

## Medical Policy



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

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

### DESCRIPTION

Cancers of unknown primary 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 to direct treatment may improve health outcomes.

**OBJECTIVE**

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

**BACKGROUND****Cancers of Unknown Primary**

Cancers of unknown primary, 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.<sup>1</sup>

Most cancers of unknown primary are adenocarcinomas or undifferentiated tumors; less commonly, they may be squamous carcinomas, melanoma, soft tissue sarcoma, or neuroendocrine tumors. Osteo- and chondrosarcomas rarely produce cancers of unknown primary. The most common primary sites of cancers of unknown primary 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 cancer of unknown primary 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.<sup>2</sup>

**Diagnosis and Classification**

Cancers of unknown primary can be classified into 4 categories. Adenocarcinomas compose approximately 70% of cancers of unknown primary. Neuroendocrine tumors compose approximately 1%, squamous cell carcinomas 5%, and poorly differentiated cancer 20% to 25% of cancers of unknown primary.

Biopsy of a cancer of unknown primary with detailed pathology evaluation may include immunohistochemical analysis of the tumor. Immunohistochemical analysis 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 immunohistochemical analysis may provide a narrow differential of possible sources of a tumor's origin, but not necessarily a definitive answer.

**Treatment Selection and Health Outcomes**

Treatment is based on the histologic type and clinical features. About 20% of patients with cancer of unknown primary have features that guide treatment. However, about 80% of patients with cancer of unknown primary have a poor prognosis with a survival of 3 to 6 months despite a variety of chemotherapeutic combinations. Multiple sites of involvement are observed in about 50% of patients, commonly in the lungs, liver, bones and lymph nodes. The premise of tissue of origin testing in cancers of unknown primary is that identifying a likely primary tumor site will inform treatment selection leading to improved survival and other outcomes.

### Tests Reviewed in This Report

Selected gene expression profiling tests are described in 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 <sup>a</sup>	Vyant Bio, Inc	Oligonucleotide microarray	2000	15
CancerTYPE ID	Biotheranostics	RT-qPCR	92	54

Adapted from Agwa et al (2013).<sup>3</sup>

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

<sup>a</sup> Formerly PathWork and ResponseDX: Tissue of Origin.

### REGULATORY STATUS

In 2008, the PathWork® Tissue of Origin Test™ (Response Genetics was acquired by Cancer Genetics, Cancer Genetics merged with StemoniX in 2020 and was renamed Vyant Bio, Inc. in 2021) was cleared for marketing with limitations (see below) by the U.S. Food and Drug Administration (FDA) through the 510(k) process (FDA product code: OIW), with subsequent clearances for expanded applications in 2010 and minor modifications in 2012. FDA determined that the test was substantially equivalent to existing tests for use in measuring the degree of similarity between the RNA expression pattern in a patient's fresh-frozen tumor and the RNA expression patterns in a database of tumor samples (poorly differentiated, undifferentiated, metastatic cases) that were diagnosed according to current clinical and histopathologic practice.

Limitations to the clearance were as follows:

- The PathWork® Tissue of Origin Test is not intended to establish the origin of tumors that cannot be diagnosed according to current clinical and pathologic practice (eg, a cancer of unknown primary).
- It is not intended to subclassify or modify the classification of tumors that can be diagnosed by current clinical and pathologic practice or to predict disease course, or survival or treatment efficacy, or to distinguish primary from metastatic tumor.
- Tumor types not in the PathWork® Tissue of Origin Test database may have RNA expression patterns similar to RNA expression patterns in tumor types in the database, leading to indeterminate results or misclassifications.

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 (CLIA). CancerTYPE ID® (Biotheranostics, San Diego, CA) is available under the auspices of the CLIA. Laboratories that offer laboratory-developed tests 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.

## POLICY

Gene expression profiling is considered **experimental / investigational** to evaluate the site of origin of a tumor of unknown primary, or to distinguish a primary from a metastatic tumor.

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## RATIONALE

This evidence review has been updated regularly with searches of the PubMed database. The most recent literature update was performed through January 16, 2024.

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.

