Cancers of unknown primary (CUP) represent 3% of all cancer cases in the United States. A detailed history and physical, as well as radiological and histological testing, can identify some but not all primary sources of secondary tumor. It is suggested that identifying a likely primary source and directing treatment accordingly may improve health outcomes.

Cancers of Unknown Primary

Cancers of unknown primary (CUP), or occult primary malignancies, are tumors that have metastasized from an unknown primary source; they make up approximately 3% of all cancer cases in the United States. Identifying the primary origin of a tumor can dictate cancer-specific treatment, expected outcome, and prognosis. (1)

Most 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 (CT) scans of the chest, abdomen, and pelvis; routine laboratory studies; and targeted evaluation of specific signs and symptoms. (2)

Biopsy of a cancer of unknown primary with detailed pathology evaluation may include immunohistochemical (IHC) analysis of the tumor. IHC identifies different antigens present on different types of tumors, and can usually distinguish an epithelial tumor (i.e., carcinoma) from a melanoma or sarcoma. Detailed cytokeratin panels often allow further classification of a carcinoma; however, tumors of different origins may show overlapping cytokeratin expression. The results of IHC may provide a narrow differential of possible sources of a tumor’s origin, but not necessarily a definitive answer.
The current success rate of the diagnostic workup of a cancer of unknown primary is 20%-30%, including consideration of clinical, radiologic, and extensive histopathologic methods. (3) 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 the identification of the site of origin of a cancer of unknown primary.

Molecular Classification of Cancers
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. (4-7)

Ramaswamy and co-workers, using microarray gene expression analysis of over 16,000 genes, showed 78% classification accuracy of 14 common tumor types. (4) Su and colleagues, using large-scale RNA profiling with microarrays, accurately predicted the anatomical site of tumor origin for 90% of 175 carcinomas. (5) Bloom et al combined multiple tumor microarray databases, creating a large collection of tumors, including 21 types, resulting in a molecular classification scheme that reached 85% accuracy. (8) Although microarray technology enables large numbers of genes to be evaluated at the same time, it is complex and time-consuming, and is limited in its use as mostly a research tool. (6) In addition, since formalin fixation can degrade RNA, fresh/frozen tissue is preferred for better accuracy with microarray technology; however, formalin-fixed is the standard for pathology material in current practice. (9)

One such microarray technology is the Pathwork® Pathchip. The test measures the expression of more than 1,500 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 more than 60 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 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 (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 7 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. (4)

Regulatory Status
In July 2008, test “Pathwork® Tissue of Origin” (Pathwork Diagnostics, Inc., Sunnyvale, CA) 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: 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 Pathwork® 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:
The Pathwork® 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 Pathwork® 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. 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.

Policy
Gene expression profiling using the Pathwork® Tissue of Origin test or the Pathwork® Tissue of Origin test kit-FFPE is considered investigational to evaluate the site of origin of a tumor of unknown primary, and to distinguish a primary from a metastatic tumor.

Policy Guidelines
There is no specific CPT coding for this testing.

The preparation of the probes might be coded using a combination of the molecular diagnostic codes 83890-83913 and the analysis of the probes might be coded using array-based evaluation of multiple molecular probes codes 88384-88386 based on the number of probes analyzed.

Pathwork Diagnostics states that they use 84999 (unlisted chemistry procedure).

Rationale

**Analytic Validity** (technical performance, i.e., reproducibility)

*Fresh Frozen Tumor sample*
In 2008, Dumar and colleagues analyzed performance characteristics of the Pathwork® test in a cross-laboratory comparison study of 60 poorly and undifferentiated metastatic (77%) and primary (23%) tumors. (10) Three academic and one commercial laboratory received archived frozen tissue specimens for procurement and processing at their individual sites. Steps performed by each of the four laboratories included tissue handling, RNA extraction, and
microarray-based gene expression assays using standard microarray protocol. The resulting microarray data generated at each laboratory were sent in a blinded fashion to Pathwork Diagnostics for generation of similarity scores for each type. Reports of the similarity scores were sent back (blinded) to the pathologists at the four laboratories for their use in generating an interpretation. Data were compared among the four laboratories to determine assay reproducibility. Correlation coefficients were between 0.95 to 0.97 for pathologists’ interpretations of the similarity scores, and cross-laboratory comparisons showed an average 93.8% overall concordance between laboratories in terms of final tissue diagnosis.

