



## ***Gene Expression-Based Assays for Cancers of Unknown Primary***

**Effective:** June 1, 2026

**Next Review:** February 2027

**Last Review:** April 2026

### **IMPORTANT REMINDER**

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

### **DESCRIPTION**

Gene expression tests have been developed to help identify the tissue of origin of tumors of unknown primary. These tests are proposed to assist in guiding treatment decisions.

### **MEDICAL POLICY CRITERIA**

Gene expression profiling is considered **investigational** to evaluate the site of origin of a tumor of unknown primary, and to distinguish a primary from a metastatic tumor. Gene expression tests for this indication include, but are not limited to, the Tissue of Origin Test, the Tissue of Origin test kit-FFPE, CancerTypeID®, miRview®, and RosettaGX Cancer Origin Test™.

*NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.*

### **CROSS REFERENCES**

1. [Gene-Based Tests for Screening, Detection, and/or Management of Prostate Cancer](#), Genetic Testing, Policy No. 17
2. [Genetic and Molecular Diagnostic Testing](#), Genetic Testing, Policy No. 20

3. [Assays of Genetic Expression in Tumor Tissue as a Technique to Determine Prognosis In Patients With Breast Cancer](#), Genetic Testing, Policy No. 42
4. [Evaluating the Utility of Genetic Panels](#), Genetic Testing, Policy No. 64
5. [Expanded Molecular Testing of Cancer to Select Targeted Therapies](#), Genetic Testing, Policy No. 83
6. [Investigational Gene Expression, Biomarker, and Multianalyte Testing](#), Laboratory, Policy No. 77

## BACKGROUND

Cancers of unknown primary (CUPs), or occult primary malignancies, represent 2% of cancers diagnosed in the United States. These cancers are heterogeneous, and the majority are accompanied by poor prognoses. There are five subtypes of CUP that can be determined with light microscopy:<sup>[1]</sup>

1. Well or moderately differentiated adenocarcinoma (60%)
2. Poorly differentiated adenocarcinoma (25%)
3. Squamous cell carcinoma (SCC) (5%)
4. Undifferentiated carcinoma (5%)
5. Neuroendocrine tumors (5%)

Median survival for CUP is 8-12 months. Poor prognostic indicators include male gender assigned at birth, age older than 65 years, poor performance status, multiple comorbidities, undifferentiated tumors, and multiple sites of metastasis. In general, SCC is associated with a better prognosis than other types of CUP. A small subset of SCC-CUP involves head and neck occult tumors of unknown origin found in a cervical lymph node. These tumors are usually squamous cell carcinoma and are rare, but are generally curable.<sup>[2]</sup>

Immunohistochemistry (IHC) is commonly used to determine tissue of origin. Recent advances in the understanding of gene expression in normal and malignant cells have led researchers to explore molecular classification as a way to improve upon IHC in the identification of the tissue of origin of CUP, and to detect genomic alterations that may respond to targeted systemic therapy.

The potential benefit of determining the primary site of cancers of unknown primary is to enable tumor-specific treatment. The molecular classification of cancers is based on the premise that, despite different degrees of loss of differentiation, tumors retain sufficient gene expression “signatures” related to their cell of origin, even after metastasis. Theoretically, it is possible to build a gene expression database spanning many different tumor types to compare to the expression profile of very poorly differentiated tumors or a cancer of unknown primary to aid in the identification of the tumor type and organ of origin.

One such microarray technology is the Tissue of Origin Test (Genecast.), formerly known as the Pathwork® Tissue of Origin Test and the ResponseDX Tissue of Origin Test. The test measures the expression of 2,000 genes and compares the similarity of the gene expression profile of a cancer of unknown primary to a database of known profiles from 15 tissues with 58 histologic morphologies. The report generated for each tumor consists of a “similarity score,” which is a measure of similarity of the gene expression profile of the specimen to the profile of the 15 known tumors in the database. Scores range from 0 (very low similarity) to 100 (very high similarity) and sum to 100 across all 15 tissues on the panel. If a single similarity score is greater than or equal to 30, it indicates that this is likely the tissue of origin. If every similarity

score is between 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. The test was developed by Pathwork Diagnostics, but was later purchased by Response Genetics, Inc., and by Cancer Genetics Inc. (now Genecast) in 2015.