Promotion of greater diversity and inclusion in clinical research of historically marginalized groups (e.g., People of Color [African-American, Asian, Black, Latino and Native American]; LGBTQIA (Lesbian, Gay, Bisexual, Transgender, Queer, Intersex, Asexual); Women; and People with Disabilities [Physical and Invisible]) allows policy populations to be more reflective of and findings more applicable to our diverse members. While we also strive to use inclusive language related to these groups in our policies, use of gender-specific nouns (e.g., women, men, sisters, etc.) will continue when reflective of language used in publications describing study populations.

## 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.

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 cancer of unknown primary. 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 cancer of unknown primary to aid in the identification of the tumor type and organ of origin. The feasibility of using molecular

classification schemes with gene expression profiling to classify these tumors of uncertain origin has been demonstrated in several studies.<sup>4,5,6,7,</sup>

### ***Populations***

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

### ***Interventions***

The Tissue of Origin test (formerly known as the PathWork Tissue of Origin Test and ResponseDX: Tissue of Origin; Vyant Bio, Inc) measures the expression of 2000 genes and compares the similarity of the gene expression profiling of a cancer of unknown primary 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 gene expression profiling 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.

An alternative method to measure gene expression is real-time quantitative polymerase chain reaction. Real-time quantitative polymerase chain reaction 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.<sup>4</sup> One assay that uses real-time quantitative polymerase chain reaction 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 mm 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. CancerTYPE ID is available with reflex to NeoTYPE Cancer Profile (NeoGenomics).

### ***Comparators***

Standard of care management is based on tumor type and probable site of origin (i.e., usual care without gene expression profiling). Because the site of origin is unknown in cancer of unknown primary, patients are typically treated with empiric chemotherapy.

### ***Outcomes***

Although test validity is relevant as a premise of the test, the outcomes informative of potential benefit include overall survival, disease-specific survival, progression-free survival, and quality of life. The premise of tissue of origin testing in cancers of unknown primary is that identifying a likely primary tumor site will inform treatment selection, leading to improved survival.

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

**Study Selection Criteria**

For the evaluation of clinical validity of these tests, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard

**REVIEW OF EVIDENCE****Clinically Valid**

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

Specifically, for these tests, clinical validity is the ability of a test to determine the site of origin. Demonstrating clinical validity is complicated by the lack of reference standard. Imperfect reference standards must be relied on such as the available presumptive or a reference pathologic diagnosis, known tumor types, comparisons immunohistochemistry 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 immunohistochemical. 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 U.S. Food and Drug Administration (FDA) in 2008 compared gene expression profiling 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.<sup>8</sup> 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%).

The clinical validation study for the PathWork Tissue of Origin Test Kit formalin-fixed, paraffin-embedded submitted to the FDA in 2009 compared gene expression profiling results for 25 to 57 samples with each of the 15 known tumors on the PathWork panel (mean, 31 specimens per known tumor).<sup>9</sup> 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 FDA's decision summary.

Monzon et al (2009) conducted a multicenter, blinded validation study of the PathWork test.<sup>10</sup> 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 ( $p=.04$ ). 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 immunohistochemical in formalin-fixed, paraffin-embedded 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).<sup>11</sup> 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 cancer of unknown primary 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. Immunohistochemical 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.

Handorf et al (2013) reported on a clinical validation study of formalin-fixed, paraffin-embedded metastatic cancer specimens of known primary tumors representing the 15 tissue types on the PathWork test panel.<sup>12</sup> PathWork's diagnostic performance was compared with immunohistochemical in 160 tumor samples. Overall concordance with known diagnoses (i.e., accuracy) was 89% for PathWork vs 83% for immunohistochemical ( $p=.013$ ). In 51 poorly differentiated and undifferentiated tumors, PathWork accuracy was 94%, and immunohistochemical accuracy was 79% ( $p=.016$ ). In 106 well-differentiated and moderately differentiated tumors, PathWork and immunohistochemical performance were similar (87% and 85% accuracy, respectively;  $p=.52$ ). These results are based on 157 specimens for which both PathWork and immunohistochemical 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 studies 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 immunohistochemical. Reported sensitivities varied according to tumor type generally ranged from 80% to over 90%.

