Technology Assessment

Technology Assessment Program

Technology Assessment on Genetic Testing or Molecular Pathology Testing of Cancers with Unknown Primary Site to Determine Origin

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Peer Reviewers

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Structured Abstract

Objective: This technology assessment reports the results of our review of the existing literature on commercially available genetic tests that are used to identify the tissue of origin (TOO) of the cancer in patients with cancer of unknown primary (CUP) site. CUP is a case of metastatic tumor for which the primary TOO remains unidentified after comprehensive clinical and pathologic evaluation. This review focused on analytical and clinical validity of the tests and their utility in guiding the diagnosis and treatment of CUP and improving health outcomes.

Data Sources: The scope of the review was limited to tests that are commercially available in the United States. We identified genetic or molecular TOO tests by searching GeneTests.org, the NIH Genetic Testing Registry, GAPP Knowledge Base, and the following Food and Drug Administration databases: Premarket Notifications (510(k)), Premarket Approvals, and Clinical Laboratory Improvement Amendments. We conducted focused searches of PubMed, Embase, and the Cochrane Library. We also searched the Internet, and once the tests were identified, we conducted a grey literature search of the manufacturer's Web sites.

Review Methods: We included systematic reviews, randomized controlled trials, nonrandomized controlled trials, prospective and retrospective cohort studies, case-control studies, and case series published from 1990 to present. We excluded non-English studies; a preliminary search found very few studies published in other languages. We searched the grey literature for relevant studies but did not contact authors for additional data. We included conference presentations and posters when they presented data not published elsewhere. Studies were rated for methodological quality. The results were synthesized across studies for each test using a meta-analytic approach when appropriate.

Results: We reviewed cytogenetic analysis and three genomic TOO tests (CancerTypeID, miRview, and PathworkDx) for analytical validity, clinical validity, and clinical utility. The published evidence in each of these areas is variable. Some data on analytic performance were available for all of the genomic TOO tests, but the evidence was sufficient to confirm validity only for the PathworkDX test. We could not compare analytic validity across tests because different data were reported for each test. We found sufficient evidence to assess the validity of the statistical algorithms for CancerTypeID and miRview. We were unable to assess the validity of the statistical algorithm for the PathworkDx TOO. Each test has three or more publications that report on the accuracy of the tests in identifying the TOO of known tumor sites. The accuracy rates across all of the studies for each of the three tests are fairly consistent. The meta-analytic summary of accuracy for the three tests with 95% CI is as follows: CancerTypeID—85 percent (83% to 86%); miRview mets—85 percent (83% to 87%), and PathworkDx—88 percent (86% to 89%). The accuracy of the tests in CUP cases is not easily determined, because actual TOO is not identified in most cases. The evidence that the TOO tests contributed to the diagnosis of CUP was moderate. Low evidence supported the clincal usefullness of the TOO tests in making diagnosis and treatment decisions. Low evidence also supported the length of survival

amnog CUP patients who received the test. The evidence was insufficient to answer other key questions on the effect of the tests on treatment or outcomes.

Conclusions: The clinical accuracy of all the three tests is similar, ranging from 85 percent to 88 percent. The evidence that the tests contribute to identifying a TOO is moderate. We do not have sufficient evidence to assess the effect of the tests on treatment decision and outcomes.

Future Research: Most studies included in the current review were funded wholly or partially by the manufacturers of the tests. The most urgent need in the literature is to have the clinical utility of the tests evaluated by research groups that have no evident conflict of interest. Given the difficulty of assessing the accuracy of the TOO in CUP cases, future research should focus on the benefits from the test to the patient in terms of effect on treatment decisions and resulting outcomes. These studies will help assess the clinical value of the TOO tests.

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Executive Summary

Introduction

The purpose of this technology assessment is to assess the evidence on the analytical validity, clinical validity, and clinical utility of commercially available genetic tests for identifying the tissue of origin (TOO) of the cancer in patients with cancer of unknown primary (CUP) site. This report was requested by the Centers for Medicare & Medicaid Services and was conducted through the Evidence-based Practice Center (EPC) Program at the Agency for Healthcare Research and Quality (AHRQ).

Methods

The review focused on five key questions (KQs) identified in the protocol, assessed the quality of evidence for each question, and synthesized the results across studies for the same test using a meta-analytic approach when there was sufficient information across the studies. The KQs were as follows:

1. What genetic or molecular TOO tests are available for clinical use in the United States and what are their characteristics?
2. What is the evidence on the analytic validity of the TOO tests?
3. What is the evidence regarding the accuracy of genetic TOO tests in classifying the origin and type of CUP?
    a. Does it differ by tumor origin?
    b. Does it differ by patient age, sex, race, or ethnicity?
4. What is the evidence that genetic TOO tests change treatment decisions and improve clinical outcomes?
5. Is the evidence regarding genetic TOO tests relevant to the Medicare population?

Approach to Evaluating the Literature

The scope of the review was limited to tests that are commercially available in the United States. We identified genetic or molecular TOO tests by searching GeneTests.org, the NIH Genetic Testing Registry, the GAPP Knowledge Base, and the following Food and Drug Administration databases: Premarket Notifications (510(k)), Premarket Approvals, and Clinical Laboratory Improvement Amendments. We also searched the Internet for tests to identify TOO in patients with CUP. We defined a "commercially available" test as one for which an Internet search or test directory identified a mechanism for a physician or laboratory to order the test or to buy a kit to perform the test.

We included systematic reviews, randomized controlled trials, nonrandomized controlled trials, prospective and retrospective cohort studies, case-control studies, and case series published from 1990 to present. We excluded non-English studies; a preliminary search found very few studies published in other languages. We searched the grey literature for relevant studies but did not contact authors for additional data. We included conference presentations and posters when they presented data not published elsewhere.

Quality Assessment

We assessed the risk of bias in the reviewed studies using the criteria described in the Methods Guide for Medical Test Reviews.[1] For studies of analytic or clinical validity (KQs 2 and 3), we used the QUADAS[2] to assess the potential for bias due to flaws in the sample selection, testing protocol, reference standards, verification procedures, interpretation, and analysis. We used Simon[3] criteria to assess the validity of the development of statistical classification algorithms. For studies of clinical utility (KQ 4), we used questions from the RTI International Question Bank[4] to assess the potential for bias from sample selection, study performance, attrition, detection of outcomes, and reporting. Quality assessment results were summarized as good, fair, or poor, correlating with a low, medium, or high risk of bias.

Data Synthesis

We summarized the evidence for each KQ in the evidence tables. For each of the three genomic tests, two or more studies assessed the ability of the test to correctly identify tumors of known origin. Given the consistency of the accuracy rates across the studies and overlapping confidence intervals, we did a meta-analysis using a fixed effects model to estimate a summary measure of accuracy.

Although more than three studies assessed the accuracy of the test in identifying the site of the primary tumor for true CUP cases for all three tests, the standards used to judge the accuracy of the TOO call varied among studies, and the validity of some standards were questionable. Therefore, we did not include a summary measure of accuracy across these studies.

Grading the Evidence for Each Key Question

We graded the overall strength of evidence according to the guidance established for the EPC Program.[1, 5] This approach incorporated four key domains: risk of bias (including study design and aggregate quality), consistency, directness, and precision of the evidence. Grades reflect the strength of the body of evidence to answer the KQs on the validity and efficacy of the interventions in this review. Two senior reviewers assessed each domain and the overall grade for each key outcome listed in the framework. Conflicts were resolved by discussing until consensus was reached. For KQs 2 and 3, the strength of evidence was graded for each test. For KQ 3a, we graded the evidence that the statistical algorithm was valid using the Simon[3] criteria for the development of statistical classification algorithms. For KQ 4, the strength of evidence was graded for each outcome (e.g., treatment change, clinical outcomes).

Results

We reviewed four tests—cytogenetic analysis and three molecular tests (CancerTypeID, miRview mets, and PathworkDx)—for analytical validity, clinical validity, and clinical utility (Table A). The published evidence in each of these areas is variable.

Table A. Genetic and molecular tests to identify tumor tissue of origin

	Pathworks TOO	CancerTYPEID	Mirview mets	Chromosomal Analysis
Analyte	mRNA	mRNA	microRNA	Chromosomes
Panel size	TOO-FFPE: 1,550 TOO-FRX: 2,000 Endometrial: 316	92	Mets: 48 Mets[2]: 62	46
Laboratory methods	Expression microarray analysis	Quantitative real time PCR	Quantitative real time PCR	High resolution G-banded
Statistical methods	Pairwise comparison, machine learning algorithm	Kohonen neural network (KNN)	Binary decision tree and KNN	NA
No. sites identified	FFPE and FRZ: 15 Endometrial: 2	28	Mets: 22 Mets[2]: 24	NA
Reported results	Similarity score	Probability of each type	Predicted tumor type from each algorithm	Karyotype

Some data on analytic performance were available for all of the three molecular TOO tests, but only the PathworkDx test had sufficient evidence to assess its analytic validity (Table B). The evidence that PathworkDx was analytically valid was high.

Table B. Analytic validity

	Pathworks TOO	CancerTYPEID	miRview
Number of studies	4	1	5
Total tumors	640	487	1,546
Marker accuracy	Coefficient of reproducibility: 32.48+/-3.97	Reproducibility (Ct values): + controls: 1.7% - controls: 1.3%	Interlaboratory concordance: >0.95%

Two out of three tests have sufficient information included in publications to allow one to assess the validity of the statistical algorithms used in the TOO. The manuscripts describing the PathworkDx TOO test do not have the same degree of detail, so it is difficult to assess the validity of the algorithm with the same degree of confidence (Table C).

Table C. Statistical validity of algorithm development

	Pathworks TOO	CancerTYPEID	miRview
Normalization	Total expression	Housekeeping genes	Total expression
Dimension reduction	Not enough detail to assess	Clustering with GLM to assess predictive value	Logistic regression to assess predictive value
Classification rule supervision	Supervised Not enough detail to assess	Supervised	Supervised
Internal validation	Yes	Yes	Yes
External validation	Yes	Yes	Yes
Criteria met?	Mostly – classification and dimension reduction not evaluable	Yes	Yes

At least three studies for each test reported on the ability of the test to identify the primary site for tissues of known origin (Table D). The accuracy rates across all of the studies for each of the three tests are fairly consistent. The summarized accuracy rate for CancerTypeID was 0.85

with 95% confidence interval (CI) (0.83 to 0.86), for miRview mets it was 0.85 with 95% CI (0.83 to 0.87), and for PathworkDx it was 0.88 with 95% CI (0.86 to 0.89).

Table D. Clinical validity

	Pathworks TOO	CancerTYPEID	miRview
Number of studies	9	6	4
Total tumors	1,243	1,478	1,198
Comparison standards	Cancers of known origin/IHC	Final diagnosis	Known origin
Percent accuracy (range)	74-97	82-95	85-88
Percent indeterminate (range)	5-18	NR	NR
Meta-analysis estimate of accuracy	0.88 95% CI, 86 to 89%	0.85 95% CI, 83 to 86%	0.85 95% CI, 83 to 87%

The ability of the tests to detect CUP cases is not as yet easily determined. The primary tumor site in CUP cases is often never identified. Therefore, only a few reports of test performance in CUP cases have independent confirmation of TOO. Fifteen studies, all rated fair, looked at the clinical utility of the TOO tests. Tables E to G demonstrate the findings in regard to clinical utility in regard to diagnosis, treatment decisions and improving outcomes respectively.

Table E. Clinical utility-diagnosis

Outcome	Number of Styles	Summary of Results
TOO predicted	17	57-100% >90% in 9 studies
TOO confirmed	9	48-88%
Test changed or resolved diagnosis	5	44-81%
Test reported to be clinically useful	1	66-67%

Methods of confirmation: Identification of primary site after test, clinicopathological features at test or at end of follow-up.

Table F. Clinical utility for treatment decisions

Outcome	Number of Studies	Summary of Results
Treatment changed	4	26% - 81%
Increase in site specific treatment	1	23% increase
Difference in treatment response	4	TOO-based: 41-74% Empiric: 17%

Table G. Clinical utility for improving outcomes

Outcome	Number of Studies	Summary of Results
Survival (months) TOO-based treatment vs. empiric treatment	2	2.5 – 3.4 month increase
Survival (months) total sample	3	12.9 – 21
Projected increase in survival (months)	1	3.6 months
Projected increased adjusted for quality of life	1	2.7 months
Stable disease	1	32%

We also examined the applicability of the evidence on these tests to the Medicare population. As showin in Table H, the majority of the studies included patients 65 years and older, the core Medicare population, and included patients of both sexes.

Table H. Applicability to Medicare patients

Characteristic	Number
Studies of clinical utility	19
Total patients	2,398
Studies with patients 65 or older	13
Both sexes	14

Given the lack of a gold standard in some reports, we judged the strength of the evidence as moderate that the tests accurately detect the TOO. The strength of evidence was also low that TOO tests affect treatment decisions and length of survival. The evidence was insufficient to answer KQs on the effect of the tests on outcomes.

Summary of Findings

We assessed four tests in this review: cytogenetic analysis, CancerTypeID, miRview mets, and PathworkDx. Of the three molecular tests, CancerTypeID has the broadest panel with the ability to detect 28 different tumor types; miRview mets has 24 tumor sites on its panel and PathworkDx has 15 tumor sites in its panel. Cytogenetic analysis can only detect a TOO associated with a specific cytogenetic abnormality. Table I summarizes our findings.

The literature on molecular genetic tests for CUP is in its infancy. The published manuscripts that we reviewed suggest that the tests have a high accuracy rate when the TOO is known or in studies where there is a well-defined, valid measure of accuracy. The literature available on the use of these tests in the diagnosis of actual CUP cases is very limited because of the lack of a consensus measure on determining the accuracy of the test's call. Given the nature of CUP, a standard for identifying the accuracy of the TOO test may not be available in many cases.

In the absence of a good measure of accuracy of the test call, a proxy measure of the utility of the test is its effect on treatment decisions and patient outcomes. The literature on the effect of the test on treatment decisions is very limited. There is low evidence that the test alters the treatment course from empiric therapy usually used in CUP to tissue-specific therapy. The effect of this change in therapy on outcomes is limited to four papers that used CancerTypeID as the TOO test and two on PathworkDx. All but one of the studies used historical controls to determine benefit of therapy. The one study.[6] that compared survival between site specific and empiric therapy had a sample size of less than 100 and was focused on CRC. Thus there is no study with a sufficient sample size that compared outcomes between patients who received tissue-specific therapy and those who did not.

One of the concerns is that all but one of the manuscripts reviewed were funded wholly or partly by the manufacturers of the tests. It is not possible at this time to rule out a possibility of publication bias in the available literature.

Table I. Overview of study outcomes

Key Question	Number of Studies	Conclusion	Strength of Evidence
KQ 2. Analytic validity: CancerTypeID	1	Only one study that was conducted by manufacturer of test. Limited measures of analytic validity reported and impossible to assess consistency of those measures across studies.	Insufficient
KQ 2. Analytic validity: miRview miRview mets	4	Four studies each reported different measures of analytic validity. Impossible to evaluate consistency of reported measures of analytical validity across studies.	Insufficient
KQ 2. Analytic validity: PathworkDx	3	Three papers reported measures of analytic validity. There is moderate consistency between studies of interlaboratory reproducability. The reported precision in these three papers is high.	High
KQ 2. Analytic validity: PathworkDx Endometrial	1	One study reported on the analytic validity of a PathworkDx Endometrial test. The reported precision of the test is high, but there is insufficient evidence to assess analytic validity.	Insufficient
KQ 3a. Adherence to Simon guidelines: CancerTypeID	2	Report on the development of the algorithm has sufficient detail on development and validation to assess the validity of the process. Development met the Simon[3] criteria.	High
KQ 3a. Adherence to Simon guidelines: miRview mets	2	Report on development of algorithm has sufficient detail on development and validation to assess the validity of the process. Development met the Simon[3] criteria.	High
KQ 3a. Adherence to Simon guidelines: PathworkDx	2	Report on development of algorithm does not have sufficient detail on development and validation to assess the validity of the process.	Low
KQ 3a. Adherence to Simon guidelines: PathworkDX Endometrial	1	Report on development of algorithm does not have sufficient detail on development and validation to assess the validity of the process.	Low
KQ 3b. Accuracy of the TOO test in classifying the origin and type of tumors of known primary site: CancerTypeID	7	Seven studies representing 1,476 tissue samples compared the ability of tests to identify origin of tumor in tissues of known origin. All report accuracy of inclusion. Accuracy of exclusion reported in two studies.	High
KQ 3b. Accuracy of the TOO test in classifying the origin and type of tumors of known primary site: miRview mets	4	Two independent studies representing 898 tissue samples tested the ability of the miRview to identify site of origin in tissues of known origin. Accuracies of inclusion and exclusion are reported for both.	High
KQ 3b. Accuracy of the TOO test in classifying the origin and type of tumors of known primary site: PathworkDx	9	Nine studies representing 1,247 tissue samples report on the ability of the test to identify the origin of tumor in tissues of known origin. Accuracy of included tissue is reported in all studies. Accuracy of excluded tissues reported in two studies.	High
KQ 4. Percentage of cases for which a TOO test identified a TOO	17	Fifteen studies rated fair provided evidence on this question. The ability of the test to identify a TOO was judged using different criteria in different studies.	Moderate
KQ 4. Percentage of CUP cases for which the TOO identified by the test was independently confirmed	6	Five studies all rated fair provided evidence for this question. Gold standard varied across studies and precision across studies was moderate. Accuracy ranged from 57% to 100%.	Low
KQ 4: Percentage of CUP cases where TOO modified diagnosis	6	Four studies rated fair and one poster rated good provided evidence for this question. Some explored potential changes; others actual changes. The reported percentage of changes ranged from 65% to 81%. Response rates in the survey were fairly low.	Low

Table I. Overview of study outcomes (continued)

Key Question	Number of Studies	Conclusion	Strength of Evidence
KQ 4: Percentage of CUP cases where test was considered clinically useful by physician or researcher	6	All studies found test clinically useful in a proportion of cases. Clinical usefulness was measured differently in each study. Wide estimate in the proportion of cases where test was useful.	Low
KQ 4: Change in treatment decisions	5	Studies have small samples, varied study designs and measures of effect on treatment decisions, making it difficult to draw conclusions on any of the tests.	Insufficient
KQ 4: Treatment response: Tissue-specific treatment based on TOO test compared with usual treatment for CUP cases	4	Small samples, insufficient studies to draw conclusions on any of the tests. Only one study has a control; all others use historical controls.	Insufficient
KQ 4: Change in survival	5	Small samples, insufficient studies to draw conclusions on any of the tests. Most use historical controls.	Low
KQ 4: Change in disease progression	1	Small samples, insufficient studies to draw conclusions on any of the tests.	Insufficient
KQ 5: Applicability	22	Almost all studies included patients age 65 and older and both male and female patients. The studies that reported race included mostly Caucasian patients.	High

Future Research

The most urgent need in the literature is to have the tests be evaluated by research groups that have no evident conflict of interest.

Given the difficulty in identifying a valid measure of accuracy of the test in true CUP cases, it seems the most fruitful research would focus on the benefits from the test to the patient in terms of effect on treatment decisions and resulting outcomes.

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Introduction

Background and Objectives for the Systematic Review

A Brief Overview of Cancer

Cancer is one of the leading causes of death in the United States. The National Cancer Institute (NCI) estimates 1,596,670 new cases of cancer, excluding melanomas, will be diagnosed in 2011.[1] Over half a million deaths in 2011 were expected to be attributed to cancer.

Cancer begins when the normal processes of cell division and death get interrupted and cells in a part of the body begin to grow uncontrollably. The abnormal cells multiply and interrupt normal function of the tissue. Hanahan and Weinberg[2] suggest that malignant growth is a result of a series of genetic changes in cells, which cause the following six essential modifications in cell physiology: self-sufficiency in growth signals, inaccuracy in growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis.[2]

Cancers are most commonly classified by the primary site of occurrence, but they are also secondarily grouped by the type of cell that the cancer is formed from. In this classification, a carcinoma is a cancer that begins in the epithelial cells that line the inside or outside of an organ. The two most common forms of carcinomas are squamous cell carcinomas and adenocarcinomas. Squamous cell cancers are formed by flat cells that resemble cells normally found on the surface of the skin or the linings of the throat, esophagus, lungs, anus, cervix, vagina, etc.; adenocarcinomas are cancers that develop from gland cells. Most cancers in the stomach and intestines are adenocarcinomas. The four most commonly occurring cancers in the United States—prostate cancer in men, breast cancer in women, lung cancer, and colorectal cancer—are all carcinomas. Cancer that develops from cells of the immune system found in the lymph nodes and other organs are called lymphomas; melanomas develop from cells that produce the skin's tan; sarcomas develop from connective tissue cells that are usually present in tendons, ligaments, muscle, fat, bones, cartilage, and related tissue; and germ cell tumors develop in the testes for men or ovaries for women or in the parts of the body where these organs developed in the fetus.

In most cases, cancer cells form solid tumors. The diagnostic work-up in a patient newly diagnosed with cancer usually includes an assessment of the stage of the cancer. Cancer staging is a way to describe the severity of the disease. It also determines the treatment modality. Most tumors can be classified as Stage 0, Stage I, Stage II, Stage III, or Stage IV. Increasing stage denotes increasing severity, with Stage IV indicating that the cancer has spread to distant organs or metastasized.

The choice of a treatment for cancer varies with both the site of primary origin and the type of cancer. Most common cancer treatments consist of combinations of three components: (1) surgery to remove as much of the cancerous tissue as possible, (2) radiation to kill or slow the growth of cancerous tissue that cannot be accessed safely through surgery, and (3) tissue-specific chemotherapy to kill cancer tissue through a system-wide administration of "anticancer" drugs. Chemotherapy is always a part of the regimen when a patient has metastatic cancer (i.e., their disease has spread from the primary organ to a distant organ). Patients who are diagnosed as having Stage III or Stage IV cancer usually have metastatic cancer. There are curative treatments for some metastatic cancers; if a curative treatment is not available, it is still possible to treat the

metastatic cancer and slow the progression of the cancer to increase survival or relieve symptoms and improve quality of life.

As the understanding of the molecular functioning of cancer cells increases, targeted therapies designed to attack specific characteristics of a cancer cell are being added to treatment regimens. Drugs used in these regimens are targeted to attack specific functions of cancer cells and leave healthy cells alone. Cancer cell type and primary site both affect the efficacy of targeted therapies. In order to design the most effective treatment regimen for a patient with metastatic cancer, it is therefore important to know the site of the primary tumor or at least the cancer cell type.

Cancer of Unknown Primary Site

A metastasized tumor shares some molecular characteristics, such as chromosomal rearrangements and expressed proteins, with the primary tumor. Thus, a metastatic breast cancer in the lung will have characteristics of breast cancer, not lung cancer. It is still considered and treated as breast cancer. The most likely sites of metastasis of various primary tumors are well known (Table 1).[3] Some cancer patients present in clinic with metastatic tumors in one or more sites without a primary tumor. These cancers are called cancers of unknown primary (CUP) site.

Table 1. Common sites of metastasis for different primary sites

Primary Cancer	Main Sites of Metastasis
Breast	Lung, liver, bones
Colon	Liver, peritoneum, lung
Kidney	Lung, liver, bones
Lung	Adrenal gland, liver, lung
Melanoma	Skin, muscle, liver
Ovary	Liver, peritoneum, lung
Pancreas	Liver, lung, peritoneum
Prostrate	Lung, liver, bones
Rectum	Liver, peritoneum, adrenal gland
Stomach	Liver, peritoneum, lung
Thyroid	Lung, liver, bones
Uterus	Liver, peritoneum, lung

Source: National Cancer Institute.

Naresh[4] hypothesized that in CUP the primary tumor is not able to develop a good supply of blood and nutrients for itself (angiogenic incompetence), leading to marked cell death and cell turnover. The primary tumor remains microscopic or disappears after seeding the metastasis. Naresh[4] suggests that the metastatic potential of the cells is not activated until the cells evolve and develop into angiogenic-competent cells. This results in a biologically advanced tumor that acquires a metastatic phenotype. Once this type of cell has developed, there is a rapid growth of the metastatic tumor. Emerging data suggest that the propensity to metastasize might be hardwired early in the disease process,[5] which suggests that in CUP the primary tumor has a "poor prognosis" signature and is unable to establish itself but can metastasize to various organs.

The American Cancer Society estimated that more than 30,000 cases of CUP were diagnosed in 2011.[1] Tong et al.[6] concluded that the number may actually be as much as 53,000 new cases of CUP among Medicare patients a year. Some CUP patients may be treated as "known primaries" for pragmatic reasons, even though the primary site is not conclusively identified. If these patients are included in the count, Greco[7] suggests that there may be as many

as 100,000 cases of CUP per year in the United States. Prognosis for CUP patients is generally poor; the median survival for all types of CUP is about 9 to 12 months.

Diagnosis and Treatment of CUP

A patient should be diagnosed as having CUP only after a thorough examination and history have ruled out a primary site. NCI guidelines call for the initial evaluation of a CUP to include a tumor biopsy; a thorough history; a complete physical examination that includes head and neck, rectal, pelvic, and breast examinations; chest x-rays; a complete blood cell count; urinalysis; and examination of the stool for occult blood.[8]

Chemotherapy is the most common option used to treat CUP. As more targeted and effective therapy for specific cancer types becomes available (renal, lung, breast, colorectal, stomach, and others), identification of the primary site of a CUP could improve staging and prognosis for many patients.[7] It can identify clinical trials for which the patient is eligible. A more accurate test for CUP would also decrease the need for further diagnostic procedures and reduce the time between excision of the cancer and initiation of the treatment. Traditional methods of identifying the tissue of origin (TOO) for CUP have had limited success,[9] and considerable research has been done to improve available techniques and develop new techniques.

Tests to Identify Primary Site

The most commonly used techniques to identify TOO include light microscopy, immunohistochemistry (IHC) staining, and computed tomography (CT) or positron emission tomography (PET) imaging.

Light Microscopy

CUP cancer tissue specimens from a fine needle aspiration or core needle biopsy are subjected to light microscopic examination after they are stained with hematoxylin and eosin or other histologic or cytologic stains. After light microscopy examination, approximately 60 percent of CUP cases are reported as adenocarcinomas and 5 percent as squamous cell carcinomas. In the remaining 35 percent, light microscopy allows less definitive conclusions—poorly differentiated adenocarcinoma, poorly differentiated carcinoma, or poorly differentiated neoplasm.[9]

IHC Tests

IHC stains are an important complement to light microscopy in the evaluation of CUP. IHC staining methods include use of fluorophore-labeled (immunofluorescence) and enzyme-labeled (immunoperoxidase) antibodies to identify proteins and other molecules in cells. IHC is used in surgical pathology to determine cancer cell types, cancer subtype classifications, and possible cell of origin in metastatic cancer of unknown or undetermined primary site.[9] IHC markers help define tumor lineage by identifying antigens expressed in the tumor that are specific to tumor type. Cytokeratin subtype is a commonly used IHC marker for identifying the primary site in CUP. The 20 subtypes of cytokeratins have different expression profiles in various cell types and tumors. Cytokeratins are typically expressed in carcinomas, whereas other immunohistochemical markers characterize sarcomas, melanomas, and hematologic malignancies. For example, cytokeratin 20 (CK20) is normally expressed in the gastrointestinal (GI) epithelium, urothelium, and Merkel cells, while cytokeratin 7 (CK7) is found in tumors of the lung, ovary, endometrium, and breast. Figure 1[9] demonstrates the approach to IHC testing using CK7 and CK20 in CUP.

This initial IHC test narrows the possible sites, then sequential testing with the additional markers listed in Table 2[9] further narrow the probable site of the primary. A recent meta-analysis found that IHC staining correctly identified the site of origin of 82 percent of blended primary and metastatic tumors and 66 percent of metastatic cancers.[10] Dennis et al.[11] demonstrated that an IHC panel of 10 marker stains correctly classified the site of origin in 88 percent of adenocarcinomas.

Figure 1. Using presence and absence of CK7 and CK20 to narrow the field of possible primary sites in CUP patients

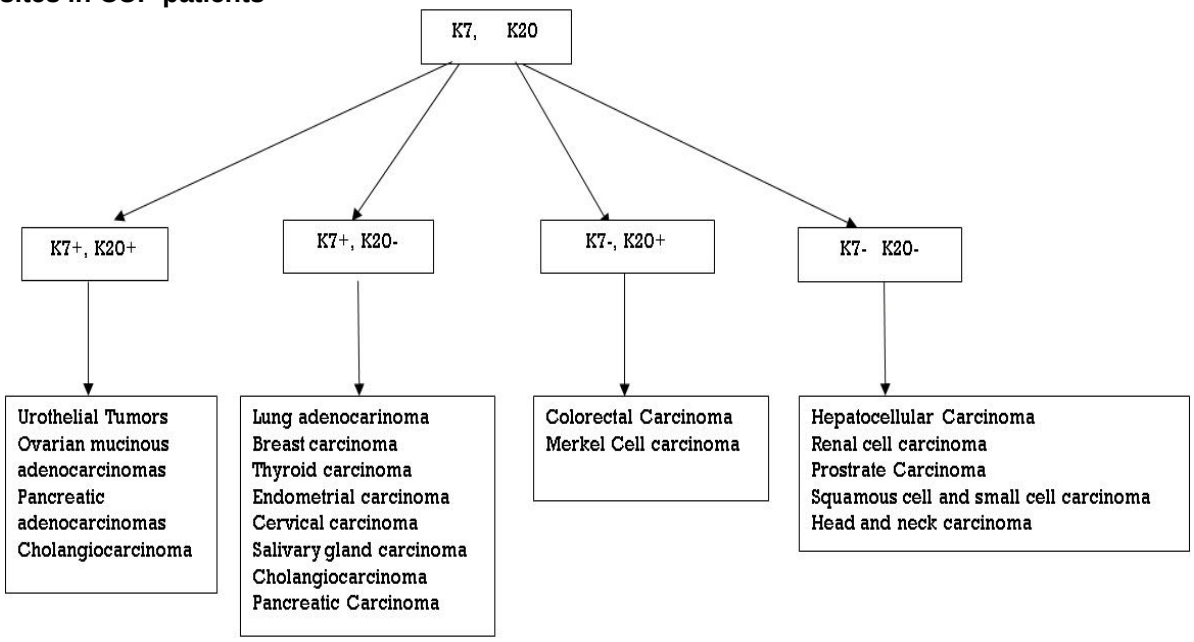


Table 2. Markers used to narrow possible primary sites after tissue is tested for CK7 and CK20

Site	IHC Marker
Urothelial tumors	UROIII, THR, HMWCK
Breast carcinoma	GCDFP-15, ER, PR
Lung(mainly adenocarcinoma)	TTF-1, surfactant A and B
Medullary thyroid carcinoma	TTF-1, calcitonin
Merkel cell carcinoma	CD117
Hepatocellular carcinoma	Hep par-1
Prostate carcinoma	PSA, PAP
Cholangiocarcinoma	CK19
Mesothelioma	Calcetrin

Abbreviations: ER = estrogen receptor; GCDFP-15 = gross cystic disease fluid protein-15; HMWCK = high molecular weight cytokeratin; PAP = prostate acid phosphatase; PR = progesterone receptor; PSA = prostate specific antigen; THR = thrombomodulin; TTF-1 = thyroid transcription factor-1; UROIII = uroplakin III.

CT Scans

CT scans combine X-rays taken from various angles into three-dimensional images, providing better views of organ structure, and can therefore detect much smaller tumors than a chest x-ray. The vast majority of CUPs that are identified are lung or pancreatic carcinomas, so chest and abdomen CT scans are routinely done to detect occult primary tumors. For women, the routine workup for a CUP also includes mammograms and pelvic CT scans. Physical exam, a

thorough medical history, and knowledge of common primary-metastasis relationships can suggest areas for additional CT scans.

PET Scan

A PET scan uses radioactive materials to measure body functions, such as blood flow, oxygen use, and sugar (glucose) metabolism. The most common radiotracer in use today is 18F-fluorodeoxyglucose (18F-FDG), which is a radio-labeled sugar (glucose) molecule. Imaging with 18F-FDG PET is used to determine sites of abnormal glucose metabolism and can be used to characterize and localize many types of tumors.[12] PET imaging alone can detect 8 percent to 53 percent of CUP primary sites; it has been most effective in detecting occult primaries in the head and neck.[12] Trials that have used PET for patients with suspected occult primaries in the head and neck detected a primary tumor in 20 percent to 31 percent of the cases with a false positive rate of about 20 percent.[12] A few centers have used whole body PET/CT scans to detect the primary site in patients with CUP, with a success rate of 21 percent to 35 percent.[12, 13]

Molecular and Genetic Tests

A clinically detectable cancerous lesion is the result of a succession of genetic changes that disrupts the normal process of cell decay and death. Cytogenetic analysis is the traditional method of identifying these changes and associating them with specific cancer types.[14] Ramaswamy et al.[15] found that a gene expression signature distinguished primary from metastatic adenocarcinomas. Similar studies by other groups suggested that gene expression profiles in metastatic tissue could identify the site of the primary tumor by comparing the known profile of different primary cancers to the one expressed by the CUP tissue and identifying the primary with the closest match.[16]

New molecular genetic TOO tests use molecular marker panels that measure patterns of gene expression and regulation. Test analytes, methodology, and panel composition (i.e., the specific markers included) and size (i.e., number of markers) differ between tests. The measurement of interest for a specific marker may be qualitative (presence or absence of the marker) or quantitative (the total estimated number of copies of the marker or the number of copies relative to another marker). Statistical algorithms analyze the pattern of markers and estimate the likelihood the tumor is derived from a specific tissue and is of a specific type. Several issues are still to be determined with respect to the validity and the utility of these tests.

Objectives of the Review

In this technology assessment we report the results of our review of the existing literature based on the key questions (KQs) identified in the protocol, assess the quality of evidence for each question, and synthesize the results across studies for the same test using a meta-analytic approach when there is sufficient information across the studies. The approach used for the meta-analysis of the results in the review is described below. No medical devices are included in this review.

Meta-Analysis

Synthesis of medical test data typically focuses on measurements of test performance, that is, its sensitivity (identifying true positives); specificity(identifying true negatives); positive predictive value (probability that an individual actually has the disease if the test is positive); negative predictive value (probability that an individual does not have the disease if the test is

negative); positive likelihood ratio (ratio of people with disease who have a positive test to those without disease who have a positive test), and negative likelihood ratio (ratio of people without disease who have a negative test to those with disease who have a negative test). The Agency for Healthcare Research and Quality (AHRQ) Methods Guide for Medical Test Reviews (MGMTR)[17] recommends summarizing sensitivity and specificity across studies and then calculating the other test performance measures using the summary sensitivity and specificity.

Sensitivity and specificity can be summarized as a summary point when the estimates across do not differ widely. In cases where the estimates vary widely or when the test threshold varies by study, the summary may be most usefully represented by a line that describes how the average sensitivity varies with average specificity. In both cases, the MGMTR recommends using a multivariate meta-analytic approach to obtain summary estimates of sensitivity and specificity. This is because although sensitivity and specificity of a test are independent within a study, they are not independent quantities across studies; they are usually negatively correlated especially when different thresholds for positivity are used in different studies. This suggests that aggregating these values across studies without allowing for correlation across studies is likely to produce biased summary estimates.

The MGMTR recommends two families of hierarchical models: the bivariate model and the "hierarchical summary receiver operating characteristic" (HSROC) model. In the absence of covariates, these families are mathematically equivalent. The covariates in these models would represent variables that contribute to the heterogeneity of the estimates (e.g., differences in patient selection, methods of verification and interpretation of results, clinical setting and disease severity).

The traditional definitions of sensitivity and specificity used in the context of diagnostic medical tests do not quite fit the use of the genetic tests used to detect the TOO in CUP cases. The test is designed to include or exclude a tissue as the TOO. The test result is the probability that the origin of a tumor is from a particular site, so the call either includes or excludes a site of origin. The sum total of probability calls from this test adds up to 1. In a typical study, sensitivity and specificity are independent, because sensitivity is measured in cases and specificity is measured in controls. In TOO tests, sensitivity and specificity are not independent, because the probability of identifying the TOO is not independent of excluding another tissue on the test's panel of tissues. The most appropriate test performance measure in this case is the proportion of tissue identified accurately with confidence bands around this measure of accuracy. The studies in the review used the terms *sensitivity* and *accuracy* interchangeably. Although a few studies report specificity, it is used as a measure of the accuracy of exclusion of a tissue and is correlated to the accuracy of inclusion. The issue of different thresholds across various studies is not applicable in this case. A tissue is included, excluded, or indeterminate.

