Technology Assessment

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

Technology Assessment Program

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We wish to acknowledge individuals listed below for their review of this report. This report has been reviewed in draft form by individuals chosen for their expertise and diverse perspectives. The purpose of the review was to provide candid, objective, and critical comments for consideration by the EPC in preparation of the final report. Synthesis of the scientific literature presented here does not necessarily represent the views of individual reviewers.

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

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 site (CUP). 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 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, miReview, 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 insufficient to confirm validity. 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 MiReview. 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 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 the accuracy (with 95% CI) of the three tests in classifying tumors of known origin were: CancerType 83 percent (78% to 86%); miReview 85 percent (83% to 88%) and for PathworkDx it was 87 percent (86% to 89%). The accuracy of the tests in CUP cases is not easily determined, since actual TOO is not identified in most cases. The evidence that the TOO tests contributed to the diagnosis of CUP was moderate. The evidence was insufficient to answer key questions (KQs) on the effect of the tests on treatment or outcomes.

Conclusions: The clinical accuracy of all the three tests is similar, ranging from 83 percent to 87 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 test be evaluated by research groups that have no evident conflict of interest. Publication bias cannot be ruled out. 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 cost effectiveness 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 and 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:

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 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 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. All three genomic tests had more than two studies included in the review that assessed the ability of the tests to correctly identify tests of known origin. Meta-analytic techniques were used to generate summary measures across these studies.

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.

The PathworkDx TOO test also had four studies that assessed its ability to detect the TOO in true CUP cases. 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. The other two tests only had two studies that estimated the accuracy of the test in CUP patients.

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 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. 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 microarray tests (CancerTypeID, miReview, 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 was available for all of the three microarray TOO tests, but it was insufficient to assess the analytic validity of the tests. Two out of three tests have sufficient information included in publication to allow one to assess the validity of the statistical algorithms used in the TOO. PathworkDx TOO manuscripts 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. There are at least three studies that report on the ability of each of the three microarray tests to identify the primary site for tumors of known origin. The accuracy rates across all of the studies for each of the three tests are fairly consistent.

The summarized accuracy rate for CancerTypeID was 0.83 with 95% CI (0.78 to 0.86), for miReview it was 0.85 with 95% CI (0.83 to 0.88), and for PathworkDx it was 0.87 with 95% CI (0.86 to 0.89). 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. In two studies with independent confirmation of TOO, the test identified the correct TOO in 75% of cases. Given the lack of a gold standard in most reports, we have judged the strength of the evidence as low that the tests accurately detect the TOO. The evidence that TOO tests affect treatment decisions was also low. 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, miReview, and PathworkDx. Of the three microarray tests, CancerTypeID has the broadest panel with the ability to detect 29 different tumor sites; miReview has 25 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 A summarizes our findings.

The literature on genetic microarray 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 two papers that used CancerTypeID as the TOO test. One focused on colorectal cancer and the other on all CUP cases. There is no study with a sufficient sample size that compares outcomes between patients who received tissue-specific therapy and those who did not.

As mentioned above, 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.

Future Research:

The most urgent need in the literature is to have the test 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.

Table A. 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: MiReview	3	Three 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	1	Two papers from the same multi-site study reported different measures of analytic validity. Impossible to evaluate consistency of reported measures of analytical validity across studies	Insufficient
KQ 3a. Adherence to Simon guidelines: CancerTypeID	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: MiReview	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 3b – 3f. Accuracy of the TOO test in classifying the origin and type of tumors of known primary site: CancerTypeID	3	Three studies with relatively small sample sizes have compared the ability of tests to identify origin of tumor in tissues of known origin. All report accuracy of inclusion. Accuracy of exclusion is reported only in one MS.	Moderate
KQ 3b – 3f. Accuracy of the TOO test in classifying the origin and type of tumors of known primary site: MiReview	3	Two independent studies with over a hundred specimens each tested the ability of the miReview to identify site of origin in tissues of known origin. Accuracy of inclusion and exclusion are both reported.	Moderate
KQ 3b – 3f. Accuracy of the TOO test in classifying the origin and type of tumors of known primary site: PathworkDx	7	Seven studies including a prospective blinded study with over 500 specimens report of 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. Percent of cases with for which a TOO test identified a TOO	11	All studies reviewed found the test provided useful guidance in diagnosis. TOO tests indicate a TOO almost all cases.	Moderate
KQ 4:Percent of cases with for which a TOO test identified a TOO call	2	Only two studies, one for PathworkDx and one for CancerTypeID had independent confirmation of the site of origin identified by the TOO test. When independent confirmation of the TOO test was available, 75% of the calls were confirmed.	Low
KQ 4: Percent of CUP cases where test was considered clinically useful by physician or researcher	4	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	4	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 to usual treatment for CUPS cases	1	Small samples, insufficient studies to draw conclusions on any of the tests.	Insufficient
KQ 4: Change in Survival	2	Small samples, insufficient studies to draw conclusions on any of the tests.	Insufficient
KQ 4: Change in Disease Progression	1	Small samples, insufficient studies to draw conclusions on any of the tests.	Insufficient

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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.[6] Over half a million deaths in 2011 are 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[7] suggest that malignant growth is a result of a series of genetic changes in cells, which cause six essential modifications in cell physiology. These are: 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.[7]

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 "anti-cancer" drugs. Chemotherapy is always a part of the regimen when a patient has metastatic cancer. Patients who are diagnosed as having Stage III or Stage IV cancer usually have metastatic cancer (i.e., their disease has spread from the primary organ to a distant organ). 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 or at least the cancer cell type.

Cancer of Unknown Primary

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).[8] 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, Lungs
Kidney	Lung, Liver, Bones
Lungs	Adrenal Gland, Liver, Lungs
Melanoma	Skin, Muscle, Liver
Ovary	Liver, Peritoneum, Lungs
Pancreas	Liver, Lungs, Peritoneum
Prostrate	Lung, Liver, Bones
Rectum	Liver, Peritoneum, Adrenal Gland
Stomach	Liver, Peritoneum, Lungs
Thyroid	Lung, Liver, Bones
Uterus	Liver, Peritoneum, Lungs

Source: National Cancer Institute.

Naresh[9] 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 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,[10] 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 estimates that more than 30,000 cases of CUP will be diagnosed in 2011.[6] Tong et al.[11] 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[12] 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 cancer of unknown primary 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.[13]

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.[12] Identification of the primary can also 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,[14] 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.[14]

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.[14] IHC markers help to define tumor lineage by identifying antigens expressed in the tumor that are specific to tumor type. Keratin subtype is a commonly used IHC marker for identifying the primary site in CUP. The 20 subtypes of keratins have different expression profiles in various cell types and tumors. For example, Keratin 20 (K20) is normally expressed in the gastrointestinal (GI) epithelium, urothelium, and Merkel cells, while Keratin 7 (K7) is found in tumors of the lung, ovary, endometrium, and breast. Figure 1demonstrates the approach to IHC testing using K7 and K20 in CUP.[14] This initial IHC test narrows the possible sites, then sequential testing with additional

markers in Table 2 further narrow the probable site of the primary.[14] 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.[15] Dennis et al.[16] 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 K7 and K20 to narrow the field of possible primary sites in CUP patients

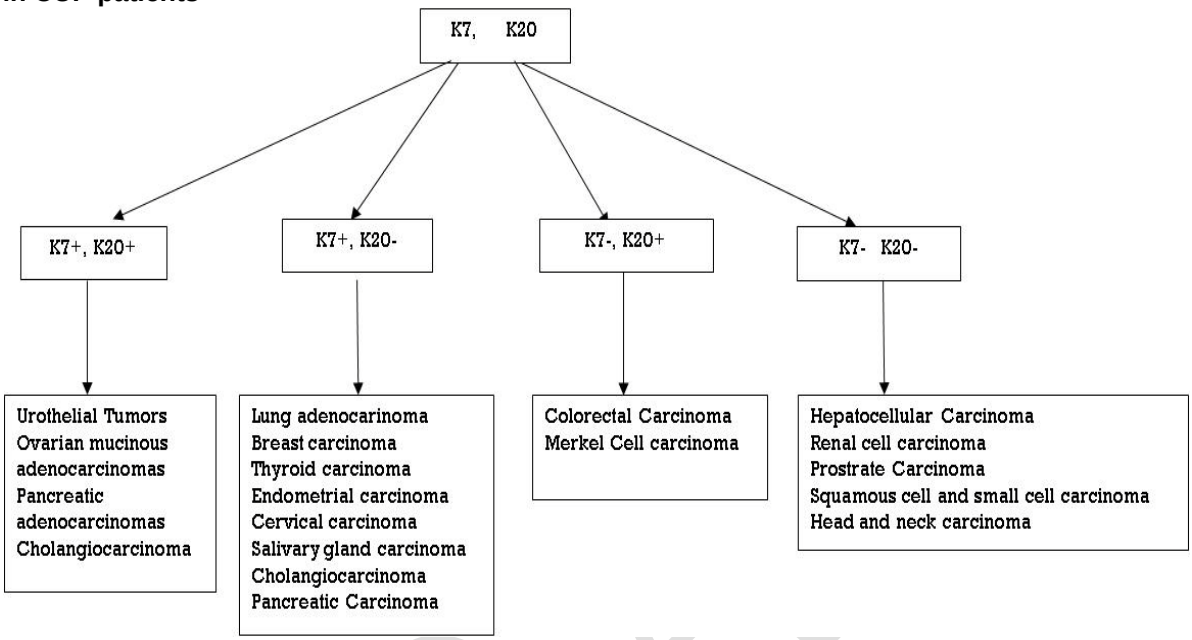


Table 2. Markers used to narrow possible primary sites after tissue is tested for K7 and K20

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.

