Title: Gene Expression-Based Assays for Cancers of Unknown Primary

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**DESCRIPTION**

Cancers of unknown primary (CUP) represent 3% to 4% of cancers diagnosed in the United States. These cancers are heterogeneous and many accompanied by poor...
prognoses. A detailed history and physical combined with imaging and tissue pathology 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 gene expression profiling to direct treatment may improve health outcomes.

Background

CANCERS OF UNKNOWN PRIMARY

Cancers of unknown primary (CUPs), or occult primary malignancies, are tumors that have metastasized from an unknown primary source; they make up approximately 3% to 4% of all cancer cases in the United States. Identifying the primary origin of a tumor can dictate cancer-specific treatment, expected outcome, and prognosis.1

Most CUPs are adenocarcinomas or undifferentiated tumors; less commonly, they may be squamous carcinomas, melanoma, soft tissue sarcoma, or neuroendocrine tumors. Osteo- and chondrosarcomas rarely produce CUPs. The most common primary sites of CUPs 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

Diagnosis and Classification

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 (ie, 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. Results of IHC may provide a narrow differential of possible sources of a tumor’s origin, but not necessarily a definitive answer.

Recent advances in the understanding of gene expression in normal and malignant cells have led researchers to explore molecular classification to improve the identification of the site of origin of a CUP. 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 with the expression profile of very poorly differentiated tumors or a CUP 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.3-6
Tissue of Origin Testing, Treatment Selection, and Health Outcomes

Patients with CUP have generally poor prognoses. For example, patients with disease limited to lymph nodes have a median survival of 6 to 9 months, and those with disease that is extranodal 2 to 4 months. The premise of tissue of origin testing in CUPs is that identifying a likely primary tumor site will inform treatment selection leading to improved survival and other outcomes or as a predictive test. To evaluate whether treatment selection can be improved, the ability of test to suggest a likely site of origin (clinical validity) must be first be shown. But demonstrating clinical validity may be problematic because patients with CUPs have no identified primary tumor for a reference standard. Imperfect reference standards must be relied on such as the available presumptive or a reference pathologic diagnosis, known tumor types, or comparisons IHC. A primary tumor diagnosed during follow-up might also be used as a reference standard, but its use would be subject to potential selection bias. Therefore, even substantial evidence supporting the ability of a test to suggest a likely site of origin will be insufficient to infer benefit. Convincing evidence for benefit requires demonstrating that using a test to select treatment will improve outcomes.

Tests Reviewed in This Report

Evidence on the analytic validity, clinical validity, and clinical utility for 3 GEP tests is reviewed in this report (see Table 1).

Table 1. Gene Expression Profiling Tests for CUP

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CUP: cancer of unknown primary; RT-qPCR: real-time quantitative polymerase chain reaction.

a Formerly PathWork® and ResponseDX: Tissue of Origin™.
b Formerly miRview® met2.

The Tissue of Origin test (formerly known as the PathWork Tissue of Origin Test and ResponseDX Tissue of Origin; Cancer Genetics). The test measures the expression of 2000 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 comprises a “similarity score,” which is a measure of similarity of 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 30 or more, 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. PathWork Diagnostics developed the test, but the company filed for bankruptcy in early 2013; Response Genetics purchased its assets, and it, in turn, was acquired by Cancer Genetics in late 2015.
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 polymerase chain reaction (RT-PCR) have been reported to be as high as 87%, but lower (71%) the more undifferentiated the tumor tested. One assay that uses RT-qPCR is the CancerTYPE ID® (biotheranostics) assay, which measures the expression of messenger RNA in a CUP tissue sample. Samples for this are formalin-fixed, paraffin-embedded (FFPE) tissue sections or unstained 10 micron sections on glass slides. 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.

miReview mets is another RT-qPCR test that uses microRNAs (miRNA), small noncoding, single-stranded RNA molecules that regulate genes posttranscription, as a signature for tumor differentiation. Expression levels of these miRNAs have been shown to be a sensitive biomarker across various pathologic conditions. Samples for this test are FFPE tissue. The miReview test utilizes 48 panel markers to detect 22 tumor types in a known database of 336 tumors, with a range of 1 to 49 tumors per type. Results from the test provided 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 RosettaGX Cancer Origin Test (formerly miRview mets² and ProOnc Tumor Source), has also been developed; this test expands the number of tumor types to 42 primary origins with a panel of 64 miRNAs.