An alternative method to measure gene expression is real-time quantitative polymerase chain reaction (RT-PCR). RT-PCR 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 RT-PCR have been reported to be as high as 87%, but less so (71%) the more undifferentiated the tumor tested.<sup>[3]</sup> One assay that uses qRT-PCR is the CancerTypeID® (CancerTypeID; bioTheranostics, Inc., San Diego, CA) assay, which measures the expression of messenger RNA in a cancer of unknown primary (CUP) tissue sample. Samples for this are FFPE tissue sections or unstained 10 micron sections on glass slides. The expression levels of 92 genes (87-tumor associated genes and five reference genes for normalization) are used to detect 27 tumor types in a known database of 578 tumors with a range of 5 to 49 tumors per type. The report generated is the probability for the main cancer type, possible subtypes, tumor types not able to be excluded, and those ruled out with 95% confidence calculated by K nearest neighbor analysis.

## REGULATORY STATUS

In July 2008, the ResponseDX®: Tissue of Origin Test (Response Genetics, Inc., Los Angeles, CA, now Genecast), formerly known as the Pathwork® Tissue of Origin Test, was cleared with limitations\* for marketing by the FDA through the 510(k) process. The FDA determined that the test was substantially equivalent to existing tests for use in measuring the degree of similarity between the RNA expression pattern in a patient's fresh-frozen tumor and the RNA expression patterns in a database of tumor samples (poorly differentiated, undifferentiated and metastatic cases) that were diagnosed according to current clinical and pathological practice. The database contains examples of RNA expression patterns for fifteen common malignant tumor types including bladder, breast, colorectal, gastric, hepatocellular, kidney, non-small cell lung, ovarian, pancreatic, prostate, and thyroid carcinomas, melanoma, testicular germ cell tumor, non-Hodgkins lymphoma (not otherwise specified), and soft tissue sarcoma (not otherwise specified). The ResponseDX®: Tissue of Origin Test result is intended for use in the context of the patient's clinical history and other diagnostic tests evaluated by a qualified clinician.

\*Limitations to the clearance were as follows:<sup>[4]</sup>

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

In June 2010, the Pathwork® Tissue of Origin Test Kit-FFPE (Pathwork Diagnostics) was cleared for marketing by the FDA through the 510(k) process.<sup>[4, 5]</sup> The 2010 clearance is an expanded application which allows the test to be run on a patient's formalin-fixed, paraffin-

embedded (FFPE) tumor, and has the same indications and limitations. As of late 2015, the Tissue of Origin Test is distributed by Genecast (formerly Cancer Genetics).

A December 2017 modification to the Pathwork® Tissue of Origin Test Kit-FFPE received FDA 510(k) approval with no changes to the indications or limitations.<sup>[6]</sup> This modification included edits to the microarray processing software as well as to the amplification and purification procedures.

To date, the CancerTypeID®, or RosettaGX Cancer Origin Test™ tests have not been submitted to the FDA for approval.

## EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature<sup>[7]</sup> is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

The focus of this review is on evidence related to the ability of test results to:

- Guide decisions in the clinical setting related to either treatment, management, or prevention, and
- Improve health outcomes as a result of those decisions.

The analytical and clinical validity of gene expression profiling to evaluate cancers of unknown primary (CUPs) has already been established. Therefore, the evidence review below will focus on the clinical utility of these tests.

The Agency for Healthcare Research and Quality (AHRQ) published a technology assessment in 2013 to review commercially available genetic tests used to identify the tissue of origin (TOO) of the cancer in patients with cancer of unknown primary (CUP).<sup>[8]</sup> AHRQ reviewed three genomic TOO tests (CancerTypeID, miRview, and PathworkDx) for analytical validity, clinical validity, and clinical utility. The review found very little evidence that supported the clinical usefulness of any of the three tests in making diagnosis and treatment decisions. The review also found very little evidence that the use of any of the three tests increased the length of survival among CUP patients who received the test. AHRQ determined that the evidence was insufficient to assess the ability of the tests to impact treatment or outcomes. Several of the key studies assessed in the AHRQ review, as well as studies published after the review, are described below.