Computed Tomographic (CT) Scans

CT scans combine X-rays taken from various angles into 3D images, providing better views of organ structure, and can therefore detect much smaller tumors than a chest x-ray. The vast majority of CUP that are identified are lung or pancreatic carcinomas, so chest and abdomen CT scans are routinely done to detect occult primary tumors. For women, mammograms and pelvic CT scans are included in the routine work up. Physical exam, a thorough medical history, and knowledge of common primary-metastasis relationships can suggest areas for additional CT scans.

Positron Emission Tomographic (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.[17] PET imaging alone can detect 8 to 53 percent of CUP primary sites; it has been most effective in detecting occult primaries in the head and neck.[17] Trials that have used PET for patients with suspected occult primaries in the head and neck detected a primary tumor in 20 to 31 percent of the cases with a false positive rate of about 20 percent.[17] 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 to 35 percent.[17, 18]

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.[19] Ramaswamy et al.[20] 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.[21]

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, i.e., 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 Health

Care Quality and Research Methods Guide for Medical Test Reviews (MGMTR)[1] 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 studies do not differ widely. In cases where the estimates vary widely or when the test threshold varies by study, the summary maybe most usefully represented by a line that describes how the average sensitivity varies with average specificity. In both cases, the MGMTR recommend using a multivariate meta-analytic approach in order 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.

There are two families of hierarchical models that the MGMTR recommends: The bivariate model or 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. Thus unlike a typical study within which sensitivity is measured on cases and specificity is measured in controls and are independent, 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 intervals around this measure of accuracy. The studies in the review have 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 choose to represent a single summary accuracy measure and its 95% CI across the various studies. We have done this only for clinical accuracy. Eight studies are summarized to obtain a summary measure of test accuracy in classifying tumors of known tissue for PathworkDx; the corresponding numbers for miReview and CancerTypeID are four and three respectively. PathworkDx TOO was the only test that 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 four studies. We therefore decided against creating a summary estimate.

Key Questions (KQs) 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 summarize 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 describe 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 inter-laboratory 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 multi-marker 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.[3] and on the accuracy and specificity of the genetic TOO test when compared to 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 improved 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 KQ 1 – KQ 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 KQ 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 the identification of tumor TOO in addition to or instead of other methods such as IHC staining or PET imaging.

Comparators for KQ 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 KQ 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

Follow up of any length after test results received

Setting

Includes studies conducted in the United States or internationally
Includes testing on patients admitted to 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 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 because a preliminary search indicated that there were very few non-English studies.. This limitation may bias the report if publication patterns changed after the preliminary search. Complete search strategies are provided in Appendix B.

Table 4. Illustrative search strategies (PubMed)

MeSH Heading Search		
Search	**Queries**	**Number of Citations**
#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 will update the literature review by repeating the initial search 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 will be investigated and, if appropriate, incorporated into the final review.

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.[1] 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[3] criteria to assess studies of diagnostic accuracy. For studies of clinical utility (KQ 4), we used questions from the RTI 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. The ratings are defined in Appendix D.

A study was rated as good if it was well designed, 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 it 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 did not identify any studies that were 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. All three tests had more than two studies included in the review that assessed the ability of the tests to identify tests of known origin. Meta-analytic techniques were used to generate summary measures across these studies.

The PathworkDx TOO also had four studies that assessed the ability of the test to detect the TOO in true CUP cases. Since 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 measure across these studies. The other two tests 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. In order 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. As Figures 4, 5, and 6 demonstrate, the sensitivities were homogeneous across studies for the same test. 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 "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 EPC Program.[1, 5] 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[3] 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 EPC 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 to the core Medicare population.

Results

Study Identification and Characteristics

The PRISMA diagram summarizes the results of the literature searches (electronic databases) (Figure 2) and Web searches (Figure 3). The two database searches yielded a total of 522 records for title and abstract review. We conducted full text review of 90 articles from the database searches and an additional 63 articles, posters, and abstracts from manufacture's Web sites and other sources. Thirty-three 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

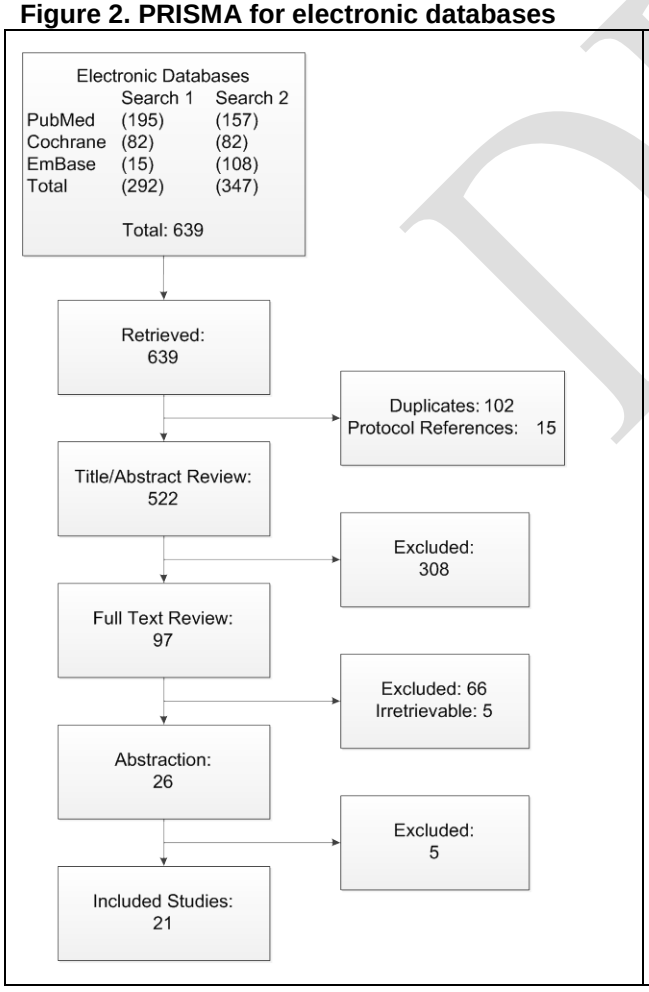
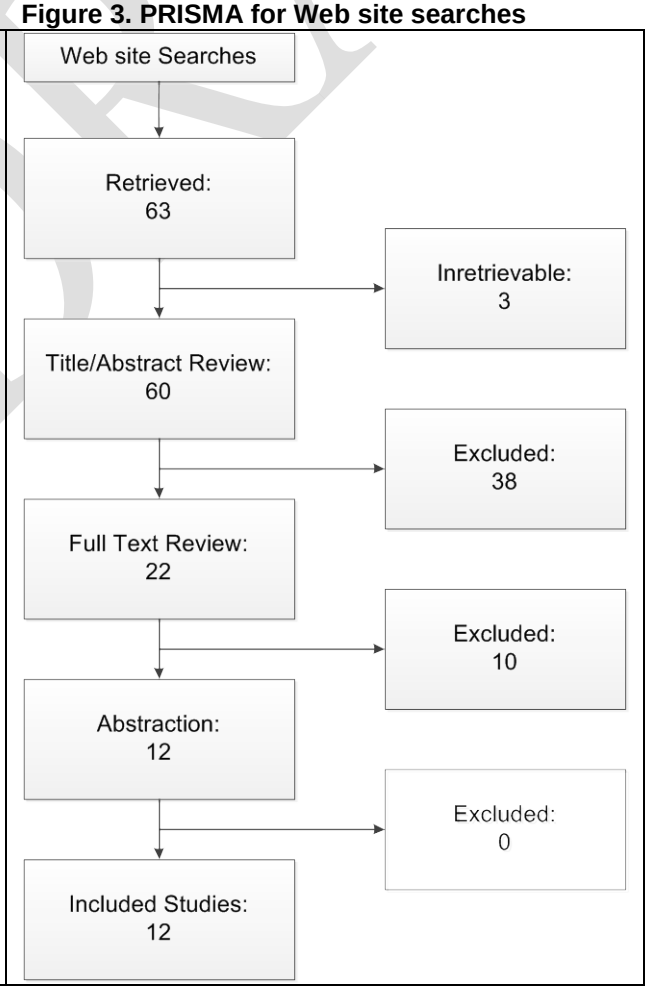


Figure 3. PRISMA for Web site searches



KQ 1. What genetic or molecular 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 site.