Formalin-fixed, paraffin-embedded (FFPE) Tumor sample
Analytical performance characteristics of the Pathwork® test for FFPE were analyzed in a cross-laboratory comparison study of 60 poorly and undifferentiated metastatic (45%) and primary (35%) tumors. Each of the 15 tumor tissue types were represented by 4 specimens each, with the exception of breast (n =3) and soft tissue sarcoma (n =5). Samples were distributed among 3 laboratories for procurement and processing at their individual sites. Data were compared among the 3 laboratories to determine assay reproducibility. Correlation coefficients were between 0.92 to 0.93 for pathologists’ interpretations of the similarity scores, and cross-laboratory comparisons showed an average 82.1% overall concordance between laboratories in terms of final tissue diagnosis. A detailed summary of the data can be found at http://www.accessdata.fda.gov/cdrh_docs/reviews/K080896.pdf. Additional analyses of the analytic performance of the test have produced similar results. (11, 12)

Clinical Validity (sensitivity and specificity)

Fresh Frozen Tumor sample
The clinical validation study for the Pathwork® Tissue of Origin test that was submitted to the U.S. Food and Drug Administration (FDA) involved a comparison of the gene expression profiles of 25 to 69 samples to each of the 15 known tumors on the Pathwork® panel (average 36 specimens per known tumor). The specimens included poorly differentiated, undifferentiated, and metastatic tumors. A similarity score was given to 545 specimens and then compared to 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 92.9% (95% confidence interval [CI]: 90.3 –95.0), and the probability that a true negative tissue call was made when a similarity score of 5 or less was reported was 99.7% (95% CI: 99.6 –99.8%). Overall, the Pathwork® performance comparing the profiles of the 545 specimens to the panel of 15 known tumor types showed a positive percent agreement of 89.4% (95% CI: 86.5–91.8%), negative percent agreement of 99.6% (95% CI: 98.6–100%), non-agreement of 6.2% (95% CI: 4.4 –8.6%), and indeterminate of 4.4% (95% CI: 2.8–6.5%).

Monzon and colleagues conducted a multicenter blinded validation study of the Pathwork® test. (12) The 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 to the original pathology report that accompanied the specimen. Overall, the Pathwork® performance comparing the profiles of the 547 specimens to the panel of 15 known tumor types showed an overall agreement of 87.8% (95% CI: 84.7–90.4%) with the reference diagnosis. Sensitivity and specificity were 87.8% (95% CI: 84.7–90.4%) and 99.4% (95% CI: 98.3–99.9%), respectively, with the original pathology report acting as the reference standard. The authors acknowledged that since there was no independent confirmation of the original pathology, using the pathology reports as the reference standard could introduce errors.
into the study results. Agreement differed by site: 94.1% for breast, 72% for both gastric and pancreatic. Performance differences between tissue sites were statistically different (chi-squared=42.02; p =0.04; degrees of freedom [df] =28; n =547). Rates of agreement between test result and reference diagnosis varied by site: 88%, 84.4%, 92.3%, and 89.7% for Clinical Genomics facility, Cogenics, Mayo Clinic, and the International Genomics Consortium, respectively, but these differences were not statistically significant.

Formalin-fixed, paraffin-embedded (FFPE) Tumor sample
The clinical validation study for the Pathwork® Tissue of Origin test Kit-FFPE that was submitted to the U.S. Food and Drug Administration (FDA) involved a comparison of the gene expression profiles of 25 to 57 samples to each of the 15 known tumors on the Pathwork® panel (average 31 specimens per known tumor). The specimens included poorly differentiated, undifferentiated, and metastatic tumors. A similarity score was given to 462 specimens and then compared to 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 was 88.5% (95% CI: 85.3–91.3), and the probability that a true negative tissue call was made when a similarity score of 5 or less was reported was 99.8% (95% CI: 99.7–99.9%). Overall, the Pathwork® performance comparing the profiles of the 462 specimens to the panel of 15 known tumor types showed a positive percent agreement of 88.5% (95% CI: 85.3–91.3%), negative percent agreement of 99.1% (95% CI: 97.6–99.7%), non-agreement of 11.5% (95% CI: 8.7–14.7%). Further details of these data can be found at [http://www.accessdata.fda.gov/cdrh_docs/reviews/K080896.pdf](http://www.accessdata.fda.gov/cdrh_docs/reviews/K080896.pdf).