Erlander et al (2011)<sup>13</sup>, revised the original classifier algorithm<sup>4</sup>, using 2,206 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.<sup>14</sup> Approximately half of formalin-fixed, paraffin-embedded 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).<sup>15</sup> For 75 included tumors, assay sensitivities were 99% (95% CI, 93% to 99%) for classification of neuroendocrine tumor type (eg, neuroendocrine, germ cell) and 95% (95% CI, 87% to 98%) for subtype (site of origin). Positive predictive values ranged from 83% to 100% for individual subtypes. A report by Brachtel et al (2016)<sup>16</sup> 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 cancer of unknown primary after a clinical diagnostic workup (i.e., before immunohistochemical).<sup>17</sup> The study evaluated the accuracy of gene expression profiling (CancerTYPE ID) by verifying results with latent primary tumor sites found months after initial presentation (24 patients) or with immunohistochemical and/or clinicopathologic findings (147 patients). Minimum test performance thresholds were prespecified. Tumor specimens adequate



for gene expression profiling 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 immunohistochemical testing and subsequent gene expression profiling, diagnoses matched in 40 (77%). When immunohistochemical suggested 2 or 3 possible primary sites (97 patients), CancerTYPE ID diagnosis matched 1 of the proposed diagnoses in 43 (44%). Among 35 patients with discordant immunohistochemical and CancerTYPE ID diagnoses, clinicopathologic correlates and subsequent immunohistochemical supported the CancerTYPE ID diagnoses in 26 (74%). The authors concluded that gene expression profiling “complements standard pathologic evaluation” of cancer of unknown primary.

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 cancer of unknown primary and poorly differentiated neoplasms.<sup>18</sup> This subset of patients with cancer of unknown primary is considered potentially treatment sensitive but comprised a small number (4%) of the 751 cancer of unknown primary patients evaluated from 2000 through 2012 at Tennessee centers. A primary site was identified in 2 patients. A diagnosis was assigned by gene expression profiling 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.

### **Section Summary: Clinically Valid**

To evaluate whether treatment selection can be improved, the ability of a test to suggest a likely site of origin (clinical validity) would typically be the first step in evaluation. Using different reference standards, these tests have reported sensitivities or concordances generally high (eg, 80% to 90% or more). However, demonstrating clinical validity may be problematic because patients with cancers of unknown primary 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 immunohistochemical comparisons. 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.

### **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 (RCTs). One published RCT and 1 conference presentation have been identified.

Hayashi et al (2019) randomized 130 patients with cancer of unknown primary to gene expression profiling directed therapy based on the predicted tissue of origin or to empirically-

directed chemotherapy with paclitaxel and carboplatin (see Table 2 ).<sup>19</sup> A total of 101 patients received the assigned treatment and were included in the analysis. There was no significant difference between the 2 groups in the 1-year survival rate, overall survival, or progression-free survival (see Table 3). For example, the 1-year survival rate was 44.0% for patients who received gene expression profiling directed treatment and 54.9% for patients who received empirical chemotherapy ( $p=.264$ ). The identification of more-responsive and less-responsive tissue types was prognostic for overall survival (16.7 vs 10.6 months;  $p=.116$ ) and progression-free survival (5.5 vs 3.9 months;  $p=.018$ ). There were several limitations to this trial which included the high percentage of patients who did not receive the assigned treatment (see Tables 4 and 5). A major limitation in interpretation of these results is that during the trial period there were few treatments that were site specific, so there was minimal difference in the actual treatments given to the 2 groups.

The second is the Randomised Phase III Trial Comparing a Strategy Based on Molecular Analysis to the Empiric Strategy in Patients With Carcinoma of an Unknown Primary (CUP) (GEFCAPI 04) study that was presented at the 2019 Congress of the European Society for Medical Oncology in Barcelona.<sup>20</sup> The majority of patients in the experimental group were assessed with Cancer TYPE ID. For the entire group of experimental and control patients analyzed ( $n=223$ ), there was no significant difference in overall survival (hazard ratio: 0.92,  $p=.71$ ) or progression-free survival (hazard ratio: 0.95,  $p<.71$ ) between patients who received site-directed therapy or empirically directed therapy of cisplatin and gemcitabine. There were 60 patients who had a gene expression profiling test with a predicted site of origin that was likely to be insensitive to cisplatin and gemcitabine, among whom overall survival for the site-directed and control groups was also not significantly different (hazard ratio [HR]: 0.74,  $p=.33$ ). However, the study was underpowered for this subgroup analysis. Median overall survival in the subgroup was not improved by gene expression profiling testing (9.1 months; 95% CI, 5.65 to 14.62 ) compared to the control group (10.87 months; 95% CI, 3.45 to 11.73 ). As in the study by Hayashi et al, using a molecular test followed by tailored systemic treatment did not improve outcomes in the total population of patients with cancer of unknown primary.