Tissue of Origin (TOO) Tests for Cancer of Unknown Primary Site (CUP)

We identified four genetic or molecular tests to determine TOO in CUP cases, cytogenetic analysis and three genomic assays: PathworkDx from Pathwork Diagnostics, CancerTypeID from Biotheranostics, and miRview from Rosetta Genomics (Table 5). 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. miRview was previously marketed in the United States as ProOnc Tumor Source.

All three 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. The Food and Drug Administration does not regulate laboratory services, only the sale of medical devices, i.e., a kit or chip for conducting the test. Hence, PathworkDx has been cleared by the FDA, but CancerTypeID and miRview have not submitted their tests for FDA review.

Analytic and statistical analyses vary between these tests. The PathworkDx and CancerTypeID panels are genes; the assays measure the expression of these genes (i.e. the amount of messenger RNA (mRNA)). miRview uses microRNAs (miRNA), which are small, non-coding, single-stranded RNA molecules that regulate genes post-transcription. PathworkDx measures expression of 1,550 genes and uses a pairwise comparison based on a machine learning algorithm to classify tumors as one of 15 tumor types. Results are reported as similarity scores for each of the 15 tumor types. CancerTypeID analyzes expression levels for 92 genes to detect 27 tumor types. Classification uses the K-nearest neighbor statistical methodology. miRview predicts 42 tumor types using 48 microRNAs. miRview uses two classification methodologies, a decision tree and a K-nearest neighbor algorithm.

The type of tumors covered by the three tests varied. All three tests claim to identify cancer of the bladder, breast, kidney, melanoma, lung, ovary, pancreas, prostate, sarcoma, testis, or thyroid (Table 6). Four primary sites identified by all three tests (lung, pancreas, ovary, and prostate) account for 51 percent of primary sites identified at autopsy in series published from 1980-2000.[22]

Cytogenetic analysis of tumor cells by G-banded karyotype may also provide information on the TOO in tumors of unknown primary site.[19] While cytogenetic abnormalities are common in tumors of all types, certain abnormalities are pathonomic of specific types of cancer.[23] These abnormalities, when found, can be used to diagnose the TOO of metastatic tumors.[19]

Table 5. Available genetic or molecular TOO tests for identifying cancers of unknown primary site and their characteristics

Name of test	Manufact-urer	How Marketed?	FDA Approval	Sample Requirements	Laboratory Analysis Method	Analyte	Panel size	Number of Tumors Identified	Number of Tumors in Reference Database (range by tumor type)	Reported Results	Statistical Analysis Method
PathworkDx	Pathwork Diagnostics http://www.pathworkdx.com/	Service	Cleared	Formulin-fixed paraffin embedded or unstained slides. 1 mm^2 tumor tissue. 30 ng total RNA	Gene expression microarray analysis using cDNA	mRNA	1,550[a] genes	15	2,039 (41-444)	Similarity scores	Pairwise comparisons by machine learning algorithm
Cancer-TypeID	Biotheranostics	Service	Not submitted	Formulin-fixed paraffin embedded tissue sections or unstained 10 micron sections on glass slides. At least 300 – 500 viable tumor cells.	Gene expression microarray analysis using cDNA	mRNA	92	27	578 (5-49)	Probability for each cancer type	KNN
miRview	Rosetta Genomics	Service	Not submitted	Formulin-fixed paraffin embedded tissue. 2.5 mm^2 tissue	microRNA expression using microarray platform	Micro-RNA	48	22	336 (1-49)	Tumor origin. May list multiple possibili-ties.	Binary decision tree and KNN classifier
Cytogenetic analysis	Multiple cytogenetic laboratories	Service	NA	Fresh tissue	High resolution banded chromosomes		NA	NA	NA	Karyotype	NA

Different references cite different panel sizes. More recent references seem to agree on 1,550 genes.

Abbreviations: cDNA = Complementary Deoxyribonucleic Acid; GIST = gastrointestinal stromal tumor; KNN = Kohonen neural network; microRNA = micro ribonucleic acid; mRNA = messenger ribonucleic acid; NA = not applicable; RNA = ribonucleic acid.

Table 6. Primary tumor sites identified by molecular TOO tests

Primary Site	PathworkDx	CancerTypeID	miRview
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 Lymphoma	•		
Ovary	•	•	•
Pancreas	•	•	•
Prostate	•	•	•
Sarcoma	•	•	•
Testicular Germ Cell	•	•	•
Thymus		•	•
Thyroid	•	•	•

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

Five studies rated good provided evidence to answer this KQ. Although the number of studies was small, at least some data on analytic performance was available for all of the genomic TOO tests. Analytic validity cannot be compared across tests because different data was 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] Inter-assay 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 to the positive control was 1.69 percent. Compared to the negative controls, the mean CV was 1.25 percent. For the five 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 (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 which 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 five 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

Two papers[25, 26] and a poster,[27] all rated good, provided information on the analytic validity of the miRview test. During development, the performance of the microarray platform was validated against RT-PCR analysis.[25] The expression distributions and diagnostic roles of the miRNAS were maintained across the platforms.[25] The developers of the test also confirmed that RNA quality and quantity was similar for fresh-frozen, formalin-fixed, and formalin-fixed paraffin-embedded samples. miRNA profiles were stable in FFPE for up to 11 years.[25] One hundred and seventy nine samples were independently tested at both the Rosetta Genomics research and development laboratory and the CLIA-approved clinical laboratory.[27] Interlaboratory concordance of miRNAs expression levels was greater than 95 percent for 160 of 174 samples.

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 identify of the expressed microRNAs ($C_t < 38$) and the average C_t of the measured microRNAs.[26]

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, Ma 2011[24232828] NR Multinational, US 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 Crossreaction 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 percent 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 Chajut, 2011[27] 2011 Multinational, US Good	Age: NR Female: NR N: 179	NR	Range of accuracy: interlaboratory concordance: > 0.95% in 160 samples Range of Specificity: NR Number of Outliers: NR Crossreaction with normal tissue: NR	NR	Required percent of valid markers: NR QC Standards for Assay: NR Changes in panel precision and accuracy over time: NR
miRview Rosenfeld, 2008[25] NR Multinational, not US Good	Age: NR Female: NR N: 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	Range of accuracy: NR Range of Specificity: NR Number of Outliers: NR Crossreaction with normal tissue: NR	NR	Required percent of valid markers: NR QC Standards for Assay: Array platform validated by RT-PCR. miRNSS 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 Varadhachary, 2011[28] 2010 US 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 Crossreaction with normal tissue: NR	NR	Required percent of valid markers: 87/104 samples passed tumor content criteria; 74/87 passed all QA criteria QC Standards for Assay: Controls: No sample; no RNS; external positives. Quality parameters for RNS 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 Dumar, 2008[29] NR US only Fair	Age: NR Female: NR N: 60	Breast, colorectal, con-small-cell lung, non-Hodgkins'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 to 0.87 Coefficient of reproducibility: 32.48 +/- 3.97 Range of Specificity: NR Number of Outliers: 19/227 Crossreaction with normal tissue: NR	All samples with adequate RNS quantity and quality produced sufficient cRNS for hybridization 31/227 samples required >1 labeling reaction Data verification algorithm addresses RNS quality, inadequate amplification, insufficient quantity of labeled RNS, inadequate hybridization time or temperature 218/227 gene expression data files passed verification All 9 failed files showed evidence of RNS degradation	Required percent 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 Pillai, 2010[30, 31] NR US 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 non-small cell lung cancer, as well as non-Hodgkin's lymphoma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, thyroid cancer, and sarcoma	Range of accuracy: overall interlaboratory concordance: 133/149 Range of Specificity: NR Number of Outliers: NR Crossreaction with normal tissue: NR	NR	Required percent of valid markers: percent 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

Abbreviations: NR = not reported, US = United States

PathworkDx

Two studies[30, 32] both rated as good, provided information on the analytic validity of the PathworkDx test. Performance is not affected by the age of the sample.[30] 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.[32] Assay data quality is assessed by three statistics; overall signal, percent present, and regional discontinuity. The overall signal is the mean summarized expression value of all probes; it must be ≥10. The percent present is the percentage of probes sets assigned a present call by the Affymetrix MAS 5.0 algorithm; it must be ≥5. The 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.[30] A study of inter-laboratory reliability conducted at four laboratories found that 218 of 227 (96%) gene expression data files passed verification.[32] The nine failed files all showed evidence of RNA degradation.

Inter-laboratory correlation was high.[32] Pearson correlation coefficients of between laboratory Affymetrix normalized gene expression values were 0.65 – 0.82. After standardization with the PathworkDx TOO algorithm, correlation coefficients ranged from 0.81 to 0.87. Calculated Similarity Scores 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 (κ>0.86). The Bland-Altman analysis found a high level of agreement (coefficient of reproducibility 32.48 $\pm$ 3.97) and indication of no systematic bias (<10% of specimens outside 95% limit of agreement).