In the meta-analysis presented here, we therefore chose to represent a single summary accuracy measure and its 95% CI across the various studies. We have done this only for clinical accuracy. Nine studies are summarized to obtain a summary measure of test accuracy in classifying tumors of known tissue for PathworkDx; the corresponding numbers for miRview mets and CancerTypeID are four and six, respectively. PathworkDx TOO and CancerTypeID had multiple studies from which the data might have been summarized with a meta-analysis with respect to the accuracy of diagnosis in CUP cases. However, the diagnosis used as a gold standard was inconsistent and incomparable among the studies. We therefore decided against creating a summary estimate.

Key Questions and Population, Intervention, Comparator, Outcome, Timing, and Setting (PICOTS)

The KQs were revised during protocol development from those included in our original scope of work. The revisions aimed to (1) rephrase questions such that they can be answered by an evidence review, (2) incorporate assessment of the statistical algorithms described above, and (3) revise or remove questions not relevant in the context of genetic TOO tests. In conducting our review, we also identified some literature on genetic testing to diagnosis small round cell tumors. This literature and our findings are discussed in the brief report titled ***Genetic Testing in the Diagnosis of Ewing Sarcoma (Appendix G)***.

KQ 1. What genetic or molecular TOO tests are available for clinical use in the United States and what are their characteristics?

We searched the Internet and the peer-reviewed literature to identify genetic or molecular TOO tests. For each test, we reported the marketer of the test, whether it is marketed as a laboratory service (test performed only at the developing laboratory) or as a testing kit (test can be performed by hospital or other labs), and its FDA status and availability in the United States. We summarized its sample requirements, number and type of analytes, the types of tumors that can be identified by the test, and how many tumors of each type are included in the reference database for the test. Finally, we described how the results are reported and how the laboratory results are translated into reported results.

KQ 2. What is the evidence on the analytic validity of the TOO tests?

To answer this question, we considered the tissue sample acceptance/rejection criteria, the measurement accuracy for individual markers (mRNAs or microRNAs) used in the test, the accuracy and specificity for each marker at the assay conditions, the degree of interlaboratory agreement, the uniqueness of the panel markers used in the panel and their robustness to contamination, and whether any antibodies used are monoclonal and their robustness. We also considered the quality control steps used for the individual markers and for the overall assay; quality control measures included the proportion of the probes or antibodies that must return a valid result for the assay to be considered valid and the stability of the multimarker panels' precision and accuracy across time.

KQ 3. What is the evidence regarding the accuracy of genetic TOO tests in classifying the origin and type of CUP?

a. Does it differ by tumor origin?

b. Does it differ by patient age, sex, race, or ethnicity?

We based our answer to this question on how closely the experimental process used to develop the statistical classification models adhered to the guidelines published by Simon et al.[18] and on the accuracy and specificity of the genetic TOO test when compared with a gold standard of cancers of known primary sites, such as IHC staining, imaging studies, or other methods in current clinical use.

KQ 4. What is the evidence that genetic TOO tests change treatment decisions and improve clinical outcomes?

We considered clinical trials and epidemiology studies that compare treatment decisions and health outcomes when genetic TOO tests are used instead of or in addition to other methods of identifying the primary site of the tumor.

KQ 5. Is the evidence regarding genetic TOO tests relevant to the Medicare population?

We compared the characteristics of participants in the studies of genetic TOO tests to the core Medicare population (i.e., individuals 65 years and older) in terms of patient age, race, and primary diagnosis. We also considered whether studies of TOO tests include cancers that occur in the core Medicare population.

PICOTS

Population for Key Question 1 through Key Question 4

Patients of any age whose cancer is first diagnosed from a metastatic tumor for which the primary site cannot be found and the TOO is unknown.

Population for Key Question 5

Patients 65 and older whose cancer is first diagnosed from a metastatic tumor for which the primary site cannot be found and the TOO is unknown.

Interventions

The use of genetic or molecular tests for identifying the tumor's TOO in addition to or instead of other methods such as IHC staining or PET imaging.

Comparators for Key Question 3

The comparison standard used in the included studies, such as the ability of tests to correctly classify cancers of known origin or the determination of TOO by IHC staining, PET imaging, or other methods.

Comparators for Key Question 5

Treatment or health outcome for patients that did not have genetic or molecular TOO testing or that had a different test.

Outcomes, Intermediate

- Treatment or management decisions

Outcomes, Health

- Response to treatment (remission or tumor shrinkage)
- Recurrence
- Length of survival
- Mortality
- Quality of life

Timing

- Followup of any length after test results received

Setting

- Includes studies conducted in the United States or internationally
- Includes testing on patients admitted to the hospital or treated as outpatients

Methods

Literature Search Strategies

The scope of the review was limited to tests that are commercially available in the United States. We identified genetic or molecular tissue-of-origin (TOO) tests by searching GeneTests.org, the NIH Genetic Testing Registry, the GAPP Knowledge Base, and the following the Food and Drug Administration databases; Premarket Notifications (510(k)), Premarket Approvals, and Clinical Laboratory Improvement Amendments databases. We also searched the Internet using the search strategy shown in Table 3. We defined a "commercially available" test as one for which an Internet search or test directory identified a mechanism for a physician or laboratory to order the test or to buy a kit to perform the test.

Table 3. Google search strategy for TOO tests

Search	Queries
#1	"tissue of origin" OR "cancer of unknown" OR "tumors of unknown" laboratory test
#2	Limited to pages in English, updated in last year

We included systematic reviews, randomized controlled trials, nonrandomized controlled trials, prospective and retrospective cohort studies, case-control studies, and case series published from 1990 to present. We excluded non-English studies; a preliminary search found very few studies published in other languages. The inclusion and exclusion are listed in Appendix A. We searched the grey literature for relevant studies but did not contact authors for additional data. We included conference presentations and posters when they presented data not published elsewhere.

We conducted targeted searches for unpublished or grey literature relevant to the review. We identified grey literature relevant to the key questions (KQs) through review of Lexus Nexus, the test developers' Web sites, ClinicalTrials.gov, Health Services Research Projects in Progress, and the European Union Clinical Trials Register. We included studies that met all of the inclusion criteria and that contained enough information on the study methods to assess the risk of bias.

We systematically searched, reviewed, and analyzed the scientific evidence for each KQ. To identify articles for this review, we conducted focused searches of PubMed, Embase, and the Cochrane Library. The search was conducted in three stages. First, an experienced research librarian used a predefined list of search terms and medical subject headings (MeSH). The search terms and limits for PubMed are listed in Table 4, MESH Heading Search. We also reviewed the test manufacturers' Web sites and the reference lists of identified papers and reviews for previously unidentified relevant papers. Following the review of the manufacturer's Web sites, we conducted a second search of the databases using text words, shown in Table 4, Text Word Search. We limited the search to studies published in English based on limited resources; this may bias the report to include more studies from English-speaking countries. Complete search strategies are provided in Appendix B.

Table 4. Illustrative search strategies (PubMed)

Search	Queries	Number of Citations
MeSH Heading Search		
#1	Search Neoplasms, Unknown Primary[mh]	2,398
#2	Search ("Gene Expression Profiling"[MeSH]) OR "Microarray Analysis/methods"[Majr]	64,521
#3	Search #1 AND #2	38
#4	Search Pathwork diagnostics OR Agendia OR CancerTypeID OR miRview mets test OR Rosetta Genomics OR AviaraDX OR Quest Diagnostics	18,686
#5	Search #1 AND #4	6
#6	Search #3 OR #5	39
#7	Search #3 OR #5 Limits: Humans, English, Publication Date from 2000	35
#8	Search Neoplasms/genetics[mh]	229,992
#9	Search Neoplasms/classification[Majr] OR Neoplasms/diagnosis[Majr]	607,681
#10	Search #8 AND #9	39,811
#11	Search ("Reproducibility of Results"[Mesh]) OR "Accuracy and Specificity"[Mesh]	478,953
#12	Search #10 AND #11	2,966
#13	Search Oligonucleotide Array Sequence Analysis/methods[Majr]	8,345
#14	Search #12 AND #13	102
#15	Search #12 AND #13 Limits: Humans, English, Publication Date from 2000	96
#16	Search #10 AND #4 Limits: Humans, English, Publication Date from 2000	72
#17	Search #3 OR #15 OR #16 Limits: Humans, English, Publication Date from 2000	195
Text Word Search		
#1	Search "tissue of origin" and "cancer"	188
#2	Search neoplasms, unknown primary	6,765
#3	Search neoplasms, unknown primary/genetics	82
#4	Search #1 OR #3	259
#5	Search #1 OR #3 limits: humans, English	219
#6	Search #1 OR #3 limits: humans, English, publication date from 2000	157

We updated the literature review by repeating the initial searches and reviewing the publication list on the manufacturers' Web sites concurrent with the peer review process. Any literature suggested by peer reviewers or public comment respondents was investigated and, if appropriate, incorporated into the final review. The review includes all identified literature published or e-published between January 1, 2000, and November 7, 2012.

Study Eligibility Criteria

Two trained members of the research team independently reviewed all identified titles and abstracts for eligibility against our inclusion/exclusion criteria. Studies marked for possible inclusion by either reviewer underwent full-text review. For studies without adequate information to determine inclusion or exclusion, we retrieved the full text and then made the determination. Each article included in the full-text review was independently reviewed by two investigators. If both reviewers agreed that a study did not meet the eligibility criteria, the study was excluded. Conflicts were resolved by discussion and consensus. We recorded the reason each excluded full-text publication did not satisfy the eligibility criteria studies.

Data Management

EndNote was used to organize and track retrieved citations.

Data Abstraction

For each included study, data were abstracted into a standard abstraction table by an abstractor trained in genetics and epidemiology or biostatistics. A senior investigator reviewed each abstraction. The data abstraction form gathered information on the study populations, settings, interventions, comparators, study designs, methods, and results.

Quality Assessment

We assessed the risk of bias in the reviewed studies using the criteria described in the Methods Guide for Medical Test Reviews.[17] For studies of analytic or clinical validity (KQs 2 and 3), we considered the potential for bias due to flaws in the sample selection, testing protocol, reference standards, verification procedures, interpretation, and analysis. We used the QUADAS[19] criteria to assess studies of diagnostic accuracy. For studies of clinical utility (KQ 4), we used questions from the RTI Question Bank[20] to assess the potential for bias from sample selection, study performance, attrition, detection of outcomes, and reporting. Studies that included information on both analytic and clinical validity and on clinical utility received separate ratings for each domain, each based on the relevant assessment instrument.

Quality assessment results were summarized as good, fair, or poor, correlating with a low, medium, or high risk of bias. The ratings are defined in Appendix D.

A study was rated as good if it had a strong design, measured outcomes appropriately, used appropriate statistical and analytical methods, reported low attrition, and reported methods and outcomes clearly and precisely. As a result, the reviewers have a high degree of confidence that the reported results reflect minimal bias and that the reported effect or correlation is similar in direction and magnitude to the actual relationship. For studies to have been rated good, the source and selection criteria of the tumors or participants in the study had to be clearly explained with no obvious source of bias and the study had to include an appropriate comparison group. If the tumor origin was known, the interpretation of the TOO had to be made without knowledge of the tumor origin. The reported results had to account for all tumors or participants included in the study. A fair study does not meet all criteria of a good study, but its flaws are not likely to cause major bias in the results. The reviewers had a high degree of confidence that the reported relationship is in the same direction as the actual relationship but only moderate confidence that the reported relationship is of the reported magnitude.

Poor studies have at least one flaw in the study's design, conduct, or analysis that could invalidate the results. We identified one study[21] that was rated poor. Two senior investigators trained in epidemiology and statistics independently assessed the quality of each study. Disagreements between the two reviewers were resolved by discussion until consensus was reached.

Data Synthesis

We summarized the evidence for each KQ in evidence tables.

Meta-Analysis

Meta-analytic techniques were used to generate summary measures across studies when there were more than two studies using the same test that addressed the same KQ. For each test, three or more studies addressed KQ 3b, the ability of the tests to identify tests of known origin. Meta-analysis was used to estimate summary measures across these studies.

The CancerTypeID and PathworkDx TOO tests also had more than three studies that assessed the ability of the tests to detect the TOO in true CUP cases. Because the criteria for assessing the accuracy of the test varied widely across these studies and several of these criteria seemed inappropriate, we decided not to summarize the accuracy measures across these studies. The miRview mets test only had two studies that estimated the accuracy of the test in CUP patients.

As discussed above, for the tests described in this review the only appropriate summary measure is a single measure of accuracy. To determine whether summarizing the measure across the studies was appropriate, we assessed heterogeneity of accuracy estimates across studies by using forest plots to display the reported accuracy (correct identification of the TOO) for TOOs. Given the homogeneity of estimates across the studies, it was determined that the most appropriate way to combine the estimate across studies was to use a fixed effects model to estimate the population parameter that the estimates from the various studies represent.

Test performance is measured in terms of accuracy. In the studies of known tissue, accuracy is defined as the proportion of test results that agree with the known TOO. We used a univariate fixed-effects model for meta-analysis. The model in the *i*th study is

(1)

where β_{fixed} is the average effect under fixed-effects model and e_i is the error term. Variance of e_i is v_i, which was estimated from the data. We used the "metaSEM" package available in "R" statistical language (R Development Core Team 2011) to perform meta-analysis using fixed-effects model.

Grading the Evidence for Each Key Question

For KQs 2, 3b, and 4, we graded the overall strength of evidence according to the guidance established for the Evidence-based Practice Center Program.[17, 22] This approach incorporates four key domains: risk of bias (including study design and aggregate quality), consistency, directness, and precision of the evidence. Grades reflect the strength of the body of evidence to answer the KQs on the validity and efficacy of the interventions in this review. We used Simon[18] criteria to assess the validity of the development of statistical classification algorithms. We assessed four criteria: the validity of the normalization methods; the validity of the statistical classification method, in particular; whether the assessment was based on supervised or unsupervised classification; and the risk of bias in the validation methods. We rated the first three criteria as valid or invalid as follows: valid normalization method: normalized based on housekeeping genes or total expression levels; validity of the statistical methods: supervised classification. We graded the overall strength of evidence according to the guidance established for the Evidence-based Practice Center Program. Two senior reviewers assessed each domain and the overall grade for each key outcome listed in the framework. Conflicts were resolved by discussing until consensus was reached. For KQs 2 and 3, strength of evidence was graded for each test. The risk of bias was rated as low, moderate, or high. It was graded as low if the validation was conducted using a completely separate validation sample or using leave-one-out validation analysis. For KQ 4, the strength of evidence was graded for each outcome (e.g., treatment change, clinical outcomes).

Assessing Applicability

KQ 5 evaluates the applicability of the genetic TOO tests to the core Medicare populations (i.e., individuals 65 years and older) in terms of patient age, race, and primary diagnosis. We assessed KQ 5 by considering the characteristics of the sample for the studies and compared them with the core Medicare population.

Results

Study Identification and Characteristics

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagrams summarize the results of the literature searches. Electronic databases are shown in Figure 2, and hand searches (comprised of Web searches and articles, abstracts, or posters suggested by peer or public reviewers) are shown in Figure 3. The four database searches yielded a total of 632 records for title and abstract review. We conducted full-text review of 123 articles from the database searches and an additional 27 articles, posters, and abstracts from manufacturers' Web sites and other sources. A total of 49 articles, posters, or conference abstracts provided evidence for the key questions (KQs) and are included in the review. Appendix C lists the studies and their characteristics. Appendix D lists the quality rating for each study. Appendix E lists studies excluded from the review.

Figure 2. PRISMA for electronic databases

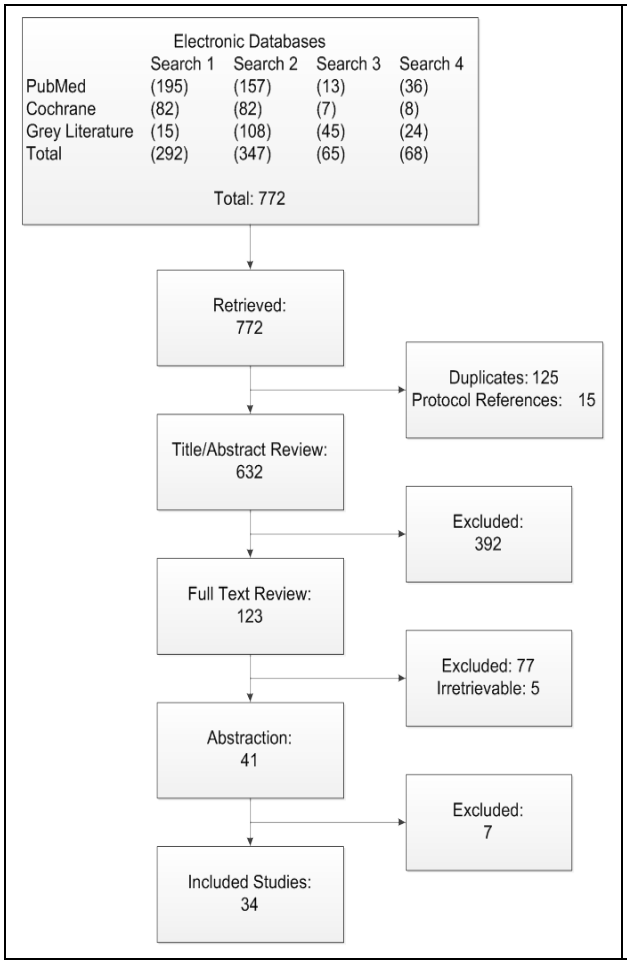
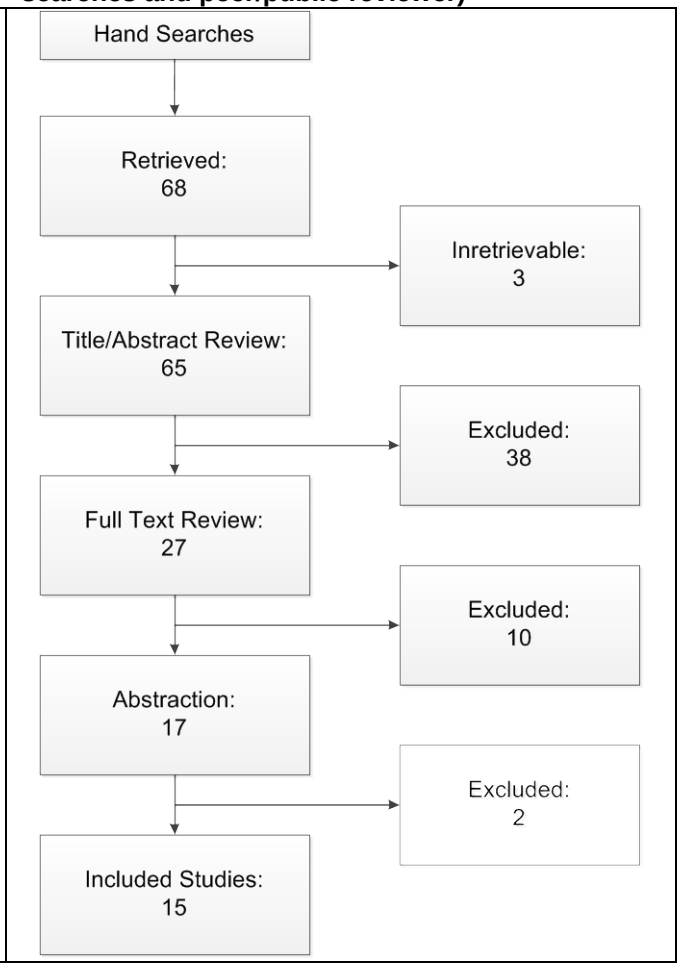


Figure 3. PRISMA for hand searches (Web searches and peer/public reviewer)



Key Question 1. What genetic or molecular tissue of origin (TOO) tests are available for clinical use in the United States and what are their characteristics?

We examined commercially available genomic tests that are used to identify the TOO of cancers of unknown primary (CUP) site.

TOO Tests for CUP

We identified four genetic or molecular tests to determine TOO in CUP cases, one cytogenetic analysis and three genomic assays: PathworkDx from Pathwork Diagnostics, CancerTypeID from Biotheranostics, and miRview mets (original test) and mets^2 (current test) from Rosetta Genomics (previously marketed in the United States as ProOnc Tumor Source) (Table 5). We also included literature on the Pathwork Tissue of Origin Endometrial Test, used in conjunction with the PathworkDx TOO test to differentiate between tumors of endometrial or ovarian origin. We excluded two TOO tests, CupPrint and Veridex, from the review because they are not available for clinical use in the United States. The CupPrint assay by Agendia was previously available in Europe but is no longer available, and it was never available in the United States. The CUP assay by Veridex was never released for clinical use. We did not include the Caris "Target Now!" because the test identifies likely effective treatment regimens based on biomarkers, not the tumor site of origin.

All TOO tests are currently conducted as a laboratory service: the sample is sent to the test developer, who does the testing and returns a result. The manufacturer of PathworkDx states that kits will be available in the future for labs who wish to do the gene expression analysis in-house and send the data to Pathwork for analysis. Historically, the Food and Drug Administration (FDA), using its enforcement discretion, has regulated very few laboratory-developed tests. Instead, it has focused on regulating tests as medical devices (i.e., a kit or chip for conducting the test). Although FDA is considering modifying the policy,[23] it does not currently require companies to apply for FDA approval prior to marketing laboratory-developed tests. PathworkDx requested and received FDA clearance. CancerTypeID, miRview mets, and miRview mets^2 have not been submitted for FDA review. Although PathworkDx received FDA clearance, the clearance includes limitations on the claims that can be made about the test. These limitations include that the TOO test is not intended: to establish the origin of tumors that cannot be diagnosed according to current clinical and pathological practice; to subclassify or modify the classification of such tumors; to predict disease course, survival, or treatment efficacy; to distinguish primary from metastatic tumors; or to distinguish tumor types in the database from tumor types not in the database.

Analytic and statistical analyses vary between these tests. The PathworkDx and CancerTypeID panels are genes; the assays measure the expression of these genes, the amount of messenger RNA (mRNA). miRview uses microRNAs (miRNAs), which are small, noncoding, single-stranded RNA molecules that regulate genes posttranscription.

PathworkDx uses microarray analysis to measure the expression levels of two large gene panels: a 1,550-gene panel for frozen specimens and a 2,000-gene panel for formalin-fixed paraffin-embedded (FFPE) specimens. The statistical analysis uses pairwise comparisons based on a machine learning algorithm to determine the similarity scores for 15 tumor types. The identified TOO is the tissue type with the highest score above 20.

Table 5. Available genetic or molecular TOO tests for identifying CUPs site and their characteristics

Name of Test	Manufact-urer	How Marketed?	FDA Approval	Sample Requirements	Laboratory Analysis Method	Analyte	Panel Size	Number of Tumor Sites Identified	Number of Tumors in Reference Database (Range by Tumor Type)	Reported Results	Statistical Analysis Method
Cancer-TypeID	Biotheranos-tics	Service	Submitted	FFPE tissue sections or unstained 10 micron sections on glass slides. At least 300–500 viable tumor cells	Quantitative RT-PCR	mRNA	92	28	2,206 (26–228)[24]	Probability for each cancer type	KNN
Cytogenetic analysis	Multiple cytogenetic laboratories	Service	NA	Fresh tissue	High resolution banded chromosomes		NA	NA	NA	Karyotype	NA
miRview mets	Rosetta Genomics	Service	Not submitted	Unstained slides, sections in tubes, or FFPE tissue. 2.5 mm^2 tissue	Microarray platform	miRNA	mets: 48 mets[2]: 64	mets: 22 mets[2]: 24	mets: 336 (1–49) mets[2]: 1,282 (NR)	Tumor origin. May list multiple possibili-ties	Binary decision tree and KNN classifier
PathworkDx TOO Test	Pathwork Diagnostics http://www.pathworkdx.com/	Service	Cleared	Frozen specimens FFPE or unstained slides. 1 mm^2 tumor tissue. 30 ng total RNA	Gene expression microarray analysis using cDNA	mRNA	Frozen: 1,550 genes; FFPE: 2,000 genes	15	2,039 (41–444)	Similarity scores	Pairwise comparisons by machine learning algorithm
PathworkDx Endometrial Test	Pathwork Diagnostics http://www.pathworkdx.com/	Service									

Abbreviations: cDNA = complementary deoxyribonucleic acid; FFPA = formalin-fixed paraffin-embedded; GIST = gastrointestinal stromal tumor; KNN = Kohonen neural network; miRNA = micro ribonucleic acid; mRNA = messenger ribonucleic acid; NA = not applicable; RNA = ribonucleic acid; RT-PCR = reverse-transcriptase polymerase chain reaction; TOO = tissue of origin.

CancerTypeID analyzes the expression levels of 92 genes using reverse-transcriptase polymerase chain reaction (RT-PCR). The classification algorithm uses the K-nearest neighbor statistical methodology to determine the probability that a tumor is one of 28 tumor types. The tissue with the highest probability is reported as the most likely TOO with the estimated probability.

The miRview mets used an RT-PCR platform to analyze the expression levels of 48 miRNAs to identify any of 22 tumor sites. The most recent version of the test, miRview mets[2], used 64 miRNAs to identify 24 tumor types. Both miRview tests use two classification methodologies, a decision tree and a K-nearest neighbor algorithm. If both methodologies give the same result, this result is reported as the TOO. If the two methodologies give different results, both possible TOOs are reported, with the most likely one indicated as the TOO and the other as a less likely TOO.

The type of tumors covered by the three tests varied. All three tests claim to identify cancer of the bladder, breast, kidney, lung, melanoma, ovary, pancreas, prostate, sarcoma, testis, or thyroid (Table 6). Four primary sites identified by all three tests (lung, ovary, pancreas, and prostate) account for 51 percent of primary sites identified at autopsy in series published from 1980 to 2000.[25]

Table 6. Primary tumor sites identified by molecular TOO tests

Primary Site	PathworkDx[26]	CancerTypeID[26]	miRview mets[21]
Adrenal		•	•
Bladder	•	•	•
Brain		•	•
Breast	•	•	•
Cervix		•	
Cholangiocarcinoma		•	•
Colorectal	•	•	•
Endometrium	•	•	
Esophagus		•	
Gallbladder		•	
Gastric	•	•	•
GIST		•	•
Head and neck		•	•
Hepatocellular	•	•	•
Intestine		•	•
Kidney	•	•	•
Lung	•	•	•
Lymph node			•
Melanoma	•	•	•
Meningioma		•	
Mesothelioma		•	•
Neuroendocrine		•	
Non-Hodgkin's lymphoma	•		•
Ovary	•	•	•
Pancreas	•	•	•
Prostate	•	•	•
Sarcoma	•	•	•
Testicle	•	•	•
Thymus		•	•
Thyroid	•	•	•

Abbreviations: GIST = gastrointestinal stromal tumor; TOO = tissue of origin.

Cytogenetic analysis of tumor cells by G-banded karyotype may also provide information on the TOO in tumors of unknown primary site.[14] While cytogenetic abnormalities are common in tumors of all types, certain abnormalities are pathonomic of specific types of cancer.[27] These abnormalities, when found, can be used to diagnose the TOO of metastatic tumors.[14]

Key Question 2. What is the evidence on the analytic validity of the TOO tests?

Ten studies,[24, 28-36] all rated good, provided evidence to answer this KQ. Some data on analytic performance were available for all of the genomic TOO tests. Analytic validity cannot be compared across tests because different data were reported for each test. Results are displayed in Table 7.

CancerTypeID

One study, rated good, provided information on the analytic validity of the CancerTypeID test.[24] Interassay reproducibility was high. Across 194 independent runs with four operators, the mean percentage coefficient of variation (CV) in observed C_t for the 92 assay genes compared with the positive control was 1.69 percent. Compared with the negative controls, the mean CV was 1.25 percent. For the 5 normalization genes, the mean CV was 2.19 percent for the positive controls and 1.66 percent for the negative controls.

The variation in the assay across different tumors of the same type was also assessed. Across six tumor types (breast, adrenal, intestine, kidney, thyroid, and prostate), the mean CV for the 92 assay genes was 33 percent; and the mean CV for the 5 normalization genes was 3.16 percent. Each assay includes one sample of known origin. In 32 assays that included three tumor types, the mean CV for the 92 assay genes was 1.58 percent (range 1.41% to 1.69%), and for the 5 normalization genes, it was 1.04 percent (range 0.85% to 1.79%). The assays were 100 percent concordant for the tumor of origin prediction for these samples.

miRview mets and mets^2

Four papers,[32, 34-36] all rated good, provided information on the analytic validity of the miRview mets and mets^2 tests. During development of the mets assay, the performance of the microarray platform was validated against RT-PCR analysis.[34] The expression distributions and diagnostic roles of the miRNAs were maintained across the platforms. The developers of the test also confirmed that RNA quality and quantity was similar for fresh-frozen, formalin-fixed, and FFPE samples. miRNA profiles were stable in FFPE for up to 11 years.[34]

Assay quality control measures include a sample with no RNA as a negative control and a well-characterized RNA sample as a positive control. The positive control must meet defined C_t ranges. The quality of each well is assessed using the fluorescence amplification curve with thresholds on the linear slope of the curve as a function of the measured C_t and maximum fluorescence. The quality of the assay of each sample is assessed on the number and identity of the expressed miRNAs ($C_t < 38$) and the average C_t of the measured miRNAs.[35, 36] For the second generation assay, the mets^2 test, the correlation coefficient of multiple assays of the same sample was 0.99. A total of 179 samples were independently tested at both the Rosetta Genomics research and development laboratory and the Clinical Laboratory Improvement Amendments-approved clinical laboratory. Of the 174 samples that passed quality control criteria at both laboratories, 160 samples had an interlaboratory concordance of miRNA expression levels above 95 percent. The laboratories agreed on a diagnosis in 175 of the 179 cases.[32]

Table 7. Evidence of the analytic validity of the TOO tests

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Characteristics	Cancer Types	Validity of Marker Measurement	QC Measures for Markers	Assay Accuracy and Precision
CancerTypeID Erlander, 2011[24] NR Multinational, U.S. Good	Age: Total: 300 Mean (SD): 62 (13) Female: 53% N: Training dataset: N=2,206; Independent sample set: N=187; Clinical cases: N=300	Adrenal, brain, breast, cervix, cholangiocarcinoma, endometrium, esophagus (squamous cell), gallbladder, gastroesophageal (adenocarcinoma), germ cell, GIST, head/neck, intestine, kidney (renal cell carcinoma), liver, lung, lymphoma, melanoma, meningioma, mesothelioma, neuroendocrine, ovary, pancreas, prostate, sarcoma, sex cord stromal tumor, skin, thymus, thyroid, urinary/bladder	Range of accuracy: NR Range of specificity: NR Number of outliers: NR Cross-reaction with normal tissue: NR	Assay reproducibility (expressed as mean percentage coefficient of variation): Ct values using positive controls (194 independent runs, 4 operators): 92 assay genes: 1.69%; 5 normalization genes: 2.19% Ct values using negative controls (194 independent runs, 4 operators): 92 assay genes:1.25%; 5 normalization genes:1.66% Assays of known tumor types (32 assays, 3 tumor types, 4 scientists) : Mean percentage CVs: 1.58% (range 1.41%–1.69%) for the 92 genes and 1.04% (range 0.85%–1.79%) for the 5 normalization genes 100% concordance for tumor of origin prediction Across tumor type (6 tumor types, 3 setups, 2 operators): 92 assay genes: 3.33%; 5 normalization genes: 3.16%	Required percentage of valid markers: NR QC standards for assay: NR Changes in panel precision and accuracy over time: NR

Table 7. Evidence of the analytic validity of the TOO tests (continued)

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Characteristics	Cancer Types	Validity of Marker Measurement	QC Measures for Markers	Assay Accuracy and Precision
miRview mets[2] Meiri, 2012[32] 1990–2010 NR Good	Age: NR Female: NR N: 509 Interlaboratory correlation: 179	Adrenal, anus, biliary tract, bladder, brain, breast, cervix, colon/rectum, gastrointestinal, liver, lung, head and neck, esophagus, lymphoma, mesothelioma, ovary, pancreas, prostate, sarcoma, skin, testis, thymus, thyroid	Range of accuracy: NR Range of specificity: NR Number of outliers: NR Cross-reaction with normal tissue: NR	Positive control QA measures: Number of miRNAs with expression > 300 (dynamic range); 98th percentile expression level of the miRNA Pearson correlation between sample and reference hybridization spikes Number of miRNAs with consistent triplicate signals Mean correlations coefficient of multiple assays of same sample: 0.99 Sensitivity: signal at 0.1 fmol with linear dynamic range of 10^3 Specificity to nucleotide mismatches: 10–100 fold for 1–4 mismatches	Interlaboratory correlation: >0.95 in 89% of samples. Interlaboratory agreement on diagnosis: 175/179 (98%)
miRview mets Rosenfeld, 2008[34] NR Multinational, not U.S. Good	Age: NR Female: NR N: 80	Bladder, brain, breast, colon, endometrium, head and neck, kidney, liver, lung, lung pleura, lymph node, melanocytes, meninges, ovary, pancreas, prostate, sarcoma, stomach, GIST, testis, thymus, thyroid	Range of accuracy: NR Range of specificity: NR Number of outliers: NR Cross-reaction with normal tissue: NR	NR	Required percentage of valid markers: NR QC standards for assay: Array platform validated by RT-PCR. miRNA maintained expression distributions and diagnostic roles Changes in panel precision and accuracy over time: NR

Table 7. Evidence of the analytic validity of the TOO tests (continued)

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Characteristics	Cancer Types	Validity of Marker Measurement	QC Measures for Markers	Assay Accuracy and Precision
miRview mets Rosenwald 2008[35] NR Multinational, U.S. Good	Age: NR Female: NR N: 853	Biliary tract, brain, breast, colon, esophagus, head and neck, kidney, liver, lung, melanoma, ovary, pancreas, prostate, stomach, testis, thymus, thyroid	Range of accuracy: NR Range of specificity: NR Number of outliers: NR Cross-reaction with normal tissue: NR	Number and identity of microRNAs (C<38) and average Ct of measured microRNAs. microRNA results consistent across 3 platforms (spotted arrays, custom commercial arrays, and qRT-PCR). Between-lab correlation of qRT-PCR signals per sample=0.98. Labs disagreed on 3 samples; 8 agreed on one answer; 61 matched perfectly. N=72	Negative control: no RNA sample. Positive control: specific RNA sample that should meet defined range in assay QA based on fluorescence amplification
miRview mets Varadhachary, 2011[36] 2010 U.S. only Good	Age: Range: 20–83 Median: 58 Female: 66 N: 104	Lymph nodes, liver, lung, bone, pelvic mass/adnexae, skin/subcutaneous, omentum/peritoneum, adrenal, other	Range of accuracy: NR Range of specificity: NR Number of outliers: NR Cross-reaction with normal tissue: NR	NR	Required percentage of valid markers: 87/104 samples passed tumor content criteria; 74/87 passed all QA criteria QC standards for assay: Controls: No sample; no RNA; external positives. Quality parameters for RNA amplification Changes in panel precision and accuracy over time: NR

Table 7. Evidence of the analytic validity of the TOO tests (continued)

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Characteristics	Cancer Types	Validity of Marker Measurement	QC Measures for Markers	Assay Accuracy and Precision
PathworkDx Dumur, 2008[30] NR U.S. only Good	Age: NR Female: NR N: 60	Breast, colorectal, nonsmall-cell lung, non-Hodgkin's lymphoma, lymphoma, pancreas, bladder, gastric, germ cell, hepatocellular, kidney, melanoma, ovarian, prostrate, soft tissue, sarcoma, thyroid	Range of accuracy: Pre-standardization 1-to-1 lab correlation, Pearson correlation coefficients 0.65–0.82; Post standardization 1-to-1 lab correlation: 0.81–0.87 Coefficient of reproducibility: 32.48 +/− 3.97 Range of specificity: NR Number of outliers: 19/227 Cross-reaction with normal tissue: NR	All samples with adequate RNA quantity and quality produced sufficient cRNA for hybridization 31/227 samples required >1 labeling reaction Data verification algorithm addresses RNA quality, inadequate amplification, insufficient quantity of labeled RNA, inadequate hybridization time or temperature 218/227 gene expression data files passed verification All 9 failed files showed evidence of RNA degradation	Required percentage of valid markers: NR QC standards for assay: No evidence of bias; similarity score interlaboratory correlation: 0.95; Concordance of physician guided conclusion: 89.4% (range, 87.0–92.5); Kappa>0.86 Changes in panel precision and accuracy over time: NR

Table 7. Evidence of the analytic validity of the TOO tests (continued)

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Characteristics	Cancer Types	Validity of Marker Measurement	QC Measures for Markers	Assay Accuracy and Precision
PathworkDx Dumur, 2011[29] NR U.S. only Good	Age: NR Female: NR N: 43 total	Mixed (7 CUP, 6 known off panel, 30 known on panel)	Range of accuracy: NR Range of specificity: NR Number of outliers: NR Cross-reaction with normal tissue: NR	NR	Required percentage of valid markers: ≥40 Percentage of present probes: Mean: 60.6 Range: 49.9–69.3% QC standards for assay: 3´/5´ ratio for glyceraldehyde-3-phosphate dehydrogenase ≤3.0[a] Mean 3´/5´ GAPDH ratio: 1.2 Range: 0.9–3.0 PathworkDx TOO test report: “Acceptable” data quality

Table 7. Evidence of the analytic validity of the TOO tests (continued)

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Characteristics	Cancer Types	Validity of Marker Measurement	QC Measures for Markers	Assay Accuracy and Precision
PathworkDx Pillai, 2011[33] NR U.S. only Good	Age: 10–20: 1 20–30: 19 30–40: 44 40–50: 79 50–60: 133 60–70: 104 70–80: 63 ≥80: 14 Female: 257 Ethnicity: African American:3 Asian/Pacific-Islander: 68 European: 314 N: 462	Bladder, breast, colorectal, gastric, testicular germ cell, kidney, hepatocellular, nonsmall cell lung cancer, non-Hodgkin's lymphoma, melanoma, ovarian, pancreatic, prostate, thyroid, and sarcoma	Range of accuracy: overall interlaboratory concordance: 133/149 Range of specificity: NR Number of outliers: NR Cross-reaction with normal tissue: NR	NR	Required percentage of valid markers: Percentage present ≥5 Overall signal (mean summarized expression value of all probes) ≥10, Regional discontinuity (correlation between intensity of probe and mean of two adjacent probes) ≤0.84 QC standards for assay: between-laboratory reproducibility of results: Overall concordance between SS scores: 89.3; Correlation coefficients for SS scores: 0.92–0.93; Slopes: 0.93–0.96; Kappa analysis of intersite agreement: 0.85–0.92; Bland-Altman analysis for systematic bias: <10% of specimens outside 95% limit of agreement; Overall signal ≥10 Changes in panel precision and accuracy over time: No change in test performance by age of specimen

Table 7. Evidence of the analytic validity of the TOO tests (continued)

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Characteristics	Cancer Types	Validity of Marker Measurement	QC Measures for Markers	Assay Accuracy and Precision
Pathwork Tissue of Origin Endometrial Test Lal, 2012[31] NR U.S. only Good	Age, n of subjects in age range 30–50: 9 50–60: 24 60–70: 28 70–90: 14 Female: NR N: 75	Ovarian, endometrial	NR	NR	Intrasite reproducibility=46/46 Concordance percentage: 100% (95% CI: 92.3–100) Intersite reproducibility=83/88 Concordance percentage: 94.3% (95% CI: 87.2–98.1) Median coefficient of variation (CV%) of the similarity score: 1.38 (range 0.32–8.79) Kappa (K) statistic: 1.0 for all three pairwise comparisons. Changes in panel precision and accuracy over time: Test performance 90.5% for specimens >2 years old

[a]Article states 3.0 or more, but usual standard is 3.0 or less.