Saibil (2025) conducted a systematic review of the evidence on the value of four different types of molecular tools, including gene expression tests, for the diagnosis and treatment of CUP.<sup>[9]</sup> Of eight included studies three focused on gene expression using microarray, next generation sequencing (NGS), or PCR-based platforms. Two included studies used mixed methods that included gene expression analysis. The certainty of the evidence results for both gene expression analyses and mixed methods were “very low.” The authors concluded that while the use of molecular profiling (not gene expression analysis) shows promise in identifying potentially targetable mutations, little has been published on survival outcomes. A need for

high quality studies that compare survival with and without molecularly guided treatments was identified.

Posner (2023) published a retrospective comparison of gene expression profiling (GEP), using microarray-based tests (CUPGuide and NanoString nCounter) to DNA mutational profiling, in which a panel of 386 cancer-associated genes were sequenced to guide targeted therapy.<sup>[10]</sup> Out of 215 patients, 191 (89%) had GEP; 201 (93%) had DNA mutational profiling, and 177 (82%) had both tests. Of note, in 49 cases (23%) a latent diagnosis or tissue of origin (TOO) assignment was made during clinical follow-up or retrospective review using conventional clinical, pathologic, and radiologic methods. Of the remaining 166 unresolved CUPs, DNA mutational profiling supported a TOO diagnosis in 51 cases (31%), but GEP prediction was useful in only 21/166 cases (13%). The authors concluded that DNA sequencing may be of greater diagnostic value in CUPs than GEP.

Ding (2022) conducted a systematic review and meta-analysis comparing empiric therapy for CUP to site-specific therapy.<sup>[11]</sup> Five studies involving 1114 patients were included. All patients were diagnosed with CUP based on clinical, radiological, and IHC standards, and a gene assay was used to predict tissue of origin. Two of the studies; Hayashi (2019) and Hainsworth (2012), are detailed below.<sup>[12, 13]</sup> In the five studies analyzed, overall survival (OS) was not significantly different using pooled estimates (hazard ratio [HR] 0.75, 95% confidence interval [CI] 0.55-1.03,  $p=0.069$ ). In two studies that reported progression free survival (PFS) there was no significant difference in PFS between patients undergoing empiric therapy compared to site-specific treatment (HR 0.93; 95% CI 0.74-1.17,  $p=0.534$ ).

A randomized phase 2 trial published by Hayashi (2019) was conducted to assess whether site-specific therapy directed by CUP improves outcomes compared with empirical chemotherapy in previously untreated patients with CUP. Patients were randomly assigned to receive standard site-specific therapy ( $n=50$ ) or empirical paclitaxel and carboplatin (PC) ( $n=51$ ). Cancer types most commonly predicted were pancreatic (21%), gastric (21%), and lymphoma (20%). The 1-year survival rate was 44.0% and 54.9% for site-specific treatment and empirical PC ( $p = 0.26$ ), respectively. Median overall and progression-free survival were 9.8 and 5.1 months, respectively, for site-specific treatment versus 12.5 and 4.8 months for empirical PC ( $p = 0.90$  and  $0.55$ , respectively). The authors concluded that no significant improvement in 1-year survival was observed for site-specific treatment based on microarray profiling compared with empirical PC therapy.

## **TISSUE OF ORIGIN TEST AND ROSETTAGX CANCER ORIGIN™**

Yoon (2016) reported results of a multicenter phase 2 trial evaluating combined use of carboplatin, paclitaxel, and everolimus in patients with CUP.<sup>[14]</sup> The primary outcome was objective response, and the study a two-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 (PFS; 6.4 months vs 3.5 months,  $p=0.026$ ; hazard ratio (HR), 0.47; 95% CI, 0.24 to 0.93), and longer OS (median, 17.8 months vs 8.3

months;  $p=0.005$ ;  $HR=0.37$ ; 95% CI, 0.18 to 0.76). The results suggest a tissue of origin test might identify platinum/taxane-sensitive tumors. However, the study was not designed to evaluate predictive use of the test, tissue of origin data were missing for 17% of patients, and severe adverse effects were common.

Nystrom 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.<sup>[15]</sup> Prior to the test, physicians had no suspected diagnosis for 54 patients (41%), which declined to 17 (16%) after testing. Changes in management were reported in 70 patients (65%). Physicians reported test results were helpful with regard to diagnosis, choosing therapy, and triaging. However, the low physician participation rate and lack of a concurrent comparator group limits any implications of these results. The study was supported by PathWork Diagnostics and two authors company employees.

