Protocol

Gene Expression-Based Assays for Cancers of Unknown Primary

(20454)

Medical Benefit		**Effective Date**: 04/01/18	**Next Review Date**: 01/20
Preauthorization	No	**Review Dates**: 05/09, 03/10, 01/11, 01/12, 01/13, 01/14, 01/15, 01/16, 01/17, 01/18, 01/19	

This protocol considers this test or procedure investigational. If the physician feels this service is medically necessary, preauthorization is recommended.

The following protocol contains medical necessity criteria that apply for this service. The criteria are also applicable to services provided in the local Medicare Advantage operating area for those members, unless separate Medicare Advantage criteria are indicated. If the criteria are not met, reimbursement will be denied and the patient cannot be billed. Please note that payment for covered services is subject to eligibility and the limitations noted in the patient's contract at the time the services are rendered.

Populations	Interventions	Comparators	Outcomes
Individuals: • With cancer of unknown primary	Interventions of interest are: • Gene expression profiling	Comparators of interest are: • Clinical workup, including imaging and pathology, without gene expression profiling	Relevant outcomes include: • 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 can identify some, but not all, primary sources of secondary tumors. It is suggested that identifying the likely primary source with gene expression profiling (GEP) to direct treatment may improve health outcomes.

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 three 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 (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.

POLICY

Gene expression profiling is considered **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 protocol 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

ACMG: 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.

MEDICARE ADVANTAGE

For Medicare Advantage the following tests will be considered **medically necessary**:

Cancer TYPE ID (Biotheranostics).

Tissue of Origin (Cancer Genetics Incorporated).

Molecular testing, using the ROSETTA Cancer Origin Test™ (PROG), is considered **medically necessary** in the pathologic diagnoses of CUP when a conventional surgical pathology/imaging work-up is unable to identify a primary neoplastic site. Other applications of this technology are considered **investigational** in the use of diagnosis of specific tumor types such as NSCLC and renal cancers.

BACKGROUND

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.[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 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 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.[3-6]

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 six to nine months, and those with a disease that is extranodal two to four 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 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).[8]

RT-qPCR: real-time quantitative polymerase chain reaction.

[a]Formerly PathWork and ResponseDX: Tissue of Origin.

[b]Formerly miRview met[2]

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 zero (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 five and 30, the test result is considered indeterminate, and a similarity score of less than five 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 seven 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.[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 tissue sections or unstained 10 µm sections on glass slides. Expression levels of 92 genes (87 tumor-associated genes and five reference genes for normalization) are used to detect 27 tumor types in a known database of 578 tumors with a range of five 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 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 tissue. The miRview test used 48-panel markers to detect 22 tumor types in a known database of 336 tumors, with a range of one 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.

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 (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 (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 the CLIA. Laboratories that offer LDTs must be licensed by the CLIA for high-complexity testing. To date, the FDA has chosen not to require any regulatory review of this test.

Services that are the subject of a clinical trial do not meet our Technology Assessment Protocol criteria and are considered investigational. *For explanation of experimental and investigational, please refer to the Technology Assessment Protocol.*

It is expected that only appropriate and medically necessary services will be rendered. We reserve the right to conduct prepayment and postpayment reviews to assess the medical appropriateness of the above-referenced procedures. **Some of this protocol may not pertain to the patients you provide care to, as it may relate to products that are not available in your geographic area.**

REFERENCES

We are not responsible for the continuing viability of web site addresses that may be listed in any references below.