Abbreviations: CI = confidence interval; cRNA = complementary RNA; Ct = cycle threshold; CV = coefficient of variation; n = number; N = number; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; GIST = gastrointestinal stromal tumor; NR = not reported; mRNA = micro ribonucleic acids; QA = quality assurance; QC = quality control; qRT-PCR = quantitative reverse transcriptase polymerase chain reaction; RNA = ribonucleic acid; RT-PCR = reverse transcriptase polymerase chain reaction; SD = standard deviation; SS = similarity score; TOO = tissue of origin; U.S. = United States.

PathworkDx

Three studies,[29, 30, 33] all rated as good, provided information on the analytic validity of the PathworkDx test. The PathworkDx data verification algorithm assesses assay quality, including quality of RNA, adequacy of RNA amplification, quantity of labeled RNA, and adequacy of hybridization time and temperature.[30] Assay data quality is assessed by three statistics: overall signal, percent present, and regional discontinuity. Overall signal is the mean summarized expression value of all probes; it must be ≥ 10. Percent present is the percentage of probes sets assigned a present call by the Affymetrix MAS 5.0 algorithm; it must be ≥ 5. Regional discontinuity is the correlation between a probe's intensity and the mean intensity of the two adjacent probes. Regional discontinuity must be ≤ 0.84. The age of the sample did not affect assay performance.[33] In their clinical validation study, Dumur et al.[29] assessed assay quality by the percent of present probes and the 3'/5' ratio of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The mean percentage of present probes was 60.6 (range: 49.9 to 69.3), and the mean 3'/5' GAPDH ratio was 1.2 (range: 0.9 to 3.0).

Two multisite studies[30, 33] assessed analytic validity and interlaboratory correlation. In the study by Dumur et al.,[30] 218 of 227 (96%) gene expression data files passed verification. All 9 failed files showed evidence of RNA degradation. Interlaboratory correlation was high; the Pearson correlation coefficients of between-laboratory Affymetrix normalized gene expression values were 0.65 to 0.82. After standardization with the PathworkDx TOO algorithm, the correlation coefficients ranged from 0.81 to 0.87. The calculated similarity scores (SS) were even more highly correlated; all comparisons had a Pearson correlation coefficient above 0.95. The overall between-laboratory concordance on the final TOO call was 89.4 percent (range: 87.0 to 92.5), and the kappa analysis indicated that agreement was very good ($\kappa > 0.86$). The Bland-Altman analysis found a high level of agreement (coefficient of reproducibility 32.48 ± 3.97) and indication of no systematic bias (<10% of specimens outside 95% limit of agreement).[30] Pillai et al.[33] also found strong correlation between laboratories. The overall concordance in SS between the three laboratories was 89.3 percent. The correlation coefficients for the SS ranged from 0.92 to 0.93, and the kappa statistic for interlaboratory agreement ranged from 0.85 to 0.92.

Cytogenetic Analysis

We found no papers that specifically examined the analytic validity of cytogenetic analysis in the context of tumors of unknown primary site.

Key Question 3a: What is the evidence on the accuracy of the TOO test in classifying the origin and type of the tumor? Did the statistical methods adhere to the guidelines published by Simon et al. (2003)?

Six studies graded as good provided evidence on this question. We were able to assess the validity of the statistical algorithm for CancerTypeID and miRview but not for PathworkDx TOO test. Figures 4 through 6 display the accuracy estimates with 95% confidence intervals (CIs) for the three tests. The results are displayed in Table 8.

Table 8. Evidence of accuracy of TOO test in classifying the origin and type of the tumor and statistical methods' adherence to Simon guidelines

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancer Types	Normalization Methodology	Dimension Reduction Methodology	Classification Rule Supervision	Internal and External Validation Methods
CancerTypeID Ma, 2006[37] NR U.S. only	Age: NR Female: NR N: 578	Adrenal, brain, breast, intestine, cervix, endometrium, gallbladder, ovary, gastrointestinal, kidney, leiomyosarcoma, liver, lung, lymphoma, meningioma, mesothelioma, osteosarcoma, pancreas, prostate, skin, small and large bowel, soft tissue, stomach, testis, thyroid, urinary/bladder	Total expression: Raw Cy5/Cy3 ratios normalized using nonlinear local regression, without background adjustment	Hypothesis tests: Logistic regression Ranking: NR Clustering : NR	Supervised: K-nearest neighbors (KNN)	Internal: Leave-one-out cross-validation to select genes; Multiclass weighted KNN classification algorithm to classify genes; Gene selection also done via genetic algorithm External: Validation study of 187 FFPE tumor samples
miRview mets Rosenfeld, 2008[34] NR Multinational, not U.S. Good	Age: NR Female: NR N: 253 learning set 83 validation set	Bladder, brain, breast, colon, endometrium, head and neck, kidney, liver, lung, lung pleura, lymph node, melanocytes, meninges, ovary, pancreas, prostate, sarcoma, stomach, GIST, testis, thymus, thyroid	Total expression: Based on median expression level for each probe across all samples	Hypothesis tests: NR Ranking: NR Clustering : NR	Supervised: Decision-tree KNN	Internal: Leave-one-out cross-validation within the training set External: Blinded test set with independent set of 83 samples

Table 8. Evidence of accuracy of TOO test in classifying the origin and type of the tumor and statistical methods' adherence to Simon guidelines (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancer Types	Normalization Methodology	Dimension Reduction Methodology	Classification Rule Supervision	Internal and External Validation Methods
miRview mets Rosenwald, 2010[35] NR Multinational, U.S. Good	Age: NR Female: NR N: 649 learning set 204 validation	Biliary tract, brain, breast, colon, esophagus, head and neck, kidney, liver, lung, melanoma, ovary, pancreas, prostate, stomach or esophagus, testis, thymus, thyroid	Total expression: Average expression of all microRNAs + scaling constant (the average expression over the entire sample set)	Hypothesis tests: NR Ranking: Decision tree algorithm selected 48 miRNAs through feature selection Clustering: NR	Supervised: Binary decision tree KNN	Internal: Leave-one-out cross validation within the training set External: Test performance assessed using independent set of 204 validation samples
PathworkDx Dumur, 2008[30] NR U.S. only Good	Age: NR Female: NR N: 60	Breast, colorectal, nonsmall-cell lung, non-Hodgkin's lymphoma, lymphoma, pancreas, bladder, gastric, germ cell, hepatocellular, kidney, melanoma, ovarian, prostrate, soft tissue, sarcoma, thyroid	Total expression: NR Selected housekeeping: 121 mRNA markers stably expressed across cell types	Hypothesis tests: NR Ranking: NR Clustering : NR	Unsupervised: NR Supervised: NR	Internal: NR External: Validated by Pillai et al. [33]
PathworkDx Pillai, 2011[33] NR U.S. only Good	Age: 10–20: 1 20–30: 19 30–40: 44 40–50: 79 50–60: 133 60–70: 104 70–80: 63 ≥80: 14 Female: 257 N: 462	Bladder, breast, colorectal, gastric, testicular germ cell, kidney, hepatocellular, nonsmall cell lung cancer, non-Hodgkin's lymphoma, melanoma, ovarian, pancreatic, prostate, thyroid, sarcoma	Total expression: NR Selected housekeeping: 121 mRNA markers stably expressed across cell types	Hypothesis tests: NR Ranking: NR Clustering : NR	Unsupervised: NR Supervised: NR	Internal: NR External: Multisite validation study

Table 8. Evidence of accuracy of TOO test in classifying the origin and type of the tumor and statistical methods' adherence to Simon guidelines (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancer Types	Normalization Methodology	Dimension Reduction Methodology	Classification Rule Supervision	Internal and External Validation Methods
PathworkDx Lal, 2012[31] NR Multisite Good	Age: NR Female: NR N: 849	Ovarian and endometrial	Total expression: NR Selected housekeeping: 59 genes	Hypothesis tests: NR Ranking: NR Clustering : NR	Unsupervised: Machine learning	Internal: NR External: Validated with blinded test set of 75 tumors

Abbreviations: FFPE = formula fixed paraffin embedded; GIST = gastrointestinal stromal tumor; KNN = K-nearest neighbor; mRNA = microRNA; N = number; NR = not reported; TOO = tissue of origin; U.S. = United States.

CancerTypeID

Normalization

Five of the 92 genes in the assay are reference genes that are used to normalize expression levels of the 87 genes used to classify tissues into the 39 tumor types. One study,[37] graded good, reported that the selection of the 5 reference genes for normalization was based on the relatively constant expression levels of these genes across all tumor types. As discussed in Czechowski et al. (2005),[38] although this is a common way to normalize gene expression data, unless the genes were identified as stable across multiple types of tissues and experimental conditions, it is difficult to know whether these genes are indeed stable across all conditions. There is not sufficient information in the publication to assess if the stability of the genes were assessed in this way.

Dimension Reduction

The dimension reduction algorithm developed used a combination of k-nearest neighbor (KNN) and a genetic algorithm to identify clusters of related genes. The identified genes were then used in a general linear model to assess the predictive ability of the cluster. Multiple iterations of the process resulted in the set of 87 genes used in the final test. This method uses repeated combinations of unsupervised and supervised clustering to identify a subset of gene profiles that would perform well in clusters.

Classification

The classification algorithm used here is a logistic regression model that is equivalent to a linear discriminant classifier. This classifier uses supervised learning and is the optimal method of classification per Simon et al.[18] First described in Ma et al.,[37] a modification of the process was described in Erlander et al.[24] (which was also graded as a good study). The modification described in Erlander et al.[24] adds the classification of the cancer subtype to the algorithm.

Validation

The algorithm for CancerTypeID was described first in Ma et al.[37] and then in Erlander et al.[24] Both studies reported internal validation using a leave one out cross validation and a separate validation in an independent test set. Simon et al.[18] suggest using at least one of the two types of validation in algorithm development and validation in an independent test set as optimal. This test meets both criteria.

miRview

Normalization

A study graded good[34] reported that a two-degree polynomial that optimized the fit between a reference vector that contained median expression levels for all miRNAs in each sample and the sample data was identified. The polynomial was used to transform the raw expression level of each probe to its normalized value.

Dimension Reduction

The algorithm used stepwise logistic regression to create a decision tree in which the nodes were the tumor types. The model assessed the predictive values of miRNAs to predict a tumor type. The process started with one miRNA and added more if the difference in the log likelihood of the new model and old model resulted in a chi-square value of 7.82 ($p<0.0005$). To mitigate the small number of samples for different tissue types, the logistic regression was repeatedly fit to bootstrapped samples that included two-thirds of the original sample. The use of logistic regression as the discriminator at nodes allowed the introduction of clinical features into the decision tree. This is an appropriate method and avoids many of the issues that are discussed by Simon et al.[18] with respect to the use of clustering methods and fold change as a means of dimension reduction.

Classification

The classification used in the algorithm also uses a decision tree with logistic regression to separate the nodes and combines the decisions of the decision tree with an unsupervised KNN classifier. The combination of a supervised and unsupervised method along with both internal and external validation meets the Simon criteria.[18]

Validation

In addition to using a leave one out cross validation, Rosenfeld et al.[34] reported validation using a blinded independent data set of 83 cases randomly selected before developing the classifier from the sample of the data. In addition, they validated the utility of the miRNAs using an RT-PCR platform and an additional 80 samples, including 65 independent samples. Simon et al.[18] suggest using at least one of the two types of validation in algorithm development and validation in an independent test set as optimal. This test meets both criteria.

PathworkDx

Normalization

As reported in Dumur et al.[30] and Pillai,[33] which were both rated as good, gene expression levels were normalized to a set of 121 stable markers that were identified as being stable across 5,000 tissue specimens processed in 11 laboratories. The process was developed as a part of the Micro Array Quality Control project and is an appropriate way to normalize microarray data.

Dimension Reduction

The dimension reduction algorithm is based on ranking genes based on expression levels. There was no description of the dimension reduction algorithm beyond that in either Dumur et al.[30] or Pillai,[33] which makes it difficult to assess its validity.

Classification

The classification uses a proprietary machine learning algorithm that compares the expression profile of the patient tissue with that of profiles of 15 cancer types. Once again, there was little detail in the publications, and it is not possible to independently assess the validity of the classifier.

Validation

Simon et al.[18] suggest using at least one of the two types of validation in algorithm development and validation in an independent test set as optimal. There was no information on the internal and external validation used during the development of the algorithm used in PathworkDx TOO. However, several retrospective studies, as well as a blinded prospective study, have validated this test. As described below, the studies reported rates of accuracy between 74 percent and 99 percent.

Pathwork Endometrial

Pathwork Endometrial is a new test similar to the PathworkDx TOO described above but designed to distinguish only between ovarian and endometrial sites of origin. Lal et al., (2012)[31] in a study graded good, described the development and validation of the test. Details on normalization, dimension reduction, and development of the classifier were scarce, making it difficult to independently assess the validity of the statistical process used to develop the tests. The validation was conducted as recommended by Simon et al.[18] in an independent sample of 75 tissues (45 endometrial and 30 ovarian).

Cytogenetic Analysis

Statistical algorithms are not used in cytogenetic analysis.

Key Question 3b. What is the evidence on the accuracy of the TOO test in classifying the origin and type of the tumor?

Twenty studies,[24, 29-35, 37, 39-49] nineteen[24, 29-35, 37, 39-44, 46-49] rated good and one rated fair,[45] provided responses to this question. Three or more studies reported on the accuracy of each of the three tests. The accuracy rates across all of the studies for each of the three tests were fairly consistent. The meta-analytic summary of accuracy for the three tests were as follows: CancerTypeID 85 percent (95% CI, 83% to 86%), miRview 85 percent (95% CI, 83% to 87%), and PathworkDx 88 percent (95% CI, 86% to 89%).

The results are displayed in Table 9, Table 10, and Table 11.

CancerTypeID

Accuracy

Six studies[24, 37, 41, 42, 47, 49] reported on the accuracy of the test. Ma et al.,[37] graded good, reported an overall accuracy of 82 percent (95% CI, 74% to 89%) in an independent test set with 119 tissues of known origin. Site-specific accuracy ranged from 0 to 100 percent (Table 11). Erlander et al.,[24] graded good, reported an overall accuracy of 83 percent (95% CI, 78% to 88%) on a test set of tissue. Site-specific accuracy (Table 11) ranged from 50 to 100 percent. In a study graded good, Kerr et al.[42] reported an overall accuracy rate of 87 percent (95% CI, 84% to 89%) in a multi-institutional cohort of 790 tissues of known origin. The diagnosis of the tissues was adjudicated prior to blinded testing with CancerTypeID. Site-specific accuracy (Table 11) ranged from 48 to 100 percent. In a separate study graded good, focused on neuroendocrine tumor subtyping, Kerr et al.[41] reported an accuracy rate of 95 percent
in a sample of 75 tissues of neuroendocrine carcinoma. CancerTypeID correctly identified the tumor subtype in 71 of the 75 tissues. In a study rated good that compared the accuracy of

immunohistochemical (IHC) staining against the accuracy of CancerTypeID, Weiss et al.[47] reported a 78 percent accuracy rate for CancerTypeID compared with 68 percent for IHC in a sample of 123 tissues. In a study, rated good, to assess the accuracy of CancerTypeID in a Chinese population, Wu et al.[49] reported an accuracy rate of 82 percent in a sample of 184 tissues that represented 23 sites of origin. Site-specific accuracy (Table 11) ranged from 64 to 100 percent. Figure 4 displays the accuracy estimates across these seven studies.

Figure 4. Point estimates and 95% confidence intervals for all the reviewed studies estimating accuracy in known tissue for CancerTypeID TOO

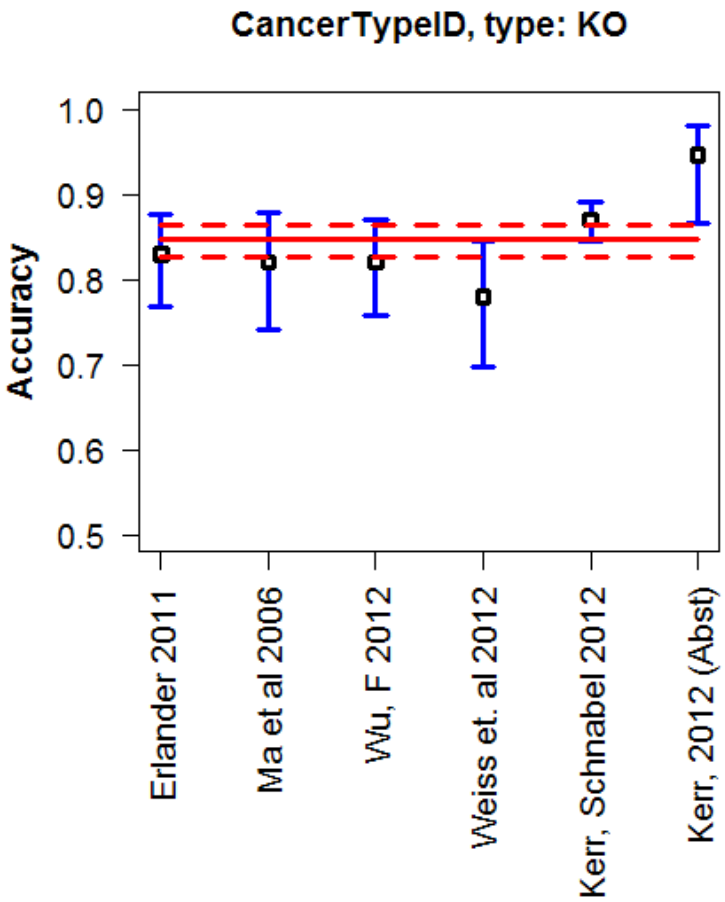


Table 9. Evidence of accuracy of the TOO test in classifying the origin when compared with gold standard

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancers Included	Gold Standard (comparison test)	Sensitivity % (95% CI)	Specificity % (95% CI)
CancerTypeID Erlander, 2011[24] NR Multinational, U.S. Good	Age: NR Female: NR N: 187	Adrenal, brain, breast, cervix, cholangiocarcinoma, endometrium, esophagus (squamous cell), gallbladder, gastroesophageal (adenocarcinoma), germ cell, GIST, head/neck, intestine, kidney (renal cell carcinoma), liver, lung, lymphoma, melanoma, meningioma, mesothelioma, neuroendocrine, ovary, pancreas, prostate, sarcoma, sex cord stromal tumor, skin, thymus, thyroid, urinary/bladder	Known origin	83 (78–88)	99 (99–99)
CancerTypeID Kerr, 2012[42] NR NR Good	Age in ranges: <50: 203 50–64: 271 >64: 316 Female: 405 N: 790	Metastatic tumors and moderately to poorly differentiated primary tumors of the following types: Adrenal, brain, breast, cervix adenocarcinoma, endometrium, gastroesophageal, germ cell, GIST, head-neck-salivary. Intestine, kidney, liver, lung-adeno/large cell, lymphoma, melanoma, meningioma, mesothelioma, neuroendocrine, ovary, pancreaticobiliary, prostate, sarcoma, sex cord stromal tumor, skin basal cell, squamous, thymus, thyroid, urinary/bladder	Diagnosis adjudicated prior to blind TOO testing	87 (84–89)	Incorrect exclusion: 5% cases
CancerTypeID Kerr, 2012[41] NR U.S. only Good	Age: NR Female: NR N: 75	Neuroendocrine carcinoma	Clinicopathologic diagnoses	95	NR

Table 9. Evidence of accuracy of the TOO test in classifying the origin when compared with gold standard (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancers Included	Gold Standard (comparison test)	Sensitivity % (95% CI)	Specificity % (95% CI)
CancerTypeID Ma, 2006[37] NR U.S. only Good	Age: NR Female: NR N: 119	Adrenal, brain, breast, intestine, cervix, endometrium, gallbladder, gastrointestinal stromal tumor of stomach, kidney, leiomyosarcoma, liver, lung, lymphoma, lymphoma, meningioma, mesothelioma, osteosarcoma, ovary, pancreas, prostate, skin, small and large bowel, soft-tissue, stomach, testis, thyroid, urinary/bladder	Known origin	82 (74–89)	NR
CancerTypeID Weiss, 2012[47] NR U.S. Good	Age: Mean (SD): 61.2 (14.4) years Female: 52% N: 123	Metastatic	Final clinicopathological diagnosis	IHC: 68 CTID: 78 p=0.017	NR
CancerTypeID Wu, 2012[49] NR China Good	Age: NR Female: 94 Ethnicity: Chinese N: 184	Adrenal, breast, endometrium, gallbladder, gastroesophageal germ cell, GIST, head and neck, intestine, kidney, liver, lung, lymphoma, melanoma, mesothelioma, neuroendocrine, ovary, pancreas, prostate, sarcoma, skin, thyroid, urinary/bladder	Clinicopathological diagnosis	82.1 (151/184)	NR

Table 9. Evidence of accuracy of the TOO test in classifying the origin when compared with gold standard (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancers Included	Gold Standard (comparison test)	Sensitivity % (95% CI)	Specificity % (95% CI)
miRview mets[2] Meiri, 2012[32] 1990–2010 NR Good	Age: NR Female: NR N: 509	Adrenal, anus, biliary tract, bladder, brain, breast, cervix, colon/rectum, gastrointestinal, liver, lung, head and neck, esophagus, lymphoma, mesothelioma, ovary, pancreas, prostate, sarcoma, skin, testis, thymus, thyroid	Known origin	Single or one of two predictions: 85.5 (418/489) Single prediction made: 89.6 (361/403)	>99
miRview mets Mueller, 2011[45] 2008 Germany Fair	Age: NR Female: NR N: 102	Biliary tract, breast, colon, head and neck, kidney, liver, lung, melanocyte, ovary, stomach or esophagus, thyroid,	Known origin	Single or one of two predictions: 84 (75/89) Single prediction: 88 (46/52)	95 Single prediction: 99
miRview mets Rosenfeld, 2008[34] NR Multinational, not U.S. Good	Age: NR Female: NR N: 83	Bladder, brain, breast, colon, endometrium, head and neck, kidney, liver, lung, lung pleura, lymph node, melanocytes, meninges, ovary, pancreas, prostate, sarcoma, stomach, stromal, testis, thymus, thyroid	Known origin	Single or one of two predictions: 86 Decision tree: 72 KNN: 72	Single or one of two predictions: 99 Decision tree: 99

Table 9. Evidence of accuracy of the TOO test in classifying the origin when compared with gold standard (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancers Included	Gold Standard (comparison test)	Sensitivity % (95% CI)	Specificity % (95% CI)
miRview mets Rosenwald, 2010[35] NR Multinational, U.S. Good	Age: NR Female: NR N: 504	Bladder, brain, breast, colon, endometrium, head and neck, kidney, liver, lung, lung pleura, lymph node, melanocytes, meninges, ovary, pancreas, prostate, sarcoma, stomach, stromal, testis, thymus, thyroid	Known origin	Single or one of two predictions: 84.6 Single prediction: 89.5	Single or one of two predictions: 96.9 Single predictions: 99.3
PathworkDx Beck, 2011[39] NR U.S. only Good	Age: NR Female: NR N: 49	Bladder, breast, colorectal, gastric, hepatocellular, liver, melanoma, ovarian, pancreatic, prostate, renal, synovial sarcoma, sarcoma, thyroid	Known origin	29/39 (74%) Indeterminate: 7/39 (18%) Incorrect: 3/39 (7%)	NR
PathworkDx Dumur, 2008[30] NR U.S. only Good	Age: NR Female: NR N: 60	Breast, colorectal, nonsmall cell lung, non-Hodgkin's lymphoma, lymphoma, pancreas, bladder, gastric, germ cell, hepatocellular, kidney, melanoma, ovarian, prostrate, soft tissue, sarcoma, thyroid	Known origin	All samples (range): 86.7% (84.9–89.3%) Samples within manufacturer's tissue quality control parameters: Average (range): 93.8 (93.3–95.5%) Indeterminate: 5.5% to 11.3%	NR

Table 9. Evidence of accuracy of the TOO test in classifying the origin when compared with gold standard (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancers Included	Gold Standard (comparison test)	Sensitivity % (95% CI)	Specificity % (95% CI)
PathworkDx Dumur, 2011[29] NR U.S. only Fair	Age: NR Female: NR N: 43	Mixed (7 CUP, 6 known off panel, 30 known on panel)	Clinicopathologic diagnosis	Agreed: Known on-panel: 97 (28/29) 95% CI (80.4–99.8)	NR
PathworkDx Grenert, 2011[40] 2000–2007 U.S. only Good	Age: Range 22–74 Median 55 Female: 20 N: 37	Bladder, breast, colorectal, gastric, testicular germ cell, kidney, hepatocellular, nonsmall cell lung, non-Hodgkin's lymphoma, melanoma, ovarian, pancreas, prostate, sarcoma, thyroid	Clinicopathologic diagnosis	95 (95% CI: 81.8–99.3)	99.60
Pathwork Tissue of Origin Test Kulkarni, 2012[43] 2007–2011 U.S. only Good	Age: NR Female: 3 N: 10	Metastatic tumors from clinically identified primary site not diagnosed by IHC alone	Final clinicopathological diagnosis	Correct diagnosis: TOO: 9/10 (90%) Overall pathologist (5) review of IHC (10 cases): 32 /50 (64%) Individual pathologists: range 5/10–8/10	NR

Table 9. Evidence of accuracy of the TOO test in classifying the origin when compared with gold standard (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancers Included	Gold Standard (comparison test)	Sensitivity % (95% CI)	Specificity % (95% CI)
PathworkDx Monzon, 2009[44] NR Multinational, U.S. Good	Age: < 50: 142 50–59: 133 60–69: 139 ≥ 70: 132 Female: 290 N: 547	Colorectal, pancreatic, nonsmall cell lung, breast, gastric, kidney, hepatocellular, ovary, sarcoma, non-Hodgkin's lymphoma, thyroid, prostate, melanoma, bladder, testicular germ cell	Known origin	480/547 (87.8) (84.7–90.4)	99.4
PathworkDx Pillai, 2011[33] NR U.S. only Good	Age: 10–20: 1 20–30: 19 30–40: 44 40–50: 79 50–60: 133 60–70: 104 70–80 : 63 ≥80: 14 Female: 257 N: 462	Bladder, breast, colorectal, gastric, testicular germ cell, kidney, hepatocellular, and nonsmall cell lung cancer, non-Hodgkin's lymphoma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, thyroid cancer, and sarcoma	Known origin	409/462 (88.5) (85.3–91.3)	99.1
PathworkDx Stancel, 2011[46] NR U.S. only Good	Age: NR Female: NR N: 20	Lung, lymphoma, colon, pancreas, breast, ovarian, gastric	Known origin	15/19 (78.9%)	NR

Table 9. Evidence of accuracy of the TOO test in classifying the origin when compared with gold standard (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancers Included	Gold Standard (comparison test)	Sensitivity % (95% CI)	Specificity % (95% CI)
PathworkDx Wu, 2010[48] NR U.S. only Good	Age: Median: 41 Range: 21–56 Female: 3 N: 15	Lung, breast, melanoma, lymphoma, sarcoma, colon, head and neck, gastric, kidney	Known origin	12/13 (92%)	NR
Pathwork Tissue of Origin Endometrial Test Lal, 2012[31] NR U.S. only Good	Age, n of subjects in age range 30–50: 9 50–60: 24 60–70: 28 70–90: 14 Female: 75 N; 75	Ovarian and endometrial	Clinical diagnosis	71/75 (94.7) (86.9–98.5) AUC: 0.997	

Abbreviations: AUC = area under receiver operating cure; CI = confidence interval; CTID = CancerTypeID; GIST = gastrointestinal stromal tumor; IHC = immunohistochemistry; n = number; N = number; NR = not reported; TOO = tissue of origin; U.S. = United States.

Table 10. Meta-analysis estimates of accuracy of TOO tests in identifying the TOO of tumors of known origin

TOO Test	Summarized Estimate of Accuracy	95% CI, Lower Bound	95% CI Upper Bound
CancerTypeID	0.85	0.83	0.86
miRview	0.85	0.83	0.87
PathworkDx	0.88	0.86	0.89

Abbreviations: CI = confidence interval; TOO = tissue of origin.

