Gene Expression-Based Assays for Cancers of Unknown Primary

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Based on review of available data, the Company considers the use of gene expression profiling (GEP) to evaluate the site of origin of a tumor of unknown primary, or to distinguish a primary from a metastatic tumor to be **investigational.***

Background/Overview

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 about 3% of all cancers in the United States. Identifying the primary origin of a tumor can dictate cancer-specific treatment, expected outcome, and prognosis.

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 scans of the chest, abdomen, and pelvis; routine laboratory studies; and targeted evaluation of specific signs and symptoms.

Diagnosis and Classification
Biopsy of a CUP with detailed pathology evaluation may include immunohistochemical (IHC) analysis of the tumor. IHC identifies different antigens present in different types of tumors and can usually distinguish an epithelial tumor (i.e., carcinoma) from melanoma or sarcoma. Detailed cytokeratin panels often allow further classification of 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 to 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.

Tissue of Origin Testing, Treatment Selection, and Health Outcomes

Patients with CUP generally have poor prognoses. For example, patients with disease limited to lymph nodes have a median survival of 6 to 9 months, and those with a 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 a 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 herein (see Table 1).

Table 1. Gene Expression Profiling Tests for Cancers of Unknown Primary

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).
RT-qPCR: real-time quantitative polymerase chain reaction.
[a] Formerly PathWork and ResponseDX: Tissue of Origin.
[b] Formerly miRview met.

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 with 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 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 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 μm 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.

miRview mets is another RT-qPCR test that uses micro ribonucleic acids (miRNAs), 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 miRview test used 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 mets^2 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.

FDA or Other Governmental Regulatory Approval

U.S. Food and Drug Administration (FDA)

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. 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 (e.g., 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 must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. CancerTYPE ID®‡ (Biotheranostics, San Diego, CA) are miRview®‡ (or RosettaGX Cancer Origin™‡; Rosetta Genomics, Philadelphia, PA) are available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the FDA has chosen not to require any regulatory review of this test.

Centers for Medicare and Medicaid Services (CMS)
A 2013 technology assessment was commission by Centers for Medicare & Medicaid for consideration by the MEDCAC panel. Studies identified evaluating CancerTYPE ID, miRview, and PathWorkDx through November 2012, were included. The report concluded that all tests had similar accuracies, ranging from 85% to 88% (9 studies of PathWorkDx, 6 of CancerTYPE ID, 4 of MiRview), but that evidence was insufficient to evaluate the effect on management and outcomes. (Following review, the MEDCAC panel voted 2 [scale of 1 = low, 3 = intermediate, and 5 = high confidence] after considering the question: “How confident are you that there is sufficient evidence to determine whether genetic testing of tumor tissue affects health outcomes (including benefits and harms) for patients with cancer whose anticancer treatment strategy is guided by the results of each of the following?”)

There are no national Medicare coverage decisions for these tests, but local Medicare coverage decisions for all 3 tests have found them to be “reasonable and necessary.” In 2011, Palmetto GBA, issued positive coverage for the PathWork Tissue of Unknown Origin Test. Because all tests are processed out of the company laboratory in California, the test will be covered for Medicare patients in the United States. In 2012, Palmetto issued a similar statement for CancerTYPE ID, and, in 2013, Novitas issued a similar statement for miRview.

Rationale/Source

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 (i.e., as a predictive test).

Patients

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

Interventions

Three 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 (i.e., 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 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 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%).

Monzon et al (2009) 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 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 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). 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 FDA 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). 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 (i.e., 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. PathWork's diagnostic performance was compared with IHC in 160 tumor samples. Overall concordance with known diagnoses (i.e., 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) revised the original classifier algorithm 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. 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 (i.e., 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). For 75 included tumors, assay sensitivities were 99% (95% CI, 93% to 99%) for classification of neuroendocrine tumor type (e.g., 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) 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 (i.e., before IHC). 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. 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 mets^2 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. 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.

Section Summary: Clinically Valid

Using different reference standards, these tests have reported sensitivities or concordances generally high (e.g., 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. 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. 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 (≥80%) colorectal site of origin. 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 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.

