

Medical Policy



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

Professional

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Populations	Interventions	Comparators	Outcomes
Individuals: <ul style="list-style-type: none"> With cancer of unknown primary 	Interventions of interest are: <ul style="list-style-type: none"> Gene expression profiling 	Comparators of interest are: <ul style="list-style-type: none"> Clinical workup, including imaging and pathology, without gene expression profiling 	Relevant outcomes include: <ul style="list-style-type: none"> Overall survival Disease-specific survival Test validity Quality of life

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.

Objective

The objective of this evidence review is to evaluate whether gene expression profiling in patients with cancers of unknown primary improves the net health outcome compared with standard of care management based on tumor type and probable site of origin.

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.⁷ 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 clinical validity and clinical utility for 3 GEP tests is reviewed in this report (see Table 1).

Table 1. Gene Expression Profiling Tests for CUP⁸

Test	Manufacturer	Platform	Genes Assayed, n	Tumor Types Assessed, n
Tissue of Origin ^a	Cancer Genetics	Oligonucleotide microarray	2000	15
CancerTYPE ID	Biotheranostics	RT-qPCR	92	54
RosettaGX Cancer Origin ^b	Rosetta Genomics	RT-qPCR (microRNA)	64	49

Adapted from Agwa et al (2013).⁸

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) 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.³ 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 formalin-fixed, paraffin-embedded (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 49 primary origins with a panel of 64 miRNAs.

Regulatory Status

In 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 product code: OIW), with subsequent clearances for expanded applications in 2010 and minor modifications in 2012. 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.

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.

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 FDA 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.

Policy Guidelines

Genetics Nomenclature Update

The Human Genome Variation Society nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). The Society's nomenclature is recommended by the Human Variome Project, the HUMAN Genome Organization, and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology—"pathogenic," "likely pathogenic,"

“uncertain significance,” “likely benign,” and “benign”—to describe variants identified that cause Mendelian disorders.

Table PG1. Nomenclature to Report on Variants Found in DNA

Previous	Updated	Definition
Mutation	Disease-associated variant	Disease-associated change in the DNA sequence
	Variant	Change in the DNA sequence
	Familial variant	Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives

Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification

Variant Classification	Definition
Pathogenic	Disease-causing change in the DNA sequence
Likely pathogenic	Likely disease-causing change in the DNA sequence
Variant of uncertain significance	Change in DNA sequence with uncertain effects on disease
Likely benign	Likely benign change in the DNA sequence
Benign	Benign change in the DNA sequence

American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

Genetic Counseling

Experts recommend formal genetic counseling for patients who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some patients; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

RATIONALE

This evidence review has been updated with searches of the MEDLINE database. The most recent literature update was performed through January 8, 2018.

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

GENE EXPRESSION PROFILING TESTS FOR CANCERS OF UNKNOWN PRIMARY

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: Tissue of Origin, CancerTYPE ID, and RosettaGX Cancer Origin (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, 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.

Simplifying Test Terms

There are 3 core characteristics for assessing a medical test. Whether imaging, laboratory, or other, all medical tests must be:

- Technically reliable
- Clinically valid
- Clinically useful.

Because different specialties may use different terms for the same concept, we are highlighting the core characteristics. The core characteristics also apply to different uses of tests, such as diagnosis, prognosis, and monitoring treatment.

Diagnostic tests detect presence or absence of a condition. Surveillance and treatment monitoring are essentially diagnostic tests over a time frame. Surveillance to see whether a condition develops or progresses is a type of detection. Treatment monitoring is also a type of detection because the purpose is to see if treatment is associated with the disappearance, regression, or progression of the condition.

Prognostic tests predict the risk of developing a condition in the future. Tests to predict response to therapy are also prognostic. Response to therapy is a type of condition and can be either a

beneficial response or adverse response. The term predictive test is often used to refer to response to therapy. To simplify terms, we use prognostic to refer both to predicting a future condition or to predicting a response to therapy.

Technically Reliable

Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review, and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Specifically, for these tests, clinical validity is the ability of a test to determine the site of origin. Demonstrating clinical validity is complicated by the lack of reference standard. Imperfect reference standards must be relied on such as the available presumptive or a reference pathologic diagnosis, known tumor types, comparisons immunohistochemistry (IHC) or primary tumor diagnosed during follow-up.

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 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 submitted to the Food and Drug Administration 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.⁹ 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%).

Monzon et al (2009) conducted a multicenter, blinded validation study of the PathWork test.¹⁰ 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 the test result and reference diagnosis varied by the testing center: 88%, 84%, 92%, and 90% for Clinical Genomics facility, Cogenics, Mayo Clinic, and the International Genomics Consortium, respectively (differences not statistically significant).

Azueta et al (2013) compared IHC in formalin-fixed, paraffin-embedded (FFPE) tissue with 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).¹¹ 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 the 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 the 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 the Food and Drug Administration in 2009 compared GEP results for 25 to 57 samples with each of the 15 known tumors on the PathWork panel (mean, 31 specimens per known tumor).¹² 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 the Food and Drug Administration's decision summary.