Table 11. Evidence of site-specific accuracy of TOO test in classifying type of the tumor

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancer/Tumor Site	Sample Size	Sensitivity	Specificity
CancerTypeID	Age:	1. Adrenal	1. 2	1. 1.00	1. 1.00
	NR	2. Brain	2. 5	2. 1.00	2. 1.00
Erlander, 2011[24]		3. Breast	3. 11	3. 1.00	3. 1.00
	Female:	4. Cholangio-carcinoma	4. 7	4. 0.71	4. 0.99
NR	NR	5. Endometrium	5. 4	5. 0.75	5. 0.99
		6. Gallbladder	6. 6	6. 0.67	6. 0.98
Multinational, U.S.	N:	7. Gastroesophageal	7. 14	7. 0.86	7. 0.97
	187	8. Germ cell	8. 6	8. 1.00	8. 0.98
Good		9. GIST	9. 1	9. 1.00	9. 1.00
		10. Head/neck	10. 13	10. 0.54	10. 0.99
		11. Intestine	11. 16	11. 0.63	11. 1.00
		12. Kidney	12. 5	12. 1.00	12. 1.00
		13. Liver	13. 7	13. 1.00	13. 1.00
		14. Lung	14. 13	14. 0.92	14. 0.98
		15. Lymphoma	15. 10	15. 1.00	15. 0.99
		16. Melanoma	16. 5	16. 0.80	16. 1.00
		17. Meningioma	17. 1	17. 1.00	17. 1.00
		18. Mesothelioma	18. 2	18. 1.00	18. 0.99
		19. Neuroendocrine	19. 7	19. 1.00	19. 1.00
		20. Ovary	20. 6	20. 0.83	20. 0.99
		21. Pancreas	21. 8	21. 0.63	21. 0.99
		22. Prostate	22. 8	22. 0.88	22. 1.00
		23. Sarcoma	23. 6	23. 1.00	23. 0.99
		24. Sex cord stromal	24. 1	24. 1.00	24. 1.00
		tumor	25. 9	25. 0.67	25. 0.99
		25. Skin	26. 2	26. 0.50	26. 1.00
		26. Thymus	27. 5	27. 1.00	27. 1.00
		27. Thyroid	28. 7	28. 0.86	28. 0.99
		28. Urinary/bladder			

Table 11. Evidence of site-specific accuracy of TOO test in classifying type of the tumor (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancer/Tumor Site	Sample Size	Sensitivity	Specificity
CancerTypeID	Age in ranges:	1. Adrenal	29. 25	57. 0.96 (0.80–1.00)	85. 0.99 (0.98–1.00)
	<50: 203	2. Brain	30. 25	58. 0.96 (0.80–1.00)	86. 1.00 (0.99–1.00)
Kerr, 2012[42]	50–64: 271	3. Breast	31. 25	59. 0.80 (0.56–0.94)	87. 1.00 (0.99–1.00)
	>64: 316	4. Cervix	32. 25	60. 0.72 (0.47–0.90)	88. 0.99 (0.99–1.00)
NR		adenocarcinoma			
	Female:	5. Endometrium	33. 25	61. 0.48 (0.26–0.70)	89. 1.00 (0.99–1.00)
NR	405	6. Gastroesophageal	34. 25	62. 0.65 (0.41–0.85)	90. 0.99 (0.99–1.00)
		7. Germ cell	35. 25	63. 0.83 (0.61–0.95)	91. 1.00 (0.99–1.00)
Good	N:	8. GIST	36. 25	64. 0.92 (0.74–0.99)	92. 1.00 (0.99–1.00)
	790	9. Head-neck-salivary	37. 25	65. 0.88 (0.68–0.97)	93. 0.99 (0.98–1.00)
		10. Intestine	38. 25	66. 0.85 (0.62–0.97)	94. 0.98 (0.97–0.99)
		11. Kidney	39. 30	67. 0.97 (0.83–1.00)	95. 1.00 (0.99–1.00)
		12. Liver	40. 25	68. 0.96 (0.80–1.00)	96. 1.00 (0.99–1.00)
		13. Lung-adeno/large	41. 25	69. 0.65 (0.43–0.84)	97. 1.00 (0.99–1.00)
		cell	42. 25	70. 0.84 (0.64–0.95)	98. 1.00 (0.99–1.00)
		14. Lymphoma	43. 25	71. 0.88 (0.69–0.97)	99. 1.00 (0.99–1.00)
		15. Melanoma	44. 25	72. 1.00 (0.80–1.00)	100. 1.00 (0.99–1.00)
		16. Meningioma	45. 25	73. 0.87 (0.66–0.97)	101. 1.00 (0.99–1.00)
		17. Mesothelioma	46. 50	74. 0.98 (0.89–1.00)	102. 1.00 (0.99–1.00)
		18. Neuroendocrine	47. 40	75. 0.86 (0.71–0.95)	103. 0.98 (0.97–0.99)
		19. Ovary	48. 30	76. 0.88 (0.68–0.97)	104. 0.99 (0.98–1.00)
		20. Pancreaticobiliary	49. 25	77. 1.00 (0.80–1.00)	105. 1.00 (0.99–1.00)
		21. Prostate	50. 60	78. 0.95 (0.86–0.99)	106. 0.99 (0.97–0.99)
		22. Sarcoma	51. 25	79. 0.80 (0.59–0.93)	107. 1.00 (0.99–1.00)
		23. Sex cord stromal			
		tumor	52. 25	80. 1.00 (0.80–1.00)	108. 1.00 (0.99–1.00)
		24. Skin basal cell	53. 30	81. 0.86 (0.68–0.96)	109. 0.98 (0.97–0.99)
		25. Squamous	54. 30	82. 0.72 (0.51–0.88)	110. 1.00 (0.99–1.00)
		26. Thymus	55. 25	83. 0.96 (0.80–1.00)	111. 1.00 (0.99–1.00)
		27. Thyroid	56. 25	84. 0.64 (0.41–0.83)	112. 0.99 (0.98–1.00)
		28. Urinary/bladder			

Table 11. Evidence of site-specific accuracy of TOO test in classifying type of the tumor (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancer/Tumor Site	Sample Size	Sensitivity	Specificity
CancerTypeID	Age:	1. Intestinal	1. 12	1. 12/12 1.00	1. 1.00
	NR	2. Lung high grade	2. 11	2. 11/10 0.91	2. 1.00
Kerr, 2012[41]		3. Lung low grade	3. 11	3. 11/10 0.91	3. 1.00
	Female:	4. Merkel cell	4. 10	4. 10/10 1.00	4. 0.97
NR	NR	5. Pancreas	5. 10	5. 10/8 0.80	5. 0.98
		6. Pheo/paraganglioma	6. 10	6. 10/10 1.00	6. 1.00
U.S. only	N:	7. Thyroid medullary	7. 11	7. 11/11 1.00	7. 1.00
	75				
Good					

Table 11. Evidence of site-specific accuracy of TOO test in classifying type of the tumor (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancer/Tumor Site	Sample Size	Sensitivity	Specificity
CancerTypeID	Age:	1. Adrenal	1. 1	1. 1.00	NR
	NR	2. Brain	2. 3	2. 1.00	
Ma, 2006[37]		3. Breast	3. 1	3. 1.00	
	Female:	4. Carcinoid–intestine	4. 2	4. 1.00	
NR	NR	5. Cervix–adeno	5. 2	5. 0.50	
		6. Cervix–squamous	6. 3	6. 0.67	
U.S. only	N:	7. Endometrium	7. 3	7. 0.67	
	119	8. Gallbladder	8. 0	8. -	
Good		9. Germ cell	9. 9	9. 0.78	
		10. GIST	10. 3	10. 1.00	
		11. Kidney	11. 4	11. 1.00	
		12. Leiomyosarcoma	12. 3	12. 0.33	
		13. Liver	13. 2	13. 1.00	
		14. Lung–adeno–large cell	14. 3	14. 0.00	
		15. Lung–small	15. 5	15. 0.40	
		16. Lung–squamous	16. 3	16. 1.00	
		17. Lymphoma	17. 10	17. 1.00	
		18. Meningioma	18. 3	18. 1.00	
		19. Mesothelioma	19. 5	19. 0.80	
		20. Osteosarcoma	20. 2	20. 1.00	
		21. Ovary	21. 5	21. 1.00	
		22. Pancreas	22. 3	22. 1.00	
		23. Prostate	23. 7	23. 1.00	
		24. Skin–basal cell	24. 4	24. 0.75	
		25. Skin–melanoma	25. 4	25. 0.75	
		26. Skin–squamous	26. 3	26. 1.00	
		27. Small and large bowel	27. 6	27. 0.83	
		28. Soft-tissue	28. 8	28. 0.88	
		29. Stomach–adeno	29. 3	29. 0.00	
		30. Thyroid–follicular–papillary	30. 3	30. 1.00	
		31. Thyroid–medullary	31. 0	31. -	
		32. Urinary/bladder	32. 6	32. 1.00	

Table 11. Evidence of site-specific accuracy of TOO test in classifying type of the tumor (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancer/Tumor Site	Sample Size	Sensitivity	Specificity
CancerTypeID Weiss, 2012[47] NR U.S. 	Age: Mean (SD): 61.2 (14.4) years Female: 52% N: 123	1. Lung 2. Urinary/bladder 3. Breast 4. GI 5. Kidney	NR	1. CTID: 0.75, IHC: 0.67 2. CTID: 0.75, IHC: 0.42 3. CTID: 0.73, IHC: 0.55 4. CTID: 0.77, IHC: 0.77 5. CTID: 0.77, IHC: 0.77	NR
Cancer TypeID Wu, 2012[49] NR China Good	Age: NR Female: 94 N: 184	1. Adrenal 2. Breast 3. GIST 4. Intestine 5. Liver 6. Lymphoma 7. Prostate 8. Thyroid 9. Germ cell 10. Neuroendocrine 11. Kidney 12. Lung 13. Skin 14. Gallbladder 15. Melanoma 16. Urinary/bladder 17. Sarcoma 18. Pancreas 19. Head and neck 20. Ovary 21. Gastroesophageal 22. Mesothelioma 23. Endometrium	1. 2 2. 10 3. 6 4. 8 5. 6 6. 5 7. 7 8. 10 9. 10 10. 9 11. 8 12. 8 13. 6 14. 5 15. 5 16. 10 17. 13 18. 7 19. 10 20. 14 21. 12 22. 7 23. 6	1. 1.000 2. 1.000 3. 1.000 4. 1.000 5. 1.000 6. 1.000 7. 1.000 8. 1.000 9 1.000 10. 0.889 11. 0.875 12. 0.875 13. 0.833 14. 0.800 15. 0.800 16. 0.800 17. 0.769 18. 0.714 19. 0.700 20. 0.643 21. 0.583 22. 0.571 23. 0.333	1. 0.995 2. 0.994 3. 1.000 4. 0.994 5. 1.000 6. 0.994 7. 1.000 8. 1.000 9. 1.000 10. 0.983 11. 0.989 12. 0.983 13. 0.994 14. 0.983 15. 0.994 16. 0.971 17. 1.000 18. 0.994 19. 1.000 20. 0.976 21. 0.988 22. 1.000 23. 0.994

Table 11. Evidence of site-specific accuracy of TOO test in classifying type of the tumor (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancer/Tumor Site	Sample Size	Sensitivity	Specificity
miRview mets	Age:	1. Billary tract	1. 1	1. 0.000	1. 1.00
	NR	2. Breast	2.18	2. 0.722	2. 0.958
Mueller, 2011[45]		3. Head and neck	3. 4	3. 1.000	3. 0.894
2008	Female:	4. Kidney	4. 17	4. 0.941	4. 0.986
	NR	5. Liver	5. 1	5. 1.000	5. 1.00
Germany		6. Lung	6. 16	6. 0.875	6. 0.781
	N:	7. Melanocyte	7. 17	7. 1.000	7. 0.903
Fair	89	8. Ovary	8. 5	8. 0.600	8. 0.976
		9. Stomach or esophagus	9. 4.	9. 1.000	9. 0.988
		10. Thyroid	10. 2	10. 0.000	10. 0.977
		11. Colon	11. 4	11. 0.750	11. 0.953
		12. Prostate	12. 12	12. 3/12	12. NR
miRview mets	Age:			Decision Tree	Decision Tree
	NR	1. Bladder	1. 2	1. 0.00	1. 1.00
Rosenfeld, 2008[34]		2. Brain	2. 5	2. 1.00	2. 1.00
	Female:	3. Breast	3. 5	3. 0.60	3. 0.97
NR	NR	4. Colon	4. 5	4. 0.40	4. 0.99
		5. Endometrium	5. 3	5. 0.00	5. 0.99
Multinational, not	N:	6. Head and neck	6. 8	6. 1.00	6. 0.99
U.S.	253	7. Kidney	7. 5	7. 1.00	7. 0.99
		8. Liver	8. 2	8. 1.00	8. 0.99
Good		9. Lung	9. 5	9. 0.80	9. 0.95
		10. Lung pleura	10. 2	10. 0.50	10. 0.99
		11. Lymph node	11. 5	11. 0.60	11. 1.00
		12. Melanocytes	12. 5	12. 60	12. 0.97
		13. Meninges	13. 3	13. 1.00	13. 0.99
		14. Ovary	14. 4	14. 0.75	14. 0.97
		15. Pancreas	15. 2	15. 0.50	15. 1.00
		16. Prostate	16. 2	16. 1.00	16. 1.00
		17. Sarcoma	17. 5	17. 0.40	17. 0.99
		18. Stomach	18. 7	18. 0.71	18. 0.96
		19. Stromal	19. 2	19. 1.00	19. 1.00
		20. Testis	20. 1	20. 1.00	20. 1000
		21. Thymus	21. 2	21. 1.00	21. 0.98
		22. Thyroid	22. 3	22. 1.00	22. 1.00

Table 11. Evidence of site-specific accuracy of TOO test in classifying type of the tumor (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancer/Tumor Site	Sample Size	Sensitivity	Specificity
miRview mets	Age:	Validation	Validation only	(1 or 2 predictions)	(1 or 2 predictions)
	NR	1. Biliary tract	1. 7	1.0.667	1. 0.94
Rosenwald, 2010[35]		2. Brain	2. 11	2. 1.00	2. 1.00
	Female:	3. Breast	3. 38	3. 0.667	3. 0.936
NR	NR	4. Colon	4. 9	4. 0.889	4. 0.944
		5. Esophagus	5. 1	5. 1.00	5. 0.984
Multinational, U.S.	N:	6. Head and neck	6. 3	6. 1.00	6. 0.924
	204	7. Kidney	7. 10	7. 0.875	7. 0.994
Good		8. Liver	8. 8	8. 1.00	8. 0.994
		9. Lung	9. 26	9. 0.913	9. 0.849
		10. Melanoma	10. 7	10. 0.857	10. 0.978
		11. Ovary	11. 13	11. 0.846	11. 1.00
		12. Pancreas	12. 6	12. 0.50	12. 0.978
		13. Prostate	13. 20	13. 0.895	13. 0.994
		14. Stomach or esophagus	14. 7	14. 0.40	14. 0.989
		15. Testis	15. 8	15. 1.00	15. 1.00
		16. Thymus	16. 6	16. 0.833	16. 0.978
		17. Thyroid	17. 24	17. 1.00	17. 0.982
PathworkDx	Age:	1. Bladder	1. 1	1. 1/1	
	NR	2. Breast	2. 1	2. 1/1	
Beck, 2011[39]		3. Colon	3. 5	3. 5/5	
	Female:	4. Gastric	4. 2	4. 0/2	
NR	NR	5. Hepatocellular	5. 2	5. 1/2	
		6. High grade sarcoma	6. 2	6. 2/2	
U.S. only	N:	7. Melanoma	7. 2	7. 2/2	
	42	8. Ovarian	8. 9	8. 6/8	
Good		9. Pancreas	9. 2	9. 1/2	
		10. Prostrate	10. 2	10. 2/2	
		11. Renal	11. 6	11. 6/6	
		12. Thyroid	12. 2	12. 1/1	
		13. Lung	13. 2	13. 0/2	
		14. Synovial sarcoma	14. 1	14. 0/1	
		15. Endometrial	15. 2	15. 0/2	

Table 11. Evidence of site-specific accuracy of TOO test in classifying type of the tumor (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancer/Tumor Site	Sample Size	Sensitivity	Specificity
PathworkDx	Age:	1. Bladder	1. 3	1. 3/3	NR
	Range 22–74	2. Breast	2. 2	2. 2/2	
Grenert, 2011[40]	Median 55	3. Colorectal	3. 3	3. 3/3	
		4. Gastric	4. 2	4. 2/2	
2000–2007	Female:	5.Testicular gem cell	5. 3	5. 2/3	
	20	6. Kidney	6. 4	6. 4/4	
U.S. only		7. Hepatocellular	7. 3	7. 3/3	
	N:	8. Nonsmall cell lung	8. 2	8. 2/2	
Good	37	9. Non-Hodgkin's lymphoma	9. 3	9. 3/3	
		10. Melanoma	10. 2	10. 2/2	
		11. Ovarian	11. 3	11. 2/3	
		12. Pancreas	12. 2	12. 2/2	
		13. Prostate	13. 1	13. 1/1	
		14. Sarcoma	14. 3	14. 3/3	
		15. Thyroid	15. 1	15. 1/1	
PathworkDx	Age:	1. Bladder	1. 28	1. 22/28; 0.78 (0.590–0.917)	1. 519/519; 1.000 (0.993–1.000)
	<50: 142	2. Breast	2. 68	2. 64/68; 0.941 (0.856–0.984)	2. 471/479; 0.983 (0.967–0.993)
Monzon, 2009[44]	50–59: 133	3. Colorectal	3. 56	3. 52/56; 0.929 (0.82.7–0.98.0)	3. 487/491; 0.992 (0.979–0.999)
	60–69: 139	4. Gastric	4. 25	4. 18/25; 0.720 (0.506–0.87.9)	4. 519/522; 0.994 (0.983–0.999)
NR	≥70: 132	5. Germ cell	5. 30	5. 22/30; 0.733 (0.541–0.877)	5. 517/517; 1.000 (0.993–1.000)
		6. Hepatocellular	6. 25	6. 23/25; 0.920 (0.740–0.990)	6. 521/522; 0.998 (0.998–1.000)
Multinational, U.S.	Female:	7. Kidney	7. 39	7. 37/39; 0.949 (0.827–0.994)	7. 507/508; 0.998 (0.989–1.000)
	290	8. Melanoma	8. 26	8. 21/26; 0.808 (0.606–0.93.4)	8. 520/521; 0.998 (0.989–1.000)
Good		9. Non-Hodgkin's lymphoma	9. 33	9. 31/33; 0.939 (0.708–0.993)	9. 511/514; 0.994 (0.983–0.999)
	N:				
	547	10. Nonsmall cell lung	10. 31	10. 27/31; .0871 (0.702–0.964)	10. 509/516; 0.986 (0.972–0.995)
		11. Ovarian	11. 69	11. 64/69; 0.928 (0.839–0.976)	11. 473/478; 0.990 (0.976–0.9970)
		12. Pancreas	12. 25	12. 18/25; 0.720 (0.506–0.879)	12. 521/522; 0.998 (0.989–1.000)
		13. Prostate	13. 26	13. 23/26; 0.885 (0.698–0.976)	13. 521/521; 1.000 (0.993–1.000)
		14. Soft tissue sarcoma	14. 31	14. 26/31; 0.839 (0.663–0.945)	14. 513/516; 0.994 (0.983–0.999)
		15. Thyroid	15. 35	15. 32/35; 0.914 (0.769–0.982)	15. 510/512; 0.996 (0.986–0.999)

Table 11. Evidence of site-specific accuracy of TOO test in classifying type of the tumor (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancer/Tumor Site	Sample Size	Sensitivity	Specificity
PathworkDx	Age:	1. Bladder	1. 29	1. 23/29; 0.793 (0.603–0.920)	NR
	10–20: 1	2. Breast	2. 57	2. 55/57; 0.965 (0.879–0.996)	
Pillai, 2011[33]	20–30: 19	3. Colorectal	3. 36	3. 33/36; 0.917 (0.775–0.982)	
	30–40: 44	4. Gastric	4. 25	4. 18/25; 0.720 (0.506–0879)	
NR	40–50: 79	5. Hepatocellular	5. 25	5. 24/25; 0.960 (0.796–0.999)	
	50–60: 133	6. Kidney	6. 28	6. 25/28; 0.893 (0.718–0.977)	
U.S. only	60–70: 104	7. Melanoma	7. 25	7. 21/25; 0.840 (0.639–0.955)	
	70–80 : 63	8. Non-Hodgkin's	8. 29	8. 26/29; 0.897 (0.726–0.978)	
Good	≥80: 14	lymphoma			
		9. Nonsmall cell lung	9. 27	9. 23/27; 0.852 (0.663–0.958)	
	Female:	10. Ovarian	10. 45	10. 40/45; 0.889 (0.759–0.963)	
	257	11. Pancreas	11. 28	11. 24/28; 0.857 (0.637–0.960)	
		12. Prostate	12. 25	12. 24/25; 0.960 (0.796–0.990)	
	N:	13. Sarcoma	13. 27	13. 24/27; 0.889 (0.708–0.976)	
	462	14. Testicular germ cell	14. 25	14. 21/25; 0.840 (0.639–0.955)	
		15. Thyroid	15. 31	15. 28/31; 0.903 (0.742–0.980)	
PathworkDx	Age:	1. Lung	1. 4	1. 3/4	NR
	NR	2. Lymphoma	2. 2	2. 2/2	
Stancel, 2011[46]		3. Colon	3. 1	3. 0/1	
	Female:	4. Pancreas	4. 1	4. 1/1	
NR	NR	5. Breast	5. 4	5. 3/4	
		6. Ovarian	6. 5	6. 5/5	
U.S. only	N:	7. Gastric	7. 2	7. 1/2	
	20				
Good					
PathworkDx	Age:	1. Lung	1. 2	1. 1/2	NR
	Median: 41	2. Breast	2. 3	2. 3/3	
Wu 2010[48]	Range: 21–56	3. Melanoma	3. 2	3. 2/2	
		4. Lymphoma	4. 3	4. 3/3	
NR	Female:	5. Sarcoma	5. 1	5. 1/1	
	3	6. Colon	6. 1	6. 1/1	
U.S. only		7. Head and neck	7. 1	7. 0/1	
	N:	8. Gastric	8. 1	8. 1/1	
Good	15	9. Kidney	9. 1	9. −/1 (failed QC)	

Table 11. Evidence of site-specific accuracy of TOO test in classifying type of the tumor (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancer/Tumor Site	Sample Size	Sensitivity	Specificity
Pathwork Tissue of Origin Endometrial Test Lal, 2012[31] NR U.S. only	Age, n of subjects in age range 30–50: 9 50–60: 24 60–70: 28 70–90: 14 Female: NR N: 75	1. Ovarian 2. Endometrial	1. 30 2. 45	1. 42/45; 0.967 (0.817–0.986) 2. 29/30; 0.933 (0.828–0.999)	NR

Abbreviations: GIST = gastrointestinal stromal tumor; n = number; N = number; NR = not reported; TOO = tissue of origin; U.S. = United States.

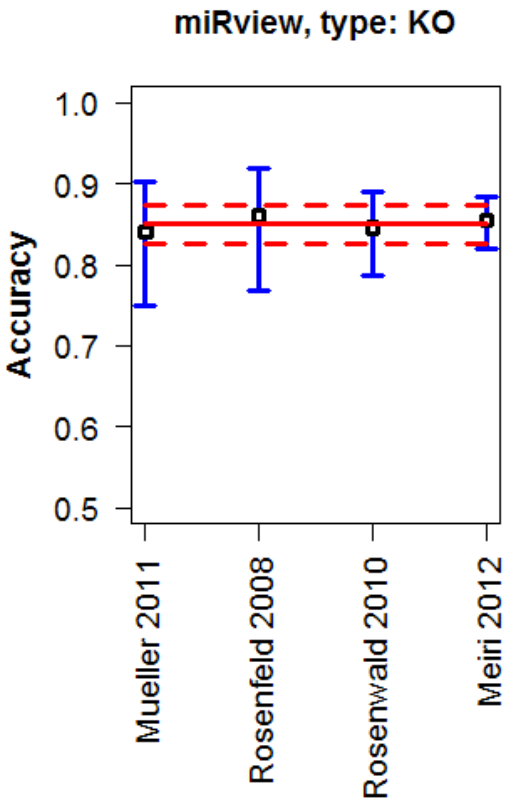
Given the consistency of the accuracy rates across the studies and overlapping confidence intervals, we did a meta-analysis using a fixed effects model to estimate a summary measure of accuracy (Table 10). We did not use any covariates to adjust for heterogeneity. The meta-analytic summarized estimate across the studies was 85 percent (95% CI, 83% to 86%).

miRview

Accuracy

The TOO call in miRview is based on the union of the calls made by the decision tree and the KNN.[34] Four studies[32, 34, 35, 45] reported on the accuracy of miRview in identifying the primary site in a tissue of known origin. Rosenfeld et al.,[34] rated good, reported on the results of a validation study on a test set of 83 specimens. The reported accuracy was 86 percent (95% CI, not reported). Rosenwald et al.,[35] rated good, reported on the results of a validation test on a sample of 188 cancers of known origin. In 159 cases (85%) either the decision tree or the KNN identified the TOO. This would suggest an overall accuracy of 85 percent. In addition, they reported that in 124 (66%) of the samples the two classification algorithms agreed. The accuracy in this group was 90 percent. The specificity in this group was reported to be over 99 percent. Mueller et al.,[45] rated fair, assessed the accuracy of the test in 101 cases of brain and central nervous system (CNS) metastases of known origin and 54 cases of brain and CNS metastases originally classified as CUP. They reported an overall accuracy of 84 percent. This estimate leaves out all 12 cases of prostate cancer, 9 of which were classified incorrectly. The authors suggest that published evidence suggests that the miRNA profiles of primary prostate tumors differ significantly from the metastatic tumors, especially in the presence of antiandrogenic therapy. They further justify the exclusion by the fact that prostate cancers form only 2 percent of CUP cases. The reported specificity for these cases was 95 percent. Figure 5 displays the accuracy rate across the four studies for this test. In a study graded good, Meiri et al.[32] reported an overall accuracy rate of 85 percent in 489 tissues of known origin, out of a sample of 509 tissues, that were assayed successfully. The sample included 149 metastatic

Figure 5. Point estimates and 95% confidence intervals for all the reviewed studies estimating accuracy in known tissue for miRview TOO



cancers. For 403 samples of these 489 tissues, miRview predicted a single site of origin with an accuracy rate of 90 percent. The specificity was above 99 percent.

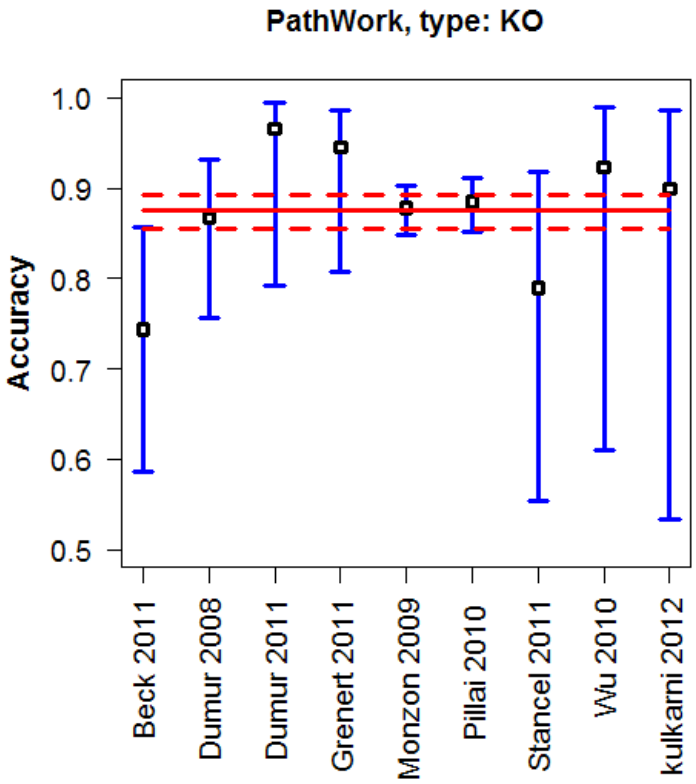
Given the consistency of the accuracy rates across the studies and overlapping confidence intervals, we did a meta-analysis using a fixed effects model to estimate a summary measure of accuracy (Table 11). We did not use any covariates to adjust for heterogeneity. The meta-analytic summarized estimate across the studies was 85 percent (95% CI, 83% to 87%).

PathworkDx

Ten studies assessed the accuracy of the PathworkDx test in samples of known primaries. Dumur et al.,[30] rated good, evaluated the accuracy of the test in tumors of known origin using 60 samples of archived snap-frozen tissue. They reported an overall accuracy rate of 87 percent. The confidence intervals were not reported. Among the 52 tissues that met all the quality assurance (QA) criteria for the test, the overall accuracy was 93.8 percent. Monzon et al.,[44] rated good, reported on a prospective validation study that assessed the accuracy of the test on 547 samples of known origin. They reported an overall accuracy of 87.8 percent (95% CI, 85% to 90%) and specificity of 99.4 percent (95% CI, 98.3% to 99.9%). Pillai et al.,[33] rated good, assessed the accuracy of the test on 462 FFPE tissue of known origin. They reported an overall accuracy rate of 89 percent (95% CI, 85% to 91%). Grenert et al.,[40] rated good, also assessed the accuracy of the TOO test on 44 FFPE samples of known origin. They reported an overall accuracy of 95 percent (95% CI, 82% to 99%) and an overall specificity of 99.6 percent. Beck et al.,[39] rated good, reported on the accuracy of 42 samples of tissues of known origin. Twenty-nine samples were tissues and morphologies included in the panel. The accuracy of the test in these tissues was 90 percent (95% CI, 73% to 97%). The accuracy for the 10 tissues with uncommon morphologies was significantly lower, 30 percent. Stancel et al.,[46] in a study rated good, reported on the accuracy of the TOO test using body fluid specimens from 19 patients with metastatic cancer. The estimated accuracy was 78 percent. This study also had 9 samples from patients with benign conditions. Seven of these tissues were diagnosed as lymphomas by the TOO test. In a study rated good, Wu et al.[48] assessed the accuracy of the test in 14 tissues of metastatic brain cancer of known origin. They reported an accuracy of 86 percent. In a study graded good, Kulkarni et al.[43] compared the performance of PathworkDx TOO in detecting the tissue of origin to IHC in a sample of 10 tissues of known origin. They reported an accuracy rate of 90 percent for the PathworkDx TOO. In a study graded fair, Dumur et al.[29] reported an accuracy rate of 97 percent (95% CI, 80% to 100%). The sample for the study included 29 tissues of known origin. Initially it appeared that the TOO test agreed with only 23 of the 29 known tissue calls. However, an examination of the 6 discordant cases revealed that the PathworkDx TOO call was accurate and the tumor site predicted by the TOO test was later confirmed by imaging or IHC tests.

Figure 6 has the accuracy rates for the nine studies for this test. Given the consistency of the accuracy rates and overlapping confidence intervals across the studies, we did a meta-analysis using a fixed effects model to estimate a summary measure of accuracy. The meta-analytic summarized accuracy rate for PathworkDx is 88 percent (95% CI, 86% to 89%).

Figure 6. Point estimates and 95% confidence intervals for all the reviewed studies estimating accuracy in known tissue for PathworkDx TOO



Pathwork Endometrial

One study, graded good, assessed the quality of the Pathwork Endometrial Test. Lal et al.[31] reported findings of a study designed to estimate the accuracy of the Pathwork Endometrial Test to distinguish between endometrial and ovarian sites of origin. In a sample of 45 endometrial cancer tissues and 30 ovarian cancer tissues, they reported an accuracy rate of 97 percent for ovarian cancers and 93 percent for endometrial cancers. They also reported an area under the curve for the test, which measures the ability of the test to discriminate between endometrial and ovarian cancers, of 0.997.

Key Question 4. How successful are TOO tests in identifying the TOO in CUP patients?

Eighteen studies,[14, 24, 29, 36, 39, 45, 50-61] eight[14, 29, 36, 50-52, 54, 60] rated good and ten[24, 39, 45, 53, 55-59, 61] rated fair provided evidence on this question. We assessed the clinical utility of TOO tests by evaluating the evidence that they accurately identify the tumor TOO and that the test results contributed to the final clinical diagnosis. The evidence is summarized in Table 12.

PathworkDx TOO and CancerTypeID had multiple studies from which the data might have been summarized with a meta-analysis. However, the comparison diagnosis used as a gold standard was inconsistent and incomparable across studies. We therefore decided against creating a summary estimate.

Table 12. Success of TOO tests in identifying the TOO in CUP patients

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	TOO Predicted Result (confirmed)	Indeterminate Results	Number of Clinically Useful Cases
CancerTypeID Erlander, 2011[24] NR Multinational, U.S. Fair	Age: Mean(± SD): 62 (13) ≥65: 44% Female: 53% N: 300	296 (Consistent with clinicopathologic impressions: 139 [47%])	4	Confirmed single suspected dx: 43/55 (78%) Confirmed one of multiple differential dx: 99/131 (74%) Provided additional information in cases of indeterminate origin: 37%
CancerTypeID Greco, 2010[51] 2000–2007 U.S. only Good	Age: 25–50: 4 50–64: 8 65+: 8 Female: 11 N: 20	18 (15)	2	NR
CancerTypeID McGee, 2009[61] 2009 US only Fair	Age: Mean: 64 ± 13 F: 40% N: 62 Clinician survey: 22 (20 responses)	62 (15 of 20 with follow up)		Guided or confirmed diagnosis: 10/20 Changed clinical diagnosis: 7/20

Table 12. Success of TOO tests in identifying the TOO in CUP patients (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	TOO Predicted Result (confirmed)	Indeterminate Results	Number of Clinically Useful Cases
CancerTypeID Schroder, 201257 NR U.S. only Fair	Age: Median: 62 years Female: 51% N: 815	91%		Pretest diagnosis unknown: CTID diagnosed 59 (93%) cases Differential diagnosis provided: CTID resolved dx in 55% cases. Provided new primary dx: 36%
CancerTypeID Thompson, 2011[58] 2008–2011 U.S. only Fair	Age: NR Female: NR N: 171	144 (by latent primary: 18/24 (75%); by IHC or clinical features: 101/144 (70%))		Confirmed 1 of 2–3 IHC differential diagnoses: 43/97
G-banded karyotype (supplemented by FISH and comparative genomic hybridization Pantou, 2003[14] 2001 Multinational, not U.S. Good	Age: <25: 1 25–50: 4 50–64: 4 65–older: 7 Mean age: 59.2 Female: 3 N: 20	5/20 identified cases 2/20 no mitoses 1/20 normal karyotype	NR	NR
miRview mets Mueller, 2011[45] 2008 Germany Fair	Age: NR Female: NR N: 54	50 (40) 10 were discordant 4 origin never diagnosed	NR	NR

Table 12. Success of TOO tests in identifying the TOO in CUP patients (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	TOO Predicted Result (confirmed)	Indeterminate Results	Number of Clinically Useful Cases
miRview mets[2] Pavlidis, 2012[60] NR NR Good	Age: NR Female: NR N: 92	84/92 (92%)	8	NR
miRview mets Varadhachary, 2011[36] 2008–2010 U.S. only Good	Age: 58 Range: 20–83 Female: Total: 66 N: 87	74 (62)	NR	IHC not helpful: 9 TOO prediction: 9 TOO consistent with clinicopathological: 7
Pathwork Tissue of Origin Test Azueta, 2012[50] 1992–2010 Spain Good	Age: NR Female: NR N: 32	Total: TOO: 32 (25/29) IHC: 28 (20/29) Peritoneal: TOO: 7 (7) IHC: 5 (5) Ovarian: TOO: 18 (22) IHC: 15 (22) Unknown: TOO: 2 IHC: 2	NR	NR

Table 12. Success of TOO tests in identifying the TOO in CUP patients (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	TOO Predicted Result (confirmed)	Indeterminate Results	Number of Clinically Useful Cases
PathworkDx Beck, 2011[39] NR U.S. only Fair	Age: NR Female: NS N: 7	4 (2 clearly incorrect)	3[52]	2
Pathwork Tissue of Origin Test Dumur, 2011[29] NR U.S. only Good	Age: Mean: 60 Range: 37–85 Female: 26 N: 40	Overall: 29/40 (28); On panel: 28/29; Off panel: 4 discordant, 9 unreported	3 inadequate sample: 1 indeterminate, 2 discordant. Off panel: 2 indeterminate	29/40. Confirmed original diagnosis: 23 Changed diagnosis: 5 Excluded suspected diagnosis: 1 TOO results obtained “several months” before IHC and imaging results.
PathworkDx Hainsworth, 2011[52] 2005 U.S. only Good	Age: Median: 56 Range: 21–86 Female: 21 N: 45	43	2	NR

Table 12. Success of TOO tests in identifying the TOO in CUP patients (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	TOO Predicted Result (confirmed)	Indeterminate Results	Number of Clinically Useful Cases
Pathwork Tissue of Origin Test Laouri, 2011[53] 2009 U.S. only Fair	Age: Median: 62 years Range: 16–69 years Female: 57% N: 284	Pretest diagnosis: Nonspecific: 172/183 Specific: 100/101 New diagnosis: 57(43)	Nonspecific: 12	Total: New posttest diagnosis: 81% (95% CI:76–85%), Confirmed suspected diagnosis: 15% Pretest diagnosis: Nonspecific: Specific posttest diagnosis: 94% B. Specific: Confirmed diagnosis: 44% New diagnosis: 55%
PathworkDx Monzon 2010[54] 2006 U.S. only Good	Age: 40–49: 1 50–59: 4 60–69: 6 70–79: 7 80–89: 3 Female: 15 N: 21	16 (10) 6 plausible	5	16

Table 12. Success of TOO tests in identifying the TOO in CUP patients (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	TOO Predicted Result (confirmed)	Indeterminate Results	Number of Clinically Useful Cases
PathworkDx Nystrom 2012[55] NR U.S. only Fair	Physician: Ordered TOO test N: 65 (of 308 eligible) Patients: Age: Mean (SD): 64 (12) Female: 61 Race: White: 95 African American: 4 Hispanic: 4 Asian American: 2 Other: 2 N: 107 patients	NR	NR	Changed diagnosis: 54/107 Provide diagnosis for patients with no working diagnosis: 27/44 No change in tissue site diagnosis: 37/107 Physician found test clinically useful: 66%
PathworkDx Pollen, 2011[56] 2009–2011 U.S. only Fair	Age: NR Female: NR N: 19	17	2	Concurred with original diagnosis: 5 Changed diagnosis: 9/17 Changed diagnosis of primary tumor to metastatic: 2 (included in 9 above)

Abbreviations: CI = confidence interval; CTID =CancerTypeID; FISH = fluorescent in situ hybridization; IHC = immunohistochemistry; n = number; N = number; NR = not reported; SD = standard deviation; TOO = tissue of origin.

CancerTypeID

Five studies,[24, 51, 57, 58, 61] one[51] rated good and four[24, 57, 58, 61] rated fair examined the success and utility of CancerTypeID in diagnosing CUP patients. Among four studies, CancerTypeID test predicted a TOO in 84 percent[58] to 99 percent[24] of the cases. Two studies[51, 58] included CUP cases for which the primary site was later identified. In both studies, the CancerTypeID diagnosis was confirmed in 75 percent of cases. Three studies[24, 57, 61] compared the TOO prediction to the suspected or diagnosed TOO based on clinicopathologic characteristics. The assay results were consistent with the clinicopathologic characteristics in 47 percent of patients in the study by Erlander et al.,[24] 77 percent of patients in the study by McGee et al.,[61] and 70 percent of patients in the study by Thompson et al.[58] McGee et al.[61] followed up with the referring physicians in 22 cases identified by CancerTypeID testing as squamous cell lung carcinoma. The final diagnosis was lung cancer in 15 of 20 cases with followup information. The assay results confirmed or guided the diagnosis in 10 cases and changed the diagnosis in 7 cases. In the study by Schroeder et al.,[57] the CancerTypeID results provided a diagnosis in 93 percent of cases with no existing diagnosis and resolved 55 percent of cases with multiple differential diagnoses at the time of testing. In 36 percent of these cases, the assay diagnosis was not any of the differential origins.

