Angeles, CA). The test measures the expression of more than 1,500 genes and compares the similarity of the GEP of a CUP to a database of known profiles from 15 tissues with more than 60 histologic morphologies. The report generated for each tumor consists of a “similarity score,” which is a measure of similarity of the GEP of the specimen to the profile of the 15 known tumors in the database. Scores range from 0 (very low similarity) to 100 (very high similarity), and sum to 100 across all 15 tissues on the panel. If a single similarity score is greater than or equal to 30, it indicates that this is likely the tissue of origin. If every similarity score is between 5 and 30, the test result is considered indeterminate, and a similarity score of less than 5 rules out that tissue type as the likely origin. The test was developed by Pathwork Diagnostics, but the company filed for bankruptcy in early 2013, and their assets were purchased by Response Genetics Inc.

MiReview mets® (Rosetta Genomics, Philadelphia, PA) is another microarray technology which uses microRNAs (miRNA), small non-coding, single-stranded RNA molecules that regulate genes post-transcription, as a signature for tumor differentiation. The expression levels of these miRNAs have been shown to be a sensitive biomarker across various pathologic conditions. Samples for this test are formalin-fixed paraffin-embedded (FFPE) tissue. The miReview test utilizes 48 panel markers used to detect 22 tumor types in a known database of 336 tumors with a range of 1 to 49 tumors per type. The results from the test provide a tumor of origin but may list multiple possibilities calculated by a binary decision tree and K nearest neighbor algorithm. A second generation test, the Rosetta Cancer Origin Test™ (formerly miRview® mets2) has recently been developed; this test expands the number of tumor types to 42 primary origins with a panel of 64 miRNAs.

An alternative method to measure gene expression is real-time quantitative polymerase chain reaction (RT-qPCR). RT-qPCR can be used at the practice level; however, it can only measure, at most, a few hundred genes, limiting tumor categorization to 7 or fewer types. Tumor classification accuracy rates using real-time quantitative polymerase chain reaction (RT-PCR) have been reported to be as high as 87%, but less so (71%) the more undifferentiated the tumor tested. (4) One assay that uses RT-qPCR is the CancerTypeID® (bioTheranostics, Inc., San Diego, CA) assay, which measures the expression of messenger RNA in a CUP tissue sample. Samples for this are FFPE tissue

sections or unstained 10 micron sections on glass slides. The expression levels of 92 genes (87-tumor associated genes and 5 reference genes for normalization) are used to detect 27 tumor types in a known database of 578 tumors with a range of 5 to 49 tumors per type. The report generated is the probability for the main cancer type, possible subtypes, tumor types not able to be excluded, and those ruled out with 95% confidence calculated by K nearest neighbor analysis.

Regulatory Status

In July 2008, test Pathwork® Tissue of Origin was cleared with limitations* for marketing by the U.S Food and Drug Administration (FDA) through the 510(k) process. FDA determined that the test was substantially equivalent to existing tests for use in measuring the degree of similarity between the RNA expression pattern in a patient's fresh-frozen tumor and the RNA expression patterns in a database of tumor samples (poorly differentiated, undifferentiated, and metastatic cases) that were diagnosed according to current clinical and pathologic practice. The database contains examples of RNA expression patterns for 15 common malignant tumor types: bladder, breast, colorectal, gastric, hepatocellular, kidney, non-small cell lung, ovarian, pancreatic, and prostate cancers; thyroid carcinomas, melanoma, testicular germ cell tumor; non-Hodgkin's lymphoma (not otherwise specified); and soft tissue sarcoma (not otherwise specified). The Pathwork® Tissue of Origin test result is intended for use in the context of the patient's clinical history and other diagnostic tests evaluated by a qualified clinician.

*Limitations to the clearance were as follows:

The Pathwork® Tissue of Origin test is not intended to establish the origin of tumors that cannot be diagnosed according to current clinical and pathologic practice, (e.g., carcinoma of unknown primary). It is not intended to subclassify or modify the classification of tumors that can be diagnosed by current clinical and pathologic practice, nor to predict disease course, or survival or treatment efficacy, nor to distinguish primary from metastatic tumor. Tumor types not in the Pathwork® Tissue of Origin test database may have RNA expression patterns that are similar to RNA expression patterns in tumor types in the database, leading to indeterminate results or misclassifications.