**Table 2. Summary of Key RCT Characteristics**

Study; Trial	Countries	Sites	Dates	Participants	Interventions	
					<i>Active</i>	<i>Comparator</i>
Hayashi et al (2019) <sup>19</sup> ,	Japan	14	2008-2017	Patients with CUP (130 who were randomized and had sufficient tissue for analysis)	GEP-directed therapy (50 analyzed)	Empirically directed chemotherapy with PC (51 analyzed)
Fizazi et al (2019) <sup>20</sup> ,	Europe	4	2012-2019	Patients with CUP (243)	GEP-directed therapy (110 mITT)	Empirically directed chemotherapy with CG (113 mITT)

CG: cisplatin and gemcitabine; CUP: cancer of unknown primary; GEP: gene expression profiling; mITT: modified intent to treat; PC: paclitaxel and carboplatin; RCT: randomized controlled trial.

**Table 3. Summary of Key RCT Results**

Study	1-yr Survival Rate	Overall Survival (95% CI) mo	Progression Free Survival (95% CI) mo
Hayashi et al (2019) <sup>19</sup> ,			
N	101	101	101
GEP-directed therapy	44.0%	9.8 (5.7 to 13.8)	5.1 (1.9 to 8.3)
Empirical-PC	54.9%	12.5 (8.9 to 16.1)	4.8 (3.3 to 6.5)
HR (95% CI)		1.028 (0.678 to 1.560)	0.884 (0.590 to 1.326)
p-Value	.264	.896	.550
Fizazi et al (2019) <sup>20</sup> ,			
N		223	223
HR (95% CI)		0.92 (0.69-1.23)	0.95 (0.72-1.25)
p-Value		.71	.71

CI: confidence interval; GEP: gene expression profiling; HR: hazard ratio; RCT: randomized controlled trial.

**Table 4. Study Relevance Limitations**

Study	Population <sup>a</sup>	Intervention <sup>b</sup>	Comparator <sup>c</sup>	Outcomes <sup>d</sup>	Follow-Up <sup>e</sup>
Hayashi et al (2019) <sup>19</sup> ,		4. There were few treatments available at the time of the study that were site specific, resulting in little difference between the site specific and empiric chemotherapy treatments			

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.

<sup>a</sup> Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.

<sup>b</sup> Intervention key: 1. Not clearly defined; 2. Version used unclear; 3. Delivery not similar intensity as comparator; 4. Not the intervention of interest.

<sup>c</sup> Comparator key: 1. Not clearly defined; 2. Not standard or optimal; 3. Delivery not similar intensity as intervention; 4. Not delivered effectively.

<sup>d</sup> Outcomes key: 1. Key health outcomes not addressed; 2. Physiologic measures, not validated surrogates; 3. No CONSORT reporting of harms; 4. Not establish and validated measurements; 5. Clinical significant difference not prespecified; 6. Clinical significant difference not supported.

<sup>e</sup> Follow-Up key: 1. Not sufficient duration for benefit; 2. Not sufficient duration for harms.

**Table 5. Study Design and Conduct Limitations**

Study	Allocation <sup>a</sup>	Blinding <sup>b</sup>	Selective Reporting <sup>c</sup>	Data Completeness <sup>d</sup>	Power <sup>e</sup>	Statistical <sup>f</sup>
Hayashi et al (2019) <sup>19</sup> ,	4. Following randomization, if the assay was completed but the results could not predict a tissue of origin, patients were transferred to the empiric treatment arm.	1, 2, 3. No blinding		1. There was high loss to follow-up with 29 patients who did not receive the assigned therapy and were not included in the analysis	2. There was insufficient power due to the high loss to follow-up.	