Cytogenetic Analysis

One study[14] reported on the ability of cytogenetic analysis to identify the TOO for tumors of unknown primary site. Five tumors (25%) had cytogenetic abnormalities that were diagnostic of a specific cancer type and primary site.

miRview mets

One good study[36] reported on the clinical utility of miRview mets TOO predictions among patients diagnosed with CUP. In the study by Varadachary[36],from 104 patients enrolled, 87 tumor samples were sufficient for analysis; of these, 74 passed quality control and returned a result. The TOO results were consistent with the final diagnosis in 62 of 74 cases, or 71 percent of the 87 cases in which profiling was attempted. Sixty-five cases had differential diagnoses based on pathology and IHC results. The TOO assay was consistent with one of the differential diagnoses in 55 of these cases. Nine cases could not be classified by IHC or pathology. The miRview TOO results matched the clinical presentation for 7 of these cases.

Two studies[45, 60] reported on the accuracy of miRview mets in diagnosing the TOO in CUP patients. Palvidis et al.,[60] in a study rated good, reported on the results of TOO testing of metastatic tumors from 92 CUP patients. The test predicted a TOO in 84/92 patients, and the TOO was consistent with the final diagnosis in 92% of the cases. Mueller et al.,[45] rated fair, reported on testing results in 54 CUP patients with brain or spinal metastases. The TOO test agreed with the best available data in 40 (74%) of these 54 cases. A clinically confirmed primary tumor was found for 28 patients. The TOO test had correctly predicted the origin in 22 of these cases. A diagnosis was suggested by pathology results but never clinically confirmed in 22 cases. The TOO results were consistent with pathology results in 18 of these 22 cases and inconsistent in 4 cases. Four cases had no suggested origin.

PathworkDx

Eight studies[29, 39, 50, 52-56] provided evidence on the utility of the PathworkDx test for diagnosis of CUP patients. Four studies[29, 50, 52, 54] were rated as good, and four[39, 53, 55, 56] as fair.

The PathworkDx test predicted a TOO in 57 percent[39] to 100 percent[50] of samples from CUP patients. Three studies[29, 50, 54] provided information on the agreement between the TOO results and the clinicopathologic characteristics or final diagnosis. Agreement ranged from 63 percent agreement with clinicopathologic characteristics[54] to 97 percent agreement with the later identified primary location[50] or the original or final diagnosis.[29] Beck et al.[39] reported on only seven CUP cases. Three cases had indeterminate results, and in two of the remaining four cases, the prediction was inconsistent with the clinical characteristics.

Six studies[29, 39, 53-56] provided information on the agreement of the TOO results with the differential diagnoses prior to testing and the utility of the TOO assay results in determining the final diagnosis. In the study by Beck et al.,[39] the TOO assay prediction matched one of the tumor types in the differential diagnoses in 2 of 4 cases with predictions. The authors commented that the test was probably clinically useful in these 2 CUP cases. In the study by Monzon et al.,[54] the assay results were included in the differential diagnosis in 10 of 16 cases with predictions. In the other 6 cases, the authors felt the assay predictions were plausible and in some cases, the assay ruled out tissues in the differential diagnosis. They felt the results in all 16 cases contributed to the management of the cases. Laouri et al.[53] reported on 284 cases, of whom 272 (96%) received a predicted TOO. The TOO test provided a new diagnosis in 94 percent (172 of 183) of cases without a specific diagnosis and in 44 percent (57 of 101) of cases that had a specific diagnosis prior to testing. Overall, 81 percent of cases had a new diagnosis after testing. Pollen et al.[56] reported on 19 difficult to diagnose cases: The TOO assay changed the diagnosis in 9 of 17 cases (53%). In 2 of these cases, a tumor thought to be a primary tumor was determined to metastatic.

Nystrom et al.[55] surveyed the referring oncologists to ask how the TOO assay results were used. They found that the assay changed the diagnosis in 53 percent of cases and provided a new diagnosis in 27 of 44 cases (61%) with no suspected diagnosis. The response rate for the survey was very low (21%), however.

Key Question 4a. What is the evidence that genetic TOO tests change treatment decisions?

The evidence regarding the effect of TOO tests on treatment decisions is very limited. Six studies[55, 58, 62-64] reported on the effect of TOO tests on treatment decisions. One study[64] was rated as good, but it was available only as a poster. Table 13 displays the results.

Three studies[55, 59, 61] examined the effect or potential effect of TOO results on patient management decisions. Laouri et al.[59] examined the TOO test results in 284 cases and compared the National Comprehensive Cancer Network (NCCN) treatment recommendations between the pre-test diagnosis and the diagnosis based on the PathworkDx TOO assay. They found that the treatment recommendations changed in 81 percent of the cases. For 178 cases, the chemotherapy regimen recommended by the NCCN guidelines was completely different after the test results. Nystrom et al.55 surveyed physicians who had ordered a PathworkDx test about their use of the test results. Among the physicians who responded, two-thirds felt the test was clinically useful, and the test resulted in a change in the management of 65 percent of the patients.

Table 13. Evidence of genetic TOO tests changing treatment decisions

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Charac-teristics	Cancer/ Tumor Types	Number Eligible	Participated/ Response	Eligibility Criteria	Indicated TOO	Treatment and Response	Treatment Change	p-value TOO vs. CUP Treatment
CancerTypeID Greco, 2012[62] NR U.S. only Fair	Age: Median: 61 years Range: 46–81 years Female: 18 N: 32	CUP	32	32	Colorectal gene expression signature	Colorectal cancer	First-line: Folfox/Folfiri 19 Other colorectal cancer drug: 4 Empiric: 8 (6/8 received CRC treatment for second-line) First-line CRC treatment: 17/23 (74%) Second-line CRC: 7/13 (54%) Any first-line treatment: 22/32 (69%) Any second-line treatment 7/13 (54%)	Not applicable: retrospective study	
CancerTypeID Hainsworth, 2010[64] NR NR Good	Age: Median: 64 Range: 26–85 Female: 33 N: 110	CUP	110	Received assay-directed treatment: 66 Evaluated: 51	Not a treatable subset of CUP No previous systematic treatment ECOG performance status 0–2 No uncontrolled brain metastases	Pancreatic: 11 Colorectal: 8 Urinary/bladder: 8 Nonsmall cell lung: 5 Ovarian: 4 Carcinoid - intestine: 4 Breast: 3 Gallbladder: 3 Liver: 3 Renal cell: 3 Skin (squamous): 3 Other: 7 No specific diagnosis: 5	Assay-directed treatment Objective treatment response: 21/51 (41%)	Changed: 66 (60%) Not changed: 44 (40%)	

Table 13. Evidence of genetic TOO tests changing treatment decisions (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Charac-teristics	Cancer/ Tumor Types	Number Eligible	Participated/ Response	Eligibility Criteria	Indicated TOO	Treatment and Response	Treatment Change	p-value TOO vs. CUP Treatment
CancerTypeID Hainsworth, 2011[63] 3/2008–8/2009 NR Fair	Age: Median: 57 Range: 35–86 Female: 27 N: 125	CUP or other identified as colorectal by TOO test	125	42 (34%)	NR	Identified by TOO test with ≥80% probability as colorectal adenocarcinoma	First-line: Advanced colorectal cancer: 12/24 Empirical for CUP: 17% (Total: 18) Second line: CRC: 8/16	Changed: 32 Not changed: NR	p=0.0257
CancerTypeID McGee [61] 2009 US only Fair	Age: Mean: 64 ± 13 F: 40% N: 22	Squamous cell lung carcinoma	22	20	Squamous cell lung carcinoma profile by CancerTypeID	Squamous cell lung carcinoma	Lung cancer therapy: 15/20 Head and neck cancer therapy: 2/20 Colorectal cancer therapy: 1.20 Unknown/ missing: 2	NR	NR
CancerTypeID Thompson, 2011[58] 2008–2011 U.S. Only Fair	Age: NR Female: NR N: 21	CUP with colorectal TOO result	NR	NR	NR	Colorectal cancer	Response: 70%		

Table 13. Evidence of genetic TOO tests changing treatment decisions (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Charac-teristics	Cancer/ Tumor Types	Number Eligible	Participated/ Response	Eligibility Criteria	Indicated TOO	Treatment and Response	Treatment Change	p-value TOO vs. CUP Treatment
PathworkDx Laouri, 2010[59] 2009 NR Fair	Age: Median: 62 Range: 16–89 Female: 161 N: 284 Nonspecific diagnosis: 183 Specific diagnosis: 101	Liver, lymph node, omentum and peritoneum, lung, soft tissue, bone, neck, brain, ovary, colon and rectum, pleura, pelvis, small bowel, other	NR	284	NR	TOO predicted: 221 (78%) Colorectal (15%) Breast (15%) Ovary (13%) Pancreas (13%) Nonsmall cell lung (11%) Hepatocellular (11%) Sarcoma, kidney, gastric, other (78%)	Change in first-line therapy: Initial nonspecific primary site: 135 major, 37 minor, 11 none Specific primary site: 43 major; 14 minor; 44 none Treatment response: NR	Changed: 229 (81%) Not changed: 55 (19%)	NR
PathworkDx Nystrom, 2012[55] NR U.S. only Fair	Age: Mean (SD): 64 (12) Female: 61 N: 316 physicians	Lymph node, soft tissue, liver, lung, bone, brain	316 physi-cians	65 physicians 107 patients	Survey			Did not change treatment: 17 Any change: 70/107 (65%, 95% CI: 58%-73%) Chemotherapy changed: 58/107 (54%, 95% CI:46%–62%) Radiation therapy: 27/107 (25%) Increase in consistency with guidelines: 23%	

Abbreviations: CI = confidence interval; CUP = cancer of unknown primary; ECOG = Eastern Cooperative Oncology Group; N = number; NR = not reported; SD = standard deviation; TOO = tissue of origin; U.S. = United States; vs. = versus.

Only 21 percent of surveyed physicians responded to the survey, however. McGee et al.[61] surveyed referring physicians in 22 cases identified by CancerTypeID testing as squamous cell lung carcinoma. Patients received therapy consistent with a lung cancer diagnosis in 13 cases with a final diagnosis of lung cancer.

Three studies[58, 62, 63] examined treatment decisions and response to treatment among cases that had a colorectal cancer gene expression profile. Hainsworth[63] found that 32 of 42 patients received first-line (24) or second-line (8) colorectal cancer treatment based on CancerTypeID results. Treatment response was much higher among patients treated with colorectal cancer specific therapy (20 of 40, 50%) than among patients who received empirical treatment for CUP (3 of 18, 17%). In the study by Greco et al.,[62] 17 of 23 (74%) of patients who received first-line colorectal cancer treatment responded to treatment. Among patients who received any first-line treatment, 22 of 32 (69%) responded to treatment. Thompson et al.[58] reported that 70 percent of patients in their study who received colorectal chemotherapy responded to treatment.

In another study, Hainsworth et al. presented preliminary results from a clinical trial that assigned treatment based on CancerTypeID results.[64] Of 110 enrolled patients, 66 received assay-directed treatment. The response to treatment was evaluated for 51 patients who received assay-directed treatment, and 21 (41%) responded to treatment.

Key Question 4b. What is the evidence that genetic TOO tests change outcomes?

The evidence for this response was limited. Seven studies,[14, 26, 55, 58, 62, 63, 65] one[14] rated as good and six[26, 55, 58, 62, 63, 65] rated fair provided evidence on this question. Table 14 has the evidence for this question.

CancerTypeID

Four studies[58, 62, 63, 65] looked at the effect of the identification of the TOO on patient outcomes. In a study graded fair, Hainsworth et al.[63] looked at the effect of TOO identification on treatment and patient outcomes. The report is based on the response to a survey that requested a response on 125 patients who had had an initial diagnosis of CUP and had the TOO identified as the colorectum. Of 125 surveys, 42 were returned. Thirty-two of these patients had received site-specific treatment for advanced colon cancer. Patients who were treated with site-specific regimens had a median survival of 8.5 months compared with 6 months in patients with empiric CUP therapy (p=0.11). In a second study graded fair, Hainsworth et al.[65] reported on a prospective trial that tested CUP patients and treated the patient based on the test. A total of 194 patients were treated with site-specific therapy. The median survival among these patients was 12.5 months, 95% CI (9.1 to 15.4 months) compared with 10.8 months in patients who received empiric therapy. Greco et al.,[62] in a study graded fair, reported on the results of a study of 32 patients for whom the TOO test indicated colorectal carcinoma as the primary cancer; 11patients were diagnosed between 2004 and 2007 at M.D. Anderson Cancer Center in Houston, Texas, using a TOO test called Veridex. The remaining 21 patients were diagnosed at the Sarah Cannon Research Institute in Nashville, Tennessee, with CancerTypeID between 2008 and 2010. The authors reported that the definitions used for CUP in both cohorts were the same. Twenty-nine of the 32 patients were treated with either first- or second-line colorectal cancer therapy. There is no detail about how many of these were from the first cohort and how many from the second. The median survival for all 32 patients was 21 months. The median survival for the 29 patients who had first- or second-line therapy was 22 months. The median survival of the 23 patients who

Table 14. Evidence that genetic TOO tests change outcomes

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteri-stics	Cancer/ Tumor Types	Number Eligible	Number Participated	Sample Requirements	Similarity Score	Indicated TOO	Outcome	Outcome by Group	Control
CancerTypeID Greco, 2012[62] NR U.S. only Fair	Age: Median: 61 years Range: 46–81 years Female: 18	CUP	32 CTID: 21 Veridex: 11	32	As marketed	NR	Colorectal cancer based on CTID or Veridex	Median survival: 2-year survival: 4-year survival:	Total sample: 21 months (95% CI: 17.1–24.9) First-line or second-line CRC treatment: (29 patients) 22 months First-line CRC treatment: 22 months Total sample: 42% Total sample: 35%	No control group

Table 14. Evidence that genetic TOO tests change outcomes (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteristics	Cancer/ Tumor Types	Number Eligible	Number Participated	Sample Requirements	Similarity Score	Indicated TOO	Outcome	Outcome by Group	Control
CancerTypeID Hainsworth, 2012[65] 2008–2011 U.S. only Fair	Age: Median: 64 Range: 26–89 Female: 136 N: 223	CUP	223 historic controls	Empiric CUP therapy: 29 Site-specific therapy: 194	NR	NR	Biliary tract, urothelium, colorectal, lung, pancreas, breast, ovary, gastroesophageal, kidney, liver, sarcoma, cervix, neuroendocrine, prostate, germ cell, skin, intestine, mesothelioma, thyroid, endometrium, melanoma, lymphoma, head and neck, adrenal	Median survival	TOO: 12.5 months (95% CI: 9.1–15.4)	Historical control: 9.1 months
CancerTypeID Hainsworth, 2011[63] 3/2008–8/2009 NR Fair	Age: NR Female: NS N: 125	CUP or other Identified by TOO as colorectal adenocarcinoma	125	42 (34%)	As marketed		Identified by TOO test with ≥80% probability as colorectal adenocarcinoma	Survival	TOO: 8.5 months p-value TOO vs. CUP: 0.11	Control: Empirical treatment: 6 months

Table 14. Evidence that genetic TOO tests change outcomes (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteri-stics	Cancer/ Tumor Types	Number Eligible	Number Participated	Sample Requirements	Similarity Score	Indicated TOO	Outcome	Outcome by Group	Control
CancerTypeID Thompson, 2011[58] 2008–2011 U.S. Only Fair	Age: NR Female: NR N: 21	CUP with colorectal TOO result		NR	NR		Colorectal cancer	Median survival	21 months	No control group
G-banded karyotype (supplemented by FISH and comparative genomic hybridization Pantou, 2003[14] 2001 Multinational, not U.S. Good	Age: < 25: 1 25–50: 4 50–64: 4 65–older: 7 Mean age: 59.2 Female: 3 N: 20	5/20 identified cases 2/20 no mitoses 1/20 normal karyotype	NR	NR				Median survival	Simple chromosomal changes: 19.7 months Complex changes: 3 months	No control group

Table 14. Evidence that genetic TOO tests change outcomes (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteri-stics	Cancer/ Tumor Types	Number Eligible	Number Participated	Sample Requirements	Similarity Score	Indicated TOO	Outcome	Outcome by Group	Control
PathworkDx Hornberger, 2012[26] NR NR Fair	Patients: Age: Mean: 64 (SD:12) Female: 61 Race: White: 95 African American: 4 Hispanic: 4 Asian American: 2 Other: 2 N (patients): 107	Any	308 treating physicians who ordered TOO test	65 (21%)	As marketed		Any	Overall survival: projected increase, 15.9–19.5 months Average estimated increase in survival adjusted for QOL: 2.7 months		No control group

Table 14. Evidence that genetic TOO tests change outcomes (continued)

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteri-stics	Cancer/ Tumor Types	Number Eligible	Number Participated	Sample Requirements	Similarity Score	Indicated TOO	Outcome	Outcome by Group	Control
Nystrom, 2012[55] NR U.S. Fair	Patients: Age: Mean: 64 (SD:12) Female: 61 Race: White: 95 African American: 4 Hispanic: 4 Asian American: 2 Other: 2 N (patients): 107	CUP						Survival 14 months 95% CI (10.2–18.6) 2-year survival 33% 3-year survival 30%	CRC (19%) Breast (14%) Sarcoma (14%) Lung (11%) Ovarian (11%) Pancreas (10%)	No control group

Abbreviations: CI = confidence interval; CRC = colorectal cancer; CTID = CancerTypeID; CUP = cancer of unknown primary; FISH = fluorescent in situ hybridization; N = number; NR = not reported; QOL = quality of life; SD = standard deviation; TOO = tissue of origin.

received first-line colorectal cancer therapy was also 22 months. The authors reported that the historical data suggest that median survival for CUP patients treated with empiric therapy was about 9 months. Two-year and 4-year survival for the 32 patients was, respectively, 42 percent and 35 percent. In a study graded fair, Thompson et al.,[58] reported a median survival of 21 months and a 70 percent response rate in patients who were treated with site-specific therapy for colon cancer.

miRview

There is no published evidence on the test changing outcomes in patients with CUP.

PathworkDx

Two studies, both graded fair, provided evidence about the effect of the identification of the TOO using PathworkDx on patient outcomes. Nystrom et al.[55] reported on a survey that was sent to 316 physicians who had ordered the PathworkDx TOO test. Sixty-five physicians (21%) responded to the survey, which gathered information on the effect of the test on decisions and patient outcomes. The reported median survival for patients who had the test ordered was 14 months from the time of the biopsy (95% CI, 10.2 to 18.6 months). Two-year survival was 33 percent and 3-year survival was 30 percent. The test is known to have changed the treatment decision in 65 percent of the patients but there is no information about the survival outcomes for the patients whose treatment changed. Hornberger et al.[26] reported on the same 107 patients described in Nystrom et al.[55] They reported that the projected benefit of site-specific therapy as a result of the TOO was an increase in survival from 15.9 months to 19.5 months. The observed benefit was a quality of life (QOL) adjusted increase in average survival of 2.7 months.

Key Question 5. Is the TOO test relevant to the Medicare population?

Twenty-two studies[14, 24, 29, 31, 33, 36, 40, 42, 44, 47-49, 51-55, 57, 62, 63, 65, 66] provided information on the age of the cases in their study (Table 15). All but one[48] included patients age 65 years or older, the Medicare core population. All of the studies of tests applicable to both cancers that provided information on the sex of the cases included both male and female cases. The studies and the TOO test panels include cancers specific to women (breast, ovarian) and to men (prostate, testicular). Only two studies[33, 49, 55] provided information on ethnicity. In both of these studies, the majority of the patients were Caucasian. Pillai et al. included 68 (15%) patients of Asian-American or Pacific Islander ancestry. One study[49] was conducted in China, so the patient population would be expected to be predominantly Chinese.

Table 15. TOO tests relevant to the Medicare population

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Size	Age	Female	Ethnicity	Cancer Types
CancerTypeID Erlander, 2011[24] NR Multinational, U.S. Good	300	Mean (SD): 62 (13)	53%	NR	Adrenal, brain, breast, cervix, cholangiocarcinoma, endometrium, esophagus (squamous cell), gallbladder, gastroesophageal (adenocarcinoma), germ cell, GIST, head/neck, intestine, kidney (renal cell carcinoma), liver, lung, lymphoma, melanoma, meningioma, mesothelioma, neuroendocrine, ovary, pancreas, prostate, sarcoma, sex cord stromal tumor, skin, thymus, thyroid, urinary/bladder
CancerTypeID Greco, 2010[51] 2000–2007 U.S. only Good	20	25–50: 4 50–64: 8 >65: 8	11	NR	CUP
CancerTypeID Greco, 2012[62] NR U.S. only Fair	32	Median: 61 Range: 46–81	18	NR	CUP

Table 15. TOO tests relevant to the Medicare population (continued)

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Size	Age	Female	Ethnicity	Cancer Types
CancerTypeID Hainsworth, 2010[64] NR NR Good	110	Median: 64 Range: 26–85	33	NR	CUP
CancerTypeID Hainsworth, 2011[63] 3/2008–8/2009 NR Fair	125	Median: 57 Range: 35–86	27	NR	Liver, bone/bone marrow, lymph nodes, peritoneum, lungs, abdominal/ retroperitoneal nodes, uterus/ovary, adrenal glands
CancerTypeID Hainsworth, 2012[65] 2008–2011 U.S. only Fair	223	Median: 64 Range: 26–89	136	NR	CUP

Table 15. TOO tests relevant to the Medicare population (continued)

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Size	Age	Female	Ethnicity	Cancer Types
CancerTypeID Kerr, 2012[42] NR NR Good	790	<50: 203 50–64: 271 >64: 316	405	NR	Metastatic tumors and moderately to poorly differentiated primary tumors of the following types: Adrenal, brain, breast, cervix adenocarcinoma, endometrium, gastroesophageal, germ cell, GIST, head-neck-salivary, intestine, kidney, liver, lung-adeno/large cell, lymphoma, melanoma, meningioma, mesothelioma, neuroendocrine, ovary, pancreaticobiliary, prostate, sarcoma, sex cord stromal tumor, skin basal cell, squamous, thymus, thyroid, urinary/bladder
CancerTypeID Schroder, 2012[57] NR U.S. only Fair	815	Median: 62 years	51%	NR	CUP
CancerTypeID Weiss, 2012[47] NR U.S. Good	123	Mean (SD): 61.2 (14.4) years	52%	NR	Metastatic

Table 15. TOO tests relevant to the Medicare population (continued)

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Size	Age	Female	Ethnicity	Cancer Types
CancerTypeID Wu, 2012[49] NS China Good	184	Age:	94	Chinese	Adrenal, breast, endometrium, gallbladder, gastroesophageal germ-cell, GIST, head and neck, intestine, kidney, liver, lung, lymphoma, melanoma, mesothelioma, neuroendocrine, ovary, pancreas, prostate, sarcoma, skin, thyroid, urinary/bladder
miRview Varadhachary, 2011[36] 2010 U.S. only Good	104	Age: Range: 20–83 Median: 58 Female:	66	NR	Lymph nodes, liver, lung, bone, pelvic mass/adnexae, skin/subcutaneous, omentum/peritoneum, adrenal, other
Pathwork Tissue of Origin Test Dumur, 2011[29] NR U.S. only Good	40	Mean: 60 Range: 37–85	26	NR	CUP

Table 15. TOO tests relevant to the Medicare population (continued)

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Size	Age	Female	Ethnicity	Cancer Types
PathworkDx Grenert, 2011[40] 2000–2007 U.S. only Good	37	Range: 22–74 Median: 55	20	NR	Bladder, breast, colorectal, gastric, testicular gem cell, kidney, hepatocellular, nonsmall cell lung, non-Hodgkin's lymphoma, melanoma, ovarian, pancreas, prostate, sarcoma, thyroid
PathworkDx Hainsworth, 2011[52] 2005 U.S. only Good	45	Median: 56 Range: 21–86	21	NR	CUP
Pathwork Tissue of Origin Test Kulkarni, 2012[43] 2007–2011 U.S. only	10	NR	3	NR	Metastatic tumors from clinically identified primary site not diagnosed by IHC alone

Table 15. TOO tests relevant to the Medicare population (continued)

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Size	Age	Female	Ethnicity	Cancer Types
PathworkDx Laouri, 2011[53] 2010 NR Fair	284	Median: 62 Range: 16–89	161	NR	Liver, lymph node, omentum and peritoneum, lung, soft tissue, bone, neck, brain, ovary, colon and rectum, pleura, pelvis, small bowel, other
PathworkDx Monzon, 2009[44] NR Multinational, U.S. Good	547	<50: 142 50–59: 133 60–69: 139 ≥70: 132	290		Colorectal, pancreatic, nonsmall cell lung, breast, gastric, kidney, hepatocellular, ovary, sarcoma, non-Hodgkin's lymphoma, thyroid, prostate, melanoma, bladder, testicular germ cell
Monzon, 2010[54] 2006 U.S. only Good	21	40–49: 1 50–59: 4 60–69: 6 70–79: 7 80–89: 3	15	NR	CUP

Table 15. TOO tests relevant to the Medicare population (continued)

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Size	Age	Female	Ethnicity	Cancer Types
PathworkDx Nystrom, 2012[55] NR U.S. only Fair	107	Mean (SD): 64 (12)	61	White: 95 African American: 4 Hispanic: 4 Asian American: 2 Other: 2	CUP
PathworkDx Pillai, 2011[33] NR U.S. only Good	462	10–20: 1 20–30: 19 30–40: 44 40–50: 79 50–60: 133 60–70: 104 70–80: 63 ≥80: 14	257	African American: 3 Asian/Pacific Islander: 68 European: 314	Bladder, breast, colorectal, gastric, testicular germ cell, kidney, hepatocellular, nonsmall cell lung, non-Hodgkin's lymphoma, melanoma, ovarian, pancreatic, prostate, thyroid, sarcoma
PathworkDx Wu, 2010[48] NR U.S. only Good	15	Median: 41 Range: 21–56	3	NR	Lung, breast, melanoma, lymphoma, sarcoma, colon, head and neck, gastric, kidney

Table 15. TOO tests relevant to the Medicare population (continued)

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Size	Age	Female	Ethnicity	Cancer Types
Pathwork Tissue of Origin Endometrial Test Lal, 2012[31] NR U.S. only Good	75	30–50: 9 50–60: 24 60–70: 28 70–90: 14	75		Ovarian, endometrial
G-banded karyotype (supplemented by FISH and comparative genomic hybridization Pantou, 2003[14] 2001 Multinational, not U.S. Good	20	<25: 1 25–50: 4 50–64: 4 65–older: 7 Mean: 59.2	3		

Abbreviations: CI = confidence interval; CUP = cancer of unknown primary; FISH = fluorescent in situ hybridization; GIST = gastrointestinal stromal tumor; IHC = immunohistochemistry; N = number; NR = not reported; SD = standard deviation; TOO = tissue of origin.

Discussion

Summary of Evidence

Table 16 summarizes our findings, which are discussed below.

Table 16. Overview of study outcomes

Key Question	Number of Studies	Conclusion	Strength of Evidence
KQ 2. Analytic validity: CancerTypeID	1	Only one study that was conducted by manufacturer of test. Limited measures of analytic validity reported and impossible to assess consistency of those measures across studies.	Insufficient
KQ 2. Analytic validity: miRview miRview mets	4	Four studies each reported different measures of analytic validity. Impossible to evaluate consistency of reported measures of analytical validity across studies.	Insufficient
KQ 2. Analytic validity: PathworkDx	3	Three papers reported measures of analytic validity. There is moderate consistency between studies of interlaboratory reproducability. The reported precision in these three papers is high.	High
KQ 2. Analytic validity PathworkDx Endometrial	1	One study reported on the analytic validity of a PathworkDx Endometrial test. The reported precision of the test is high, but there is insufficient evidence to assess analytic validity.	Insufficient
KQ 3a. Adherence to Simon guidelines: CancerTypeID	2	Report on the development of the algorithm has sufficient detail on development and validation to assess the validity of the process. Development met the Simon[3] criteria.	High
KQ 3a. Adherence to Simon guidelines: miRview mets	2	Report on development of algorithm has sufficient detail on development and validation to assess the validity of the process. Development met the Simon[3] criteria.	High
KQ 3a. Adherence to Simon guidelines: PathworkDx	2	Report on development of algorithm does not have sufficient detail on development and validation to assess the validity of the process.	Low
KQ 3a. Adherence to Simon guidelines: PathworkDX Endometrial	1	Report on development of algorithm does not have sufficient detail on development and validation to assess the validity of the process.	Low
KQ 3b. Accuracy of the TOO test in classifying the origin and type of tumors of known primary site: CancerTypeID	7	Seven studies representing 1,476 tissue samples compared the ability of tests to identify origin of tumor in tissues of known origin. All report accuracy of inclusion. Accuracy of exclusion reported in two studies.	High
KQ 3b. Accuracy of the TOO test in classifying the origin and type of tumors of known primary site: miRview mets	4	Two independent studies representing 898 tissue samples tested the ability of the miRview to identify site of origin in tissues of known origin. Accuracies of inclusion and exclusion are reported for both.	High
KQ 3b. Accuracy of the TOO test in classifying the origin and type of tumors of known primary site: PathworkDx	9	Nine studies representing 1,247 tissue samples report on the ability of the test to identify the origin of tumor in tissues of known origin. Accuracy of included tissue is reported in all studies. Accuracy of excluded tissues reported in two studies.	High
KQ 4. Percentage of cases for which a TOO test identified a TOO	17	Fifteen studies rated fair provided evidence on this question. The ability of the test to identify a TOO was judged using different criteria in different studies.	Moderate
KQ 4. Percentage of CUP cases for which the TOO identified by the test was independently confirmed	6	Five studies all rated fair provided evidence for this question. Gold standard varied across studies and precision across studies was moderate. Accuracy ranged from 57% to 100%.	Low

Table 16. Overview of study outcomes (continued)

Key Question	Number of Studies	Conclusion	Strength of Evidence
KQ 4: Percentage of CUP cases where TOO modified diagnosis	6	Four studies rated fair and one poster rated good provided evidence for this question. Some explored potential changes; others actual changes. The reported percentage of changes ranged from 65% to 81%. Response rates in the survey were fairly low.	Low
KQ 4: Percentage of CUP cases where test was considered clinically useful by physician or researcher	6	All studies found test clinically useful in a proportion of cases. Clinical usefulness was measured differently in each study. Wide estimate in the proportion of cases where test was useful.	Low
KQ 4: Change in treatment decisions	5	Studies have small samples, varied study designs and measures of effect on treatment decisions, making it difficult to draw conclusions on any of the tests.	Insufficient
KQ 4: Treatment response: Tissue-specific treatment based on TOO test compared with usual treatment for CUP cases	4	Small samples, insufficient studies to draw conclusions on any of the tests. Only one study has a control; all others use historical controls.	Insufficient
KQ 4: Change in survival	5	Small samples, insufficient studies to draw conclusions on any of the tests. Most use historical controls.	Low
KQ 4: Change in disease progression	1	Small samples, insufficient studies to draw conclusions on any of the tests.	Insufficient
KQ 5: Applicability	22	Almost all studies included patients age 65 and older and both male and female patients. The studies that reported race included mostly Caucasian patients.	High

Key Question 1: TOO Tests

Three broadly applicable tissue of origin (TOO) tests are currently available for clinical use in the United States. These tests identify from 15 (PathworkDx) to 27 (CancerTypeID) primary tumor sites. All three tests identify bladder, breast, kidney, melanoma, lung, ovary, pancreas, prostate, sarcoma, testis, or thyroid primary sites. Between 1980 and 2000, eight primary sites (lung, pancreas, kidney/adrenal, stomach, bowel, liver or bile duct, ovary or uterus, and prostate) accounted for 79 percent of primary sites identified by autopsy.[25] These sites are at least partially identified by all three tests. PathworkDx TOO does not list adrenal tumors as an identified site, and miRview mets[2] does not list endometrial tumors.

Chromosome analysis by G-banding, fluorescent in situ hybridization (FISH), or comparative genomic hybridization can be used to identify tumor types associated with specific chromosomal rearrangements. In cases of cancer of unknown primary (CUP) sites, these tests are much less likely to predict a tumor identification than any of the genomic tests (25% versus > 80%). Chromosomal analysis can be very useful when differential diagnosis includes a tumor type associated with a specific genomic rearrangement, such as in Ewing sarcoma (see Appendix G).

Key Question 2: Analytic Validity

Ten articles[24, 28-36] reported data on the analytic validity of the genomic TOO tests. The information presented in the articles suggests the tests are analytically valid and the described quality control measures for the tests are appropriate. The evidence for each test is limited to one or two studies, however. We were only able to assess the consistency of measures of analytic validity across studies for the PathworkDx TOO test. We considered the evidence of analytic

validity for the PathworkDx TOO test as high, but we felt evidence was insufficient to determine the analytic validity of the CancerTypeID and miRview mets or mets^2 tests (Appendix F). Only one article[24] reported on the analytic validity of the CancerTypeID TOO test. Three studies reported on the analytic validity of the miRview mets and one on the second-generation mets^2 test, but the different studies of the mets test did not report the same measures of analytic validity. One study[34] reports on analytic reproducibility between testing platforms and sample times. A second reports on interlaboratory reproducibility,[35] and a third[36] reports the quality control measures for the assay. Three studies[29, 30, 33] reported on the analytic validity of the PathworkDx TOO test. Two studies[30, 33] reported on interlaboratory and interassay reproducibility and quality control measures. Interlaboratory correlation between the similarity scores is high, consistent in direction, and of moderate precision.

Key Question 3a: Statistical Validity

One good study,[37] described the development of the algorithm used in CancerTypeID. The normalization uses five housekeeping genes, and although it is stated that these were found to be stable in multiple tissues, it is not clear if a formal experiment was done to test the stability of the housekeeping genes under multiple conditions. A modification of the algorithm is described in Erlander et al.[24] The methods of dimension reduction, classification, and validation are clearly stated, and overall the published evidence on the quality of the algorithm is high.

Rosenfeld et al.,[34] graded good, describe the development of the algorithm for miRview mets. All aspects of the development of the algorithm are well described, and the published evidence on the quality of the algorithm used in miRview is high.

Dumur et al.[67] and Pillai et al.,[33] graded good, both describe the normalization process used in the PathworkDx TOO. The dimension reduction algorithm and the classification are considered proprietary and not described in enough detail to determine the appropriateness of the algorithm. The published evidence on the statistical validity of the algorithm is insufficient.

Lal et al.[31] describe a modification of the PathworkDx TOO aimed at distinguishing ovarian cancers from endometrial cancers. As above, the dimension reduction algorithm and the classification are considered proprietary and not described in enough detail to determine the appropriateness of the algorithm. The published evidence on the statistical validity of the algorithm is insufficient.

Key Question 3b: Accuracy of the TOO Test in Classifying the Origin and Tissue of the Tumor

Six studies,[24, 37, 41, 42, 47, 49] representing a sample size of 1,478 tissue samples, reported on the ability of the CancerTypeID test to identify the origin of the tumor in tumors of known origin. Based on these studies, with similar estimates of accuracy and the meta-analytic summary estimate 85 percent (83%, 86%), the evidence is high that CancerTypeID correctly identifies tumor type in known tissue nearly 80 percent of the time.

Four studies,[32, 34, 35, 45] representing a total of 1,198 samples, reported on the ability of the miRview mets to identify the tissue of origin in tumors of known origin. Based on the similarity of the estimates of accuracy across studies rated both good and fair and the meta-analytic estimate of 85 percent 95% CI (83%, 87%), the evidence is high that miRview mets accurately identifies the tumor type in known tissue 85 percent of the time.

Nine studies,[29, 33, 39, 40, 43, 44, 46, 48, 67] representing a total of 1,243 tissue samples, reported on the ability of the test to identify the origin of the tumor in tumors of known origin. Based on the similarity of estimates of accuracy across studies rated good and fair and the meta-analytic summary estimate of 88 percent, 95% CI (86% to 89%) the evidence is high that the PathworkDx test correctly identifies the tumor source 88 percent of the time, 95% CI (86% to 89%).