Cytogenetic Analysis

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

KQ 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 for the responses to these questions. We were able to assess the validity of the statistical algorithm for CancerTypeID and MiReview but not for PathworkDx TOO test. Figures 4-6 display the accuracy estimates with 95% CI 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
miRview Rosenfeld 2008[25] NR Multinational, not US Good	Age: NR Female: NR N: 336	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	Total Expression: Based on median expression level for each probe across all samples Selected Housekeeping: NR	Hypothesis tests: NR Ranking: NR Clustering : NR	Unsupervised: NR Supervised: Decision-tree algorithm KNN algorithm	Internal: Leave one-out cross validation within the training set External: Blinded test set
miRview Rosenwald 2010[26] NR Multinational, US 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: Expression of each microRNS - average expression of all microRNSs of the sample + scaling constant (the average expression over the entire sample set) Selected Housekeeping: NR	Hypothesis tests: NR Ranking: Decision tree algorithm used that finally selected 48 miRNSs through feature selection Clustering : NR	Unsupervised: NR Supervised: Classifier that combines binary decision tree and K-nearest neighbors (KNN) trained on 649 patients	Internal: NR External: Test performance assessed using independent set of 204 validation 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
PathworkDx Dumur 2008[33] NR US only Good	Age: NR Female: NR N: 60	Breast, colorectal, non-small-cell lung, non-Hodgkins's lymphoma, lymphoma, pancreas, bladder, gastric, germ cell, hepatocellular, kidney, melanoma, ovarian, prostrate, soft tissue, sarcoma, thyroid	Total Expression: NR Selected Housekeeping: All raw expression based on aggregate expression levels for 121 mRNS markers stably expressed across cell types	Hypothesis tests: NR Ranking: Classification model uses 1,550 markers chosen by gene ranking Clustering : NR	Unsupervised: NR Supervised: Algorithm trained on 2,039 well-characterized tumor specimens	Internal: NR External: NR

Abbreviations: NR = not reported, US = 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,[34] graded good, report that the selection of the five reference genes for normalization, was based on the relatively constant expression levels of these genes across all tumor types. This is an appropriate way to select genes for normalization.

Dimension Reduction

The dimension reduction algorithm developed used a combination of K- Nearest Neighbors 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 which is equivalent to a linear discriminant classifier. This classifier that uses supervised learning and is the optimal method of classification as per Simon et al.[3] This is first described in Ma et al.[34] and a modification of the process is described in Erlander et al.[35] which was also graded as a good study. The modification described in Erlander et al.[35] adds the classification of the cancer sub–type to the algorithm.

Validation

The algorithm for CancerTypeID described first in Ma et al.[34] and then in Erlander et al.;[35] both report, both internal validation using a leave one out cross validation (LOOCV) and a separate validation in an independent test set. Simon et al.[3] suggest using at least one of the two types of validation in algorithm development and identifies validation in an independent test set as optimal. This test meets both criteria.

miRview

Normalization

In a study graded good, Rosenfeld et al.,[25] report that expression values were normalized using a polynomial function that optimized the fit between a vector of sample values and a reference vector had the median expression level for each miRNAs across all the samples. 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. The nodes at each decision tree were the tumor types. The model was assessing 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

($p<0.0005$). In order 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.[3] 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 being used to separate the nodes and combines the decisions of the decision tree with an unsupervised K – Nearest Neighbor classifier. The combination of a supervised and unsupervised method along with both internal and external validation meets the Simon[3] criteria.

Validation

In addition to using LOOCV, Rosenfeld et al.[25] report 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 which included 65 independent samples. Simon et al.[3] suggests using at least one of the two types of validation in algorithm development and identifies validation in an independent test set as optimal. This test meets both criteria.

PathworkDx

Normalization

As reported in Dumar et al.[32] and Pillai[30] 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 used is one that 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 is no description of the dimension reduction algorithm beyond that in either Dumur et al.[32] or Pillai[30] 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 to that of profiles of 15 cancer types. Once again there is little detail in the publications and it is not possible to independently assess the validity of the classifier.

Validation

Simon et al.[3] suggests using at least one of the two types of validation in algorithm development and identifies validation in an independent test set as optimal. There is no information on the internal and external validation used during the development of the algorithm used in PathworkDx TOO. However, there have been several retrospective studies as well as a

blinded prospective study that validated this test. The studies have all reported high rates of accuracy as described below.

Cytogenetic Analysis

Statistical algorithms are not used in cytogenetic analysis.

KQ 3b-3f. What is the evidence on the accuracy of the TOO test in classifying the origin and type of the tumor?

Thirteen studies (eleven rated good and two rated fair) provided responses to this question. There are three or more studies that report on the accuracy of each of the three tests. 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 were: CancerTypeID 83 percent (78% to 86%); miReview 85 percent (83% to 88%) and for PathworkDx it was 87 percent (86% to 89%).

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

CancerTypeID

Accuracy

Three papers report on the accuracy of the test. Ma et al.,[34] graded good, report an overall accuracy of 82 percent with 95% CI (74% to 89%) in an independent test set with 119 tissues of known origin. The site specific accuracy ranged from 0.0 to 1.0 (Table 12). Erlander et al.,[35] graded good, report an overall accuracy of 83 percent on a test set of tissue but do not report 95% CI or provide further details. Greco et al. (2010), rated good, estimated the accuracy of the test by comparing the prediction of the tests in 20 CUP patients whose true latent site was later identified. They report an accuracy of 75 percent with 95% CI (60% to 80%). Figure 4 displays the accuracy estimates across these three studies.

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 83 percent; 95% CI (78% to 86%).

miRview

Accuracy

The TOO call in miRview is based on the union of the calls made by decision tree and the KNN.[25] Rosenfeld et al.,[25] rated good, report on the results of a validation study on a test set of 83 specimens. The reported accuracy is 86 percent. The 95% CI has not been reported. In a study rated good, Rosenwald et al.[26] reports 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 report 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 is reported to be over 99 percent. Mueller et al.,[36] rated fair, assessed the accuracy of the test in 101 cases of brain and CNS metastases of known origin and 54 cases of brain and CNS metastases originally classified as CUP. They report an overall accuracy of 84 percent. This estimate leaves out all 12 cases of prostate cancer, nine of which

were classified incorrectly. The authors suggest that there is published evidence that suggests that the miRNAs profiles of primary prostate tumors differ significantly from the metastatic tumors especially in the presence of anti-androgenic 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 is 95 percent. Chajut et al.,[27] graded good, report the accuracy of miRview in classifying 489 samples of cancers of known primary including 149 metastatic cancers. They report an overall accuracy of 85 percent and specificity above 99 percent. Figure 5 displays the accuracy rate across the four studies for this test. 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. The meta-analytic summarized estimate across the studies was 0.85, 95% CI (0.83 to 0.88).Figure 6 has the accuracy rates for the eight 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 0.87 (Table 12); 95% CI (0.86 to 0.89).

Cytogenetic Analysis

We did not find any studies that examined the accuracy of cytogenetic analysis in identifying the TOO of tumors of known origin.

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	Cancertypes	Sample size	Gold standard (comparison test)	Sensitivity	Specificity
CancerTypeID Erlander, 2011,[35] Ma 2011[24232828] NR Multinational, US 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	187	Known origin	0.83 (numbers not reported)	0.99
CancerTypeID Ma, Patel et al., 2006[34333838] NR US only Good	Age: NR Female: NR N: NR	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, menigioma, 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	Validation: 119 FFPE	Known origin	"Accuracy" Overall (95% CI): 82% (74% - 89%) Unclassifiable - 8 (6.7%)	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	Cancertypes	Sample size	Gold standard (comparison test)	Senstivity	Specificity
miRview Chajut, 2011[27262727] 2011 Multinational, US Good	Chajut Age: NR Female: NR N: 509	Adrenocortical carcinoma, pheochromocytoma, squamous cell carcinoma (anus or skin), cholangioca. or adenoca. of extrahepatic biliary tract, urothelial carcinoma, astrocytic tumor, oligodendroglioma, adenocarcinoma of the breast, squamous cell carcinoma (uterine cervix), colorectal adenocarcinoma, carcinoid (gastrointestinal tract), gastrointestinal stromal tumor (gist), renal cell carcinoma (chromophobe), renal cell carcinoma (clear cell), renal cell carcinoma (papillary), hepatocellular carcinoma, lung, large cell or adenocarcinoma, lung (small cell carcinoma), carcinoid (lung), squamous cell carcinoma (lung, head&neck, or esophagus), pleural mesothelioma, lymphoma, ovarian primitive germ cell tumor, pancreatic adenocarcinoma, pancreatic adenocarcinoma, prostatic adenocarcinoma, ewing sarcoma, chondrosarcoma, malignant fibrous histiocytoma (mfh) or fibrosarcoma, osteosarcoma, rhabdomyosarcoma, synovial sarcoma, liposarcoma, melanoma, gastric or esophageal adenocarcinoma, non-seminomatous testicular germ cell tumor, seminomatous testicular germ cell tumor, thymoma/thymic carcinoma, thyroid carcinoma (follicular), thyroid carcinoma (papillary), thyroid medullary	509; 489 processed successfully	Known origin	418/489	> 99% (numbers not shown)
miRview Mueller, 2011[36354141] 2008 Germany Fair	Age: NR Female: NR N: NR	Billary tract, breast, head & neck, kidney, liver, lung, melanocyte, ovary , stomach or esophagus, thyroid, colon	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%