**Regulatory Status**
In July 2008, the PathWork® Tissue of Origin Test™ (Response Genetics; now Cancer Genetics) was cleared for marketing with limitations (see below) 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, metastatic cases) that were diagnosed according to current clinical and histopathologic practice. The database contains examples of RNA expression patterns for 15 common malignant tumor types.
A PathWork® Tissue of Origin® Test result was 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, (eg, a cancer 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, or to predict disease course, or survival or treatment efficacy, or to distinguish primary from metastatic tumor.
- Tumor types not in the PathWork® Tissue of Origin Test database may have RNA expression patterns 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 FDA through the 510(k) process. The 2010 clearance was an expanded application, which permitted 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 FDA through the 510(k) process.

The test is now offered by Cancer Genetics, as the Tissue of Origin® test.

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 Clinical Laboratory Improvement Amendments (CLIA). CancerTYPE ID® (Biotheranostics, San Diego, CA) are miRview® (or RosettaGX Cancer Origin™; Rosetta Genomics, Philadelphia, PA) are available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.
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.

RATIONALE

This evidence review has been updated with searches of the MEDLINE database. The most recent literature review was performed for the period through January 25, 2017; Biotheranostics also provided a list of potential publications without date restrictions for consideration (see Appendix Table 1 for genetic testing categories).

Clinical Context and Test Purpose

The purpose of tissue of origin testing is to identify a likely primary tumor type and by doing so inform treatment selection that might lead to improved health outcomes (ie, as a predictive test).

Patients

The target populations are patients with a cancer of unknown primary (CUP) and no identified primary tumor following a standard evaluation (eg, history, physical, imaging, pathology).

Interventions

Three gene expression profiling (GEP) tests currently available in the United States are the primary focus of this review (see Table 1).

Comparators

The comparator of interest is standard of care management based on tumor type and probable site of origin—ie, usual care without GEP.

Outcomes

Although test validity is relevant as a premise of the test, the outcomes informative of potential benefit include overall survival (OS), disease-specific survival, and quality of life.

Timing

Given the generally poor survival experience of patients with CUP, outcomes assessed over a follow-up of 1 to 2 years are relevant.

Setting

Both community and academic settings are of interest.

Analytic Validity

Tissue of Origin Test

Fresh Frozen Tumor Sample

In 2008, Dumur et al analyzed the 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. Three academic and 1 commercial laboratories 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 Tumor Sample
Analytic performance characteristics of the PathWork test for formalin-fixed, paraffin-embedded (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 was 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 Food and Drug Administration’s (FDA) decision summary. Additional analyses of the analytic performance of the test have produced similar results.

CancerTYPE ID
Erlander et al (2011) assessed the analytic performance characteristics of the 92-gene CancerTYPE ID test. Reproducibility was assessed using positive and negative controls. A total of 194 independent runs that included 4 operators provided the overall mean percentage coefficient of variation 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 coefficient of variation was 1.25% and 1.66% for the 92 genes and 5 normalization genes, respectively.

RosettaGX Cancer Origin
FFPE Tumor Sample
A 2011 study by Rosenwald 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 clinical laboratory approved under Clinical Laboratory Improvement Amendments to determine the concordance of the microRNA (miRNA) profiles. Interlaboratory concordance was greater than 95% in 160 (92%) of 174 samples.

Section Summary: Analytic Validity
Evidence for the analytic validity of the 3 tests pertains primarily to assay reproducibility, quality control, detection limit, and specificity (effect of interfering substances). While some available evidence supports aspects of analytic validity for all tests, only the Tissue of Origin Test has been cleared by FDA.
Clinical Validity

Tissue of Origin Test

Five included studies reported evidence that the Tissue of Origin Test can predict a likely site of origin using a variety of reference standards: reference or available diagnosis, a primary tumor identified during follow-up, and immunohistochemistry (IHC). Concordance rates in the range of 85% to 90% were reported compared with the reference standards employed.