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.

<sup>a</sup> Allocation key: 1. Participants not randomly allocated; 2. Allocation not concealed; 3. Allocation concealment unclear; 4. Inadequate control for selection bias.

<sup>b</sup> Blinding key: 1. Not blinded to treatment assignment; 2. Not blinded outcome assessment; 3. Outcome assessed by treating physician.

<sup>c</sup> Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.

<sup>d</sup> Data Completeness key: 1. High loss to follow-up or missing data; 2. Inadequate handling of missing data; 3. High number of crossovers; 4. Inadequate handling of crossovers; 5. Inappropriate exclusions; 6. Not intent to treat analysis (per protocol for noninferiority trials).

<sup>e</sup> Power key: 1. Power calculations not reported; 2. Power not calculated for primary outcome; 3. Power not based on clinically important difference.

<sup>f</sup> Statistical key: 1. Analysis is not appropriate for outcome type: (a) continuous; (b) binary; (c) time to event; 2. Analysis is not appropriate for multiple observations per patient; 3. Confidence intervals and/or p values not reported; 4. Comparative treatment effects not calculated.

### Tissue of Origin Test

Nystrom et al (2012) enrolled 65 physicians (from 316 approached) caring for 107 patients with cancer of unknown primary in 2009 to participate in a study of management changes following a tissue of origin test.<sup>21</sup> 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 cancer of unknown primary 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 were 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 cancer of unknown primary.<sup>22</sup> 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%). 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%,  $p=.097$ ), accompanied by longer progression-free survival (6.4 months vs 3.5 months,  $p=.026$ ; HR, 0.47; 95% CI, 0.24 to 0.93), and longer overall survival (median, 17.8 months vs 8.3 months;  $p=.005$ ; 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 cancer of unknown primary evaluated with a CancerTYPE ID assay between 2008 and 2009, Hainsworth et al (2012) identified those with a probable ( $\geq 80\%$ ) colorectal site of origin.<sup>23</sup> 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 cancer of unknown primary 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;  $p=.11$ ). 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 cancer of unknown primary undergoing tissue of origin testing.

Hainsworth et al (2013) published a multisite prospective case series of the 92-gene CancerTYPE ID assay.<sup>24</sup> Formalin-fixed, paraffin-embedded 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 cancer of unknown primary patients who received standard empirical therapy at the same center. Although these results are consistent with possible benefit from gene expression profiling testing in cancer of unknown primary, 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.<sup>9,25</sup>

**Section Summary: Clinically Useful**

Direct evidence of clinical utility is provided by studies that compare health outcomes for patients managed with and without the test. The benefit would be most convincingly demonstrated through a trial randomizing patients with cancer of unknown primary to receive treatment based on gene expression profiling results or usual care. One published RCT and 1 conference presentation with this design were identified. These trials did not find a survival benefit for patients with cancer of unknown primary who received treatment based on the site of origin as determined by molecular testing. A limitation in interpretation of the published trial results is that there were few treatments that were site specific, so there was minimal difference in the actual treatments given to the 2 groups. In the second RCT, most cancers responded to the control treatments. Therefore, the possibility remains that if more site-specific treatments are developed, molecular testing to determine the site of origin in patients with CUP may have clinical utility. The absence of convincing evidence from RCTs prevents conclusions about clinical utility.

**SUPPLEMENTAL INFORMATION**

The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

**Practice Guidelines and Position Statements**

Guidelines or position statements will be considered for inclusion in 'Supplemental Information' if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

**National Comprehensive Cancer Network**

Current National Comprehensive Cancer Network (NCCN) guidelines for the workup of an occult primary malignancy (v. 1.2024) address the use of molecular methods to classify tumors.<sup>26</sup> Gene sequencing to predict tissue of origin is not recommended. The guidelines later note:

"In an attempt to identify the tissue of origin, biopsy specimens are often analyzed by immunohistochemistry (IHC). Gene expression profiling (GEP) assays have also been developed to attempt to identify the tissue of origin in patients with occult primary cancers. Both methodologies offer a similar range of accuracy in tumor classification (approximately 75%). While there may be diagnostic benefit of GEP, a clinical benefit has not been demonstrated."