Handorf et al (2013) 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.¹³ PathWork's diagnostic performance was compared with IHC in 160 tumor samples. Overall concordance with known diagnoses (ie, accuracy) was 89% for PathWork vs 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 were 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)¹⁴ revised the original classifier algorithm³ using 2206 samples derived 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%).

Kerr et al (2012) reported on a multicenter study of the 92-gene CancerTYPE ID test conducted to assess the test's clinical validity.¹⁵ 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 by 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, the 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 subgroup 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).¹⁶ 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 report by Brachtel et al (2016)¹⁷ examined a subset of 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%), consistent with the larger sample. From the 644 cases, a sensitivity of 87% (95% CI, 84% to 89%) was estimated.

Greco et al (2013) published a retrospective, single-center study of 171 patients diagnosed with CUP after a clinical diagnostic workup (ie, before IHC).¹⁸ The study evaluated 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 the 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.¹⁹ 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 the benefit was obtained cannot be assessed.

RosettaGX Cancer Origin

Meiri et al (2012) assessed the clinical validity of the miRview mets² test in 509 FFPE specimens.²⁰ Four hundred eighty-nine of these samples were successfully processed, and results were compared with the known origin of the specimen. The sensitivity 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.²¹⁻²³

Section Summary: Clinically Valid

Using different reference standards, these 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.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

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.²⁴ 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 suggested was longer than 9 months for unselected chemotherapy treated CUP patients. However, the low physician participation rate and lack of a concurrent comparator group limit any implications of these results. The study was supported by PathWork Diagnostics and 2 authors company employees.

Yoon et al (2016) reported on results of a multicenter phase 2 trial evaluating combined use of carboplatin, paclitaxel, and everolimus in patients with CUP.²⁵ The primary outcome was an

objective response, and the study's 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%, $p=0.097$), accompanied by longer progression-free survival (6.4 months vs 3.5 months, $p=0.026$; hazard ratio, 0.47; 95% CI, 0.24 to 0.93), and longer overall survival (median, 17.8 months vs 8.3 months; $p=0.005$; HR=0.37; 95% CI, 0.18 to 0.76). The results suggested the Tissue of Origin Test might identify platinum/taxane-sensitive tumors. However, the trial was not designed to evaluate the predictive use of the test, the Tissue of Origin data was missing for 17% of patients, and severe adverse events were common.

CancerTYPE ID

From patients with CUP evaluated with a CancerTYPE ID assay between 2008 and 2009, Hainsworth et al (2012) identified those with a probable ($\geq 80\%$) colorectal site of origin.²⁶ A total of 125 patients (of 1544 results) were predicted to have primary colorectal cancer. 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 colorectal cancer 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 empirical CUP regimens with a response rate of 17%. For first-line therapies, physician-assessed progression-free survival was longer following colorectal cancer regimens (8.5 months vs 6 months; $p=0.11$). 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.

Hainsworth et al (2013) published a multisite prospective case series of the 92-gene CancerTYPE ID assay.²⁷ 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 empirical 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.^{28,29}

RosettaGX Cancer Origin

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

Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

An indirect argument to support the use of GEP testing to determine the likely source of the primary tumor cannot be constructed.

Section Summary: Clinically Useful

There is limited indirect evidence from nonrandomized studies for two 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 about clinical utility. The benefit would be most convincingly demonstrated through a marker strategy designed trial randomizing patients with CUPs to receive treatment based on GEP results or usual care.

SUMMARY OF EVIDENCE

For individuals who have CUP who receive GEP, the evidence includes studies of clinical validity, and limited evidence on potential clinical utility. Relevant outcomes are overall survival, disease-specific survival, test validity, and quality of life. Of the 3 commercially available tests reviewed, one has been cleared by the Food and Drug Administration (Tissue of Origin). For these tests, the clinical validity is the ability of a test to determine the site of origin. 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 who had CUP with 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.1.2018) 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... 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 determining 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 cancer classifier assays (gene signature profiling) at this time for the identification of tissue of origin as standard management in the diagnostic workup of patients with CUP [cancer of unknown primary]. -Furthermore, 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.³⁰ 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, as relates to use of GEP assays to identify tissue of origin in patients with cancer of unknown primary, “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.”)³¹ 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

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT03278600	The Value of Tissue-of-origin Profiling in Predicting Primary Site and Directing Therapy in Patients With Cancer of Unknown Primary: a Prospective Randomized Controlled Study	172	Sep 2020

NCT No.	Trial Name	Planned Enrollment	Completion Date
Unpublished			
NCT01540058	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)	223	Oct 2017 (unknown)

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.

<u>REVISIONS</u>	
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:

REVISIONS	
	<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." ▪ 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
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: <ul style="list-style-type: none"> ▪ Added CPT Code: 81540 ▪ Updated Coding notations
04-19-2017	Description section updated
	In Policy section: <ul style="list-style-type: none"> ▪ 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: <ul style="list-style-type: none"> ▪ Coding notations updated
	References updated
05-09-2018	Description section updated
	In Policy section: <ul style="list-style-type: none"> ▪ Policy Guidelines added on Genetic Nomenclature and Genetic Counseling
	Rationale section updated
	References updated
	Appendix removed

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