The clinical validation study for the PathWork Tissue of Origin Test that was submitted to FDA in 2008 compared GEP tests for 25 to 69 samples with each of the 15 known tumors on the PathWork panel (mean, 36 specimens per known tumor). Specimens included poorly differentiated, undifferentiated, and metastatic tumors. A similarity score was assigned to 545 specimens and then compared with 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 93% (95% confidence interval [CI], 90% to 95%), and the probability that a true-negative tissue call was made when a similarity score of 5 or less was reported was 100% (95% CI, 100% to 100%). Overall PathWork performance comparing the profiles of the 545 specimens with the panel of 15 known tumor types showed a positive percent agreement of 90% (95% CI, 87% to 92%), negative percent agreement of 100% (95% CI, 99% to 100%), nonagreement of 6% (95% CI, 4% to 9%), and indeterminate of 4% (95% CI, 3% to 7%).

In 2009, Monzon et al conducted a multicenter blinded validation study of the PathWork test. 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 with the original pathology report that accompanied the specimen. The PathWork performance comparing the profiles of the 547 specimens with the panel of 15 known tumor types showed overall sensitivity (positive percent agreement with reference diagnosis) of 88% (95% CI, 85% to 90%) and overall specificity (negative percent agreement with reference diagnosis) of 99% (95% CI, 98% to 100%), with the original pathology report acting as the reference standard. The authors noted that because there was no independent confirmation of the original pathology, using the pathology reports as the reference standard could introduce error into study results. Agreement differed by cancer type: 94% for breast and 72% for both gastric and pancreatic; these differences were statistically significant (p=0.04). 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 (differences not statistically significant).

In 2013, Azueta et al compared 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). The primary site of origin was determined by clinical follow-up in 29 (83%) patients and was considered the criterion 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 CUP were required to contain at least 60% tumor and less than 20% necrotic tissue. PathWork concordance was 86% (25/29 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% (23/29 diagnoses); 4 cases were indeterminate, and 2 cases had 2 possible diagnoses.
diagnoses of 2 of 3 cases of unknown primary after clinical follow-up matched the PathWork diagnoses.

The clinical validation study for the PathWork Tissue of Origin Test Kit-FFPE submitted to FDA in 2009 compared GEP results for 25 to 57 samples to each of the 15 known tumors on the PathWork panel (mean, 31 specimens per known tumor). Specimens included poorly differentiated, undifferentiated, and metastatic tumors. A similarity score was assigned to 462 specimens and then compared with 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 89% (95% CI, 85% to 91%), 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% (95% CI, 98% to 100%). The proportion of nonagreement (false negatives) was 12% (95% CI, 9% to 15%). Further details of these data are available in FDA's decision summary.

In 2013, Handorf et al reported on a clinical validation study of FFPE metastatic cancer specimens of known primary tumors representing the 15 tissue types on the PathWork test panel. PathWork's diagnostic performance was compared with IHC in 160 tumor samples. Overall concordance with known diagnoses (ie, accuracy) was 89% for PathWork versus 83% for IHC (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 testing were performed; 3 specimens from the original set of 160 were considered nonevaluable by PathWork (similarity score, <20) and were excluded.

**CancerTYPE ID**

Results derived from 4 samples reported evidence for supporting the ability of CancerTYPE ID to predict a likely site of origin. Reference standards included a known tumor type, reference diagnosis, a primary tumor identified during follow-up, and IHC. Reported sensitivities varied according to tumor type generally ranged from 80% to over 90%.