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	Cancertypes	Sample size	Gold standard (comparison test)	Sensitivity	Specificity
miRview Rosenfeld, 2008[25] NR Multinational, not US Good	Age: NR Female: NR N: NS	Bladder, brain, breast, colon, endometrium, head & neck, kidney, liver, lung, lung pleura, lymph node, melanocytes, meninges, ovary, pancreas, prostate, sarcoma, stomach, stromal, testis, thymus, thyroid	83 (blinded test set)	Known origin	Combined accuracy using DecisionTree & KNN = 86%	Combined value not available Decision Tree=99% KNN=NR
miRview Rosenwald, 2010[26] NR Multinational, US Good	Age: NR Female: NR N: NR	Bladder, brain, breast, colon, endometrium, head & neck, kidney, liver, lung, lung pleura, lymph node, melanocytes, meninges, ovary, pancreas, prostate, sarcoma, stomach, stromal, testis, thymus, thyroid	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[37364242] NR US only Good	Age: NR Female: NR N: NR	Bladder, breast, colorectal, gastric, hepatocellular, melanoma, liver, synovial sarcoma, sarcoma, ovarian, pancreatic, prostate, renal, thyroid	42 (39 on panel)	known origin	29/39 Indeterminate: 7/39 Incorrect: 3/39	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	Cancertypes	Sample size	Gold standard (comparison test)	Sensitivity	Specificity
PathworkDx Dumar, 2008[29] NS US only Good	Age: NS Female: NS N: 60	Breast, colorectal, non-small-cell lung, non-Hodgkins's lymphoma, lymphoma, pancreas, bladder, gastric, germ cell, hepatocellular, kidney, melanoma, ovarian, prostrate, soft tissue, sarcoma, thyroid NS	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, Blevins et al., 2008[29] NS US only Fair	Age: NS Female: NS N: 20	Mixed (1 CUP, 2 off panel)	20	Pathology report or IHC	Agreed: 14/17 Indeterminate: 1/17 Discordant with pathology: 2	NR
PathworkDx Grenert et al., 2011[38374343] 2000-2007 US only Good	Age: Range 22 -74 Median 55 Female: 20 N: 37	Bladder, breast, colorectal, gastric, testicular gem cell, kidney, hepatocellular, non-small cell lung, non-hodgkin's lymphoma, melanoma, ovarian, pancreas, prostate, sarcoma, thyroid	37	95% (81.8 - 99.3)	99.60%	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	Cancertypes	Sample size	Gold standard (comparison test)	Sensitivity	Specificity
PathworkDx Monzon, 2009[39384444] NS Multinational, US Good	Age: < 50: 142 50-59: 133 60-69: 139 ≥ 70: 132 Female: 290 N: 547	Colorectal, pancreatic, non-small cell lung, breast, gastric, kidney, hepatocellular, ovary, sarcoma, non-hodgkin's lymphoma, thyroid, prostate, melanoma, bladder, testicular germ cell	547	Known origin	480/547 (87.8%) Non-agreement: 39/547 Indeterminate: 28/547	99.40%
PathworkDx Pillai, 2011[30] NS US 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 non-small cell lung cancer, as well as non-Hodgkin's lymphoma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, thyroid cancer, and sarcoma.	462	Known origin	Overall agreement: 409/462. Accuracy: 88.5	99.1
PathworkDx Stancel, 2011[40394545] NS US only Good	Age: NS Female: NS N: 20	Lung, lymphoma, colon, pancreas, breast, ovarian, gastric	20 Passed QA: 19	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	Cancertypes	Sample size	Gold standard (comparison test)	Sensitivity	Specificity
PathworkDx Wu 2010[41404646] NS US only Good	Age: Median: 41 Range: 21-56 Female: 3 N: 15	Lung, breast, melanoma, lymphoma, sarcoma, colon, head & neck, gastric, kidney	15 Results, 1 off-panel specimen: 14 Sample size = 13	Known origin	12/13	NR

Abbreviations: NR = not reported, US = 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
CancerType ID	0.82	0.78	0.86
miRview	0.85	0.83	0.88
PathworkDx	0.87	0.86	0.89

Abbreviations: CI=confidence interval

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

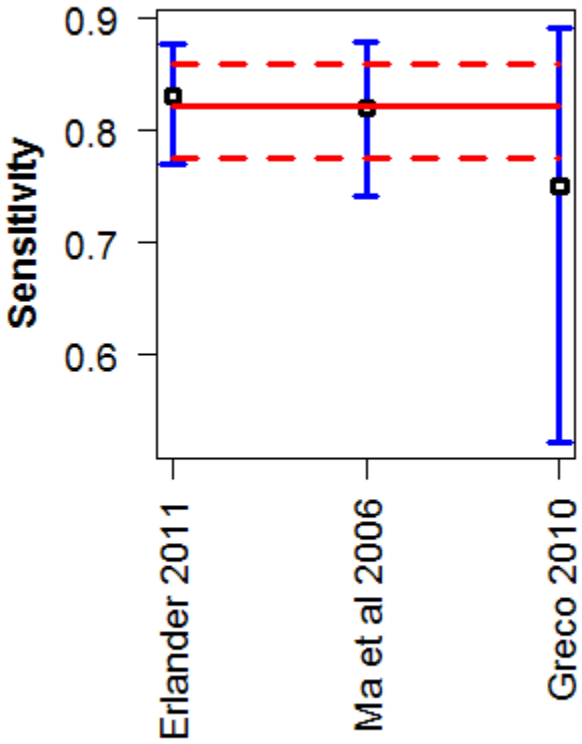


Figure 5. Point estimates and 95% Confidence intervals for all the reviewed studies estimating accuracy in known tissue for miReview TOO

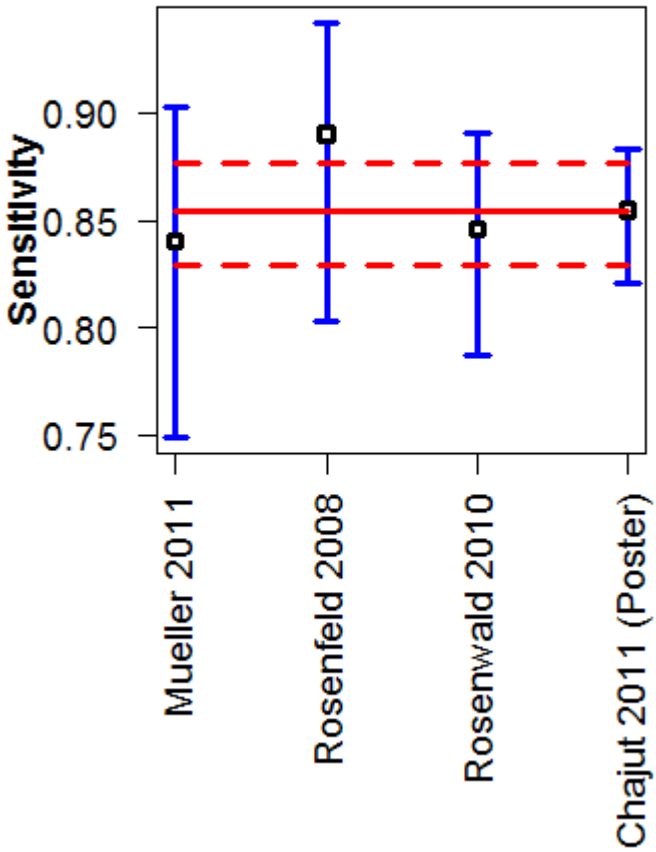


Figure 6. Point estimates and 95% Confidence intervals for all the reviewed studies estimating accuracy in known tissue for PathworkDx TOO