1. PDQ Adult Treatment Editorial Board. Carcinoma of Unknown Primary Treatment (PDQ®). 2018; https://www.ncbi.nlm.nih.gov/books/NBK65811/. Accessed February 27, 2018.
2. Oien KA, Evans TR. Raising the profile of cancer of unknown primary. J Clin Oncol. Sep 20 2008;26(27):4373-4375. PMID 18802148

3. Ma XJ, Patel R, Wang X, et al. Molecular classification of human cancers using a 92-gene real-time quantitative polymerase chain reaction assay. Arch Pathol Lab Med. Apr 2006;130(4):465-473. PMID 16594740
4. Ramaswamy S, Tamayo P, Rifkin R, et al. Multiclass cancer diagnosis using tumor gene expression signatures. Proc Natl Acad Sci U S A. Dec 18 2001;98(26):15149-15154. PMID 11742071
5. Su AI, Welsh JB, Sapinoso LM, et al. Molecular classification of human carcinomas by use of gene expression signatures. Cancer Res. Oct 15 2001;61(20):7388-7393. PMID 11606367
6. Tothill RW, Kowalczyk A, Rischin D, et al. An expression-based site of origin diagnostic method designed for clinical application to cancer of unknown origin. Cancer Res. May 15 2005;65(10):4031-4040. PMID 15899792
7. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology: occult primary (cancer of unknown primary [CUP]). Version 1.2018. http://www.nccn.org/professionals/physician_gls/pdf/occult.pdf. Accessed January 16, 2018.
8. Agwa E, Ma PC. Overview of various techniques/platforms with critical evaluation of each. Curr Treat Options Oncol. Dec 2013;14(4):623-633. PMID 24243164
9. U.S. Food and Drug Administration. 510(k) Substantial Equivalence Determination Decision Summary: Pathwork Tissue of Origin Test. 2008; https://www.accessdata.fda.gov/cdrh_docs/reviews/K080896.pdf. Accessed February 27, 2018.
10. Monzon FA, Lyons-Weiler M, Buturovic LJ, et al. Multicenter validation of a 1,550-gene expression profile for identification of tumor tissue of origin. J Clin Oncol. May 20 2009;27(15):2503-2508. PMID 19332734
11. Azueta A, Maiques O, Velasco A, et al. Gene expression microarray-based assay to determine tumor site of origin in a series of metastatic tumors to the ovary and peritoneal carcinomatosis of suspected gynecologic origin. Hum Pathol. Jan 2013;44(1):20-28. PMID 22939961
12. U.S. Food and Drug Administration. 510(k) Substantial Equivalence Determination Decision Summary: Pathwork Tissue of Origin Test Kit-FFPE. 2010; https://www.accessdata.fda.gov/cdrh_docs/reviews/K092967.pdf. Accessed February 27, 2018.
13. Handorf CR, Kulkarni A, Grenert JP, et al. A multicenter study directly comparing the diagnostic accuracy of gene expression profiling and immunohistochemistry for primary site identification in metastatic tumors. Am J Surg Pathol. Jul 2013;37(7):1067-1075. PMID 23648464
14. Erlander MG, Ma XJ, Kesty NC, et al. Performance and clinical evaluation of the 92-gene real-time PCR assay for tumor classification. J Mol Diagn. Sep 2011;13(5):493-503. PMID 21708287
15. Kerr SE, Schnabel CA, Sullivan PS, et al. Multisite validation study to determine performance characteristics of a 92-gene molecular cancer classifier. Clin Cancer Res. Jul 15 2012;18(14):3952-3960. PMID 22648269
16. Kerr SE, Schnabel CA, Sullivan PS, et al. A 92-gene cancer classifier predicts the site of origin for neuroendocrine tumors. Mod Pathol. Jan 2014;27(1):44-54. PMID 23846576
17. Brachtel EF, Operana TN, Sullivan PS, et al. Molecular classification of cancer with the 92-gene assay in cytology and limited tissue samples. Oncotarget. May 10 2016;7(19):27220-27231. PMID 27034010
18. Greco FA, Lennington WJ, Spigel DR, et al. Molecular profiling diagnosis in unknown primary cancer: accuracy and ability to complement standard pathology. J Natl Cancer Inst. Jun 5 2013;105(11):782-790. PMID 23641043
19. Greco FA, Lennington WJ, Spigel DR, et al. Poorly differentiated neoplasms of unknown primary site: diagnostic usefulness of a molecular cancer classifier assay. Mol Diagn Ther. Apr 2015;19(2):91-97. PMID 25758902
20. Meiri E, Mueller WC, Rosenwald S, et al. A second-generation microRNA-based assay for diagnosing tumor tissue origin. Oncologist. May 2012;17(6):801-812. PMID 22618571
21. Mueller WC, Spector Y, Edmonston TB, et al. Accurate classification of metastatic brain tumors using a novel microRNA-based test. Oncologist. Jan 2011;16(2):165-174. PMID 21273512
22. Rosenfeld N, Aharonov R, Meiri E, et al. MicroRNAs accurately identify cancer tissue origin. Nat Biotechnol. Apr 2008;26(4):462-469. PMID 18362881