Key Question 4: Diagnosis

Moderate evidence supports the ability of TOO tests to predict the TOO and to affect the diagnosis of CUP cases. As noted in the introduction, CUP cases by definition have not been diagnosed even after extensive clinical and pathological evaluation. The TOO of many cases of CUP are not diagnosed even at autopsy.[25] Therefore, a test may be useful in evaluating CUP cases even if it provides a diagnosis in a minority of cases. The genomic tests predicted a TOO in 57 percent[39] to 100 percent[52] of cases. G-banded chromosomes predicted a TOO in 25 percent[14] of CUP cases. The TOO test provided a diagnosis in 29 percent to 94 percent of cases without a pretest diagnosis[24, 39, 53, 55, 57] and confirmed one of multiple differential diagnoses in 44 percent to 74 percent of cases.[24, 57, 58] It changed the diagnosis in 17 percent to 55 percent of cases with a specific diagnosis prior to testing.[29, 53, 55-57] In 26 percent to 79 percent of cases, the test did not change the pretest diagnosis.[24, 29, 36, 53, 55-57]

Low evidence supports the accuracy of the TOO prediction in CUP cases and the overall clinical utility of the tests. A confirmatory diagnosis was available in 10 studies,[24, 29, 36, 39, 45, 50, 51, 53, 54, 58] and the predicted diagnosis was confirmed in 48 percent[24] to 96 percent[29] of cases. One study[55] surveyed physicians and found that two-thirds of physicians reported the test was useful clinically. In two studies, the study authors assessed the utility of the case results and reported the case would have been clinically useful in 2 of 7 (29%)[39] and 16 of 21 (76%)[54] cases.

Key Question 4a: Treatment

Low evidence supports the premise that TOO results affect treatment decisions. Laouri[59] reported that TOO test results changed the treatment recommendations (based on National Comprehensive Cancer Network guidelines) in 81 percent of cases. Nystrom et al.[55] surveyed physicians who had ordered a TOO test result and found that the test results had changed management decisions in 65 percent of cases. Only 21 percent of surveyed physicians responded to the survey, however.

In a preliminary report of treatment response among patients who received assay-directed treatment, 41 percent responded to therapy; no comparison group was available. Three studies[58, 62, 63] examined treatment decisions and response among cases with a colorectal cancer gene expression profile. Only one study[63] included a comparison group; they found much higher response to treatment among patients who received colorectal-specific treatment compared with patients who received empiric therapy. The other two studies found response to colorectal-specific therapy was around 70 percent, but these studies had no control group.[58, 62]

Key Question 4b: Outcomes

The evidence for this question was limited. Seven studies,[14, 26, 55, 58, 62, 63, 65] one[14] rated as good and six[26, 55, 58, 62, 63, 65] rated fair, provided evidence on this question. There is low evidence that changing from empiric CUP therapy to site-specific therapy post TOO increases survival by

between 2 to 3 months. Most of the evidence is based on surveys of doctors or retrospective analysis. As discussed by Hainsworth et al.,[65] in many cancers, empiric CUP therapy and site-specific CUP therapy is identical and the subpopulation of patients with CUP who would benefit from site-specific therapy is very small. Therefore, it is unlikely that there will be a prospective study that will be large enough to assess the effect of the TOO test and subsequent management on patient outcomes.

Key Question 5: Applicability

Twenty-two studies[14, 24, 29, 31, 33, 36, 40, 42, 44, 47-49, 51-55, 57, 62, 63, 65, 66] provided information on the age of the cases in their study (Table 15). All but one[48] included patients age 65 years or older, the Medicare core population. All of the studies of tests applicable to both cancers that provided information on the sex of the cases included both male and female cases. The studies and the TOO test panels include cancers specific to women (breast, ovarian) and to men (prostate, testicular). Only two studies[33, 49, 55] provided information on ethnicity. In both of these studies, the majority of the patients were Caucasian. Pillai et al. included 68 (15%) patients of Asian-American or Pacific Islander ancestry. One study[49] was conducted in China, so the patient population would be expected to be predominantly Chinese.

Summary of Accuracy

A majority of the studies that contributed to the assessment of accuracy of the tests in identifying the origin of the tumor when the tissues were of known origin were rated good. Twenty studies were graded good and one was graded fair. The estimates of accuracy were consistent across all the studies, and the method used to determine accuracy was valid. The summary estimates of accuracy in known tissue for all the tests were 84 percent for CancerTypeID, 87 percent for miRview mets, and 87 percent for PathworkDx.

The accuracy of the TOO call by the tests in CUP cases is not as easily determined. The studies that examined the accuracy of the TOO call variously used the following gold standards: (a) the discovery of a latent tumor post TOO test that confirmed the TOO result,[51, 58] (b) improved response to tissue-specific treatment regimens,[63, 64] or (c) consistency between the clinicopathological presentation and evidence of accuracy of the test.[24, 29, 36, 50, 54, 57] The first of these is clearly a legitimate way to assess accuracy but will only rarely be available. Response to a specific treatment regimen is often used by clinicians to indicate differential diagnosis, particularly when a diagnosis is difficult to make. Response to treatment is not a valid gold standard for a diagnostic test, however. Similarly, consistency with clinicopathological features is of questionable validity, because the TOO test would not have been ordered if the clinicopathological features definitely identified the TOO. Given the rarity of cases for which a true gold standard is available, the accuracy of the TOO call by these tests will always be difficult to assess directly. The greater need is to determine if the tests are providing added benefit in diagnosing true CUP cases.

Gaps and Issues in the Literature on TOO Tests

Overall, the studies of the TOO tests were well designed and of fair to good quality. The primary concern with this body of literature is that all but one[39] of the published studies either included authors employed by the test manufacturers or were funded by the manufacturers. Studies with findings that questioned the value of these tests may not have been published. It is notable that the study by Beck et al.,[39] which received only equipment and assay materials from

the test manufacturer, presented the most negative view of the utility of the test. We included abstracts and poster presentations in our review to increase the likelihood of identifying studies with negative results, but we cannot rule out the possibility of bias toward publication of positive studies.

The dominence of studies funded or conducted by the test manufacturers is due in part to the relative immaturity of the literature on these tests. Most published studies examined analytic validity or clinical validity; such studies can be expensive to conduct and are unlikely to be independently funded. Studies of clinical utility need to be free of potential conflicts of interest, it may also be easier to obtain independent funding for studies of clinical utility than studies of test validity. If funding by the test manufacturers is necessary, the credability of the studies would be increased if the funding mechanism and requirements were as transparent as possible.

The evidence for the analytic validity for each test is limited to one or two studies. The information presented in the articles suggests the tests are analytically valid and the described quality control measures for the tests are appropriate. We were only able to assess the consistency of measures of analytic validity across studies for the PathworkDx TOO test. We considered the evidence of analytic validity for the PathworkDx TOO test as high, but we felt evidence was insufficient to determine the analytic validity of the CancerTypeID and miRview mets or mets[2] tests (Appendix F). Only one article[24] reported on the analytic validity of the CancerTypeID TOO test. In addition, the published information[30, 33] on the development of the statistical algorithm used in the PathworkDx text was too limited to assess whether the development process met the Simon[18] criteria.

The greater gap was in the data on clinical utility. Low evidence supports the premise that TOO results affect treatment decisions, response to therapy, or survival outcomes. All but one[63] of these studies either do not have control groups or use historical controls. As discussed above, it is also acknowledged[65] that the CUP test would affect treatment in a relatively small number of cases; therefore, a large-scale clinical trial, if ethical, is likely to be impractical. Nonrandomized prospective studies similar to the study by Hainsworth et al.[65] that compare patients in empiric therapy to patients who opt for tissue-specific therapy as a result of a TOO test would be useful in improving evidence on utility. The clinical utility of these tests is still uncertain.

Summary of Findings

We assessed four tests in this review: cytogenetic analysis, CancerTypeID, miRview mets, and PathworkDx TOO. Of the three tests, CancerTypeID has the broadest panel with the ability to detect 29 different tumor sites. miRview mets has 25 tumor sites on its panel and PathworkDx has 15 tumor sites in its panel.

The literature on genetic tests for CUP is in its infancy. In studies comparing TOO test results to known tumor site or a well-defined, valid measure of accuracy, the tests demonstrated a high degree of accuracy. The available literature on the application of these tests to actual CUP cases is very limited, and some studies are difficult to interpret because they lack a gold standard for accuracy of the test's call. Given the nature of the CUP, a diagnostic gold standard may not always be available.

In the absence of a good measure of the diagnostic accuracy of the test, a proxy measure of the utility of the test is its effect on treatment decisions and patient outcomes. The literature on the effect of the test on treatment decisions is very limited. There is some evidence that the test alters the treatment course from empiric therapy usually used in CUP to tissue-specific therapy.

Site-specific therapy and targeted therapy are not different for all cancers. This is true in the case of cancers such as breast, nonsmall cell lung cancer, ovarian, and bladder where the first-line empiric therapy for CUP and the target specific therapy are the same. As a result, the subset of CUP patients expected to benefit from target-specific therapy is expected to be very small.[65]

The effect of this change in therapy on outcomes is limited to five papers. There is low evidence that the site-specific therapies change outcomes. Although some research suggests there is a modest increase in survival (approximately 3 months), only one study with a control group compares outcomes between patients who received tissue-specific therapy and those who did not. This one focuses on colorectal cancer and does not have a large enough sample size.

As mentioned, one of the concerns is that all but one of the manuscripts reviewed had a possibility of publication bias in the available literature.

Future Research

Most studies included in the current review were funded wholly or partly by the manufacturers of the tests. The most urgent need in the literature is to have research groups that have no evident conflict of interest evaluate the clinical utlity of the tests.

Because tissue-specific therapy would be the standard of care for a patient with a tissue-specific diagnosis, ethical considerations would probably rule out a controlled trial that randomized patients with tissue-specific diagnoses from a TOO test into empiric therapy or tissue-specific therapy. A prospective trial such as the one reported by Monzon et al.[44] and Hainsworth et al.,[65] but with an appropriate comparison group, may be the best design available.

Given the lack of a true gold standard to assess the clinical accuracy of a TOO, future research should focus on the benefits from the test to the patient in terms of effect on treatment decisions and resulting outcomes. These studies will help assess clinical value of the TOO tests.

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Appendix A. Inclusion and Exclusion Criteria

Inclusion Criteria

- **TEST TYPE:** Genetic or molecular test used for the identification tumor tissue-of-origin that are commercially available (one for which an internet search or test directory identified a mechanism for a physician or laboratory to order the test or to buy a kit to perform the test) in the United States.
- **STUDY TYPE:** Systematic reviews randomized controlled trials, nonrandomized controlled trials, prospective and retrospective cohort studies, case-control studies, and case series published from 1990 to present that were relevant to at least one of the key questions. Conference presentations and posters from 1990 to present with presented data not published elsewhere.

Exclusion Criteria

- Non-English studies

Appendix B. Search Strategies

Search Strategy #1

PubMed

#1	Search Neoplasms, Unknown Primary[mh]	2398
#2	Search ("Gene Expression Profiling"[MeSH]) OR "Microarray Analysis/methods"[Majr]	64521
#3	Search #1 AND #2	38
#4	Search Pathwork diagnostics OR Agendia OR CancerTypeID OR MiRview Mets test OR Rosetta Genomics OR AviaraDX OR Quest Diagnostics	18686
#5	Search #1 AND #4	6
#6	Search #3 OR #5	39
#7	Search #3 OR #5 Limits: Humans, English, Publication Date from 2000	35
#8	Search Neoplasms/genetics[mh]	229992
#9	Search Neoplasms/classification[Majr] OR Neoplasms/diagnosis[Majr]	607681
#10	Search #8 AND #9	39811
#11	Search ("Reproducibility of Results"[Mesh]) OR "Sensitivity and Specificity"[Mesh]	478953
#12	Search #10 AND #11	2966
#13	Search Oligonucleotide Array Sequence Analysis/methods[Majr]	8345
#14	Search #12 AND #13	102
#15	Search #12 AND #13 Limits: Humans, English, Publication Date from 2000	96
#16	Search #10 AND #4 Limits: Humans, English, Publication Date from 2000	72
#17	Search #3 OR #15 OR #16 Limits: Humans, English, Publication Date from 2000	195

PubMed = **195 unique citations.**

Similar search terms were used for the Cochrane Database = **82 unique citations**.

Grey literature databases: EMBASE (was searched specifically for the tests by name). Biosis Previews, Academic Search Premier, Business Source Premier, Health Source, NexisLexis, Academic OneFile, and Scirus) = **15 unique citations.**

When all results were combined and duplicates removed, the total database contained **292 records.**

Search Strategy #2

PubMed

#13 Search ”tissue of origin” and “cancer”	188
#14 Search neoplasms, unknown primary	6765
#15 Search neoplasms, unknown primary/genetics	82
#18 Search #13 OR #15	259
#19 Search #13 OR #15 Limits: Humans, English	219
#20 Search #13 OR #15 Limits: Humans, English, Publication Date from 2000	157

PubMed = **157 unique citations.**

Similar search terms were used for the Cochrane Database = **82 unique citations.**

Grey literature databases: EMBASE (was searched specifically for the tests by name). Biosis Previews, Academic Search Premier, Business Source Premier, Health Source, NexisLexis, Academic OneFile, and Scirus) = **108 unique citations.**

When all results were combined and duplicates removed, the total database contained **347 records**.

The two search strategies were combined yielding 522 unique citations for title and abstract review.

Search Strategy #3

PubMed

#1Search “tissue of origin” AND “cancer” 201

#2Search Neoplasms, Unknown Primary/Genetics 93

#4Search #1 OR #2 282

#5Search #1 OR #2 Filters: Humans 255

#6Search #1 OR #2 Filters: Humans; English 245

#9Search (“2011/10/01”[Date - Entrez] : “3000”[Date - Entrez]) Field: Date - Entrez Filters: Humans; English 198318

#11Search #6 AND #9 Field: Date - Entrez Filters: Humans; English 13

PubMed = **13 unique citations.**

Similar search terms were used for the Cochrane Database = **7 unique citations**.

Grey literature databases: EMBASE (was searched specifically for the tests by name). Biosis Previews, Academic Search Premier, Business Source Premier, Health Source, NexisLexis, Academic OneFile, and Scirus) = **45 unique citations.**

When all results were combined and duplicates removed, the total database contained **45 records.**

Search Strategy #4

PubMed

#1 Search (Neoplasms, Unknown Primary[mh]) AND ((“Gene Expression Profiling”[MeSH]) OR “Microarray Analysis/methods”[Majr]) Filters: Humans; English 47

#2 Search (Neoplasms, Unknown Primary[mh]) AND ((“Gene Expression Profiling”[MeSH]) OR “Microarray Analysis/methods”[Majr]) Filters: English 47

#3 Search (Neoplasms, Unknown Primary[mh]) AND (Pathwork diagnostics OR Agendia OR CancerTypeID OR MiRview Mets test OR Rosetta Genomics OR AviaraDX OR Quest Diagnostics) Filters: English 8

#4 Search (#2 OR #3) Filters: English 47

#5 Search (#2 OR #3) Filters: Publication date from 2012/08/01 to 2012/11/07; English 0

#6 Search (#2 OR #3) Schema: all Filters: Publication date from 2012/08/01 to 2012/11/07; English 0

#7 Search (Neoplasms/genetics[mh]) AND (Neoplasms/classification[Majr] OR Neoplasms/diagnosis[Majr]) Filters: English 43921

#8 Search (“Reproducibility of Results”[Mesh]) OR “Sensitivity and Specificity”[Mesh] Filters: English 521700

#9 Search Oligonucleotide Array Sequence Analysis/methods[Majr] Filters: English 9024

#10 Search (#7 and #8 and #9) Filters: English 104

#11 Search (#7 and #8 and #9) Schema: all Filters: Not Human, English 4

#12 Search ((“tissue of origin” AND “cancer”)) AND Neoplasms, Unknown Primary/Genetics Filters: Publication date from 2012/08/01 to 2012/11/07; English 0

#13 Search ((“tissue of origin” AND “cancer”)) AND Neoplasms, Unknown Primary/Genetics Schema: all Filters: Publication date from 2012/08/01 to 2012/11/07; English 0

#15 Search ((“tissue of origin” AND “cancer”)) or Neoplasms, Unknown Primary/Genetics Filters: English 284

#17 Search ((“tissue of origin” AND “cancer”)) or Neoplasms, Unknown Primary/Genetics Filters: Publication date from 2012/08/01 to 2012/11/07; English 9

#18 Search ((“tissue of origin” AND “cancer”)) or Neoplasms, Unknown Primary/Genetics
Filters: Humans; English 251

#19 Search (#15 NOT #18) 33

PubMed = **36 unique citations.**

Similar search terms were used for the Cochrane Database = **8 unique citations.**

Grey literature databases: Biosis Previews, Academic Search Premier, Business Source Premier, Health Source, NexisLexis, Academic OneFile, and Scirus) = **24 unique citations.**

When all results were combined and duplicates removed, the total database contained **65 records**.

Appendix C. Evidence Tables

Table C-1. Study characteristics of included studies

Tissue of Origin Test, Author, Year Study Years, Region	Race/ Ancestry	Age Group of Patients	Age, Median, Range or Distribution	Female, n (%)	Sample Size, N	Cancer/Tumor Types
CancerTypeID Erlander 2011[1] NS Multinational, U.S.	NR	Multiple age groups	≥65: 44% Mean(± SD): 62 (13)	53%	Training dataset: N=2206; Independent sample set: N=187; Clinical cases: N=300	Adrenal, brain, breast, cervix, cholangiocarcinoma, endometrium, esophagus (squamous cell), gallbladder, gastroesophageal (adenocarcinoma), germ cell, gist, head/neck, intestine, kidney (renal cell carcinoma), liver, lung, lymphoma, melanoma, meningioma, mesothelioma, neuroendocrine, ovary, pancreas, prostate, sarcoma, sex cord stromal tumor, skin, thymus, thyroid, urinary/bladder
CancerTypeID Greco 2010[2] 2000-2007 U.S. only	NR	Multiple age groups	25 - 50: 4 50-64: 8 65+: 8	11	28	Breast, primary peritoneal, ovary, colon, nonsmall cell lung cancer, gastric, melanoma, pancreas
CancerTypeID Greco 2012[3] NR U.S. only	NR		Median: 61 years Range: 46–81 years	18	32	CUP

Table C-1. Study characteristics of included studies (continued)

Tissue of Origin Test, Author, Year Study Years, Region	Race/ Ancestry	Age Group of Patients	Age, Median, Range or Distribution	Female, n (%)	Sample Size, N	Cancer/Tumor Types
CancerTypeID Hainsworth, Schnabel et al. 2011[4] 3/2008-8/2009 NS NS	NR	Multiple age groups	Median: 57 Range: 35-86	27	125	Liver, bone/bone marrow, lymph nodes, peritoneum, lungs, abdominal/ retroperitoneal nodes, uterus/ovary, adrenal glands
CancerTypeID Hainsworth 2010[5] [Disease] NS NS	NR	Multiple age groups	Median: 64 Range: 26-85	33	110	CUP
CancerTypeID Hainsworth 2010[5] [Survival] NS NS	NR	Multiple age groups	Median: 64 Range: 26-85	33	110	CUP
CancerTypeID Hainsworth, 2012[6] 2008–2011 U.S. only	NR		Median: 64 Range: 26–89	136	223	CUP

Table C-1. Study characteristics of included studies (continued)

Tissue of Origin Test, Author, Year Study Years, Region	Race/ Ancestry	Age Group of Patients	Age, Median, Range or Distribution	Female, n (%)	Sample Size, N	Cancer/Tumor Types
CancerTypeID Kerr 2012[7] NR NR	NR	Multiple age groups	<50: 203 50–64: 271 >64: 316	405	790	Metastatic tumors and moderately to poorly differentiated primary tumors of the following types: Adrenal, brain, breast, cervix adenocarcinoma, endometrium, gastroesophageal, germ cell, GIST, head-neck-salivary. Intestine, kidney, liver, lung-adeno/large cell, lymphoma, melanoma, meningioma, mesothelioma, neuroendocrine, ovary, pancreaticobiliary, prostate, sarcoma, sex cord stromal tumor, skin basal cell, squamous, thymus, thyroid, urinary/bladder
CancerTypeID Kerr 2012[8] NR NR	NR		NR	NR	75	Neuroendocrine carcinoma

Table C-1. Study characteristics of included studies (continued)

Tissue of Origin Test, Author, Year Study Years, Region	Race/ Ancestry	Age Group of Patients	Age, Median, Range or Distribution	Female, n (%)	Sample Size, N	Cancer/Tumor Types
CancerTypeID Ma 2006[9] NR U.S. only	NR	NR	NR	NR	578	Adrenal, brain, breast, carcinoid–intestine, cervix–adeno, cervix–squamous, endometrium, gallbladder, germ–cell–ovary, gist (gastrointestinal stromal tumor of stomach), kidney, leiomyosarcoma, liver, lung–adeno–large cell, lung–small, lung–squamous, lymphoma–b cell, lymphoma–Hodgkin, lymphoma–t cell, meningioma, mesothelioma, osteosarcoma, ovary–clear, ovary–serous, pancreas, prostate, skin–basal cell, skin–melanoma, skin–squamous, small and large bowel, soft-tissue–liposarcoma, soft-tissue–mfh, soft-tissue–sarcoma–synovial, stomach–adeno, testis–other, testis–seminoma, thyroid–follicular–papillary, thyroid–medullary, urinary/bladder
CancerTypeID McGee et la, 2011[10] 2009 U.S. only	NR		64 ±13	40%	62 Clinician survey 22 (20 responses)	Squamous cell lung cancer
CancerTypeID Schroder, 2012[11] NR U.S. only	NR		Median: 62 years	51%	815	

Table C-1. Study characteristics of included studies (continued)

Tissue of Origin Test, Author, Year Study Years, Region	Race/ Ancestry	Age Group of Patients	Age, Median, Range or Distribution	Female, n (%)	Sample Size, N	Cancer/Tumor Types
CancerTypeID Thompson, 2011[12] 2008–2011 U.S. only	NR		NR	NR	Total N: 171 CRC patients (data for KQ 4b): 21	CUP with colorectal TOO result
CancerTypeID Weiss, 2012[13] NR U.S.	NR		Mean (SD): 61.2 (14.4) years	52%	123	Metastatic
CancerTypeID Wu, 2012[14] NR China	Chinese		NR	94	184	Adrenal, breast, endometrium, gall bladder, gastroesophageal germ cell, GIST, head and neck, intestine, kidney, liver, lung, lymphoma, melanoma, mesothelioma, neuroendocrine, ovary, pancreas, prostate, sarcoma, skin, thyroid, urinary/bladder
G-banded karyotype (supplemented by FISH and comparative genomic hybridization Pantou 2003[15] 2001 Multinational, not U.S.	NR	Multiple age groups	< 25 (1) 25-50 (4) 50-64 (4) 65-older (7) Mean age: 59.2	3	34	CUP

Table C-1. Study characteristics of included studies (continued)

Tissue of Origin Test, Author, Year Study Years, Region	Race/ Ancestry	Age Group of Patients	Age, Median, Range or Distribution	Female, n (%)	Sample Size, N	Cancer/Tumor Types
miRview mets[2] Meiri 2012[17] 1990–2010 NR	NR	NR	NR	NR	509 Interlaboratory correlation: 179	Adrenal, anus, biliary tract, bladder, brain, breast, cervix, colon/rectum, gastrointestinal, liver, lung, head and neck, esophagus, lymphoma, mesothelioma, ovary, pancreas, prostate, sarcoma, skin, testis, thymus, thyroid
miRview Mueller 2011[18] 2008 Germany	NR	NR	NR	NR	102	Billary tract, breast, head and neck, kidney, liver, lung, melanocyte, ovary, stomach or esophagus, thyroid, colon

Table C-1. Study characteristics of included studies (continued)

Tissue of Origin Test, Author, Year Study Years, Region	Race/ Ancestry	Age Group of Patients	Age, Median, Range or Distribution	Female, n (%)	Sample Size, N	Cancer/Tumor Types
miRview Pavlidis 2012[19] NR NR Good	NR	NR	NR	NR	92	CUP
miRview Rosenfeld 2008[20] NS Multinational, not U.S.	NR	NR	NR	NR	80	Bladder, brain, breast, colon, endometrium, head & neck, kidney, liver, lung, lung pleura, lymph node, melanocytes, meninges, ovary, pancreas, prostate, sarcoma, stomach, gist, testis, thymus, thyroid
miRview Rosenwald 2010[21] NS Multinational, U.S.	NR	NR	NR	NR	649 Learning set. 204 Validation	Biliary tract, brain, breast, colon, esophagus, head and neck, kidney, liver, lung, melanoma, ovary, pancreas, prostate, stomach or esophagus, testis, thymus, thyroid
miRview Varadhachary 2011[22] 2008-2010 U.S. only	NR	NR	Median: 58 Range: 20-83	Total: 66 Results: 45	104	lymph nodes, liver, lung, bone, pelvic mass/adnexae, skin/subcutaneous, omentum/peritoneum, adrenal, other

Table C-1. Study characteristics of included studies (continued)

Tissue of Origin Test, Author, Year Study Years, Region	Race/ Ancestry	Age Group of Patients	Age, Median, Range or Distribution	Female, n (%)	Sample Size, N	Cancer/Tumor Types
Pathwork Tissue of Origin Test Azueta, 2012[23] 1992–2010 Spain	NR		NR	NR	32	Ovarian, peritoneal carcinomatosis
PathworkDx Beck 2011[24] NS U.S. only	NR	NR	NR	NR	49	Bladder, breast, colorectal, gastric, hepatocellular, melanoma, liver, synovial sarcoma, sarcoma, ovarian, pancreatic, prostate, renal, thyroid
PathworkDx Dumar 2008[25] NS U.S. only	NR	NR	NR	NR	60	Breast, colorectal, nonsmall-cell lung, non-Hodgkin's lymphoma, lymphoma, pancreas, bladder, gastric, germ cell, hepatocellular, kidney, melanoma, ovarian, prostrate, soft tissue, sarcoma, thyroid
PathworkDx Grenert 2011[26] 2000-2007 U.S. only	NR	Multiple age groups	Median: 55 Range: 22 -74	20	45	Bladder, breast, colorectal, gastric, testicular gem cell, kidney, hepatocellular, nonsmall cell lung, non-Hodgkin's lymphoma, melanoma, ovarian, pancreas, prostate, sarcoma, thyroid

Table C-1. Study characteristics of included studies (continued)

Tissue of Origin Test, Author, Year Study Years, Region	Race/ Ancestry	Age Group of Patients	Age, Median, Range or Distribution	Female, n (%)	Sample Size, N	Cancer/Tumor Types
PathworkDx Hainsworth, 2011[27] 2005 U.S. only	NR	Multiple age groups	Range: 21-86 Median: 56	21	48	CUP
PathworkDx Hornberger, 2012[28] NR NR	Race: White: 95 African American: 4 Hispanic: 4 Asian American: 2 Other: 2 N	Patients: Mean: 64 (SD:12)	61	Patients: 107	Any
Pathwork Tissue of Origin Test Kulkarni, 2012[29] 2007–2011 U.S. only	NR	NR	3	10	Metastatic tumors from clinically identified primary site not diagnosed by IHC alone

Table C-1. Study characteristics of included studies (continued)

Tissue of Origin Test, Author, Year Study Years, Region	Race/ Ancestry	Age Group of Patients	Age, Median, Range or Distribution	Female, n (%)	Sample Size, N	Cancer/Tumor Types
Pathwork Tissue of Origin Endometrial Test Lal, 2012 [30] NR U.S. only	NR		30–50: 9 50–60: 24 60–70: 28 70–90: 14	75	75	Ovarian, endometrial
Pathwork Tissue of Origin Test Laouri, 2011[31] 2009 U.S. only	NR		Median: 62 years Range: 16–69 years	161 57%	284	Liver, lymph node, omentum and peritoneum, lung, soft tissue, bone, neck, brain, ovary, colon and rectum, pleura, pelvis, small bowel, other
PathworkDx Monzon 2010[32] 2006 U.S. only	NR	Multiple age groups	40-49: 1 50-59: 4 60-69: 6 70-79: 7 80-89: 3	15	21	CUP

Table C-1. Study characteristics of included studies (continued)

Tissue of Origin Test, Author, Year Study Years, Region	Race/ Ancestry	Age Group of Patients	Age, Median, Range or Distribution	Female, n (%)	Sample Size, N	Cancer/Tumor Types
PathworkDx Monzon 2009[33] NS Multinational, U.S.	NR	Multiple age groups	< 50 (142 50-59 (133) 60-69 (139) >= 70 (132)	290	547	Colorectal, pancreatic, nonsmall cell lung, breast, gastric, kidney, hepatocellular, ovary, sarcoma, non-Hodgkin's lymphoma, thyroid, prostate, melanoma, bladder, testicular germ cell
PathworkDx Nystrom 2012[34] NR U.S. only	Race: White: 95 African American: 4 Hispanic: 4 Asian American: 2 Other: 2		Mean (SD): 64 (12)	61	Physician: Ordered TOO test N: 65 (of 308 eligible) N: 107 patients N: 316 physicians	Lymph node, soft tissue, liver, lung, bone, brain CUP
PathworkDx Pillai 2011[35] NS U.S. only	NR	Multiple age groups	10-20: 1 20-30: 19 30-40: 44 40-50: 79 50-60: 133 60-70: 104 70-80: 63 ≥ 80: 14	257	462	Bladder, breast, colorectal, gastric, testicular germ cell, kidney, hepatocellular, and nonsmall cell lung cancer, as well as non-Hodgkin's lymphoma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, thyroid cancer, and sarcoma.
Pathwork Tissue of Origin Test Pollen, 2011[36]	NR		NR	NR	19	NR

Table C-1. Study characteristics of included studies (continued)

Tissue of Origin Test, Author, Year Study Years, Region	Race/ Ancestry	Age Group of Patients	Age, Median, Range or Distribution	Female, n (%)	Sample Size, N	Cancer/Tumor Types
PathworkDx Stancel 2011[37] NS U.S. only	NR		NR	NR	27	Lung, lymphoma, colon, pancreas, breast, ovarian, gastric
PathworkDx Wu 2010[38] NS U.S. only	NR	Multiple age groups	Range: 21-56 Median: 41	3	15	Lung, breast, melanoma, lymphoma, sarcoma, colon, head and neck, gastric, kidney

Abbreviations: CUP = cancer of unknown primary; GIST = gastrointestinal stromal tumor; MFH = malignant fibrous histiocytoma; N= number; NR = not reported; NS = not significant; SD = standard deviation; U.S. = United States.

Table C-2. KQ 2. Evidence of analytic validity of tissue-of-origin tests

Tissue of Origin Test, Author, Year	Range of Sensitivity and Specificity for Measuring the Markers	Number of Outliers, Cross Reaction of Markers With Normal Tissue	Antibodies Monoclonal, Robustness of Antibodies	QC Measures for Markers	Required % of Valid Markers	QC Standards for Assay	Changes in Panel Precision and Accuracy Over Time
CancerTypeID Erlander, Ma 2011[1]	Sensitivity NR Specificity NR	NR	NR	Assay reproducibility (expressed as mean percentage coefficient of variation): Ct values using positive controls (194 independent runs, 4 operators): 92 assay genes- 1.69%; 5 normalization genes -2.19%. Ct values using negative controls (194 independent runs, 4 operators): 92 assay genes-1.25%; 5 normalization genes -1.66%. Assays of known tumor types (32 assays, 3 tumor types, 4 scientists): The mean percentage CVs were 1.58% (range 1.41%-1.69%) for the 92 genes and 1.04% (range, 0.85% to 1.79%) for the 5 normalization genes. 100% concordance for tumor of origin prediction. Across tumor type (6 tumor types, 3 setups, 2 operators): 92 assay genes - 3.33%; 5 normalization genes - 3.16%.	NR	NR	NR

Table C-2. KQ 2. Evidence of analytic validity of tissue-of-origin tests (continued)

Tissue of Origin Test, Author, Year	Range of Sensitivity and Specificity for Measuring the Markers	Number of Outliers, Cross Reaction of Markers With Normal Tissue	Antibodies Monoclonal, Robustness of Antibodies	QC Measures for Markers	Required % of Valid Markers	QC Standards for Assay	Changes in Panel Precision and Accuracy Over Time
miRview mets[2] Meiri 2012[17]	Range of accuracy: NR Range of specificity: NR	Number of outliers: NR Cross-reaction with normal tissue: NR	NR	Positive control QA measures: Number of miRNAs with expression >300 (dynamic range); 98th percentile expression level of the miRNA Pearson correlation between sample and reference hybridization spikes Number of miRNAs with consistent triplicate signals Mean correlations coefficient of multiple assays of same sample: 0.99	NR	NR	Interlaboratory correlation: >0.95 in 89% of samples. Interlaboratory agreement on diagnosis: 175/179 (98%)
miRview Rosenfeld 2008[20]	Sensitivity NR Specificity NR	NR NR	NR NR	NR	NR	Array platform validated by RT-PCR. miRNAs maintained expression distributions and diagnostic roles.	NR
miRview Rosenfeld 2010[21]	Sensitivity NR Specificity NR	NR NR	NR NR	Number and identity of microRNAs (C<38) and average Ct of measured microRNAs. microRNA results consistent across 3 platforms (spotted arrays, custom commercial arrays, and qRT-PCR). Between-lab correlation of qRT-PCR signals per sample=0.98. Labs disagreed on 3 samples; 8 agreed on one answer; 61 matched perfectly. N=72	NR	NR.	Negative control: no RNA sample. Positive control: specific RNA sample that should meet defined range in assay QA based on fluorescence amplification

Table C-2. KQ 2. Evidence of analytic validity of tissue-of-origin tests (continued)

Tissue of Origin Test, Author, Year	Range of Sensitivity and Specificity for Measuring the Markers	Number of Outliers, Cross Reaction of Markers With Normal Tissue	Antibodies Monoclonal, Robustness of Antibodies	QC Measures for Markers	Required % of Valid Markers	QC Standards for Assay	Changes in Panel Precision and Accuracy Over Time
miRview Varadhachary 2011[22]	Sensitivity NR Specificity NR	NR NR	NR NR	NR	87/104 samples passed tumor content criteria. 74/87 passed all QA criteria.	Controls: No sample; no RNA; external positives. Quality parameters for RNA amplification.	NR
PathworkDx Dumar 2008[25]	Sensitivity Pre-standardization 1-to-1 lab correlation: Pearson correlation coefficients 0.65-0.82 Post standardization 1-to-1 lab correlation: 0.81 to 0.87 Coefficient of reproducibility: 32.48 +/- 3.97 Specificity NR	19/227 NR	NR NR	All samples with adequate RNA quantity and quality produced sufficient cRNA for hybridization. 31/227 samples required >1 labeling reaction. Data verification algorithm addresses RNA quality, inadequate amplification, insufficient quantity of labeled RNA, inadequate hybridization time or temperature. 218/227 gene expression data files passed verification. All 9 failed files showed evidence of RNA degradation.	NR	No evidence of bias. Similarity Score interlaboratory correlation: 0.95. Concordance of Physician Guided Conclusion: 89.4% (range, 87.0 - 92.5). Kappa > 0.86.	NR

Table C-2. KQ 2. Evidence of analytic validity of tissue-of-origin tests (continued)

Tissue of Origin Test, Author, Year	Range of Sensitivity and Specificity for Measuring the Markers	Number of Outliers, Cross Reaction of Markers With Normal Tissue	Antibodies Monoclonal, Robustness of Antibodies	QC Measures for Markers	Required % of Valid Markers	QC Standards for Assay	Changes in Panel Precision and Accuracy Over Time
PathworkDx Dumur, 2011[39]	Range of accuracy: NR Range of specificity: NR	Number of outliers: NR Cross-reaction with normal tissue: NR		NR	NR	NR	Required percentage of valid markers: ≥40 Percentage of present probes: Mean: 60.6 Range: 49.9–69.3% QC standards for assay: 3´/5´ ratio for glyceraldehyde-3-phosphate dehydrogenase ≤3.0* Mean 3´/5´ GAPDH ratio: 1.2 Range: 0.9–3.0 PathworkDX TOO test report: “Acceptable” data quality

Table C-2. KQ 2. Evidence of analytic validity of tissue-of-origin tests (continued)

Tissue of Origin Test, Author, Year	Range of Sensitivity and Specificity for Measuring the Markers	Number of Outliers, Cross Reaction of Markers With Normal Tissue	Antibodies Monoclonal, Robustness of Antibodies	QC Measures for Markers	Required % of Valid Markers	QC Standards for Assay	Changes in Panel Precision and Accuracy Over Time
Pathwork Tissue of Origin Endometrial Test Lal, 2012[30]	NR	NR	NR	NR	NR	NR	Intrasite reproducibility=46/46 Concordance percentage: 100% (95% CI: 92.3–100) Intersite reproducibility=83/88 Concordance percentage: 94.3% (95% CI: 87.2–98.1) Median coefficient of variation (CV%) of the similarity score: 1.38 (range 0.32–8.79) Kappa (K) statistic: 1.0 for all three pairwise comparisons. Changes in panel precision and accuracy over time: Test performance 90.5% for specimens >2 years old

Abbreviations: CI = confidence interval; Ct = cycle threshold; CV = coefficient of variation; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; microRNA = micro ribonucleic acid; miRNA = micro ribonucleic acid; NR = not reported; QA = quality assurance; qRT-PCR = quantitative reverse transcriptase polymerase chain reaction; RNA = ribonucleic acid; RT-PCR = reverse-transcriptase polymerase chain reaction; TOO = tissue of origin.