### **CancerTypeID®**

In 2013, Hainsworth conducted a multi-site prospective case-series of the 92-gene CancerTypeID assay<sup>[16]</sup>. The molecular profiling assay predicted a tissue of origin in 247 (98%) of 252 patients. One-hundred nineteen assay predictions were made with  $\geq 80\%$  similarity score and the rest were below 80% probability. Twenty-nine patients did not remain on study due to decreasing performance, brain metastases, or patient and physician decision. Of the remaining 223 patients, 194 (87%) received assay-directed chemotherapy, and 29 received standard empiric therapy. The median overall survival of the 194 patients receiving assay-directed chemotherapy was 12.5 months, which was found to be within the *a priori*-specified improvement target of 30% compared with historical trial data on 396 performance-matched CUP patients receiving standard empiric therapy at the same center. Due to potential biases introduced by the nonrandomized design, confounding variables, such as use of subsequent lines of empirical therapy, and heterogeneity of unknown primary cancers, conclusions that can be drawn are limited.

From patients with CUP who had undergone a CancerTYPE ID assay between March 2008 and August 2009, Hainsworth (2012) identified those with a probable (80% or greater) colorectal site of origin.<sup>[13]</sup> A total of 125 patients (of 1544 results) were predicted to have a primary colorectal cancer (CRC). Physicians caring for patients were sent questionnaires with a request for deidentified pathology reports and 42 (34%) responded. A total of 32 patients were given colorectal cancer regimens (16 first-line therapy only, eight first- and second-line therapy, eight second-line therapy only) with a reported response rate of 50% following first-line and 50% following second-line therapy; 18 patients were given empiric CUP regimens with a response rate of 17%. For first-line therapies, physician assessed progression-free survival (PFS) was longer following CRC regimens, 8.5 months versus six 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.

Saller (2022) published a retrospective case series of four patients with neuroendocrine tumors of unknown primary origin who had tumor analysis with CancerTypeID®.<sup>[17]</sup> Despite a

variety of tumor presentations including brain, liver, and soft tissue mass, all four cases were determined to be of probable lung origin with CancerTypeID®. The study states the CancerTypeID impacted treatment, and affected outcomes in two of the cases; but the study does not provide specific information on the changes in treatment course or differences in outcome due to the CancerTypeID test.

## PRACTICE GUIDELINE SUMMARY

### NATIONAL COMPREHENSIVE CANCER NETWORK

The National Comprehensive Cancer Network (NCCN) guidelines (v.1.2026) for the workup of an occult primary malignancy for adenocarcinoma, carcinoma not otherwise specified, and squamous cell carcinoma address the use of molecular methods in the classification of tumors.<sup>[1]</sup> The guideline states that “Tissue of origin studies are not recommended.” Further, the NCCN states that, “While there may be a diagnostic benefit to GEP [compared to immunohistochemistry], a clinical benefit has not been demonstrated. Consequently, the Panel does not currently recommend use of gene sequencing to predict tissue of origin.”

The NCCN Guidelines for Treatment of Neuroendocrine and Adrenal Tumors (v3.2025) state, for poorly differentiated extrapulmonary neuroendocrine carcinoma, “Consider molecular profiling of tumor tissue” if disease is unresectable/metastatic and patient is a candidate for anti-cancer therapy.<sup>[18]</sup> Specific tests to consider include *NTRK* fusions, *RET* fusions, *BRAF* V600E mutation, microsatellite instability, mismatch repair deficiency, and tumor mutational burden. The use of gene-expression-based assays is not addressed.

The NCCN guidelines for Head and Neck Cancers (v1.2026) do not include the use of gene expression-based assays for head and neck tumors of unknown primary.<sup>[2]</sup>

## SUMMARY

There is not enough research to show that tumor gene expression testing can improve survival or other health outcomes for people with cancer of unknown primary. Also, there are no clinical guidelines based on research that recommend the use of this testing to classify cancers of unknown primary. Therefore, gene expression profiling is considered investigational to identify cancers of unknown primary.

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| <b>Codes</b> | <b>Number</b> | <b>Description</b>                                                                                                                                                                                                                                                                                                          |
|--------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| CPT          | 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                                                                                                                                                                                                                                                                       |
|              | 84999         | Unlisted chemistry procedure                                                                                                                                                                                                                                                                                                |
| HCPCS        | None          |                                                                                                                                                                                                                                                                                                                             |

**Date of Origin:** January 2011