In June 2010, the Pathwork® Tissue of Origin Test Kit-FFPE was cleared for marketing by the FDA through the 510(k) process. The 2010 clearance is an expanded application, which allows the test to be run on a patient's formalin-fixed, paraffin-embedded (FFPE) tumor and has the same indications and limitations. In May 2012, minor modifications to the "Pathwork® Tissue of Origin Test Kit-FFPE" were determined to be substantially equivalent to the previously approved device by the U.S. Food and Drug Administration (FDA) through the 510(k) process.

Neither CancerType ID® nor miRview® (or Rosetta Cancer Origin™) have been submitted to the FDA for approval.

POLICY

Gene expression profiling is considered **experimental / investigational** to evaluate the site of origin of a tumor of unknown primary, or to distinguish a primary from a metastatic tumor.

Examples of such tests include:

- Pathwork® Tissue of Origin Test
- Pathwork® Tissue of Origin Test Kit-FFPE
- CancerTypeID®
- Rosetta Cancer Origin™ (formerly miRview® mets2)

RATIONALE

Pathwork® Tissue of Origin Test

Analytic Validity (technical performance, ie, reproducibility)

Fresh frozen tumor sample

In 2008, Dumur et al. analyzed performance characteristics of the Pathwork® Tissue of Origin test in a cross-laboratory comparison study of 60 poorly and undifferentiated metastatic (77%) and primary (23%) tumors.(10) Three academic and one commercial laboratory received archived frozen tissue specimens for procurement and processing at their individual sites. Steps performed by each of the 4 laboratories included tissue handling, RNA extraction, and microarray-based gene expression assays using standard microarray protocol. The resulting microarray data generated at each laboratory were sent in a blinded fashion to Pathwork Diagnostics for generation of similarity scores for each type. Reports of the similarity scores were sent back (blinded) to the pathologists at the 4 laboratories for their use in generating an interpretation. Data were compared among the 4 laboratories to determine assay reproducibility. Correlation coefficients were between 0.95 and 0.97 for pathologists' interpretations of the similarity scores, and cross-laboratory comparisons showed an average 93.8% overall concordance between laboratories in terms of final tissue diagnosis.

Formalin-fixed, paraffin-embedded (FFPE) tumor sample

Analytical performance characteristics of the Pathwork® test for FFPE were analyzed in a cross-laboratory comparison study of 60 poorly and undifferentiated metastatic (45%) and primary (35%) tumors. Each of the 15 tumor tissue types were represented by 4 specimens each, with the exception of breast (n=3) and soft tissue sarcoma (n=5). Samples were distributed among 3 laboratories for procurement and processing at their individual sites. Data were compared among the 3 laboratories to determine assay reproducibility. Correlation coefficients were between 0.92 and 0.93 for pathologists' interpretations of the similarity scores, and cross-laboratory comparisons showed an average 82.1% overall concordance between laboratories in terms of final tissue diagnosis. A detailed summary of the data is available in the FDA's decision summary online at:

<http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm?ID=27502>. Additional analyses of the analytic performance of the test have produced similar results.(11,12)

Clinical Validity (sensitivity and specificity)

Fresh frozen tumor sample

The clinical validation study for the Pathwork® Tissue of Origin test that was submitted to the U.S. Food and Drug Administration (FDA) in 2008 involved a comparison of the gene expression profiles of 25 to 69 samples to each of the 15 known tumors on the Pathwork® panel (average 36 specimens per known tumor). The specimens included poorly differentiated, undifferentiated, and metastatic tumors. A similarity score was given to 545 specimens and then compared to the available specimen diagnosis. Based on the 545 results, the probability that a true tissue of origin call was obtained when a similarity score of 30 or more was reported was 92.9% (95% confidence interval [CI], 90.3 to 95.0%), and the probability that a true-negative tissue call was made when a similarity score of 5 or less was reported was 99.7% (95% CI, 99.6 to 99.8%). Overall, the Pathwork® performance comparing the profiles of the 545 specimens to the panel of 15 known tumor types showed a positive percent agreement of 89.4% (95% CI, 86.5 to 91.8%), negative percent agreement of 99.6% (95% CI, 98.6 to 100%), nonagreement of 6.2% (95% CI, 4.4 to 8.6%), and indeterminate of 4.4% (95% CI, 2.8 to 6.5%).