Erlander et al (2011) revised the original classifier algorithm using 2206 samples created from multiple tumor banks and commercial sources. These samples expanded on the standard CancerTYPE ID algorithm to increase tumor coverage and depth across 30 main cancer types and 54 histologic subtypes. Sensitivity of the classifier for the main cancer type based on internal validation (leave-one-out cross validation) was 87% (95% CI, 85% to 88%) and, for the histologic subtype, 85% (95% CI, 83% to 86). In an independent test set of 187 samples, sensitivity was 83% (95% CI, 78% to 88%).

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. Approximately half of FFPE specimens for this study were from metastatic tumors of any grade, and the remainder 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 sex, performed diagnostic adjudication on 790 tumors, across 28 tumor types. Each specimen was then classified according to class or main type and subtype with the 92-gene assay. A similarity score of 85% or greater 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 adjudicated diagnoses, 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 6% remained unclassifiable. Test specificity was uniformly high in all tumor types, ranging from 98% to 100%. Positive predictive values ranged from 61% to 100% and exceeded 90% in 16 of 28 tumor types. In an analysis of covariance, assay performance was found to be unaffected by tissue type (ie, metastatic or primary), histologic grade, or specimen type. A 2014 sub-study of this dataset evaluated primary (41%) and metastatic (59%) tumors considered to have neuroendocrine differentiation (Merkel cell carcinoma, medullary thyroid carcinoma, pheochromocytoma, paraganglioma, pulmonary neuroendocrine carcinoma, pancreatic neuroendocrine carcinoma, gastrointestinal neuroendocrine carcinoma). For 75 included tumors, assay sensitivities were 99% (95% CI, 93% to 99%) for classification of neuroendocrine tumor type (eg, neuroendocrine, germ cell) and 95% (95% CI, 87% to 98%) for subtype (site of origin). Positive predictive values ranged from 83% to 100% for individual subtypes. A 2016 report by Brachtel et al examined a subset of samples from 109 patients with limited tissue studied by Kerr et al (2012) and 644 other consecutive cytology samples. In the 109 patients, sensitivity for tumor classification was 91% (95% CI, 84% to 95%) or consistent with the larger sample. From the 644 cases, a sensitivity of 87% (95% CI, 84% to 89%) was estimated.

In 2013, Greco et al published a retrospective, single-center study of 171 patients diagnosed with CUP after a clinical diagnostic workup (ie, before IHC). The purpose of the study was to evaluate the accuracy of GEP (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 GEP were obtained in 149 (87%) patients, 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 testing and subsequent GEP, 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 GEP “complements standard pathologic evaluation” of CUP.

Consistent with other clinical validity data, Greco et al (2015) retrospectively reported on the use of CancerTYPE ID on archived samples from 30 patients with CUP and poorly differentiated neoplasms. This subset of patients with CUP is considered potentially treatment sensitive, but comprised a small number (4%) of the 751 CUP patients evaluated from 2000 through 2012 at Tennessee centers. A primary site was identified in 2 patients. A diagnosis was assigned by GEP in 25 (83%) of the samples. Although 7 recently evaluated patients received treatment based on the diagnosis provided, and 5 reportedly had "favorable" outcomes, whether benefit was obtained cannot be assessed.

**RosettaGX Cancer Origin**

**FFPE Tumor Sample**

In 2012, Meiri et al assessed the clinical validity of the miRview mets test in 509 FFPE specimens. Four hundred eighty-nine of these samples were successfully processed, and results were compared with 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, with ranges of 84% to 86% and 95% to 99%, respectively.\textsuperscript{25-27}

\textbf{Section Summary: Clinical Validity}

Using different reference standards, the tests have reported sensitivities or concordances generally high (eg, 80% to 90% or more). However, clinical validity evidence does not provide support for potential benefit.

\textbf{Clinical Utility}

\textbf{Tissue of Origin Test}

Nystrom et al (2012) enrolled 65 physicians (from 316 approached) caring for 107 patients with CUP in 2009 to participate in a study of management changes following a tissue of origin test.\textsuperscript{28} Prior to the test, physicians had no suspected diagnosis for 54 (41%) patients, which declined to 17 (16%) after testing. Changes in management were reported in 70 (65%) patients. Physicians reported test results were helpful with regard to diagnosis, choosing therapy, and triaging. Median survival was 14 months, which the authors suggest longer than 9 months for unselected chemotherapy treated CUP patients. However, the low physician participation rate and lack of a concurrent comparator group limits any implications of these results. The study was supported by PathWork Diagnostics and 2 authors company employees.