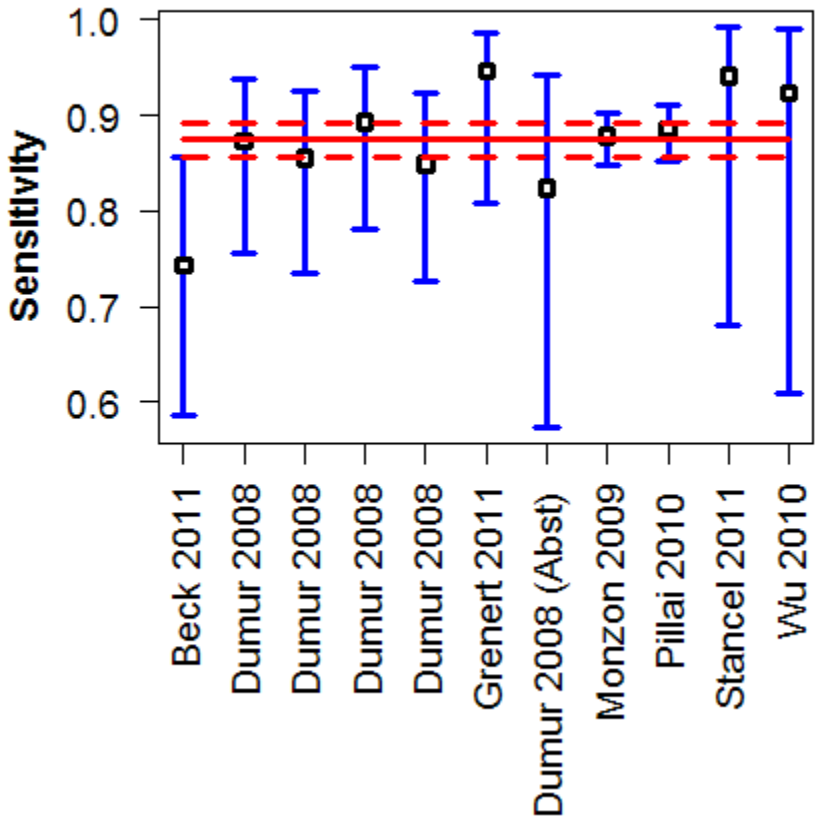


Table 5. Evidence of site-specific accuracy of TOO test in classifying type of the tumor

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[24232828]		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, US	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 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

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	Accuracy	Specificity
CancerTypeID	Age:	1. Adrenal	1. 1	1. 1.00	NR
	NR	2. Brain	2. 3	2. 1.00	
Ma et al., 2006[34333838]		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	
US only	N:	7. Endometrium	7. 3	7. 0.67	
	NR	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	
		33. Overall	33. 119	33. 0.82	

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	Age:	1. Billary tract	1. 1	1. 0%	1. 100%
	NR	2. Breast	2.18	2. 72.2%	2. 95.8%
Mueller, 2011[36354141]		3. Head & Neck	3. 4	3. 100%	3. 89.4%
	Female:	4. Kidney	4. 17	4. 94.1%	4. 98.6%
2008	NR	5. Liver	5. 1	5. 100%	5. 100%
		6. Lung	6. 16	6. 87.5%	6. 78.1%
Germany	N:	7. Melanocyte	7. 17	7. 100%	7. 90.3%
	89 (samples with	8. Ovary	8. 5	8. 60%	8. 97.6%
Fair	known TOO)	9. Stomach or esophagus	9. 4.	9. 100%	9. 98.8%
		10. Thyroid	10. 2	10. 0%	10. 97.7%
		11. Colon	11. 4	11. 75%	11. 95.3%
		12. Overall (excluding prostate)	12. 89	12. 84%	12. 95%
		13. Prostate	13. 12	13. 3/12	13. NR
miRview	Age:			Decision Tree	Decision Tree
	NR	1. Bladder	1. 2	1. 0	1. 100
Rosenfeld, 2008[25]		2. Brain	2. 5	2. 100	2. 100
	Female:	3. Breast	3. 5	3. 60	3. 97
NR	NR	4. Colon	4. 5	4. 40	4.99
		5. Endometrium	5. 3	5. 0	5.99
Multinational, not US	N:	6. Head & neck	6. 8	6. 100	6.99
	NR	7. Kidney	7. 5	7. 100	7.99
Good		8. Liver	8. 2	8. 100	8.99
		9. Lung	9. 5	9. 80	9.95
		10. Lung pleura	10. 2	10. 50	10. 99
		11. Lymph node	11. 5	11. 60	11. 100
		12. Melanocytes	12. 5	12. 60	12. 97
		13. Meninges	13. 3	13. 100	13. 99
		14. Ovary	14. 4	14. 75	14. 97
		15. Pancreas	15. 2	15. 50	15. 100
		16. Prostate	16. 2	16. 100	16. 100
		17. Sarcoma	17. 5	17. 40	17. 99
		18. Stomach	18. 7	18. 71	18. 96
		19. Stromal	19. 2	19. 100	19. 100
		20. Testis	20. 1	20. 100	20. 100
		21. Thymus	21. 2	21. 100	21. 98
		22. Thyroid	22. 3	22. 100	22. 100

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	Age:	Validation	Validation only	(1 or 2	(1 or 2
	NR	1. Biliary tract	1. 7	predictions)	predictions)
Rosenwald, 2010[26]		2. Brain	2. 11	1. 66.7%	1. 94%
	Female:	3. Breast	3. 38	2. 100%	2. 100%
NR	NR	4. Colon	4. 9	3. 66.7%	3. 93.6%
		5. Esophagus	5. 1	4. 88.9%	4. 94.4%
Multinational, US	N:	6. Head and neck	6. 3	5. 100%	5. 98.4%
	NR	7. Kidney	7. 10	6. 100	6. 92.4%
Good		8. Liver	8. 8	7. 87.5%	7. 99.4%
		9. Lung	9. 26	8. 100%	8. 99.4%
		10. Melanoma	10. 7	9. 91.3%	9. 84.9%
		11. Ovary	11. 13	10. 85.7%	10. 97.8%
		12. Pancreas	12. 6	11. 84.6%	11. 100%
		13. Prostate	13. 20	12. 50%	12. 97.8%
		14. Stomach or esophagus	14. 7	13. 89.5%	13. 99.4%
		15. Testis	15. 8	14. 40%	14. 98.9%
		16. Thymus	16. 6	15. 100%	15. 100%
		17. Thyroid	17. 24	16. 83.3%	16. 97.8%
				17. 100%	17. 98.2%
PathworkDx	Age:	1. Bladder	1. 1	1. 100%	1. 100%
	NR	2. Breast	2. 1	2. 100%	2. 100%
Beck, 2011[37364242]		3. Colon	3. 5	3. 100%	3. 100%
	Female:	4. Gastric	4. 2	4. 0%	4. 50%
NR	NR	5. Hepatocellular	5. 2	5. 50%	5. 100%
		6. High grade sarcoma	6. 2	6. 100%	6. 100%
US only	N:	7. Melanoma	7. 2	7. 100%	7. 100%
	NR	8. Ovarian	8.67%	8. 83%	8. 83%
Good		9. Pancreas	9. 2	9. 50%	9. 50%
		10. Prostrate	10. 2	10. 100%	10. 100%
		11. Renal	11. 6	11. 100%	11. 100%
		12. Thyroid	12. 2	12. 100%	12. 100%
		13. Lung	13. 2	13. 0%	13. –
		14. Synovial sarcoma	14. 1	14. 0%	14. –
		15. Endometrial	15. 2	15. 0%	15. -

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[38374343]	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	
US only		7. Hepatocellular	7. 3	7. 3/3	
	N:	8. Non-small 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	1. 519/519
	< 50: 142	2. Breast	2. 68	2. 64/68	2. 471/479
Monzon, 2009[39384444]	50-59: 133	3. Colorectal	3. 56	3. 52/56	3. 487/491
	60-69: 139	4. Gastric	4. 25	4. 18/25	4. 519/522
NR	≥ 70: 132	5. Germ Cell	5. 30	5. 22/30	5. 517/517
		6. Hepatocellular	6. 25	6. 23/25	6. 521/522
Multinational, US	Female:	7. Kidney	7. 39	7. 37/39	7. 507/508
	290	8. Melanoma	8. 26	8. 21/26	8. 520/521
Good		9. Non-Hodgkin's lymphoma	9. 33	9. 31/33	9. 511/514
	N:	10. Non–small cell lung	10. 31	10. 27/31	10. 509/516
	547	11. Ovarian	11. 69	11. 64/69	11. 473/478
		12. Pancreas	12. 25	12. 18/25	12. 521/522
		13. Prostate	13. 26	13. 23/26	13. 521/521
		14. Soft tissue sarcoma	14. 31	14. 26/31	14. 513/516
		15. Thyroid	15. 35	15. 32/35	15. 510/512

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	Accuracy	Specificity
PathworkDx Pillai, 2011[30293232] NR US 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	1. Bladder 2. Breast 3. Colorectal 4. Gastric 5. Hepatocellular 6. Kidney 7. Melanoma 8. Non-Hodgkin's lymphoma 9. Non-small 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[40394545] NR US only Good	Age: NR Female: NR N: 20	1. Lung 2. Lymphoma 3. Colon 4. Pancreas 5. Breast 6. Ovarian 7. Gastric	1. 4 2. 2 3. 1 4. 1 5. 4 6. 5 7. 2	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[41404646] NR US only Good	Age: Median: 41 Range: 21-56 Female: 3 N: 15	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	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: NR = not reported, US = United States

PathworkDx

Several studies assessed the accuracy of the PathworkDx test in samples of known primaries, Dumur et al., 2008[32] 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 are not reported. Among the 52 tissues that met all the QA criteria for the test, the overall accuracy was 93.8 percent. Monzon et al.,[39] rated good, report on a prospective validation study that assessed the accuracy of the test on 547 samples of known origin. They report an overall accuracy of 87.8 percent; 95% CI (84.7 to 90.4); and specificity of 99.4 percent (95% CI, 98.3 to 99.9). Pillai et al.,[30] rated good, assessed the accuracy of the test on 462 formalin fixed paraffin embedded (FFPE) tissue of known origin. They report an overall accuracy rate of 89 percent; 95% CI (85 to 91). Grenert et al.,[38] rated good, also assessed accuracy of the TOO test on 44 FFPE samples of known origin. They report an overall accuracy of 95 percent; 95% CI (81.8 to 99.3) and an overall specificity of 99.6 percent. Beck et al.,[37] rated good, report 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. One study,[29] rated fair and only available as an abstract, reported that the accuracy of the test was 82 percent in 17 samples of tissues of known origin; 95 % CI were not reported. Stancel et al.,[40] in a study rated good, report on the accuracy of the TOO test using body fluid specimens from 19 patients with metastatic cancer. The estimated accuracy was 78.5 percent. This study also had nine 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.[41] assessed the accuracy of the test in 14 tissues of metastatic brain cancer of known origin. They report an accuracy of 85.7 percent. Figure 2c has the accuracy rates for the eight 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 0.87; 95% CI (0.86 to 0.89).