In 2009, Monzon et al. conducted a multicenter blinded validation study of the Pathwork® test.⁽¹³⁾ Specimens included poorly differentiated, undifferentiated, and metastatic tumors. A total of 351 frozen specimens and electronic files of microarray data on 271 specimens were obtained, with 547 meeting all inclusion criteria. A similarity score was given to the specimens, which was then compared to the original pathology report that accompanied the specimen. The Pathwork® performance comparing the profiles of the 547 specimens to the panel of 15 known tumor types showed overall sensitivity (positive percent agreement with reference diagnosis) of 87.8% (95% CI, 84.7 to 90.4%) and overall specificity (negative percent agreement with reference diagnosis) of 99.4% (95% CI, 98.3 to 99.9%), with the original pathology report acting as the reference standard. The authors acknowledged that since there was no independent confirmation of the original pathology, using the pathology reports as the reference standard could introduce errors into the study results. Agreement differed by cancer type: 94% for breast and 72% for both gastric and pancreatic; these differences were statistically significant ($\chi^2=42.02$; $p=0.04$; degrees of freedom = 28; $n=547$). Rates of agreement between test result and reference diagnosis varied by testing center: 88%, 84%, 92%, and 90% for Clinical Genomics facility, Cogenics, Mayo Clinic, and the International Genomics Consortium, respectively, but these differences were not statistically significant.

In 2012, Azueta et al. compared immunohistochemical (IHC) in FFPE tissue and the Pathwork® test in archived fresh-frozen tissue in a series of 32 metastatic tumors of suspected gynecologic origin (25 metastatic to the ovary, 7 peritoneal metastases).⁽¹⁴⁾ The primary site of origin was determined by clinical follow-up in 29 patients (83%) and was considered the gold standard. All peritoneal metastases originated from the ovary, and metastases to the ovary originated from the colon (11 cases), breast (5 cases), stomach (4 cases), endometrium (1 case), and an angiosarcoma (1 case). Eligible frozen sections from these cases and 3 with cancer of unknown primary (CUP) were required to contain at least 60% tumor and less than 20% necrotic tissue. Pathwork® concordance was 86% (25 of 29 diagnoses); in 2 cases, diagnoses were incorrect, and 2 cases had 2 possible diagnoses. Pathwork® diagnosed 2 of 3 cases of unknown primary after clinical follow-up. IHC concordance was 79% (23 of 29 diagnoses); 4 cases were indeterminate, 2 cases had 2 possible diagnoses, and diagnoses of 2 of 3 cases of unknown primary after clinical follow-up matched the Pathwork® diagnoses.

Formalin-fixed, paraffin-embedded (FFPE) tumor sample

The clinical validation study for the Pathwork® Tissue of Origin test Kit-FFPE that was submitted to the FDA in 2009 involved a comparison of the gene expression profiles of 25 to 57 samples to each of the 15 known tumors on the Pathwork® panel (average 31 specimens per known tumor). The specimens included poorly differentiated, undifferentiated, and metastatic tumors. A similarity score was given to 462 specimens and then compared to the available specimen diagnosis. Based on the 462 results, the probability that a true tissue of origin call was obtained when a similarity score was reported (positive percent agreement) was 88.5% (95% CI, 85.3 to 91.3%), and the probability that a true negative (ie, unknown) tissue call was made when a similarity score of 5 or less was reported (negative percent agreement) was 99.1% (95% CI, 97.6 to 99.7%). The proportion of nonagreement (false negatives) was 11.5% (95% CI, 8.7 to 14.7%). Further details of these data are available in the FDA's decision summary online at: <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmnm.cfm?ID=32236>.