Yoon et al (2016) reported results of a multicenter phase 2 trial evaluating combined use of carboplatin, paclitaxel, and everolimus in patients with CUP.\textsuperscript{29} The primary outcome was objective response, and the study a 2-stage design with 11 or more responses in 50 assessable patients at the second stage considered success. There were 16 partial responses (objective response rate, 36%; 95% CI, 22% to 51%). Grade 3 or 4 adverse events occurred in 40 (87%) patients. Results from the PathWork Tissue of Origin Test were used post hoc to examine any association with response to therapy. In 38 of 46 patients, the test was successfully obtained and 10 different tissues of origin were predicted. In 19 patients with a tissue of origin where platinum/taxane therapy might be considered standard therapy, objective response rates were higher compared with other patients (53% vs 26%, \textit{p}=0.097), accompanied by longer progression-free survival (PFS; 6.4 months vs 3.5 months, \textit{p}=0.026; hazard ratio [HR], 0.47; 95% CI, 0.24 to 0.93), and longer OS (median, 17.8 months vs 8.3 months; \textit{p}=0.005; HR=0.37; 95% CI, 0.18 to 0.76). The results suggested a tissue of origin test might identify platinum/taxane-sensitive tumors. However, the study was not designed to evaluate predictive use of the test, tissue of origin data were missing for 17% of patients, and severe adverse events were common.

\textbf{CancerTYPE ID}

From patients with CUP who had undergone a CancerTYPE ID assay between March 2008 and August 2009, Hainsworth et al (2012) identified those with a probable (\textgeq 80%) colorectal site of origin.\textsuperscript{30} A total of 125 patients (of 1544 results) were predicted to have a primary colorectal cancer (CRC). Physicians caring for patients were sent questionnaires with a request for deidentified pathology reports—42 (34%) responded (physicians were paid $250). The date of questionnaire mailing was not reported. A total of 32 patients were given CRC regimens (16 first-line therapy only, 8 first- and second-line therapy, 8 second-line therapy only) with a reported response rate of 50% following first-line and 50% following second-line therapy; 18 patients were given empiric CUP regimens with a response rate of 17%. For first-line therapies, physician-assessed PFS was longer following CRC regimens—8.5 months versus 6 months (\textit{p}=0.11). The
authors concluded that “Molecular tumor profiling seems to improve survival by allowing specific therapy in this patient subgroup....” However, conclusions are limited by significant potential biases: low physician response rates and potential selection bias; unverified physician-reported retrospective assessment of progression, response, or death; absence of information on patient performance status to assess between-group prognostic differences; and the post hoc subgroup definition of uncertain generalizability to patients with CUP undergoing tissue of origin testing.

In 2013, Hainsworth et al published a multisite prospective case series of the 92-gene CancerTYPE ID assay.31 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 (48%) assay predictions were made with a similarity score of 80% or greater, and the rest were below 80% probability. Twenty-nine (12%) patients did not remain in the study due to decreasing performance status, brain metastases, or patient and physician decision. Of the remaining 223 patients, 194 (87%) received assay-directed chemotherapy and 29 (13%) 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 exceeded a prespecified improvement threshold of 30% compared with historical trial data for 396 performance-matched CUP patients who received standard empiric therapy at the same center. Although these results are consistent with possible benefit from GEP testing in CUP, potential biases accompany the nonrandomized design—confounding variables, use of subsequent lines of empirical therapy, heterogeneity of unknown primary cancers, comparison with historical controls—and limit conclusions that can be drawn.32,33

RosettaGX Cancer Origin
No published data on the clinical utility of RosettaGX Cancer Origin test and impact on patient treatment decision or diagnosis were identified in the literature.