23. Rosenwald S, Gilad S, Benjamin S, et al. Validation of a microRNA-based qRT-PCR test for accurate identification of tumor tissue origin. Mod Pathol. Jun 2010;23(6):814-823. PMID 20348879
24. Nystrom SJ, Hornberger JC, Varadhachary GR, et al. Clinical utility of gene-expression profiling for tumor-site origin in patients with metastatic or poorly differentiated cancer: impact on diagnosis, treatment, and survival. Oncotarget. Jun 2012;3(6):620-628. PMID 22689213
25. Yoon HH, Foster NR, Meyers JP, et al. Gene expression profiling identifies responsive patients with cancer of unknown primary treated with carboplatin, paclitaxel, and everolimus: NCCTG N0871 (alliance). Ann Oncol. Feb 2016;27(2):339-344. PMID 26578722
26. Hainsworth JD, Schnabel CA, Erlander MG, et al. A retrospective study of treatment outcomes in patients with carcinoma of unknown primary site and a colorectal cancer molecular profile. Clin Colorectal Cancer. Jun 2012;11(2):112-118. PMID 22000811
27. Hainsworth JD, Rubin MS, Spigel DR, et al. Molecular gene expression profiling to predict the tissue of origin and direct site-specific therapy in patients with carcinoma of unknown primary site: a prospective trial of the Sarah Cannon research Institute. J Clin Oncol. Jan 10 2013;31(2):217-223. PMID 23032625
28. Varadhachary GR, Raber MN. Cancer of unknown primary site. N Engl J Med. Aug 21 2014;371(8):757-765. PMID 25140961
29. 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
30. National Institute for Health and Care Excellence (NICE). Metastatic malignant disease of unknown primary origin in adults: diagnosis and management [CG104]. 2010; https://www.nice.org.uk/guidance/CG104. Accessed February 27, 2018.
31. Fizazi K, Greco FA, Pavlidis N, et al. Cancers of unknown primary site: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. Sep 2015;26(Suppl 5):v133-138. PMID 26314775
32. Meleth S, Whitehead N, Evans TS, et al. Genetic Testing or Molecular Pathology Testing of Cancers with Unknown Primary Site to Determine Origin. AHRQ Technology Assessments. Rockville, MD: Agency for Healthcare Research and Quality; 2013.
33. Medicare Evidence Development & Coverage Advisory Committee. MEDCAC Meeting 5/1/2013 - Genetic Tests for Cancer Diagnosis. 2013; https://www.cms.gov/Regulations-andGuidance/Guidance/FACA/downloads/id67a.pdf. Accessed February 27, 2018.
34. Novitas Solutions, Inc. (Primary Geographic Jurisdiction - Arkansas, Louisiana, Mississippi, Colorado, New Mexico, Oklahoma, Texas, Delaware, District of Columbia, Maryland, New Jersey, Pennsylvania) Local Coverage Determination (LCD): Biomarkers for Oncology (L35396), Revision Effective Date for services performed on or after 10/04/2018.
35. Noridian Healthcare Solutions, LLC, (Jurisdiction E - California - Entire State, American Samoa, Guam, Hawaii, Northern Mariana Islands, Nevada) Local Coverage Determination (LCD): MolDX: Molecular Diagnostic Tests (MDT) (L35160), Revision Effective Date for services performed on or after 06/21/2018.