In 2013, Handorf et al. reported a clinical validation study of 160 FFPE metastatic cancer specimens of known primary tumors representing the 15 tissue types on the Pathwork® test panel. (15) Pathwork® diagnostic performance was compared to IHC in 160 tumor samples. Overall concordance with known diagnoses (ie, accuracy) was 89% for Pathwork® and 83% for IHC, a statistically significant difference ($p=0.013$). In 51 poorly differentiated and undifferentiated tumors, Pathwork® accuracy was 94%, and IHC accuracy was 79% ($p=0.016$). In 106 well-differentiated and moderately differentiated tumors, Pathwork® and IHC performance was similar (87% and 85% accuracy, respectively; $p=0.52$). These results are based on 157 specimens for which both Pathwork® and IHC were performed; 3 specimens from the original set of 160 were considered nonevaluable by Pathwork® (similarity score <20) and were excluded.

Clinical Utility (impact on patient outcomes)

Two clinical trials are currently recruiting patients to test the direct clinical utility and the clinical application of gene expression profiling (GEP) to patient management and tumor site-specific therapy of the Pathwork® Tissue of Origin test. See the Clinical Trials summary below for further information.

CancerType ID®

Analytic Validity (technical performance, ie, reproducibility)

Formalin-fixed, paraffin-embedded (FFPE) tumor sample

Erlander et al. analyzed the analytic performance characteristics of the 92-gene CancerType ID® test. (16) A training set of 2,557 tumor samples was created from multiple tumor banks and commercial sources with 2206 samples included in the final internal validation dataset. These samples expanded on the standard CancerType ID® algorithm to increase tumor coverage and depth across 30 main cancer types and 54 histologic subtypes. Reproducibility was calculated from the observed cycle time for the 92 genes and 5 normalization genes using positive and negative controls. A total of 194 independent runs that included 4 operators provided the overall mean percentage coefficient of variation (CV) for the positive controls, which were 1.69% and 2.19% for the 92-genes and 5 normalization genes, respectively; for the negative controls the CV was 1.25% and 1.66% for the 92 genes and 5 normalization genes, respectively.

Clinical Validity (sensitivity and specificity)

Formalin-fixed, paraffin-embedded (FFPE) tumor sample

In 2013, Greco et al. published a retrospective, single-center study of 171 patients diagnosed with CUP after a clinical diagnostic work-up (ie, before IHC).⁽¹⁷⁾ The purpose of the study was to evaluate the accuracy of gene expression profiling (CancerType ID®) by verifying results with latent primary tumor sites found months after initial presentation (24 patients) or with IHC and/or clinicopathologic findings (147 patients). Minimum test performance thresholds were prespecified. Tumor specimens adequate for gene expression profiling were obtained in 149 patients (87%), and diagnoses were made in 144 (96%). Of 24 patients with latent primary tumor sites, CancerType ID® diagnoses were accurate in 18 (75%), and IHC diagnoses were accurate in 6 (25%). Of 52 patients with diagnosis made by IHC and subsequent gene expression profiling, diagnoses matched in 40 (77%). When IHC suggested 2 or 3 possible primary sites (97 patients), CancerType ID® diagnosis matched one of the proposed diagnoses in 43 (44%). Among 35 patients with discordant IHC and CancerType ID® diagnoses, clinicopathologic correlates and subsequent IHC supported the CancerType ID® diagnoses in 26 (74%). The authors concluded that gene expression profiling “complements standard pathologic evaluation” of CUP.