Few other studies have analyzed the clinical validity of using microarray gene expression technology. One study used microarray technology (CupPrint, Agenda, Amsterdam, the Netherlands), that used FFPE tumor samples. The study analyzed 495 genes in 84 patients with tumors of known origin and 38 patients with cancer of unknown primary (CUP) to assess the potential contribution to patient management. (3) Sixteen of the patients with CUP had their primary site of tumor origin identified by standard laboratory techniques. Molecular testing identified the correct site of tumor origin in 94% of cases of CUP and 83% of the tumors of known origin. Ferracin and colleagues published a report of MicroRNA profiling using 101 FFPE tumor samples from primary cancers and metastases. (14) Forty samples, of 10 cancer types, were used to build a cancer-type-specific microRNA signature. This signature was then used to predict the primary site of metastatic cancer. Overall accuracy was 100% for primary cancers and 78% for metastatic cancers in the cohort sample. The signature was then applied to a published set of 170 samples where the prediction rates were consistent with the cohort results.

**Clinical Utility** (Impact on patient outcomes)

No clinical trials have been conducted that would provide direct evidence of the clinical utility of the Pathwork® Tissue of Origin test, nor has the clinical application of gene expression profiling to direct patient management and tumor site-specific therapy been demonstrated in prospective studies.

One small study using microarray technology (not Pathwork®) on formalin-fixed paraffin-embedded tumor, retrospectively analyzed the gene expression profile of tumors from 21 patients with cancer of unknown primary. The clinical relevance and implications of the results on patient management were reviewed. (15) In the 21 patients, standard methods had failed to determine a primary tumor origin. Results of gene expression profiling were reviewed in the context of tumor histology and clinical suspicion of tumor origin. Gene expression profiling
confirmed the clinical suspicion in 16 of 21 cases, with a clinical/gene expression profile inconsistency in 4 of 21 and a pathological/gene profile inconsistency in 1 patient. The authors concluded that the use of gene expression profiling would have influenced patient management in 12 of 21 of the cases.

Summary

Limited data have been published on the clinical impact of the Pathwork® test. Without knowledge of how this test would affect clinical practice and clinical health outcomes (clinical utility) for patients diagnosed through the use of this test, the investigational policy statement remains unchanged. A trial where patients with a cancer of unknown primary were randomized to receive treatment based on the results of the Pathwork® Tissue of Origin test or based on standard diagnostic procedures would be useful to determine the clinical utility of the Pathwork test.

Clinical Trials

In June 2009, final data collection for the primary outcomes was completed in a study aimed at the identification of the tissue of origin in patients with metastatic tumors of unknown primary site. As of October 2010, no results have been published in the peer-reviewed literature.

An October 2011 search of the National Cancer Institute and clinicaltrials.gov databases returned no ongoing phase II or III studies investigating the use of molecular gene expression profiling with microarray technology in patients with cancer of unknown primary.

Practice Guidelines and Position Statements

National Comprehensive Cancer Network guidelines for the workup of an occult primary malignancy address the use of molecular methods in the classification of tumors. They conclude that there is insufficient data to confirm whether gene expression profiling can be used in choosing treatment options which would improve the prognosis of patients with occult primary cancers. Therefore the panel does not recommend the testing as a part of routine evaluation of a cancer of unknown primary origin. (16)

Medicare National Coverage

No national coverage determination.

References:


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**Type of Service**
Pathology/Laboratory

**Place of Service**
Laboratory/Reference Laboratory

**Index**
Pathwork Tissue of Unknown Origin