Section Summary: Clinical Utility
There is limited indirect evidence from nonrandomized studies for 2 of the tests concerning clinical utility and studies had significant limitations including comparisons with historical controls and possible selection bias. The absence of either convincing evidence from an unbiased nonrandomized study or randomized controlled trials prevents conclusions concerning clinical utility. Benefit would be most convincingly demonstrated through a marker strategy designed trial randomizing patients with cancers of unknown primary to receive treatment based on gene expression profiling results or usual care.

SUMMARY OF EVIDENCE
For individuals who have a cancer of unknown primary (CUP) who receive gene expression profiling, the evidence includes studies of analytic validity, clinical validity, and limited evidence on potential clinical utility. Relevant outcomes are overall survival, disease-specific survival, test validity, and quality of life. For the 3 commercially available tests reviewed, there is some evidence to support relevant aspects of analytic validity; 1 test has been cleared by the Food and Drug Administration. Using different reference standards (known tumor type, reference diagnosis, a primary tumor identified during follow-up, immunohistochemical analysis) for the tissue of origin, the tests have reported sensitivities or concordances generally high (eg, 80% to 90% or more). However, evidence for clinical validity does not support potential benefit. There is
limited indirect evidence from nonrandomized studies on clinical utility, and all studies had significant limitations. Benefit would be most convincingly demonstrated through a marker strategy–designed trial randomizing patients with a CUP to treatment based on expression profiling results or to usual care. The evidence is insufficient to determine the effects of the technology on health outcomes.

PRACTICE GUIDELINES AND POSITION STATEMENTS

National Comprehensive Cancer Network
Current National Comprehensive Cancer Network (NCCN) guidelines for the workup of an occult primary malignancy (v.2.2017) address the use of molecular methods to classify tumors.7 The guidelines state, “Tumor sequencing and Gene signature profiling for tissue of origin is not recommended for standard management at this time.” A footnote acknowledges that “there may be diagnostic benefit, though not necessarily clinical benefit. The use of gene signature profiling is a category 3 recommendation [based on any level of evidence, there is major NCCN disagreement that the intervention is appropriate].” The guidelines later note:

“In an attempt to identify the tissue of origin, biopsy specimens are often analyzed by immunohistochemistry (IHC). In addition, gene expression profiling (GEP) assays have been developed to attempt to identify the tissue of origin in patients with occult primary cancers. It is noteworthy that thus far the literature on this approach, as with the literature on IHC application in the workup of occult primary tumors, has focused far more on establishing a tissue of origin than on establishing whether such identification leads to better outcomes in patients. Thus, while there is diagnostic benefit of GEP, a clinical benefit has not been demonstrated. Consequently, the panel does not recommend tumor sequencing and gene signature profiling for the identification of tissue of origin as standard management in the diagnostic workup of patients with occult primary tumors. Overall, the panel believes that neither IHC, a diagnostic tool in widespread use, nor GEP should be used indiscriminately.”

National Institute for Health and Care Excellence
A 2010 clinical guidance from the National Institute for Health and Care Excellence recommended against the use of gene expression profiling (GEP) to identify primary tumors in patients with cancers of unknown primary (CUPs).34 This recommendation was based on “limited evidence that gene-expression based profiling changes the management of patients with CUP and no evidence of improvement in outcome.” The guidance included a research recommendation for trials to assess the clinical utility of GEP.

European Society of Medical Oncology
The 2015 guidelines from the European Society of Medical Oncology stated that, for GEP assays to identify tissue of origin in patients with CUP, “their impact on patient outcome via administration of primary site specific therapy remains questionable and unproven in randomized trials” (Level of evidence: IV based on “retrospective cohort studies or case–control studies”; Grade of recommendation C: “insufficient evidence for efficacy or benefit does not outweigh the risk or the disadvantages.”)35,36 Rather, “Immunohistochemistry should be applied meticulously in order to identify the tissue of origin and to exclude chemosensitive and potentially curable tumors (ie, lymphomas and germ cell tumors).”