In 2012, Kerr et al. reported on a multicenter study of the 92-gene CancerType ID® test conducted to assess the test’s clinical validity.⁽¹⁸⁾ Approximately half of FFPE specimens for this study were from metastatic tumors of any grade, and the remainder were from poorly differentiated primary tumors processed within 6 years of testing. Laboratory personnel at 3 study sites, blinded to all information except biopsy site and patient gender, performed diagnostic adjudication on the 1,017 cases selected for inclusion. Adjudication failed on 60 cases and another 167 were excluded per protocol or due to quality control reasons. A total of 790 cases, across 28-tumor types, were classified according to class or main type and subtype with the 92-gene assay. Similarity score of $\geq 85\%$ was specified *a priori* as a threshold for classification with cases falling below this value determined to be unclassifiable by the test. When results of the 92-gene test were compared with the adjudicated diagnosis, overall sensitivity of the 92-gene assay was 87% (95% CI, 84 to 89%) with a range of 48% to 100% within tumor types. The reference diagnosis was incorrectly ruled out in 5% of cases, and 5.9% remained unclassifiable. Test specificity was uniformly high in all tumor types, ranging from 98% to 100%. Positive predictive values exceeded 90% in 16 of 28 tumor types (57%), with an overall range of 61% to 100%. In ANCOVA sub-group analysis, assay performance was found to be unaffected by tissue type (ie, metastatic or primary), histologic grade, or specimen type.

In 2011, Erlander et al. investigated the clinical performance characteristics of the 92-gene CancerType ID® test.⁽¹⁶⁾ A training set of 2557 tumor samples was created from multiple tumor banks and commercial sources. After excluding samples for inadequate tumor content, inconsistent or inconclusive pathologic information, cycle threshold (cycle number at which the generated fluorescence exceeds the fluorescence threshold) >28 , or independent pathologic review, 2206 samples were included in the final internal validation dataset. These samples all underwent real-time polymerase chain reaction (RT-qPCR) with the 92-gene assay primer-probe to serve as inputs for a modification to the standard CancerType ID® classification algorithm. Overall sensitivity of the CancerType ID® test determined by cross validation was 87% (95% CI, 85 to 88%) for main tumor type with a specificity of 100% (95% CI, 99 to 100%). Positive predictive value for main type accuracy was 87%, and negative predictive value was 100%. For

tumor subtypes these values were similar with a sensitivity of 85% (95% CI, 83 to 86%), specificity of 100% (95% CI, 100 to 100%), positive predictive value of 85%, and negative predictive value of 100%. One hundred eighty-seven independently collected tissue samples with specimens derived from formalin-fixed, paraffin-embedded blocks (84%) and snap-frozen tissues (16%) also were used to test the performance of the new algorithm. This test set included 28 of the 30 main cancer types and had an overall sensitivity of 83% and ranged from 50% to 100% across individual tumor types.

Clinical Utility (impact on patient outcomes)

In 2013, Hainsworth et al. published a multisite prospective case-series of the 92-gene CancerType ID® assay.(19) FFPE biopsy specimens for this study included adenocarcinoma, poorly differentiated adenocarcinoma, poorly differentiated carcinoma, or squamous carcinoma. A total of 289 patients were enrolled, and 252 (87%) had adequate biopsy tissue for the assay. The molecular profiling assay predicted a tissue of origin in 247 (98%) of 252 patients. One hundred nineteen assay predictions (48%) were made with $\geq 80\%$ similarity score, and the rest were below 80% probability. Twenty-nine patients (12%) did not remain on study due to decreasing performance, brain metastases, or patient and physician decision. Of the remaining 223 patients, 194 (87%) received assay-directed chemotherapy, and 29 received standard empiric therapy. Median overall survival of the 194 patients who received assay-directed chemotherapy (67% of the original patient sample) was 12.5 months, which was found to exceed a prespecified improvement threshold of 30% compared with historical trial data on 396 performance-matched CUP patients who received standard empiric therapy at the same center.

Rosetta Cancer Origin™ (formerly miRview® mets2)

Analytic Validity (technical performance, ie, reproducibility)

Formalin-fixed, paraffin-embedded (FFPE) tumor sample

A 2011 study by Chajut et al. provided information on the analytic validity of the miRview® mets2 test. One hundred seventy-four FFPE specimens were independently tested by Rosetta Genomics research and development laboratory and by a CLIA-approved clinical laboratory to determine concordance of the microRNA (miRNA) profiles. Inter-laboratory concordance was greater than 95% in 160 of 174 samples (92%).(20)

Clinical Validity (sensitivity and specificity)

Formalin-fixed, paraffin-embedded (FFPE) tumor sample

In 2012, Meiri et al. assessed the clinical validity of the miRview® mets2 test in 509 FFPE specimens.(21) Four hundred eighty-nine of these samples were successfully processed, and results were compared to the known origin of the specimen. Sensitivity of the test was 86%, and specificity exceeded 99%. Three smaller clinical validation studies testing 83 to 204 samples reported similar sensitivity and specificity, ranging from 84% to 86% and 95% to 99% respectively.(22-24)

Clinical Utility (impact on patient outcomes)

No published data on the clinical utility of miRview® mets2 and impact on patient treatment decision or diagnosis has been identified in the literature.