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 FDA (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, IHC analysis) for the tissue of origin, the tests have reported sensitivities or concordances generally high (e.g., 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.

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Policy History

Original Effective Date: 10/20/2010
Current Effective Date: 05/16/2018
10/14/2010 Medical Policy Committee review

10/20/2010	Medical Policy Implementation Committee approval.
06/14/2012	Medical Policy Committee review
06/20/2012	Medical Policy Implementation Committee approval. A new test for formalin-fixed paraffin-embedded (FFPE) specimens added to the investigational statement.
01/23/2013	Coding updated
05/02/2013	Medical Policy Committee review
05/22/2013	Medical Policy Implementation Committee approval. Coverage statement changed to be generalizable to gene expression profiling and not specific to the Pathwork test.
05/01/2014	Medical Policy Committee review
05/21/2014	Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
05/07/2015	Medical Policy Committee review
05/20/2015	Medical Policy Implementation Committee approval. Title change. Coverage eligibility unchanged.
08/03/2015	Coding update: ICD10 Diagnosis code section added; ICD9 Procedure code section removed.
01/01/2016	Coding update
02/03/2016	Coding update
05/05/2016	Medical Policy Committee review
05/18/2016	Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
01/01/2017	Coding update: Removing ICD-9 Diagnosis Codes
05/04/2017	Medical Policy Committee review
05/17/2017	Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
01/01/2018	Coding update
05/03/2018	Medical Policy Committee review
05/16/2018	Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
08/07/2018	Coding update
10/29/2018	Coding update

Next Scheduled Review Date: 05/2019

Coding

The five character codes included in the Blue Cross Blue Shield of Louisiana Medical Policy Coverage Guidelines are obtained from Current Procedural Terminology (CPT®)‡, copyright 2017 by the American Medical Association (AMA). CPT is developed by the AMA as a listing of descriptive terms and five character identifying codes and modifiers for reporting medical services and procedures performed by physician.

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Gene Expression-Based Assays for Cancers of Unknown Primary

Policy # 00271
Original Effective Date: 10/20/2010
Current Effective Date: 05/16/2018

Codes used to identify services associated with this policy may include (but may not be limited to) the following:

Code Type	Code			
CPT	81406, 81479, 81504, 81540			
HCPCS	No codes			
ICD-10 Diagnosis	C0.00-C0.09	C01	C02.0-C02.9	C02.30-C02.39
	C04.0-C04.9	C05.0-C05.9	C0.60-C06.2	C06.80
	C06.89	C06.9	C07	C08.0-C08.9
	C08.90-C09.9	C10.0-C11.9	C12	C13.0-C13.9
	C14.0	C14.2	C14.8	C15.3-C15.9
	C16.0-C16.9	C17.0-C17.9	C18.0-C18.9	C19
	C20	C21.0-C21.8	C22.0-C22.9	C23
	C24.0-C24.9	C25.0-C25.9	C26.0-C26.9	C30.0-C30.1
	C31.0-C31.9	C32.0-C32.9	C33	C34.00-C34.02
	C34.10-C34.12	C34.2	C34.30-C34.32	C34.80-C34.82
	C34.90-C34.92	C37	C38.0-C38.8	C40.00-C40.02
	C40.10-C40.12	C40.20-C40.22	C40.30-C40.32	C40.80-C40.82
	C40.90-C40.92	C41.0-C41.9	C43.10-C43.12	C43.20-C43.22
	C43.30-C43.39	C43.4	C43.51-C43.59	C43.60-C43.62
	C43.70-C43.72	C43.8	C43.9	C44.00-C44.09
	C44.191-C44.199	C44.201-C44.209	C44.211-C44.219	C44.221-C44.229
	C44.291-C44.299	C44.300-C44.319	C44.320-C44.329	C44.390-C44.399
	C44.40-C44.49	C44.500-C44.519	C44.520-C44.529	C44.590-C44.599
	C44.601-C44.619	C44.621-C44.629	C44.691-C44.699	C44.701-C44.709
	C44.711-C44.719	C44.721-C44.729	C44.791-C44.799	C44.80-C44.89
	C44.90-C44.99	C45.0-C45.9	C45.60-C45.9	C46.0-C46.9
	C47.0	C47.10-C47.9	C48.0-C48.8	C49.0-C49.9
	C4A.0	C4A.10-C4A.12	C4A.20-C4A.22	C4A.30-C4A.39
	C4A.4	C4A.51-C4A.59	C4A.60-C4A.62	C4A.70-C4A72
	C4A.8	C4A.9	C50.011-C50.19	C50.021-C50.029
	C50.111-C50.119	C50.121-C50.129	C50.211-C50.219	C50.221-C50.229
	C50.311-C50.319	C50.321-C50.329	C50.411-C50.419	C50.421-C50.429
	C50.511-C50.519	C50.521-C50.529	C50.611-C50.619	C50.521-C50.529
	C50.811-C50.819	C50.821-C50.829	C50.911-C50.919	C50.921-C50.929
	C51.0-C51.9	C52	C53.0-C53.9	C54.0-C54.9
	C55	C56.1-C56.9	C57.00-C57.22	C57.3