**National Institute for Health and Care Excellence**

A 2010 clinical guidance on diagnosis and management of malignant disease of unknown primary origin from the National Institute for Health and Care Excellence (NICE) was updated in 2023. In the 2023 update, NICE withdrew recommendations on gene-expression-based profiling and added a link to the NHS Genomic Medicine Service's national genomic test directory..<sup>27</sup>

**U.S. Preventive Services Task Force Recommendations**

Not applicable.

**Ongoing and Unpublished Clinical Trials**

A currently unpublished trial that might influence this review is listed in Table 6.

**Table 6. Summary of Key Trials**

<b>NCT No.</b>	<b>Trial Name</b>	<b>Planned Enrollment</b>	<b>Completion Date</b>
<i>Ongoing</i>			
NCT03498521	A Phase II, Randomized, Active-Controlled, Multi-Center Study Comparing the Efficacy and Safety of Targeted Therapy or Cancer Immunotherapy Guided by Genomic Profiling Versus Platinum-Based Chemotherapy in Patients With Cancer of Unknown Primary Site Who Have Received Three Cycles of Platinum Doublet Chemotherapy (CUPISCO)	790	Jun 2024
<i>Unpublished</i>			
NCT01540058	A Randomised Phase III Trial Comparing a Strategy Based on Molecular Analysis to the Empiric Strategy in Patients With Carcinoma of an Unknown Primary (CUP)	243	Aug 2019 (Conference Presentation)
NCT03278600	The Value of Tissue-of-origin Profiling in Predicting Primary Site and Directing Therapy in Patients With Cancer of Unknown Primary: a Prospective Randomized Controlled Study	172	March 2021 (status unknown)

NCT: national clinical trial.

**CODING**

**The following codes for treatment and procedures applicable to this policy are included below for informational purposes. This may not be a comprehensive list of procedure codes applicable to this policy.**

**Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.**

**The code(s) listed below are medically necessary ONLY if the procedure is performed according to the "Policy" section of this document.**

<b>CPT/HCPCS</b>	
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

<b>REVISIONS</b>	
09-06-2011	Policy added to the bcbsks.com web site.
01-15-2013	In Coding section: <ul style="list-style-type: none"> <li>Updated coding instructions to remove reference to 83890-83913 and 88384-88386 which are no longer effective as of 12-31-2012.</li> </ul>
03-08-2013	Revised Title from "Gene Expression Testing for Cancers of Unknown Primary" to "Microarray-Based Gene Expression Testing for Cancers of Unknown Primary".
	Description section updated.
	In Policy section: <ul style="list-style-type: none"> <li>Clarified policy statement from, "Gene expression profiling using the Pathwork® Tissue of Origin test or the Pathwork® Tissue of Origin test kit-FFPE is considered experimental / investigational to evaluate the site of origin of a tumor of unknown primary, and or to distinguish a primary from a metastatic tumor." To "Gene expression is considered experimental / investigational to evaluate the site of origin of a tumor of unknown primary, or to distinguish a primary from a metastatic tumor."</li> <li>This policy statement change was made to be generalizable to gene expression profiling and not specific to the Pathwork test, so is not a change of policy position.</li> </ul>
	Rationale section updated
	References updated
01-21-2014	Description section updated
	In Policy section: <ul style="list-style-type: none"> <li>Updated examples of tests.</li> </ul>
	Rationale section updated
	In Coding section:



<b>REVISIONS</b>	
	<ul style="list-style-type: none"> <li>Added CPT codes: 81504 (Eff 01-01-2014), 81479, 81599</li> <li>Removed CPT code: 84999</li> </ul>
	References updated
02-10-2015	Title revised to "Gene Expression-Based Assays for Cancers of Unknown Primary" from "Microarray-Based Gene Expression Testing for Cancers of Unknown Primary"
	Description section updated
	Rationale section updated
	References updated
01-01-2016	In Coding section: <ul style="list-style-type: none"> <li>Added CPT Code: 81540</li> <li>Updated Coding notations</li> </ul>
04-19-2017	Description section updated
	In Policy section: <ul style="list-style-type: none"> <li>Removed examples of tests, which are now listed in Table 1. Gene Expression Profiling Tests for CUP of the Rationale section.</li> </ul>
	Rationale section updated
	In Coding section: <ul style="list-style-type: none"> <li>Coding notations updated</li> </ul>
	References updated
05-09-2018	Description section updated
	In Policy section: <ul style="list-style-type: none"> <li>Policy Guidelines added on Genetic Nomenclature and Genetic Counseling</li> </ul>
	Rationale section updated
	References updated
	Appendix removed
07-01-2019	Description section updated
	Rationale section updated
	References updated
09-18-2020	Description section updated
	Rationale section updated
	In Coding section: <ul style="list-style-type: none"> <li>Removed Coding notations – no change of policy intent.</li> </ul>
	References updated
05-05-2021	Description section updated
	Rationale section updated
	References updated
05-04-2022	Updated Description Section
	Updated Rationale Section
	Updated References Section
04-25-2023	Updated Description Section
	Updated Rationale Section
	Updated Coding Section <ul style="list-style-type: none"> <li>Removed ICD-10 Diagnoses box</li> </ul>
	Updated References Section
04-23-2024	Updated Description Section
	Updated Rationale Section
	Updated References Section

**REFERENCES**

1. PDQ Adult Treatment Editorial Board. Carcinoma of Unknown Primary Treatment (PDQ). 2023; <https://www.ncbi.nlm.nih.gov/books/NBK65811/>. Accessed January 16, 2024.
2. Oien KA, Evans TR. Raising the profile of cancer of unknown primary. *J Clin Oncol*. Sep 20 2008; 26(27): 4373-5. PMID 18802148
3. Agwa E, Ma PC. Overview of various techniques/platforms with critical evaluation of each. *Curr Treat Options Oncol*. Dec 2013; 14(4): 623-33. PMID 24243164
4. 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): 465-73. PMID 16594740
5. 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): 15149-54. PMID 11742071
6. 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): 7388-93. PMID 11606367
7. 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): 4031-40. PMID 15899792
8. 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](https://www.accessdata.fda.gov/cdrh_docs/reviews/K080896.pdf). Accessed January 16, 2024.
9. 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](https://www.accessdata.fda.gov/cdrh_docs/reviews/K092967.pdf). Accessed January 16, 2024.
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): 2503-8. 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): 20-8. PMID 22939961
12. 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): 1067-75. PMID 23648464
13. 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
14. 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): 3952-60. PMID 22648269
15. 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

16. Brachtel EF, Operaña 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): 27220-31. PMID 27034010
17. 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 05 2013; 105(11): 782-90. PMID 23641043
18. 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): 91-7. PMID 25758902
19. Hayashi H, Kurata T, Takiguchi Y, et al. Randomized Phase II Trial Comparing Site-Specific Treatment Based on Gene Expression Profiling With Carboplatin and Paclitaxel for Patients With Cancer of Unknown Primary Site. *J Clin Oncol*. Mar 01 2019; 37(7): 570-579. PMID 30653423
20. Fizazi, K, Maillard, A. Penel, N. et al. A phase 3 trial of empiric chemotherapy with cisplatin and gemcitabine or systemic treatment tailored by molecular gene expression in patient with carcinomas of an unknown primary site (GEFCAPI 04). ESMO Congress presentation. 2019.
21. 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): 620-8. PMID 22689213
22. 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): 339-44. PMID 26578722
23. 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): 112-8. PMID 22000811
24. 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): 217-23. PMID 23032625
25. Prasad V, Oseran A, Fakhrejehani 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
26. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology: occult primary (cancer of unknown primary [CUP]). Version 1.2024. [http://www.nccn.org/professionals/physician\\_gls/pdf/occult.pdf](http://www.nccn.org/professionals/physician_gls/pdf/occult.pdf). Accessed January 16, 2024.
27. 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 January 16, 2024.
28. 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.
29. Medicare Evidence Development & Coverage Advisory Committee. MEDCAC Meeting 5/1/2013 - Genetic Tests for Cancer Diagnosis. 2013; <https://www.cms.gov/Regulations-and-Guidance/Guidance/FACA/downloads/id67a.pdf>. Accessed January 16, 2024.