Other Microassay Tests for CUP

Clinical Validity (sensitivity and specificity)

Formalin-fixed, paraffin-embedded (FFPE) tumor sample

Other studies have analyzed the clinical validity of using microarray gene expression technology. One 2008 study used microarray technology (CupPrint®, Agendia, Amsterdam, the Netherlands) in FFPE tumor samples. The CupPrint assay utilizes the same internal validation data set as the CancerType ID® test and is currently marketed outside of the U.S. The study analyzed 495 genes in 84 patients with tumors of known origin and 38 patients with cancer of unknown primary (CUP) to assess the potential contribution to patient management.⁽³⁾ Sixteen of the patients with CUP (48%) had their primary site of tumor origin identified by standard laboratory techniques. Molecular testing identified the correct site of tumor origin in 94% of cases of CUP and 83% of the tumors of known origin.

Ades et al. (2013) compared gene expression profiling (CupPrint) to standard clinical work-up in patients with newly diagnosed, untreated metastatic tumors.⁽²⁵⁾ The authors prespecified a minimum concordance threshold of 75% to establish the diagnostic accuracy of CupPrint. Of 67 prospectively enrolled patients, both CupPrint and clinical diagnoses were obtained in 31 (46%). Median time to diagnosis was significantly shorter with CupPrint than with clinical workup (10 days vs 48 days, respectively; $p < 0.001$). Diagnoses were concordant in 11 patients (35%). The authors concluded that the diagnostic accuracy of CupPrint is low.

Clinical Utility (impact on patient outcomes)

A small 2008 study of CupPrint retrospectively analyzed the GEP of FFPE tumor samples from 21 patients with CUP.⁽²⁶⁾ In all patients, standard methods had failed to determine a primary tumor origin. GEP results were reviewed in the context of tumor histology and clinical suspicion of tumor origin; the clinical relevance of results and implications for patient management were assessed. Gene expression profiling confirmed clinical suspicion in 16 of 21 patients (76%). There was clinical/GEP inconsistency in 4 of 21 patients (19%) and histologic/gene profile inconsistency in 1 patient (5%). The authors concluded that the use of GEP would have influenced patient management in 12 of 21 patients.

Ferracin et al. (2011) published a report of microRNA profiling in 101 FFPE tumor samples from primary cancers and metastases.⁽²⁷⁾ Forty samples of 10 cancer types were used to build a cancer-type-specific microRNA signature. This signature was then used to predict the primary site of metastatic cancer. Overall accuracy was 100% for primary cancers and 78% for metastatic cancers in the cohort sample. The signature was then applied to a published set of 170 samples; prediction rates were consistent with the cohort results.

Summary

The available literature suggests that microarray-based gene expression testing may result in a high accuracy rate of identifying cancers of unknown primary (CUP) when comparing the results to a known tissue of origin. However, without data on how these tests would alter clinical practice and clinical health outcomes (clinical utility), the investigational policy statement remains unchanged. A trial that randomizes patients with a cancer of unknown primary to receive treatment based on the results of these types of tests or based on standard diagnostic

procedures would be useful to determine the clinical utility of gene-expression testing of cancers of unknown primary.

Ongoing Clinical Trials

A September 2013 search of the National Cancer Institute and online Clinicaltrials.gov databases returned 2 clinical trials currently recruiting patients to directly test the clinical utility and clinical application of gene expression profiling (GEP) to patient management and tumor site-specific therapy.