	C57.4	C57.7-C57.9	C58	C60.0-C60.9
	C61	C62.00-C62.12	C62.90-C62.92	C63.00-C63.12
	C63.2	C63.7-C63.9	C64.1-C64.9	C65.1-C65.9
	C66.1-C66.9	C66.70-C66.9	C67.0-C67.9	C68.0-C68.8
	C69.00-C69.02	C69.10-C69.12	C69.20-C69.22	C69.30-C69.32
	C69.40-C69.42	C69.50-C69.52	C71.9	C72.0-C72.9
	C73	C74.00-C74.12	C74.90-C74.92	C75.0-C75.9
	C76.0-C76.52	C76.8	C77.0-C77.9	C78.00-C78.02
	C78.1-C78.89	C79.00-C79.9	C7A.00-C7A.8	C7B.00-C7B.09
	C7B.1-C7B.8	C80.0	C81.00-C81.99	C82.00-C82.99
	C83.32-C83.39	C83.50-C83.59	C83.70-C83.99	C84.00-C84.19
	C84.40-C84.49	C84.60-C84.69	C84.70-C84.79	C84.90-C84.99
	C84.A0-C84.A9	C84.Z0-C84.Z9	C85.10-C85.19	C85.20-C85.29
	C85.80-C85.99	C85.90-C85.99	C86.0-C86.6	C88.2-C88.9
	C90.00-C90.02	C90.10-C90.12	C90.20-C90.22	C90.30-C90.32
	C91.40-C91.42	C96.0	C96.2	C96.4
	C96.9	C96.A	C96.Z	D03.0
	D03.10-D03.12	D03.20-D03.22	D03.30-D03.39	D03.4
	D03.51-D03.59	D03.60-D03.62	D03.70-D03.72	D03.8
	D03.9			

*****Investigational – A medical treatment, procedure, drug, device, or biological product is Investigational if the effectiveness has not been clearly tested and it has not been incorporated into standard medical practice. Any determination we make that a medical treatment, procedure, drug, device, or biological product is Investigational will be based on a consideration of the following:

A. Whether the medical treatment, procedure, drug, device, or biological product can be lawfully marketed without approval of the U.S. Food and Drug Administration (FDA) and whether such approval has been granted at the time the medical treatment, procedure, drug, device, or biological product is sought to be furnished; or
B. Whether the medical treatment, procedure, drug, device, or biological product requires further studies or clinical trials to determine its maximum tolerated dose, toxicity, safety, effectiveness, or effectiveness as compared with the standard means of treatment or diagnosis, must improve health outcomes, according to the consensus of opinion among experts as shown by reliable evidence, including:
    1. Consultation with the Blue Cross and Blue Shield Association technology assessment program (TEC) or other nonaffiliated technology evaluation center(s);
    2. Credible scientific evidence published in peer-reviewed medical literature generally recognized by the relevant medical community; or
    3. Reference to federal regulations.

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