U.S. PREVENTIVE SERVICES TASK FORCE RECOMMENDATIONS
Not applicable.
ONGOING AND UNPUBLISHED CLINICAL TRIALS
A currently unpublished trial that might influence this review is listed in Table 2.

Table 2. Summary of Key Trials

<table>
<thead>
<tr>
<th>NCT No.</th>
<th>Trial Name</th>
<th>Planned Enrollment</th>
<th>Completion Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ongoing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT01540058</td>
<td>A Randomised Phase III Trial Comparing a Strategy Based on Molecular Analysis to the Empiric Strategy in Patients With Carcinoma of an Unknown Primary (CUP)</td>
<td>223</td>
<td>Oct 2017</td>
</tr>
</tbody>
</table>

NCT: national clinical trial.

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
- 81479 Unlisted molecular pathology procedure
- 81504 Oncology (tissue of origin), microarray gene expression profiling of >2000 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as tissue similarity scores
- 81540 Oncology (tumor of unknown origin), mRNA, gene expression profiling by real-time RT-PCR of 92 genes (87 content and 5 housekeeping) to classify tumor into main cancer type and subtype, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a probability of a predicted main cancer type and subtype
- 81599 Unlisted multianalyte assay with algorithmic analysis

- There is specific CPT coding for the Pathwork Tissue of Origin® Test: 81504.
- There is a CPT code specific to the CancerTYPE ID® test: 81540
- The other tests described in this policy 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:
  - 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:
- 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."
- 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:
- Updated examples of tests.

Rationale section updated

In Coding section:
- Added CPT codes: 81504 (Eff 01-01-2014), 81479, 81599
- Removed CPT code: 84999

References updated

02-10-2015 Title revised to "Gene Expression-Based Assays for Cancers of Unknown Primary" from "Microarray-Based Gene Expression Testing for Cancers of Unknown Primary"
Description section updated
Rationale section updated
References updated

01-01-2016 In Coding section:
- Added CPT Code: 81540
- Updated Coding notations

04-19-2017 Description section updated

In Policy section:
- Removed examples of tests, which are now listed in Table 1. Gene Expression Profiling Tests for CUP of the Rationale section.

Rationale section updated
In Coding section:
- Coding notations updated

References updated

REFERENCES
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20348879
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2014;371(8):757-765. PMID 25140961
33. Prasad V, Oseran A, Fakhrejahani F. The use of gene expression profiling and mutation
analysis increases the cost of care for patients with carcinoma of unknown primary; does it
also improve survival? Eur J Cancer. Feb 2016;54:159-162. PMID 26608119

Appendix Table 1.

<table>
<thead>
<tr>
<th>Category</th>
<th>Addressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Testing of an affected individual’s germline to benefit the individual</td>
<td></td>
</tr>
<tr>
<td>1a. Diagnostic</td>
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<tr>
<td>1b. Prognostic</td>
<td></td>
</tr>
<tr>
<td>1c. Therapeutic</td>
<td></td>
</tr>
<tr>
<td>2. Testing cancer cells from an affected individual to benefit the individual</td>
<td>X</td>
</tr>
<tr>
<td>2a. Diagnostic</td>
<td></td>
</tr>
<tr>
<td>2b. Prognostic</td>
<td></td>
</tr>
<tr>
<td>2c. Therapeutic</td>
<td>X</td>
</tr>
<tr>
<td>3. Testing an asymptomatic individual to determine future risk of disease</td>
<td></td>
</tr>
<tr>
<td>4. Testing of an affected individual’s germline to benefit family members</td>
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</tr>
<tr>
<td>5. Reproductive testing</td>
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<tr>
<td>5a. Carrier testing: preconception</td>
<td></td>
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<tr>
<td>5b. Carrier testing: prenatal</td>
<td></td>
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<tr>
<td>5c. In utero testing: aneuploidy</td>
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<tr>
<td>5d. In utero testing: familial variants</td>
<td></td>
</tr>
<tr>
<td>5e. In utero testing: other</td>
<td></td>
</tr>
<tr>
<td>5f. Preimplantation testing with in vitro fertilization</td>
<td></td>
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</tbody>
</table>