- A randomized European Phase 3 trial, NCT01540058, began in March 2012 with a completion date of October 2016. A treatment strategy guided by Pathwork® Tissue of Unknown Origin analysis followed by treatment for the suspected primary cancer is compared to an empiric strategy in patients with CUP. The study's primary outcome is progression from date of randomization, and secondary outcomes include tumor response rate, toxicity, and overall survival.
- A clinical trial of miRview® mets was completed in April 2012, NCT01202786, in Israel. The trial investigated the cost-effectiveness of using the miRview test compared to conventional workup of patients with cancer of unknown primary origin. Sixty participants were enrolled, and the following data were collected: cost and time of the diagnostic process from day 1 of the study to the decision on treatment program, the concordance between the miRview result compared with standard workup, treatment response, and overall survival. This trial has not been published.

Practice Guidelines and Position Statements

Current National Comprehensive Cancer Network guidelines for the workup of an occult primary malignancy address the use of molecular methods in the classification of tumors. They conclude that there are insufficient data to confirm whether gene expression profiling can be used in choosing treatment options that would improve the prognosis of patients with occult primary cancers. Therefore, the panel does not recommend the testing as a part of routine evaluation of a cancer of unknown primary origin.(28)

A 2010 clinical guideline from the National Institute for Health and Clinical Excellence recommends against the use of gene expression profiling to identify primary tumors in patients with CUP.(29) This recommendation is based on "limited evidence that gene-expression based profiling changes the management of patients with CUP and no evidence of improvement in outcome." The guideline includes a research recommendation for trials to assess the clinical utility of gene expression profiling.

CODING

The following codes for treatment and procedures applicable to this policy 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. 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.

CPT/HCPCS

81504 Oncology (tissue of origin), microarray gene expression profiling of > 2000 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as tissue similarity scores (Effective 01-01-2014)

81479 Unlisted molecular pathology procedure

81599 Unlisted multianalyte assay with algorithmic analysis

- Effective in July 2013, there is a specific CPT coding for the Pathwork Tissue of Origin test: 81504.
- Prior to July 2013, the preparation of the probes might have been coded using a combination of the molecular diagnostic codes 83890-83913 and the analysis of the probes might have been coded using array-based evaluation of multiple molecular probes codes 88384-88386 based on the number of probes analyzed.
- Prior to July 2013, Pathwork Diagnostics stated that they used 84999 (unlisted chemistry procedure).
- The other tests listed above do not have specific CPT codes.
 - If the test result is calculated using an algorithm and reported as a numeric score(s) or as a probability, the unlisted multianalyte assays with algorithmic analyses code 81599 would be reported.
 - If not, the unlisted molecular pathology code 81479 would be reported.

DIAGNOSES

Experimental / investigational for all diagnoses related to this policy.

REVISIONS

09-06-2011	Policy added to the bcbsks.com web site.
01-15-2013	In Coding section: <ul style="list-style-type: none"> ▪ Updated coding instructions to remove reference to 83890-83913 and 88384-88386 which are no longer effective as of 12-31-2012.
03-08-2013	Revised Title from "Gene Expression Testing for Cancers of Unknown Primary" to "Microarray-Based Gene Expression Testing for Cancers of Unknown Primary".
	Description section updated. In Policy section: <ul style="list-style-type: none"> ▪ Clarified policy statement from, "Gene expression profiling using the Pathwork® Tissue of Origin test or the Pathwork® Tissue of Origin test kit-FFPE is considered experimental / investigational to evaluate the site of origin of a tumor of unknown primary, and or to distinguish a primary from a metastatic tumor." To "Gene expression is considered experimental / investigational to evaluate the site of origin of a tumor of unknown primary, or to distinguish a primary from a metastatic tumor."

	<ul style="list-style-type: none"> ▪ This policy statement change was made to be generalizable to gene expression profiling and not specific to the Pathwork test, so is not a change of policy position.
	Rationale section updated
	References updated
01-21-2014	Description section updated
	In Policy section: <ul style="list-style-type: none"> ▪ Updated examples of tests.
	Rationale section updated
	In Coding section: <ul style="list-style-type: none"> ▪ Added CPT codes: 81504 (Eff 01-01-2014), 81479, 81599 ▪ Removed CPT code: 84999
	References updated

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