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

Eleven studies (six[19, 28, 42-45] rated good and five rated fair[24, 36, 37, 46, 47]) 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 affect treatment decisions or health outcomes. The results are in

Table 6.

Table 6. 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	# Cases Clinically Useful
CancerTypeID Erlander, 2011[35] Ma, 2011[24232828] NR Multinational, US Good	Age: Mean(± SD): 62 (13) ≥65: 44% Female: 53% N: 300	296 (142)	4	252
CancerTypeID Greco, 2010[42415858] 2000-2007 US only Good	Age: 25 - 50: 4 50-64: 8 65+: 8 Female: 11 N: 20	18 (15)	2	NR
G-banded karyotype (supplemented by FISH and comparative genomic hybiridization Pantou, 2003[19145959] 2001 Multinational, not US 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 Mueller, 2011[36354141] 2008 Germany Fair	Age: NR Female: NR N: 53	50 (40) 10 were discordant 4 origin never diagnosed	NR	NR
miRview Varadhachary, 2011[28276060] 2008-2010 US only	Age: 58 Range: 20-83 Female: Total: 66 Results: 45 N:	74 (62)	NR	IHC not helpful: 9 TOO prediction: 9 TOO consistent with clinicopathological: 7

Good	84			

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	# Cases Clinically Useful
PathworkDx Beck, 2011[37364242] NR US only Good	Age: NR Female: NR N: 7	4 (2 clearly incorrect)	3	2
PathworkDx Gutierrez, 2011[43] NS US only Good	Age: NR Female: 63 N: 111	Changed diagnosis, % (95% CI): 54% (46 - 62) p <0.0001	NS	67%
PathworkDx Hainsworth, 2011[48] 2005 US only Good	Age: Median: 56 Range: 21-86 Female: 21 N: 45	43	2	NR
PathworkDx Medeiros, 2008[49] NR US only Fair	Age: NR Female: NR N: 11	8 (73%) TOO results 6/8 consistent with clinicopathologic characteristics	3	NR
PathworkDx Monzon, 2010[44] 2006 US only Good	Age: 40-49: 1 50-59: 4 60-69: 6 70-79: 7 80-89: 3 Female: 15 N:	16 (10) 6 plausible	5	16

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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	# Cases Clinically Useful
PathworkDx Laouri, 2010[50] 2009 NR Fair	Age: Median: 62 Range: 16-89 Female: 161 N: 284 Non-specific Dx: 183 Specific Dx: 101	Non-specific Dx: 172 Specific Dx: 100 (New 57,;Confirm 43)	No Specific Dx: 11 Specific Dx: 1	NR

CancerTypeID

One study,[42] rated good, reported on the success of CancerTypeID in predicting TOO among CUP patients for which the primary site was later identified. A TOO was predicted in 20 of 28 patients (90%). In 15 cases (75%; 95% CI, 60% to 85%), the predicted site of origin corresponded to the latent primary site that was later identified. Three predictions were incorrect (15%), and two results were indeterminate (10%). One study,[24] rated good, reported on CancerTypeID results for 300 CUP patients. CancerTypeID predicted a TOO in 296 (99%) of them. The assay results were consistent with a suspected origin based on clinicopathologic characteristics in 142 patients (48%).

miRview

One good study[28] reported on the clinical utility of miRview TOO predictions among patients diagnosed with CUP. Of the 104 patients enrolled, sufficient tumor sample for analysis was available for 87 and 74 of these passed quality control and returned a result. The TOO results were consistent with the final diagnosis in 62 of 74 cases, 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 the one of the differential diagnosis 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.

One study,[36] 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 four cases. Four cases had no suggested origin.

PathworkDx

Six studies[37, 43-46, 51] provided evidence on the utility of the PathworkDx test for diagnosis of CUP patients. Three studies were available only as abstracts[43, 49] or posters.[51] Three studies[43-45] were rated as good, and three[37, 46, 51] as fair. The PathworkDx test predicted a TOO in 57 percent[37] to 96 percent[51, 52] of samples from CUP patients. The criteria used to determine whether the TOO test was clinically useful were not well described, and the proportion of cases

in which the TOO test was judged to be clinically useful varied widely, from 29 percent[37] to 100 percent.[39]

Monzon et al.[44] retrospectively assayed frozen tumor specimens from 21 patients with CUP. The TOO test returned a prediction in 16 (76%) cases. In 10 (48%) cases, the TOO prediction was consistent with clinicopathological suggestions. In the other six cases, the TOO prediction was unexpected, but was not in conflict with clinical or pathological information. The authors estimated that the PathworkDx findings would be clinically useful in all cases for which a prediction was made, but no independent confirmation of their judgment was reported. Gutierrez et al.[43] interviewed physicians who had ordered TOO tests on a total of 111 patients. The TOO test result changed the primary diagnosis in 54 percent (95% CI, 46 to 62) of patients. In 67 percent of the cases, the physicians felt the TOO results were clinically useful.

Three studies,[37, 46, 51] rated fair, reported on the use of TOO tests in case series of CUP patients. Laouri et al.[51] reported on 284 cases tested by PathworkDx in 2009. Of these, 272 (96%) received a predicted TOO. Among the 101 cases with a suspected TOO at time of submission, the TOO test identified a different TOO in 57 cases, and confirmed the suspected diagnosis in 43 cases. Among the 183 cases without a suspected TOO when submitted, the TOO test identified a TOO for 172. Most of the tumors studied by Beck et al.[37] were of known origin, but seven tumors were of unknown primary. Three (43%) of these cases received an indeterminate diagnosis. Two (29%) predictions were incompatible with the clinical presentation. Two predictions were included in the differential diagnosis based on clinic-pathological characteristics. The authors felt the PathworkDx results were probably clinical useful in these two (29%) cases. Medeiros et al.[46] also presented the results of a small cases series. Of 11 cases, 8 received a TOO prediction, and 6 of the 8 were consistent with the clinicopathologic characteristics of the case.

PathworkDx TOO was the only test that 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.

Cytogenetic analysis

One study[19] 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.

KQ 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. Four studies[43, 47, 53, 54] reported on the effect of TOO tests on treatment decisions. Two studies were rated as good, but one was available only as an abstract[43] and one as a poster.[54] One paper[55] and one poster[51] were rated fair. Table 13 displays the results.

Hainsworth[53] examined treatment and disease course among 42 CUP patients that were predicted by CancerTypeID to have tumors of colorectal origin. Thirty-two of these patients had received first-line (24) or second-line (8) colorectal cancer treatment based on the CancerTypeID results. Treatment response was much higher among patients who received colorectal cancer treatment (20 of 40, 50%) than patients who received empirical treatment for CUP (3 of 18, 17%). Hainsworth et al. also presented preliminary results from a clinical trial of treatment

assigned based on CancerTypeID results.[54] 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 (51%) responded to treatment.

A series of 284 CUP cases that received PathworkDx TOO tests found that the results changed first line therapy in 229 (81%) of cases. For 178 cases, the chemotherapy regimen recommended by the National Comprehensive Cancer Network (NCCN) guidelines was completely different after the test results. The test results led to a minor change in recommended chemotherapy regimen in 51 cases.

Gutierrez et al.[43] interviewed physicians who had ordered a PathworkDx test. Sixty-six physicians had ordered tests on a total of 111 patients. Treatment recommendations changed based on the TOO results in 65 percent (95% CI, 57 to 72; $p<0.0001$) of cases. Chemotherapy regimen changed for 61 (55%; 95% CI, 47 to 63; $p<0.0001$) patients. Physicians felt the results were clinically useful for 67 percent of cases.