Table C-3. KQ 3a. Evidence of accuracy of tissue-of-origin tests in classifying the origin and type of tumor and adherence of statistical methods to guidelines[40]

Tissue of Origin Test, Author, Year	Total Expression	Selected Housekeeping	Hypothesis Tests	Ranking	Clustering (e.g., PCA)	Classification	Internal	External
CancerTypeID Ma, 2006[9]	NR	Raw Cy5/Cy3 ratios per array were normalized using nonlinear local regression, without background adjustment	Logistic regression	NR	NR	Supervised Logistic Regression	Leave-one-out cross-validation to select genes; Multiclass weighted K-Nearest Neighbor Classification algorithm to classify genes; Gene selection also done via Genetic algorithm	Validation study of 187 FFPE tumor samples
miRview Rosenfeld 2008[20]	Based on median expression level for each probe across all samples	NR	NR	NR	NR	Unsupervised KNN algorithm Supervised Logistic regression decision-tree algorithm	Leave one-out cross validation within the training set	Blinded test set with independent set of 83 samples
miRview Rosenwald 2010[21]	Average expression of all microRNAs of the sample + scaling constant (the average expression over the entire sample set)	NR	NR	Decision tree algorithm used that finally selected 48 miRNAs through feature selection	NR	Supervised: Binary decision tree KNN	Leave-one-out cross validation within the training set	Test performance assessed using independent set of 204 validation samples

Abbreviations: FFPE = formalin-fixed paraffin-embedded; K = Kappa; KNN = K-nearest neighbor; microRNAs = micro ribonucleic acid; NR = not reported.

Table C-4. KQ 3b-f. Evidence of accuracy of the tissue-of-origin test in classifying the origin and type of the tumor: Accuracy of CUP compared to gold standard

Tissue of Origin Test, Author, Year	Sample Size	Gold Standard (Comparison Test)	Sensitivity	Specificity
CancerTypeID Erlander, Ma 2011[1]	187	Known origin	0.83 (numbers not reported)	0.99
CancerTypeID Kerr, 2012[8] U.S.CAP abstract	75	Clinicopathological diagnosis	71/75 (95%)	NR
CancerTypeID Kerr, Schnabel 2012[7] Clinical Cancer Research	790	Diagnosis adjudicated prior to blind TOO testing	Overall Sensitivity = 87% (95% confidence interval (CI), 84 to 89)	Incorrect exclusion 5% cases
CancerTypeID Ma, Patel, et al., 2006[9]	Validation: 119 FFPE	Known origin	"Accuracy" Overall (95% CI): 82% (74% to 89%) Unclassifiable - 8 (6.7%)	NR
CancerTypeID Weiss et al., 2012[13]	N=123	Final clinicopathological diagnosis	Accuracy: IHC=0.68 CTID=0.78 p=0.017	NR
CancerTypeID Wu F, 2012[14]	N=184	Clinicopathological diagnosis	151/184 (82.1%)	NR
miRview mets[2] Meiri 2012[17]	509	Known origin	One of two predictions accurate: 418/489 Single prediction made: 361/403 accurate	> 99%
miRview Mueller 2011[18]	89 [excludes 12 cases of prostate cancer]	Known origin	75/89 for at least one classifier For 52 with single prediction: 46/52	95% Among 52 with single prediction: 99%
miRview Rosenfeld 2008[20]	83 (blinded test set)	Known origin	Combined accuracy using DecisionTree & KNN = 86%	Combined value not available Decision Tree=99% KNN=NR

Table C-4. KQ 3b-f. Evidence of accuracy of the tissue-of-origin test in classifying the origin and type of the tumor: Accuracy of CUP compared to gold standard (continued)

Tissue of Origin Test, Author, Year	Sample Size	Gold Standard (Comparison Test)	Sensitivity	Specificity
miRview Rosenwald 2010[21]	204 validation samples; 7 metastases from patients whose primary tumor was previously profiled. 188 passed QA	Known origin	84.6% Single prediction: 89.5%	96.9 Single predictions: 99.3%
PathworkDx Beck 2011[24]	42 (39 on panel)	known origin	29/39 Indeterminate: 7/39 Incorrect: 3/39	NR
PathworkDx Dumur 2008[25]	60	Known origin	All samples (range): 86.7% (84.9-89.3%) Samples within manufacturer's tissue quality control parameters: Average (range): 93.8 (93.3-95.5%) Indeterminate: 5.5% to 11.3%	NR
PathworkDx Dumur 2011[39]	43	Clinicopathologic diagnosis	Agreed: Known on-panel: 97 (28/29) 95% CI (80.4–99.8)	NR
Pathwork Grenert 2011[26]	37	95% (81.8 - 99.3)	99.60%	NR
Pathwork Tissue of Origin Test Kulkarni, 2012[29]	N=10	Final clinicopathological diagnosis	Correct diagnosis: TOO: 9/10 (90%) Overall pathologist (5) review of IHC (10 cases): 32 /50 (64%) Individual pathologists: range 5/10 to 8/10	NR
Pathwork Tissue of Origin Endometrial Test Lal 2012 [30]	75	Clinical diagnosis	71/75 (94.7) (86.9–98.5) AUC: 0.997	NR

Table C-4. KQ 3b-f. Evidence of accuracy of the tissue-of-origin test in classifying the origin and type of the tumor: Accuracy of CUP compared to gold standard (continued)

Tissue of Origin Test, Author, Year	Sample Size	Gold Standard (Comparison Test)	Sensitivity	Specificity
PathworkDx Monzon 2009[33]	547	Known origin	480/547 (87.8%) (84.7–90.4)	99.40%
PathworkDx Pillai 2010[35]	462	Known origin	Overall agreement: 409/462. Sensitivity: 88.5	99.1
PathworkDx Stancel 2011[37]	20 Passed QA: 19	Known origin	15/19 (78.9%)	NR
PathworkDx Wu 2010[38]	15 Results, 1 off-panel specimen: 14 Sample size = 13	Known origin	12/13(92%)	NR

Abbreviations: AUC = area under receiver operating curve; CI = confidence interval; FFPE = formalin-fixed paraffin-embedded; IHC = immunohistochemistry; KNN = K-nearest neighbor; N= number; QA = quality assurance; TOO = tissue of origin.

Table C-5. KQ 3b-f. Evidence of accuracy of the tissue-of-origin test in classifying the origin and type of the tumor: Site-specific accuracy of CUP

Tissue of Origin Test, Author, Year	Sample size	Gold Standard (Comparison Test)	Site	Sample Size	Sensitivity	Specificity
CancerTypeID Erlander, 2011[1]	187	Known origin	1. Adrenal	1. 2	1. 1.00	1. 1.00
			2. Brain	2. 5	2. 1.00	2. 1.00
			3. Breast	3. 11	3. 1.00	3. 1.00
			4. Cholangio-carcinoma	4. 7	4. 0.71	4. 0.99
			5. Endometrium	5. 4	5. 0.75	5. 0.99
			6. Gallbladder	6. 6	6. 0.67	6. 0.98
			7. Gastroesophageal	7. 14	7. 0.86	7. 0.97
			8. Germ cell	8. 6	8. 1.00	8. 0.98
			9. GIST	9. 1	9. 1.00	9. 1.00
			10. Head/neck	10. 13	10. 0.54	10. 0.99
			11. Intestine	11. 16	11. 0.63	11. 1.00
			12. Kidney	12. 5	12. 1.00	12. 1.00
			13. Liver	13. 7	13. 1.00	13. 1.00
			14. Lung	14. 13	14. 0.92	14. 0.98
			15. Lymphoma	15. 10	15. 1.00	15. 0.99
			16. Melanoma	16. 5	16. 0.80	16. 1.00
			17. Meningioma	17. 1	17. 1.00	17. 1.00
			18. Mesothelioma	18. 2	18. 1.00	18. 0.99
			19. Neuroendocrine	19. 7	19. 1.00	19. 1.00
			20. Ovary	20. 6	20. 0.83	20. 0.99
			21. Pancreas	21. 8	21. 0.63	21. 0.99
			22. Prostate	22. 8	22. 0.88	22. 1.00
			23. Sarcoma	23. 6	23. 1.00	23. 0.99
			24. Sex cord stromal tumor	24. 1	24. 1.00	24. 1.00
			25. Skin	25. 9	25. 0.67	25. 0.99
			26. Thymus	26. 2	26. 0.50	26. 1.00
			27. Thyroid	27. 5	27. 1.00	27. 1.00
			28. Urinary bladder	28. 7	28. 0.86	28. 0.99
CancerTypeID Kerr, 2012[8] USCAP abstract	75	Clinicopathological diagnosis	1. Intestinal	1. 12	1. 12/12 (1.00)	1. 1.00
			2. Lung high grade	2. 11	2. 10/11 (0.91)	2. 1.00
			3.Lung low grade	3. 11	3. 10/11 (0.91)	3. 1.00
			4. Merkel cell	4. 10	4. 10/10 (1.00)	4. 0.97
			5. Pancreas	5. 10	5. 8/10 (0.80)	5. 0.98
			6. Pheo/paraganglioma	6. 10	6. 10/10 (1.00)	6. 1.00
			7. Thyroid medullary	7. 11	7. 11/11 (1.00)	7. 1.00

Table C-5. KQ 3b-f. Evidence of accuracy of the tissue-of-origin test in classifying the origin and type of the tumor: Site-specific accuracy of CUP (continued)

Tissue of Origin Test, Author, Year	Sample size	Gold Standard (Comparison Test)	Site	Sample Size	Sensitivity	Specificity
CancerTypeID Kerr, Schnabel 2012[7] Clinical Cancer Research	790	Diagnosis adjudicated prior to blind TOO testing	Adrenal	25	0.96 (0.80–1.00)	0.99 (0.98–1.00)
			Brain	25	0.96 (0.80–1.00)	1.00 (0.99–1.00)
			Breast	25	0.80 (0.56–0.94)	1.00 (0.99–1.00)
			Cervix adenocarcinoma	25	0.72 (0.47–0.90)	0.99 (0.99–1.00)
			Endometrium	25	0.48 (0.26–0.70)	1.00 (0.99–1.00)
			Gastroesophageal	25	0.65 (0.41–0.85)	0.99 (0.99–1.00)
			Germ cell	25	0.83 (0.61–0.95)	1.00 (0.99–1.00)
			GIST	25	0.92 (0.74–0.99)	1.00 (0.99–1.00)
			Head-neck-salivary	25	0.88 (0.68–0.97)	0.99 (0.98–1.00)
			Intestine	25	0.85 (0.62–0.97)	0.98 (0.97–0.99)
			Kidney	30	0.97 (0.83–1.00)	1.00 (0.99–1.00)
			Liver	25	0.96 (0.80–1.00)	1.00 (0.99–1.00)
			Lung-adeno/large cell	25	0.65 (0.43–0.84)	1.00 (0.99–1.00)
			Lymphoma	25	0.84 (0.64–0.95)	1.00 (0.99–1.00)
			Melanoma	25	0.88 (0.69–0.97)	1.00 (0.99–1.00)
			Meningioma	25	1.00 (0.80–1.00)	1.00 (0.99–1.00)
			Mesothelioma	25	0.87 (0.66–0.97)	1.00 (0.99–1.00)
			Neuroendocrine	50	0.98 (0.89–1.00)	1.00 (0.99–1.00)
			Ovary	40	0.86 (0.71–0.95)	0.98 (0.97–0.99)
			Pancreaticobiliary	30	0.88 (0.68–0.97)	0.99 (0.98–1.00)
			Prostate	25	1.00 (0.80–1.00)	1.00 (0.99–1.00)
			Sarcoma	60	0.95 (0.86–0.99)	0.99 (0.97–0.99)
			Sex cord stromal tumor	25	0.80 (0.59–0.93)	1.00 (0.99–1.00)
			Skin basal cell	25	1.00 (0.80–1.00)	1.00 (0.99–1.00)
			Squamous	30	0.86 (0.68–0.96)	0.98 (0.97–0.99)
			Thymus	25	0.72 (0.51–0.88)	1.00 (0.99–1.00)
			Thyroid	25	0.96 (0.80–1.00)	1.00 (0.99–1.00)
			Urinary bladder	25	0.64 (0.41–0.83)	0.99 (0.98–1.00)

Table C-5. KQ 3b-f. Evidence of accuracy of the tissue-of-origin test in classifying the origin and type of the tumor: Site-specific accuracy of CUP (continued)

Tissue of Origin Test, Author, Year	Sample size	Gold Standard (Comparison Test)	Site	Sample Size	Sensitivity	Specificity
CancerTypeID Ma, 2006[9]	Validation: 119 FFPE	Known origin	1.Adrenal 2. Brain 3. Breast 4. Carcinoid–intestine 5. Cervix–adeno 6. Cervix–squamous 7. Endometrium 8. Gallbladder 9. Germ–cell 10. GIST 11. Kidney 12. Leiomyosarcoma 13. Liver 14. Lung–adeno–large cell 15. Lung–small 16. Lung–squamous 17. Lymphoma 18. Meningioma 19. Mesothelioma 20. Osteosarcoma 21. Ovary 22. Pancreas 23. Prostate 24. Skin–basal cell 25. Skin–melanoma 26. Skin–squamous 27. Small and large bowel 28. Soft-tissue 29. Stomach–adeno 30.Thyroid–follicular–papillary 31.Thyroid–medullary 32.Urinary bladder 33. Overall	1. 1 2. 3 3. 1 4. 2 5. 2 6. 3 7. 3 8. 0 9. 9 10. 3 11. 4 12. 3 13. 2 14. 3 15. 5 16. 3 17. 10 18. 3 19. 5 20. 2 21. 5 22. 3 23. 7 24. 4 25. 4 26. 3 27. 6 28. 8 29. 3 30. 3 31. 0 32. 6 33. 481	FFPE Accuracy 1. 1.00 2. 1.00 3. 1.00 4. 1.00 5. 0.50 6. 0.67 7. 0.67 8. - 9. 0.78 10. 1.00 11. 1.00 12. 0.33 13. 1.00 14. 0.00 15. 0.40 16. 1.00 17. 1.00 18. 1.00 19. 0.80 20. 1.00 21. 1.00 22. 1.00 23. 1.00 24. 0.75 25. 0.75 26. 1.00 27. 0.83 28. 0.88 29. 0.00 30. 1.00 31. - 32. 1.00 33.119	NR

Table C-5. KQ 3b-f. Evidence of accuracy of the tissue-of-origin test in classifying the origin and type of the tumor: Site-specific accuracy of CUP (continued)

Tissue of Origin Test, Author, Year	Sample size	Gold Standard (Comparison Test)	Site	Sample Size	Sensitivity	Specificity
CancerTypeID Weiss et al., 2012[13]	N=123	Final clinicopathological diagnosis	1. Lung 2. Urinary/bladder 3. Breast 4. GI 5. Kidney	NS	1. CTID=75, IHC=67 2. CTID=75, IHC=42 3. CTID=73, IHC=55 4. CTID=77, IHC=77 5. CTID=77, IHC=77	NR
CancerTypeID Wu F, 2012[14]	N=184	Clinicopathological diagnosis	1. Adrenal 2. Breast 3. GIST 4. Intestine 5. Liver 6. Lymphoma 7. Prostate 8. Thyroid 9. Germ-cell 10. Neuroendocrine 11. Kidney 12. Lung 13. Skin 14. Gallbladder 15. Melanoma 16. Urinary Bladder 17. Sarcoma 18. Pancreas 19. Head & Neck 20. Ovary 21. Gastroesophageal 22. Mesothelioma 23. Endometrium	1. 2 2. 10 3. 6 4. 8 5. 6 6. 5 7. 7 8. 10 9. 10 10. 9 11. 8 12. 8 13. 6 14. 5 15. 5 16. 10 17. 13 18. 7 19. 10 20. 14 21. 12 22. 7 23. 6	1. 1.000 2. 1.000 3. 1.000 4. 1.000 5. 1.000 6. 1.000 7. 1.000 8. 1.000 9. 1.000 10. 0.889 11. 0.875 12. 0.875 13. 0.833 14. 0.800 15. 0.800 16. 0.800 17. 0.769 18. 0.714 19. 0.700 20. 0.643 21. 0.583 22. 0.571 23. 0.333	1. 0.995 2. 0.994 3. 1.000 4. 0.994 5. 1.000 6. 0.994 7. 1.000 8. 1.000 9. 1.000 10. 0.983 11. 0.989 12. 0.983 13. 0.994 14. 0.983 15. 0.994 16. 0.971 17. 1.000 18. 0.994 19. 1.000 20. 0.976 21. 0.988 22. 1.000 23. 0.994

Table C-5. KQ 3b-f. Evidence of accuracy of the tissue-of-origin test in classifying the origin and type of the tumor: Site-specific accuracy of CUP (continued)

Tissue of Origin Test, Author, Year	Sample size	Gold Standard (Comparison Test)	Site	Sample Size	Sensitivity	Specificity
miRview Mueller 2011[18]	89 [excludes 12 cases of prostate cancer]	Known origin	89 (Samples with known TOO)	1. 0% 2. 72.2% 3. 100% 4. 94.12% 5. 100% 6. 87.5% 7. 100% 8. 60% 9. 100% 10. 0% 11. 75% Prostate: 9 of 12 incorrectly classified	1. 100% 2. 95.8% 3. 89.4% 4. 98.6% 5. 100% 6. 78.1% 7. 90.3% 8. 97.6% 9. 98.8% 10. 97.7% 11. 95.3% Overall 95%	NR

Table C-5. KQ 3b-f. Evidence of accuracy of the tissue-of-origin test in classifying the origin and type of the tumor: Site-specific accuracy of CUP (continued)

Tissue of Origin Test, Author, Year	Sample size	Gold Standard (Comparison Test)	Site	Sample Size	Sensitivity	Specificity
miRview Rosenfeld 2008[20]	83 (blinded test set)	Known origin	1. Bladder 2. Brain 3. Breast 4. Colon 5. Endometrium 6. Head & neck 7. Kidney 8. Liver 9. Lung 10. Lung pleura 11. Lymph node 12. Melanocytes 13. Meninges 14. Ovary 15. Pancreas 16. Prostate 17. Sarcoma 18. Stomach 19. Stromal 20. Testis 21. Thymus 22. Thyroid	1. 2 2. 5 3. 5 4. 5 5. 3 6. 8 7. 5 8. 2 9. 5 10. 2 11. 5 12. 5 13. 3 14. 4 15. 2 16. 2 17. 5 18. 7 19. 2 20. 1 21. 2 22. 3	Decision Tree 1. 0 2. 100 3. 60 4. 40 5. 0 6. 100 7. 100 8. 100 9. 80 10. 50 11. 60 12. 60 13. 100 14. 75 15. 50 16. 100 17. 40 18. 71 19. 100 20. 100 21. 100 22. 100	Decision Tree 1. 100 2. 100 3. 97 4.99 5.99 6.99 7.99 8.99 9.95 10. 99 11. 100 12. 97 13. 99 14. 97 15. 100 16. 100 17. 99 18. 96 19. 100 20. 100 21. 98 22. 100

Table C-5. KQ 3b-f. Evidence of accuracy of the tissue-of-origin test in classifying the origin and type of the tumor: Site-specific accuracy of CUP (continued)

Tissue of Origin Test, Author, Year	Sample size	Gold Standard (Comparison Test)	Site	Sample Size	Sensitivity	Specificity
miRview Rosenwald 2010[21]	204 validation samples; 7 metastases from patients whose primary tumor was previously profiled. 188 passed QA	Known origin	Validation 1. Biliary tract 2. Brain 3. Breast 4. Colon 5. Esophagus 6. Head and neck 7. Kidney 8. Liver 9. Lung 10. Melanoma 11. Ovary 12. Pancreas 13. Prostate 14. Stomach or esophagus 15. Testis 16. Thymus 17. Thyroid	Validation only 1. 7 2. 11 3. 38 4. 9 5. 1 6. 3 7. 10 8. 8 9. 26 10. 7 11. 13 12. 6 13. 20 14. 7 15. 8 16. 6 17. 24	(1 or 2 predictions) 1. 66.7% 2. 100% 3. 66.7% 4. 88.9% 5. 100% 6. 100 7. 87.5% 8. 100% 9. 91.3% 10. 85.7% 11. 84.6% 12. 50% 13. 89.5% 14. 40% 15. 100% 16. 83.3% 17. 100%	(1 or 2 predictions) 1. 94% 2. 100% 3. 93.6% 4. 94.4% 5. 98.4% 6. 92.4% 7. 99.4% 8. 99.4% 9. 84.9% 10. 97.8% 11. 100% 12. 97.8% 13. 99.4% 14. 98.9% 15. 100% 16. 97.8% 17. 98.2%
PathworkDx Beck 2011[24]	42 (39 on panel)	Known origin	NR	NR	1. 100% 2. 0% 3. 0%	1. 100% 2. 0% 3. 0%

Table C-5. KQ 3b-f. Evidence of accuracy of the tissue-of-origin test in classifying the origin and type of the tumor: Site-specific accuracy of CUP (continued)

Tissue of Origin Test, Author, Year	Sample size	Gold Standard (Comparison Test)	Site	Sample Size	Sensitivity	Specificity
PathworkDx Grenert, Smith 2011[26]	37	95% (81.8 - 99.3)	1. Bladder 3 2. Breast 2 3. Colorectal 3 4. Gastric 2 5.Testicular gem cell 3 6. Kidney 4 7. Hepatocellular 3 8. Nonsmall cell lung 2 9. Non-Hodgkin's lymphoma 3 10. Melanoma 2 11. Ovarian 3 12. Pancreas 2 13. Prostate 1 14 Sarcoma 3 15 Thyroid 1	1. 3 2. 2 3. 3 4. 2 5. 3 6. 4 7. 3 8. 2 9. 3 10. 2 11. 3 12. 2 13. 1 14. 3 15. 1	1. 3 2. 2 3. 3 4. 2 5. 2 6. 4 7. 3 8. 2 9. 3 10. 2 11. 2 12. 2 13. 1 14. 3 15. 1	NR
Pathwork Tissue of Origin Endometrial Test Lal, 2012[30]	75	Clinical diagnosis	1. Ovarian 2. Endometrial	1. 30 2. 45	1. 42/45; 0.967 (0.817–0.986) 2. 29/30; 0.933 (0.828–0.999)	
PathworkDx Monzon 2009[33]	547	Known origin	1. Bladder 2. Breast 3. Colorectal 4. Gastric 5. Germ Cell 6. Hepatocellular 7. Kidney 8. Melanoma 9. Non-Hodgkin's lymphoma 10. Non–small cell lung 11. Ovarian 12. Pancreas 13. Prostate 14. Soft tissue sarcoma 15. Thyroid	1. 28 2. 68 3. 56 4. 25 5. 30 6. 25 7. 39 8. 26 9. 33 10. 31 11. 69 12. 25 13. 26 14. 31 15. 35	1. 22/28 2. 64/68 3. 52/56 4. 18/25 5. 22/30 6. 23/25 7. 37/39 8. 21/26 9. 31/33 10. 27/31 11. 64/69 12. 18/25 13. 23/26 14. 26/31 15. 32/35	1. 519/519 2. 471/479 3. 487/491 4. 519/522 5. 517/517 6. 521/522 7. 507/508 8. 520/521 9. 511/514 10. 509/516 11. 473/478 12. 521/522 13. 521/521 14. 513/516 15. 510/512

Table C-5. KQ 3b-f. Evidence of accuracy of the tissue-of-origin test in classifying the origin and type of the tumor: Site-specific accuracy of CUP (continued)

Tissue of Origin Test, Author, Year	Sample size	Gold Standard (Comparison Test)	Site	Sample Size	Sensitivity	Specificity
PathworkDx Pillai 2011[35]	462	Known origin	1. Bladder 2. Breast 3. Colorectal 4. Gastric 5. Hepatocellular 6. Kidney 7. Melanoma 8. Non-Hodgkin's lymphoma 9. Nonsmall cell lung 10 Ovarian 11 Pancreas 12 Prostate 13. Sarcoma 14. Testicular germ cell 15. Thyroid	1. 29 2. 57 3. 36 4. 25 5. 25 6. 28 7. 25 8. 29 9. 27 10. 45 11. 28 12. 25 13. 27 14. 25 15. 31	1. 23/29 2. 55/57 3. 33/36 4. 18/25 5. 24/25 6. 25/28 7. 21/25 8. 26/29 9. 23/27 10. 40/45 11. 24/28 12. 24/25 13. 24/27 14. 21/25 15. 28/31	NR
PathworkDx Stancel 2011[37]	20 Passed QA: 19	Known origin	1. Lung (4) 2. Lymphoma (2) 3. Colon (1) 4. Pancreas (1) 5. Breast (4) 6. Ovarian (5) 7. Gastric (2)		Agreement: 1. 3/4 2. 2/2 3. 0/1 4. 1/1 5. 3/4 6. 5/5 7. 1/2	NR
PathworkDx Wu 2010[38]	15 Results, 1 off-panel specimen: 14 Sample size = 13	Known origin	1. Lung 2. Breast 3. Melanoma 4. Lymphoma 5. Sarcoma 6. Colon 7. Head & Neck (off-panel) 8. Gastric	1. 3 2. 3 3. 2 4. 3 5. 1 6. 1 7. 1 8. 1	Accuracy of test: 1. 1/3 2. 3/3 3. 2/2 4. 3/3 5. 1/1 6. 1/1 7. 0/1 8. 1/1	NR

Abbreviations: FFPE = formalin-fixed paraffin-embedded; GIST = gastrointestinal stromal tumor; IHC = immunohistochemistry; N= number; NR = not reported; QA = quality assurance; TOO = tissue of origin; USCAP = United States and Canadian Academy of Pathology.

Table C-6. KQ 4. Evidence of ability of tissue of origin tests to identify the primary tumor site in CUP cases

Tissue of Origin Test	First Author, Publish Year	TOO Predicted Result (Confirmed)	Indeterminate Results	# Cases Clinically Useful
CancerTypeID	Erlander, Ma 2011[1]	296 (142)	4	252
	Greco 2010[2]	18 (15)	2	NR
	Schroeder[11]	91%		Pretest diagnosis unknown: CTID diagnosed 59 (93%) cases Differential diagnosis provided: CTID resolved dx in 55% cases. Provided new primary dx: 36%
	Thompson[12]	CTID=144 (By latent primary: 18/24 (75%); by IHC or clinical features: 101/144 (70%))	5	Confirmed 1 of 2-3 IHC diagnoses: 43/97
G-banded karyotype (supplemented by FISH and comparative genomic hybridization	Pantou 2003[15]	5/20 identified cases 2/20 no mitoses 1/20 normal karyotype	NR	NR
miRview	Mueller 2011[18]	50 (40) 10 were discordant 4 origin never diagnosed	NR	NR
	Pavlidid 2012[19]	84/92 (92%)	8	NR
	Varadhachary 2011[22]	74 (62)	NR	IHC not helpful: 9 TOO prediction: 9 TOO consistent with clinicopathological: 7

Table C-6. KQ 4. Evidence of ability of tissue of origin tests to identify the primary tumor site in CUP cases (continued)

Tissue of Origin Test	First Author, Publish Year	TOO Predicted Result (Confirmed)	Indeterminate Results	# Cases Clinically Useful
Pathwork	Azueta 2012[23]	Total: TOO: 32 (25/29) IHC: 28 (20/29) Peritoneal: TOO: 7 (7) IHC: 5 (5) Ovarian: TOO: 18 (22) IHC: 15 (22) Unknown: TOO: 2 IHC: 2	NR	NR
	Beck 2011[24]	4 (2 clearly incorrect)	3	2
	Dumur 2011[39]	Overall: 29/40 (28); On panel: 28/29; Off panel: 4 discordant, 9 unreported	3 inadequate sample: 1 indeterminate, 2 discordant. Off panel: 2 indeterminate	29/40. Confirmed original diagnosis: 23 Changed diagnosis: 5 Excluded suspected diagnosis: 1 TOO results obtained "several months" before IHC and imaging results.
	Hainsworth 2011[27]	43	2	NR
	Laouri, 2011[31]	Non-specific dx: 172 Specific Dx: 100 (New 57; Confirm 43)	No Specific Dx: 11 Specific Dx: 1	NR
	Monzon 2010[32]	16 (10) 6 plausible	5	16
	Nystrom 2012[34]	NR	NR	Changed diagnosis: 54/107 Provide diagnosis for patients with no working diagnosis: 27/44 No change in tissue site diagnosis: 37/107 Physician found test clinically useful: 66%

Abbreviations: Dx/dx = diagnosis; FISH = fluorescent in situ hybridization; IHC = immunohistochemistry; NR = not reported; TOO = tissue of origin

Table C-7. KQ 4a. Evidence of genetic tissue-of-origin tests changed treatment decisions

Tissue of Origin Test, Author, Year	# Eligible	Response	Sample Requirements	Identity Score	Indicated TOO	Treatment	Treatment Response	TOO Did NOT Change Treatment	TOO Changed Treatment	P-value TOO vs. CUP
CancerTypeID Greco 2012[3]	32	32	Colorectal gene expression signature		Colorectal cancer	First-line: Folfox/Folfiri 19 Other colorectal cancer drug: 4 Empiric: 8 (6/8 received CRC treatment for second-line) First-line CRC treatment: 17/23 (74%) Second-line CRC: 7/13 (54%) Any first-line treatment: 22/32 (69%) Any second-line treatment 7/13 (54%)		Not applicable: retrospective study	Not applicable: retrospective study	

Table C-7. KQ 4a. Evidence of genetic tissue-of-origin tests changed treatment decisions (continued)

Tissue of Origin Test, Author, Year	# Eligible	Response	Sample Requirements	Identity Score	Indicated TOO	Treatment	Treatment Response	TOO Did NOT Change Treatment	TOO Changed Treatment	P-value TOO vs. CUP
CancerTypeID Hainsworth 2010[5]	110	Received assay directed treatment: 66 Evaluated: 51	Not a treatable C-subset of CUP No previous systematic treatment ECOG performance status 0–2 No uncontrolled brain metastases	NS	Pancreatic: 11 Colorectal: 8 Urinary Bladder: 8 Nonsmall cell lung: 5 Ovarian: 4 Carcinoid - interstine: 4 Breast: 3 Gallbladder: 3 Liver: 3 Renal cell: 3 Skin (squamous): 3 Other: 7 No specific diagnosis: 5	Assay directed treatment	Objective treatment response: 21	44 (40%)	66 (60%)	51% response for assay directed therapy
CancerTypeID Hainsworth, 2011[4]	125	42 (34%)	NS	NS	Identified by TOO test with ≥ 80% probability as colorectal adenocarcinoma	1. 1st line: Advanced colorectal cancer: 24 2.Empirical for CUP: 18 3. 2nd line: CRC" 16	1. 12/24 2. 17% 3. 8/16	NR	32	p=0.0257

Table C-7. KQ 4a. Evidence of genetic tissue-of-origin tests changed treatment decisions (continued)

Tissue of Origin Test, Author, Year	# Eligible	Response	Sample Requirements	Identity Score	Indicated TOO	Treatment	Treatment Response	TOO Did NOT Change Treatment	TOO Changed Treatment	P-value TOO vs. CUP
CancerTypeID McGee et la, 2011[10]	62	22	Squamous cell lung tumors	NR	Squamous cell lung cancer	Lung cancer therapy: 15/20 Head and neck cancer therapy: 2/20 Colorectal cancer therapy: 1/20 Unknown/ missing:2		Not applicable: retrospective study	Not applicable: retrospective study	
CancerTypeID Thompson, 2011[12]	NR	NR	NR	NR	Colorectal Cancer	NR	Response: 70% NR	NR	NR	NR
PathworkDx Laouri 2011[31]	284 Non-specific dx: 183 Specific Dx: 101	284	NR	SS	TOO Predicted: 221 (78%) Colorectal (15%) Breast (15%) Ovary (13%) Pancreas (13%) Nonsmall Cell Lung (11%) Hepatocellular (11%) Sarcoma, Kidney, Gastric, Other (78%)	Change in first line therapy: Initial Non-specific Primary Site: 135 Major, 37 Minor, 11 None Specific Primary site: 43 Major; 14 Minor; 44 None	NR	55 (19%)	229 (81%)	NR

Table C-7. KQ 4a. Evidence of genetic tissue-of-origin tests changed treatment decisions (continued)

Tissue of Origin Test, Author, Year	# Eligible	Response	Sample Requirements	Identity Score	Indicated TOO	Treatment	Treatment Response	TOO Did NOT Change Treatment	TOO Changed Treatment	P-value TOO vs. CUP
PathworkDx Nystrom 2012[34]	316 physi-cians	65 physicians 107 patients	Survey	NR	NR	NR	NR	17	Any change: 70/107 (65%, 95% CI: 58%-73%) Chemotherapy changed: 58/107 (54%, 95% CI:46%–62%) Radiation therapy: 27/107 (25%) Increase in consistency with guidelines: 23%	NR

Abbreviations: CI = confidence interval; CRC = colorectal cancer; CUP = cancer of unknown primary; ECOG = Eastern Cooperative Oncology Group; NR = not reported; NS = not significant; TOO = tissue of origin.

Table C-8. KQ 4b. Evidence of genetic tissue-of-origin tests changed outcomes

Tissue of Origin Test, Author, Year	# Eligible	# Partici-pated	Cancer/ Tumor Types	Sample Require-ments	Score	Indicated TOO	Outcome	Effect of TOO	p-value TOO vs. CUP
CancerTypeID Greco 2012[3]	32 CTID: 21 Veridex: 11	32	CUP	As marketed	NR	Colorectal cancer based on CTID or Veridex	Median survival:	Total sample: 21 months (95% CI: 17.1–24.9) First-line or second-line CRC treatment: (29 patients) 22 months	
							2-year survival:	First-line CRC treatment: 22 months	
							4-year survival:	Total sample: 42% Total sample: 35% No Control Group	
CancerTypeID Hainsworth, 2012[6] 2008–2011	223 historic controls	Empiric CUP therapy: 29 Site-specific therapy: 194	CUP	NR	NR	Biliary tract, urothelium, colorectal, lung, pancreas, breast, ovary, gastroesophageal, kidney, liver, sarcoma, cervix, neuroendocrine, prostate, germ cell, skin, intestine, mesothelioma, thyroid, endometrium, melanoma, lymphoma, head and neck, adrenal	Median survival	TOO: 12.5 months (95% CI: 9.1–15.4) Historical control: 9.1 months	

Table C-8. KQ 4b. Evidence of genetic tissue-of-origin tests changed outcomes (continued)

Tissue of Origin Test, Author, Year	# Eligible	# Partici-pated	Cancer/ Tumor Types	Sample Require-ments	Score	Indicated TOO	Outcome	Effect of TOO	p-value TOO vs. CUP
CancerType ID Hainsworth, 2011[4]	125	42 (34%)	CUP or other Identified by TOO as colo-rectal adeno-carcinoma	As marketed	Identified by TOO test with ≥80% probability as colorectal adenocarinoma	Colorectal adenocarinoma	Survival TOO: 8.5 months Control: Empirical CUP 6 months	NR	0.11
CancerTypeID Thompson, 2011[12]	NR	NR	CUP with colorectal TOO result	NR	NR	Colorectal cancer	Median survival	21 months No control group	
G-banded karyotype (supplemented by FISH and comparative genomic hybridization Pantou, 2003[15]	NR	NR	5/20 identified cases 2/20 no mitoses 1/20 normal karyotype	NR	NR	NR	Median survival	Simple chromosoma l changes: 19.7 months Complex changes: 3 months No control group	
PathworkDx Hornberger 2012[28]	308 treating physicians who ordered TOO test	65 (21%)	Any	As marketed	NR	NR	Overall survival: projected increase, 15.9–19.5 months Average estimated increase in survival adjusted for QOL: 2.7 months	No control group	

Table C-8. KQ 4b. Evidence of genetic tissue-of-origin tests changed outcomes (continued)

Tissue of Origin Test, Author, Year	# Eligible	# Partici-pated	Cancer/ Tumor Types	Sample Require-ments	Score	Indicated TOO	Outcome	Effect of TOO	p-value TOO vs. CUP
PathworkDx Nystrom et al., 2012[34]	NR	NR	CUP	NR	NR	NR	Survival 14 months 95% CI (10.2–18.6) 2-year survival 33% 3-year survival 30%	CRC (19%) Breast (14%) Sarcoma (14%) Lung (11%) Ovarian (11%) Pancreas (10%)	

Abbreviations: CI = confidence interval; CRC = colorectal cancer; CUP = cancer of unknown primary; FISH = fluorescent in situ hybridization; NR = not reported; QOL = quality of life; TOO = tissue of origin.

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Appendix D. Quality Assessment Ratings of Studies

Title (Year)	KQ2 and KQ3	KQ4
Barr (1995)[1]	G	NA
Beck (2011)[2]	G	F
Bridge (2006)[3]	F	F
Delattre (1994)[4]	F	F
Dumur (2008)[5]	G	NA
Dumur (2008) Assessing the impact of tumor devitalization time (poster)[6]	F	NA
Dumur (2008) Clinical verification of the Pathwork TOO Test (abstract)[7]	G	NA
Erlander (2011)[8]	G	NA
Gamberi (2011)[9]	F	NA
Greco (2010)[10]	G	NA
Grenert (2011)[11]	G	NA
Hainsworth, Pillai et al. (2011)[12]	NA	G
Hainsworth, Schnabel et al. (2011)[13]	NA	F
Hainsworth, Spigel et al. (2010) (poster)[14]	NA	G
Hornberger (2012)[15]	NA	F
Kerr (2012)[16]	G	NA
Lae (2002)[17]	G	NA
Laouri (2010)[18]	NA	F
Lewis (2007)[19]	G	NA
Ma (2006)[20]	G	NA
Mhawech-Fauceglia (2006)[21]	G	NA
Monzon (2009)[22]	G	NA
Monzon (2010)[23]	G	NA
Mueller (2011)[24]	F	F
Pantou (2003)[25]	NA	G
Patel (2005)[26]	G	NA
Pillai (2010)[27]	G	NA
Rosenfeld (2008)[28]	G	NA
Rosenwald (2010)[29]	G	NA
Stancel (2011)[30]	G	G
Varadhachary (2011)[31]	G	G
Wu (2010)[32]	G	NA
Yamaguchi (2005)[33]	F	NA
Dumur (2011)[34]	G	NA
Greco (2012)[35]	NA	F
Kerr (2012)[36]	G	NA
Lal (2012)[37]	G	NA
Laouri (2011)[38]	NA	F
McGee (2011)[39]	NA	F
Pollen (2011)[40]	NA	F
Schroeder (2012)[41]	NA	F
Wu (2012)[42]	G	NA
Nystrom (2012)[43]	NA	G
Meiri (2012)[44]	G	F
Azueta (2012)[45]	G	NA
Hainsworth (2012)[46]	NA	F
Kulkarni (2012)[47]	G	NA
Pavlidis (2012)[48]	NA	G
Thompson (2011)[49]	NA	F
Weiss (2012)[50]	G	G

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Appendix E. Excluded Articles

Exclusion Code Key

EXC1= Wrong intervention
EXC2 = Wrong publication type
EXC3 = Wrong study design
EXC4 = No relevant outcomes to Key Questions
BKG = Excluded for BKG

Excluded at Abstract Stage

1. ASCO-NCI-EORTC immunohistochemistry meta-analysis. 2008 Molecular Markers. hand search
2. . CDKN2A mutations and MC1R variants in Italian patients with single or multiple primary melanoma. Pigm Cell Melanoma R. 2008 2008/12//;21(6):700+. EXC1
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Appendix F. Strength of Evidence Assessment Table

Table F-1. Overview of study outcomes

Key Question	Number of Studies	Conclusion	Strength of Evidence
KQ 2. Analytic validity: CancerTypeID	1	Only one study that was conducted by manufacturer of test. Limited measures of analytic validity reported and impossible to assess consistency of those measures across studies.	Insufficient
KQ 2. Analytic validity: miRview miRview mets	4	Four studies each reported different measures of analytic validity. Impossible to evaluate consistency of reported measures of analytical validity across studies.	Insufficient
KQ 2. Analytic validity: PathworkDx	3	Three papers reported measures of analytic validity. There is moderate consistency between studies of interlaboratory reproducibility. The reported precision in these three papers is high.	High
KQ 2. Analytic validity: PathworkDx Endometrial	1	One study reported on the analytic validity of a PathworkDx Endometrial test. The reported precision of the test is high, but there is insufficient evidence to assess analytic validity.	Insufficient
KQ 3a. Adherence to Simon guidelines: CancerTypeID	2	Report on the development of the algorithm has sufficient detail on development and validation to assess the validity of the process. Development met the Simon[3] criteria.	High
KQ 3a. Adherence to Simon guidelines: miRview mets	2	Report on development of algorithm has sufficient detail on development and validation to assess the validity of the process. Development met the Simon[3] criteria.	High
KQ 3a. Adherence to Simon guidelines: PathworkDx	2	Report on development of algorithm does not have sufficient detail on development and validation to assess the validity of the process.	Low
KQ 3a. Adherence to Simon guidelines: PathworkDX endometrial	1	Report on development of algorithm does not have sufficient detail on development and validation to assess the validity of the process.	Low
KQ 3b. Accuracy of the TOO test in classifying the origin and type of tumors of known primary site: CancerTypeID	7	Seven studies representing 1,476 tissue samples compared the ability of tests to identify origin of tumor in tissues of known origin. All report accuracy of inclusion. Accuracy of exclusion reported in two studies.	High
KQ 3b. Accuracy of the TOO test in classifying the origin and type of tumors of known primary site: miRview mets	4	Two independent studies representing 898 tissue samples tested the ability of the miRview to identify site of origin in tissues of known origin. Accuracies of inclusion and exclusion are reported for both.	High
KQ 3b. Accuracy of the TOO test in classifying the origin and type of tumors of known primary site: PathworkDx	9	Nine studies representing 1,247 tissue samples report on the ability of the test to identify the origin of tumor in tissues of known origin. Accuracy of included tissue is reported in all studies. Accuracy of excluded tissues reported in two studies.	High
KQ 4. Percentage of cases for which a TOO test identified a TOO	17	Fifteen studies rated fair provided evidence on this question. The ability of the test to identify a TOO was judged using different criteria in different studies.	Moderate
KQ 4. Percentage of CUP cases for which the TOO identified by the test was independently confirmed	6	Five studies all rated fair provided evidence for this question. Gold standard varied across studies and precision across studies was moderate. Accuracy ranged from 57% to 100%.	Low
KQ 4: Percentage of CUP cases where TOO modified diagnosis	6	Four studies rated fair and one poster rated good provided evidence for this question. Some explored potential changes; others actual changes. The reported percentage of changes ranged from 65% to 81%. Response rates in the survey were fairly low.	Low

Key Question	Number of Studies	Conclusion	Strength of Evidence
KQ 4: Percentage of CUP cases where test was considered clinically useful by physician or researcher	6	All studies found test clinically useful in a proportion of cases. Clinical usefulness was measured differently in each study. Wide estimate in the proportion of cases where test was useful.	Low
KQ 4: Change in treatment decisions	5	Studies have small samples, varied study designs and measures of effect on treatment decisions, making it difficult to draw conclusions on any of the tests.	Insufficient
KQ 4: Treatment response: Tissue-specific treatment based on TOO test compared with usual treatment for CUP cases	4	Small samples, insufficient studies to draw conclusions on any of the tests. Only one study has a control; all others use historical controls.	Insufficient
KQ 4: Change in survival	5	Small samples, insufficient studies to draw conclusions on any of the tests. Most use historical controls.	Low
KQ 4: Change in disease progression	1	Small samples, insufficient studies to draw conclusions on any of the tests.	Insufficient
KQ 5: Applicability	22	Almost all studies included patients age 65 and older and both male and female patients. The studies that reported race included mostly Caucasian patients.	High

Appendix G. Genetic Testing in the Diagnosis of Ewing Sarcoma

Genetic Testing in the Diagnosis of Ewing Sarcoma

Introduction

Soft tissue small round cell tumors (SRCTs) are a heterogeneous group of neoplasms that predominate in childhood and adolescence and share similar morphological features, consisting of dense proliferation of small undifferentiated round cells. Rhabdomyosarcomas, peripheral neuroepitheliomas, Ewing sarcoma (ES) family, and non-Hodgkin's lymphomas are the prototypic SRCTs.[1, 2] The recently described desmoplastic SRCT and rhabdoid tumors are SRCTs. The tumors that make up this group are listed in Table G-1.[2, 3]

Table G-1. Small Round Cell Tumors

Ewing sarcoma
Peripheral neuroectodermal tumor
Rhabdomyosarcoma
Synovial sarcoma
Non-Hodgkin's lymphoma
Retinoblastoma
Neuroblastoma
Hepatoblastoma
Nephroblastoma

The most common SRCTs are the ES family. ES is a highly malignant tumor that commonly occurs in bone and soft tissues of children and adolescents but is occasionally seen in adults.[4] It is the second most common pediatric bone tumor, accounting for 30 percent of all primary bone tumors. The ES family of tumors shares common immunohistology and molecular genetics and is considered to be one single group.[5] The pathognomonic genetic marker of the ES family of tumors is the presence of the balanced translocation t(11;22)(q24;q12) that creates the EWS/FLI1 fusion gene and results in the expression of an abnormal protein. Approximately 85 percent of patients have the translocation t(11;22)(q24;q12), and 10 percent of patients have the translocation t(22;21)(q22;q12).[6, 7] Molecular identification of the specific type of translocation, as well as tumor type, has prognostic significance for SRCTs. Patients with localized ES disease and tumor expressing type 1 EWS-FLI1 fusion transcript have longer disease-free survival than those with other fusion transcript types.[8]

The annual incidence of ES for 1973 and 2004 in the United States was 2.93 cases/1,000,000.[9] ES is much more common in white populations and has a slight male predominance. In 15 percent to 30 percent of patients, metastases are present at the time of diagnosis. The most common sites for metastases are lungs (50%), bone (25%), and bone marrow (20%).[10] The presence of metastases significantly affects long-term survival of patients with ES. Although multidisciplinary care has improved the survival rate of patients with localized ES to nearly 70 percent, these advances have not significantly changed the long-term outcome for those with metastatic disease, where 5-year survival remains less than 25 percent.[11]

Other mesenchymal tumors with specific translocations that should be considered in the differential diagnosis of ES include alveolar rhabdomyosarcoma and synovial sarcoma. Rhabdomyosarcoma is predominantly a disease of children and adolescents accounting for 5 percent to 8 percent of cancers in the pediatric population. Alveolar rhabdomyosarcoma is the most aggressive type of rhabdomyosarcoma and can be distinguished from other tumors by the detection of a PAX-FKHR gene translocation.[12] Alveolar rhabdomyosarcomas containing a PAX7-FKHR translocation are usually less invasive and have a better prognosis than those with the PAX3-FKHR gene translocation.[13]

Similarly, synovial sarcomas frequently present during the second decade of life and should be considered in differential diagnosis along with other mesenchymal tumors that occur during adolescence. In synovial sarcoma, the SYT gene on chromosome 18 is fused to a member of the SSX gene family (t(X;18)(p11;q11)).[12] Ladanyi et al. have reported that cases involving the SYT-SSX1 gene fusion have a worse prognosis.[14]

The diagnostic distinctions among the SRCTs are becoming increasingly important as specific, successful treatments are developed for each tumor. However, differential diagnosis has been difficult because the histologic criteria for distinguishing the subtypes are relatively subtle, and there is no well-established immunohistological marker that is differentially expressed by these tumors. Therefore, the identification of consistent chromosomal translocations and fusion regions associated with a majority of the tumor types is an important advantage in the differentiation of the SRCTs and a base for molecular testing.[15] Molecular diagnostics, using either fluorescent in situ hybridization (FISH) to detect the fusion gene or reverse transcriptase polymerase chain reaction (RT-PCR) to detect its transcript, is developed for clinical use and is now a routine part of pathological examination. In addition to aiding in diagnosis, RT-PCR enables the detection of circulating metastatic tumor cells in blood.[10] The growing needs for efficient genetic identification have led to development and application of commercially available molecular assays for detecting the chromosomal translocations and fusions in clinical samples of the SRCTs. The commercially available assays are listed in Table G-2.

Methods

As discussed in Chapter 2, we designed the literature searches to identify papers on tissue of origin (TOO) tests for cancers of unknown primary site, which is the primary purpose of this review. The literature searches identified nine articles on genetic testing for the diagnosis of ES and other SRCTs. During the abstraction process, we became aware that a substantial portion of the literature on genetic testing for the diagnosis of SRCTs had not been identified by the searches. Upon further investigation, it became clear that the issues involved in assessing genetic testing for diagnosing small round cell tumors differed from those for CUP TOO tests. A systematic review of genetic testing for diagnosing small round cell tumors would have somewhat different key questions and required different search strategies. After discussion with Agency for Healthcare Research and Quality (AHRQ), we decided to focus resources on the CUP TOO test.

We report here on the limited review we completed on genetic testing for diagnosis of SRCTs. Literature search strategies, study eligibility, data management, data abstraction, and quality assessment of the articles were conducted as reported in the Methods chapter of the TOO report. We did not grade the strength of the evidence because the review is not comprehensive.

Table G-2. ES commercially available assays

Name of test	Manufacturer	How Marketed?	FDA Approval	Sample Requirements	Laboratory Analysis Method	Type of Tumors Identified	Number of Tumors in Reference Database	Reported Results	Statistical Analysis Method
EWSR1 (22q12) Gene rearrangement by FISH: Vysis LSI EWSR1 Dual Color Break Apart Probe	Abbot Molecular http://www.abbottmolecular.com/products/oncology/fish/vysis-ewsr1-break-apart-fish-probe-kit.html#	Kit	Approved	Formalin-fixed paraffin-embedded tissue	Fluorescence in situ DNA hybridization	ES Primitive neurodec-todermal tumor Clear cell sarcoma	NA	Signal pattern	NA
EWSR1 (22q12) gene rearrangement by FISH	Quest Diagnostics Nichols Institute http://www.questdiagnostics.com/hcp/testmenu/jsp/showTestMenu.jsp?fn=16112.html&labCode=SEA	Service	Not submitted		Fluorescence in situ DNA hybridization	ES Primitive neurodec-todermal tumor Clear cell sarcoma	NA	Signal pattern	NA
ES by RT-PCR	ARUP Laboratories http://www.aruplab.com/guides/ug/tests/0051220.jsp			Fresh frozen tumor tissue. formalin fixed paraffin-embedded tissue block, or unstained sections on charged slides. 100 mg or 0.5–2.0 cm^3	Reverse transcription polymerase chain reaction/ fluorescence monitoring	ES	NA	Not found	NA

Abbreviations: FISH = fluorescent in situ hybridization; NA = not applicable; RT-PCR = reverse transcriptase polymerase chain reaction

Tumor-specific rearrangements were first identified and tested using G-banded karotypes.[14] Over the last two decades, however, fluorescent in situ hybridization (FISH) or reverse transcriptase polymerase chain reaction (RT-PCR) has become the technique of choice for identifying such gene rearrangements. Both of these techniques rely on nucleic acid probes that bind to the sample DNA. Nucleic acid probes may differ in length and sequence, which affects the precise area of binding. Because there is variation in the splice points between the two chromosome segments, the rearrangement may have different characteristics. The probes used in the tests affect the performance of the test, so combining evidence across tests that use different probes must be done with caution.

Results

A kit is commercially available for FISH testing—the Vysis LSI EWSR1 Dual Color Break Apart Probe kit, sold by Abbott Laboratories—but some laboratories, such as the Mayo Clinic Medical Laboratories, develop their own probes. Quest Diagnostics offers FISH testing for ES as a laboratory test rather than as a kit. ARUP Laboratories offers both a FISH and an RT-PCR test for ES diagnosis.

Analytic Validity

We identified nine studies[4, 12, 15-21]that examined the analytic validity of genetic tests (RT_PCR, FISH, or karotype) for the diagnosis of ES (Table G-3). Five studies[12, 15, 18-20] were rated good, and four[4, 16, 17, 21] were rated fair. All studies that performed RT-PCR used noncommercial probes for the analysis. Three good studies[17, 19, 20] and two fair studies[16, 21] reported on experience using the commercially available FISH Dual Color Break Apart Probe produced by Vysis, Inc. Mhawech-Faucegalia et al.[19] compared the Vysis FISH probe to immunohistochemistry (IHC) analysis. They found that FISH performed poorly when tissue preservation was poor. Five of 58 analyses failed entirely, and a translocation was detected in only 50 percent of cases. The specificity of FISH was 100 percent.

Three studies compared the ability of the Vysis, Inc. break apart EWS (22q12) FISH probe and RT-PCR to detect pathonomic translocations in ES and other SRCTs. Neither test was clearly better. Patel et al.[20] found that RT-PCR analysis detected fusion transcripts in all eight of their samples, but the FISH probe detected translocations in only seven (88%) samples. Gamberi et al.[17] reported on their clinical experience. RT-PCR was performed first; FISH, using the Vysis, Inc. probes, was performed if the sample was inadequate for RT-PCR or if RT-PCR was negative. RT-PCR or FISH produced interpretable results in 188 of 222 ES cases (85%). RT-PCR detected a transcript in 121 cases, and FISH identified a translocation in 23 more cases. Bridge et al.[16] examined the sensitivity and specificity of the Vysis, Inc. FISH break apart probe, FISH fusion, and RT-PCR. The gold standard was the histopathologic diagnosis. They reported that 12 percent to 16 percent of cases were uninformative, depending on the technique, and that the concordance between FISH and RT-PCR was 67 percent, primarily due to the lower sensitivity of the RT-PCR assay.[16] Sensitivity and specificity of the two FISH assays were equivalent. The way the number of cases is reported is very unclear, however, and the proportions could not be verified from the reported data. Yamaguchi et al.[21] also compared RT-PCR with the Vysis Inc. FISH probe. FISH detected translocations in 14 of 16 ES/primitive neuroectodermal embriogenic tumor (PNET) cases. RT-PCR results were only available for seven cases; a transcript was detected for five. Each method detected one translocation that was not detected by the other method.

Table G-3. KQ 3. Evidence of analytic validity of TOO tests for ES

TOO Test, Author, Year Study Dates, Region	Sample Characteristics	Cancer/Tumor Types	Range of *Sensitivity* for Measuring the Markers	Range of *Specificity* for Measuring the Markers	QC Measures for Markers	Other
ES (RT-PCR) Barr, 1995[15] 1988–1993 U.S. only Good	Age: NR Female: NR N: 79	Alveolar rhabdomyosarcoma, embryonal rhabdomyosarcoma, ES, desmoplastic small round cell tumor, undifferentiated small round cell tumor, other	Translocations identified on karotype: 11/11 Fusions not identified by karotype: 4 Translocations identified by FISH: 11/12	RT-PCR compared with FISH: 19/19	Technical success RT-PCR: 79/80 FISH: 31/41 Karotyping: 29/74	NR
ES (RT-PCR) Bridge, 2006[16] 2004 U.S. only Fair	Age: NR Female: NR N: 66	ES/primitive neuroectodermal tumors undifferentiated round cell sarcomas, small cell carcinomas, neuroblastomas, alveolar rhabdomyosarcomas, fibrosarcomas, malignant teratoma	ES/primitive neuroectodermal tumors: FISH break apart: 20/22 FISH fusion: 20/22 RT-PCR: 7/19 [recreated from article; does not match sensitivity in Table 1]	FISH break apart: 36/36 [recreated from article] FISH fusion: 34/34 [recreated from article] RT-PCR: 85% [stated in article; unable to determine actual numbers]	Technical success of FISH break: 59/67 Technical success of FISH fusion: 56/66 Technical success of RT-PCR: 36/43	NR
ES (RT-PCR) Delattre, 1994[4] 1993 NR Fair	Age: Range: 1 to 48 Median: 13 Female: NR N: 114	Osseous ES, atypical ES, peripheral primitive neuroectodermal, not ES tumors, lacking hallmarks of specific disease	t(11:22): 23/23 RT-PCR+ complex/variant translocations: 8/9 RT-PCR+ no rearrangement of 11, 21, or 22: 6/8 RT-PCR+	NR	NR	NR

Table G-3. KQ 3. Evidence of analytic validity of TOO tests for ES (continued)

TOO Test, Author, Year Study Dates, Region	Sample Characteristics	Cancer/Tumor Types	Range of *Sensitivity* for Measuring the Markers	Range of *Specificity* for Measuring the Markers	QC Measures for Markers	Other
ES (RT-PCR) Gamberi, 2011[17] 2006–2009 Multinational, not U.S. Fair	Age: NR Female: 73 N: 222	ES family tumors	Interpretable RT-PCR or FISH: 188/222 RT-PCR +: 144 FISH+: 24	NR	Tissue quality standards: Inadequate tissue ≤ 1,000 tumor cells, poor RNA quality [A260/280 < 1.6] or negative RT-PCR results FISH: ≥ 100 tumor cell nuclei counted	Required percentage of valid markers: Positive FISH: translocation > 10% of the cell QC standards for assay: RNA integrity: Primers for β-actin. RT-PCR: Positive controls with translocation confirmed by sequencing. Negative controls: normal tissue, other types of tumors, and a water blank
ES (RT-PCR) Lae, 2002[18] 1992–2000 U.S. only Good	Age: Range: 6–54 Mean: 25 years 0–9: 1 10–24: 14 25–49: 15 50–64: 2 65+: 0 Female: 3% N: 32	Extraskeletal ES, primitive neuroectodermal tumor, rhabdomyosarcoma, intraabdominal small round cell tumor, intraabdominal carcinoma	28/29 (Southern blot confirmation) No molecular analysis: 2	1/1	NR	

Table G-3. KQ 3. Evidence of analytic validity of TOO tests for ES (continued)

TOO Test, Author, Year Study Dates, Region	Sample Characteristics	Cancer/Tumor Types	Range of *Sensitivity* for Measuring the Markers	Range of *Specificity* for Measuring the Markers	QC Measures for Markers	Other
ES (RT-PCR) Lewis, 2007[12] NR U.S. only Good	Age: Range: 1–26 0–9: 9 10–24: 39 25–49: 2 Female: 15/50 N: 69 (2 cases had multiple tumors)	ES Controls: ES, neuroblastoma, leiomyosarcoma, desmoplastic sarcoma, synovial sarcoma, rhabdomyosarcoma	NR	NR	Failed RNA extraction: 17% 4 had Cp > 35 for housekeeping control gene	QC standards for assay: Two real-time RT-PCR systems employed for detecting transcripts. As a control for cDNA synthesis and sample quality, each sample reverse transcribed and amplified for the housekeeper gene MRPL19
ES (FISH) Mhawech-Fauceglia, 2006[19] NR U.S. only Good	Age: NR Female: NR N: 58	ES	50%	100%	NR	NR
ES (RT-PCR) Patel 2005[20] NR U.S. only Good	Age: NR Female: NR N: 42	Clear cell sarcoma, malignant melanoma	Concordance between duplicate cores (Pearson coefficient): Clear cell sarcoma: 0.99 Malignant melanoma: 0.97	NR	NR	NR

Table G-3. KQ 3. Evidence of analytic validity of TOO tests for ES (continued)

TOO Test, Author, Year Study Dates, Region	Sample Characteristics	Cancer/Tumor Types	Range of *Sensitivity* for Measuring the Markers	Range of *Specificity* for Measuring the Markers	QC Measures for Markers	Other
ES (FISH and RT-PCR) Yamaguchi, 2012[21] NR Multinational, not U.S. Fair	Age: 10–24: 7 25–49: 13 50–64: 6 65+: 2 NR: 9 Female: 13 N: 37	ES/primitive neuroectodermal tumor, desmoplastic small round cell tumor, clear cell sarcoma Negative controls: Poorly differentiated synovial sarcoma, alveolar rhabdomyosarcoma, neuroblastoma	RT-PCR compared with FISH: ES/primitive neuroectodermal tumor: 4/6 Desmoplastic small round cell tumor: 6/6 Clear cell sarcoma: 5/5	RT-PCR compared with FISH: 3/9	NA	NA

Abbreviations: FISH = fluorescent in situ hybridization; NR = not reported; RT-PCR = reverse transcriptase polymerase chain reaction

Fusion transcripts were detected by both methods in all six cases of desmoplastic small-round-cell tumor (DSRCT) and in five of six cases of clear cell sarcoma.

One good study[15] and one fair study[4] compared RT-PCR, karyotype, and FISH, although not all tumors were tested with all three tests. In one study,[15] standard cytogenetic analysis (G-banded karyotype) was successful in 29 of 74 (39%) cases and identified translocations in 11 of the 29 cases. In the second study,[4] a translocation was identified by karyotype in 23 of 40 (58%) of cases. RT-PCR identified fusion transcripts in all cases with a translocation identified by karyotype, plus 4[15] and 6[4] additional cases. In the Delattre study,[4] FISH identified one translocation not identified by RT-PCR. Barr et al.[15] also tested 41 cases for the PAX3-FKHR transcript associated with alveolar rhabdomyosarcoma using RT-PCR and FISH. FISH produced definitive results in 31 cases; RT-PCR results were concordant with FISH in 30 cases. Compared with FISH, the sensitivity of RT-PCR was 92 percent (11/12) and specificity was 100 percent.

Lae et al.[18] validated RT-PCR detection of EWS-WT1 fusion transcripts by Southern blot. A fusion transcript was detected in 28 of 30 patients. Southern blot detected one transcript not detected by RT-PCR (RT-PCR sensitivity: 97%). One tumor had no tumor transcript detected by either RT-PCR or Southern blot even though its clinicopathologic and immunohistochemical profile was typical of SRCT.

Lewis et al.[12] reported relatively high failure rates for RT-PCR. Sixteen of 69 (23%) samples failed the analysis: 12 (17%) failed RNA extraction and 4 (6%) were excluded because of poor sample quality. Extraction or PCR failure was not associated with sample age.

Clinical Validity

Nine studies[4, 12, 15-21] examined the clinical validity of genetic tests (RT-PCR, FISH, or karotype) for the diagnosis of ES (Table G-4). Five studies[12, 15, 18-20] were rated good, and four were rated fair.[4, 16, 17, 21]

Four studies,[16, 17, 19, 21] two good[17, 19] and two fair,[16, 21] reported on the sensitivity and specificity of FISH to diagnose ES or other SCRT by detecting specific chromosomal translocations. The sensitivity ranged from 50 percent (19 of 38)[19] to 91 percent.[16] The two studies rated good reported lower sensitivity of FISH—50 percent[19] and 66 percent[17]—than the two studies rated fair—88 percent[21] and 91 percent.[16] In all four studies,[16, 17, 19, 21] specificity was 100 percent (15/15).

Eight studies[4, 12, 15-18, 20, 21] reported on the ability of RT-PCR to diagnosis ES and other SCRTs by detecting fusion transcripts associated with specific tumors. Four studies[12, 15, 18, 20] were rated good, and four were rated fair.[4, 16, 17, 21] In 7 of the 8 studies, the sensitivity of RT-PCR ranged from 70 percent[20] to 93 percent.[18] The exception reported RT-PCR sensitivity to be 54 percent.[16] As previously noted, the reporting for this study[16] was unclear, and the reported sensitivity could not be verified from the data presented in the article. Specificity for RT-PCR ranged from 85 percent[16] to 100 percent.[4, 12, 17, 20] The quality of the study was not related to the reported sensitivity or specificity.

Table G-4. KQ 3b–3f. Evidence of accuracy of the TOO test in ES

TOO test, Author, year Study Dates, Region	Sample Characteristics	Cancer/tumor types	Sample size	Gold standard (comparison test)	Sensitivity	Specificity
ES (FISH) Mhawech-Fauceglia, 2006[19] NR U.S. only Good	Age: NR Female: NR N: 58	ES	53	IHC	19/38 (0.50: assumes 38 is the total number of cases with successful FISH analysis, not number of rearranged, based on information in text)	15/15 (1.00)
ES (FISH) Yamaguchi, 2012[21] NR Multinational, not U.S. Fair	Age: 10–24: 7 25–49: 13 50–64: 6 65+: 2 Female: 13 N: 28	ES/primitive neuroectodermal tumor, desmoplastic small round cell tumor, clear cell sarcoma Negative controls: Poorly differentiated synovial sarcoma, alveolar rhabdomyosarcoma, neuroblastoma	37	Clinico-pathological assessment	ES/PNET: 14/16 DSRCT: 6/6 CCS : 5/6	0/9
ES (RT-PCR) Barr, 1995[15] 1988–1993 U.S. only Good	Age: NR Female: NR N: 79	Alveolar rhabdomyosarcoma, embryonal rhabdomyosarcoma, ES, desmoplastic small round cell tumor, undifferentiated small round cell tumor, other.	Expected positive: 1. Alveolar rhabdomyo-sarcoma=21 2. ES=8 3. Desmo-plastic small round cell tumor=3 Expected negative 4. Embryonal rhabdomyosarcoma=30 5. Undiffer-entiated small round cell tumor=7 6. Other=10	Clinicopathological assessment	Positives among expected positives 1. 18/21 2. 6/8 3. 3/3 Overall: 27/32 (84%)	Negatives among expected negatives 4. 28/30 5. 5/7 6. 9/10 Overall: 42/47 (89%)

Table G-4. KQ 3b–3f. Evidence of accuracy of the TOO test in ES (continued)

TOO test, Author, year Study Dates, Region	Sample Characteristics	Cancer/tumor types	Sample size	Gold standard (comparison test)	Sensitivity	Specificity
ES (RT-PCR) Bridge, 2006[16] 2004 U.S. only Fair	Age: NR Female: NR N: 66	ES/primitive neuroectodermal tumor undifferentiated round cell sarcomas, small cell carcinomas, neuroblastomas, alveolar rhabdomyo-sarcomas, fibrosarcomas, malignant teratoma	N = 67 FISH break apart method N = 66 FISH fusion method N = 43 RT-PCR	Clinico-pathological assessment	FISH break apart: 91% (actual numbers not reported, cannot be calculated from provided data) FISH fusion: 91% RT-PCR: 54%	FISH break apart: 100% FISH fusion: 100% RT-PCR: 85%
ES (RT-PCR) Delattre, 1994[4] 1993 NR Fair	Age: Range: 1 to 48 Median: 13 Female: NR N: 114	Osseous ES, atypical ES, peripheral primitive neuroectodermal, not ES tumors, lacking hallmarks of specific disease	114	Clinicopathological assessment	83/87	Non-ES: 0/12 Undifferen-tiated: 9/15
ES (RT-PCR) Gamberi, 2011[17] 2006–2009 Multinational, not U.S. Fair	Age: NR Female: 73 N: 222	ES family tumors	188	IHC	144/156	SRCT: 0/4 Non-EFT/SRCT: 0/28

Table G-4. KQ 3b–3f. Evidence of accuracy of the TOO test in ES (continued)

TOO test, Author, year Study Dates, Region	Sample Characteristics	Cancer/tumor types	Sample size	Gold standard (comparison test)	Sensitivity	Specificity
ES (RT-PCR) Lae, 2002[18] 1992–2000 U.S. only Good	Age: Range: 6–54 Mean: 25 years 0–9: 1 10–24: 14 25–49: 15 50–64: 2 65+: 0 Female: 3% N: 32	Extraskeletal ES, 32 primitive neuroectodermal tumor, rhabdomyosar-coma, intraabdominal small round cell tumor, intraabdominal carcinoma		Clinico-pathological assessment	RT-PCR = 28/30 (93%) Southern blot hybridization=29/30 (97%)	NR
ES (RT-PCR) Lewis, 2007[12] NR U.S. only Good	Age: Range: 1–26 0–9: 9 10–24: 39 25–49: 2 Female: 15/50 N: 69 (2 cases had multiple tumors)	ES Controls: ES, neuroblastoma, leiomyosarcoma, desmoplastic sarcoma, synovial sarcoma, rhabdomyosarcoma	ES: 53 non ES: 11	IHC	41/50 (82%)	0/11
ES (RT-PCR) Patel, 2005[20] NR U.S. only Good	Age: NR Female: NR N: 42	Clear cell sarcoma, malignant melanoma	Clear cell sarcoma N = 10 Malignant melanoma N = 32	Known origin	7/10 (70%)	0/32 (100%)

Table G-4. KQ 3b–3f. Evidence of accuracy of the TOO test in ES (continued)

TOO test, Author, year Study Dates, Region	Sample Characteristics	Cancer/tumor types	Sample size	Gold standard (comparison test)	Sensitivity	Specificity
ES (RT-PCR) Yamaguchi[21], 2012 NR Multinational, not U.S. Fair	Age: 10–24: 7 25–49: 13 50–64: 6 65+: 2 Female: 13 N: 37	ES/PNET, DSRCT, CCS Negative controls: Poorly differentiated synovial sarcoma, alveolar rhabdomyosarcoma, neuroblastoma	37	Clinico-pathological assessment	RT-PCR: ES/PNET: 5/7 DSRCT: 6/6 CCS: 5/5	6/9

Abbreviations: CCS =clear cell sarcoma; DSRCT = desmoplastic small-round-cell tumor; EFT =Ewing family tumor; ES = Ewing sarcoma; FISH = fluorescent in situ hybridization; IHC = immunohistochemistry; NA = not applicable; NR = not reported; PNET = primitive neuroectodermal embriogenic tumor; RT-PCR = reverse transcriptase polymerase chain reaction; SRCT = soft tissue small round cell tumors; TOO = tissue of origin

Clinical Utility

One study[17] rated good reported on the usefulness of molecular genetic tests for the diagnosis of SRCTs. Molecular analysis was informative in 188 of 222 (85%) cases with a presumptive diagnosis of ES. The molecular analysis protocol of RT-PCR analysis followed by FISH in cases that were failed or negative by RT-PCR had a sensitivity of 92 percent and specificity of 100 percent.

Discussion

This review of genetic tests for the diagnosis of SCRTs is neither complete nor systematic. The literature reviewed here was identified ancillary to our systematic review of genetic or molecular tests for CUP origin. Even this limited review suggests that molecular genetic tests are valuable aids to the challenge of diagnosing SCRTs and differentiating them from other tumors with similar morphology and pathology. Both molecular techniques had good analytic and clinical sensitivity and specificity. Only two studies[4, 15] compared molecular analysis to G-banded karyotype, but both demonstrated that the molecular techniques have much higher success and sensitivity. Neither FISH nor RT-PCR was clearly superior. Each technique missed translocations that were identified by the other technique. Although a definite conclusion cannot be made from this review, our results suggest that a combined protocol, such as that used by Gamberi et al.,[17] would provide the best sensitivity in clinical practice.

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