Table 7. Evidence of genetic TOO tests changing treatment decisions

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Charac-teristics	Cancer/ Tumor Types	# Eligible	Participated/ Response	Eligibility Criteria	Indicated TOO	Treatment and Response	Treatment Change	P-value TOO vs. CUP Treatment
CancerTypeID Hainsworth, 2010[54] NR NR Good[54]	Age: Median: 64 Range: 26-85 Female: 33 N: 110	NR	110	Received assay directed treatment: 66 Evaluated: 51	Diagnosed with CUP after standard diagnostic evaluation; Metastatic carcinoma; Exclusion: treatable subsets of CUP No previous systematic treatment Biopsy material available for assay ECOG performance status 0-2 No uncontrolled brain metastases	Pancreatic: 11 Colorectal: 8 Urinary Bladder: 8 Non-small cell lung: 5 Ovarian: 4 Carcinoid - intestine: 4 Breast: 3 GallBladder: 3 Liver: 3 Renal cell: 3 Skin (squmous): 3 Other: 7 No specific diagnosis: 5	Assay directed treatment Objective treatment response: 21	Changed: 66 (60%) Not changed: 44 (40%)	51% response for assay directed therapy
CancerTypeID Hainsworth, 2011[53] 3/2008-8/2009 NR Fair	Age: Median: 57 Range: 35-86 Female: 27 N: 125	Liver, bone/bone marrow, lymph nodes, peritoneum, lungs, abdominal/ retroperitoneal nodes, uterus/ovary, adrenal glands	125	42 (34%)	NS	Identified by TOO test with ≥ 80% probability as colorectal adenocarcinoma	1st line: Advanced colorectal cancer: 12/24 Empirical for CUP: 17% (Total: 18) 2nd line: CRC" 8/16	Changed: 32 Not Changed: NR	p=0.0257

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	# Eligible	Participated/ Response	Eligibility Criteria	Indicated TOO	Treatment and Response	Treatment Change	P-value TOO vs. CUP Treatment
PathworkDx Gutierrez, 2011[43] NR US only Good	Age: NR Female: 57% N: 111	NR	111	111	NR	NR	NR	Changed: Changed treatment recommendations: 65% (95% CI, 57 to 75) Changed chemotherapy regimen: 61 (55%, 95% CI, 47 to 63) Not changed: NR	P< 0.0001

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	# Eligible	Participated/ Response	Eligibility Criteria	Indicated TOO	Treatment and Response	Treatment Change	P-value TOO vs. CUP Treatment
PathworkDx Laouri, 2010[51506464] 2009 NR Fair	Age: Median: 62 Range: 16-89 Female: 161 N: 284 Non-specific Dx: 183 Specific Dx: 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%) Non-small 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 Treatment Response: NR	Changed: 229 (81%) Not changed: 55 (19%)	NR

Abbreviations: Dx = diagnosis, NR = not reported, US = United States

KQ 4b. What is the evidence that the genetic TOO tests change outcome?

The evidence for this response was very limited. Two studies one rated fair and one rated good provided responses for this question. Table 14 has the evidence for this question.

CancerTypeID

Two studies have looked at the effect of the identification of the TOO on patient outcomes. In a study graded fair, Hainsworth et al.[53] 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 ad an initial diagnosis of CUP and had the TOO identified as the colo-rectum. Forty–two of the 125 surveys 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 to 6 months in patients with empiric CUP therapy(p=0.11). In another study, that was rated good, 66 of 110 patients enrolled got site specific therapy based on the test.[54] Fifty one of these patients were evaluated for disease progression and overall survival. Twenty-one patients (41%) had an objective response (complete or partial response) to therapy. The specifics of the treatment responses, i.e.—how many had a partial response and how many had complete response—is not described. Twenty patients (39%) had stable disease for over 6 months. The median overall survival in patients who received site specific therapy was 12.85 months, 95% CI (10.81 to NR).

miRview

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

PathworkDx

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

Table 8. Evidence that genetic TOO tests change outcomes

TOO Test, Author, Year Study Dates, Region, Quality Rating	Sample Characteri-stics	Cancer/ Tumor Types	# Eligible	# Participated	Sample Requirements	Score	Indicated TOO	Outcome	TOO
CancerType-ID Hainsworth, 2011[53] 3/2008-8/2009 NR Fair	Age: NR Female: NS N: NR	Liver, bone/bone marrow, lymph nodes, peritoneum, lungs, abdominal/ retroperitoneal nodes, uterus/ovary, adrenal glands	125	42 (34%)	NS	Identified by TOO test with ≥ 80% proba-bility as colo-rectal adeno-carinoma	colorectal adeno-carinoma	Survival TOO: 8.5 months Control: Empirical CUP 6 months	Effect: NR p-value TOO vs. CUP: 0.11
CancerType-ID Hainsworth 2010[54] NR NR Good	Age: Median: 64 Range: 26-85 Female: 33 N: 66	NR	66	51 evaluated	Diagnosed with CUP after standard diagnostic evaluation	NR	Pancreatic: 11 Colorectal: 8 Urinary Bladder: 8 Non-small 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	Stable disease TOO: 21 Control: NR	Effect: NR p-value TOO vs. CUP: NR

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	# Eligible	# Participated	Sample Requirements	Score	Indicated TOO	Outcome	TOO
CancerType-ID Hainsworth, 2010[54] NR NR Good	Age: Median: 64 Range: 26-85 Female: 33 N: 66	NR	66	51 evaluated	Diagnosed with CUP after standard diagnostic evaluation	NS	Pancreatic: 11 Colorectal: 8 Urinary Bladder: 8 Non-small 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	Survival TOO: 12.9 months Control: NR	Effect: NR p-value TOO vs. CUP: NR

Abbreviations: NR = not reported

KQ 5. Is the TOO test relevant to the Medicare population?

Nine[24, 28, 30, 38, 39, 41, 50, 52, 53] studies provided information on the age of the cases in their study (Table 15). All but one[56] included patients age 65 years or older, the Medicare core population. All of the studies that provided information on the gender of the cases included both male and female cases, and the studies and TOO test panels include cancers specific to women (breast, ovarian) and to men (prostate, testicular). None of the studies provided information on the race or ethnicity of the cases.

Table 9. TOO tests relevant to the Medicare population

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Characteristics	Cancer Types
CancerTypeID Erlander, Ma 2011[24232828] NR Multinational, US 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
CancerTypeID Hainsworth 2010[54] NR NR Good	Age: Median: 64 Range: 26-85 Female: 33 N: 110	NR
CancerTypeID Hainsworth, 2011[53] 3/2008-8/2009 NR Fair	Age: Median: 57 Range: 35-86 Female: 27 N: 125	Liver, bone/bone marrow, lymph nodes, peritoneum, lungs, abdominal/ retroperitoneal nodes, uterus/ovary, adrenal glands
miRview Varadhachary, 2011[28276060] 2010 US 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

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

TOO Test, Author, Year Study Dates, Region Quality Rating	Sample Characteristics	Cancer Types
PathworkDx Grenert, 2011[38] 2000-2007 US only Good	Age: Range: 22 -74 Median: 55 Female: 20 N: 37	Bladder, breast, colorectal, gastric, testicular gem cell, kidney, hepatocellular, non-small cell lung, non-hodgkin's lymphoma, melanoma, ovarian, pancreas, prostate, sarcoma, thyroid
PathworkDx Laouri, 2010[50, 51] 2009 NR Fair	Age: Median: 62 Range: 16-89 Female: 161 N: 284 Non-specific Dx: 183 Specific Dx: 101	Liver, lymph node, omentum and peritoneum, lung, soft tissue, bone, neck, brain, ovary, colon and rectum, pleura, pelvis, small bowel, other
PathworkDx Monzon, 2009[39384444] NR Multinational, US Good	Age: < 50: 142 50-59: 133 60-69: 139 ≥ 70: 132 Female: 290 N: 547	Colorectal, pancreatic, non-small cell lung, breast, gastric, kidney, hepatocellular, ovary, sarcoma, non-hodgkin's lymphoma, thyroid, prostate, melanoma, bladder, testicular germ cell
PathworkDx Pillai, 2011[30293232] NR US 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 non-small cell lung cancer, as well as non-Hodgkin's lymphoma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, thyroid cancer, and sarcoma
PathworkDx Wu, 2010[41404646] NR US only Good	Age: Median: 41 Range: 21-56 Female: 3 N: 15	Lung, breast, melanoma, lymphoma, sarcoma, colon, head & neck, gastric, kidney

Discussion

Genomic Tissue of Origin (TOO) Tests

Three 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 claim to 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.[22] All three tests identify lung, pancreas, ovary, and prostate tumors, which account for 51 percent of primary tumors identified at autopsy. All three tests identify kidney tumors, but only CancerTypeID identifies adrenal tumors. Stomach, bowel, and liver or bile duct tumors are covered by at least one of the tests.

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 the manufacturer of test. Limited measures of analytic validity reported, and impossible to assess consistency of those measures across studies.	Insufficient
KQ 2. Analytic validity: MiReview	3	Three 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	1	Two papers from the same multi-site study reported different measures of analytic validity. Impossible to evaluate consistency of reported measures of analytical validity across studies.	Insufficient
KQ 3a. Adherence to Simon guidelines: CancerTypeID	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: MiReview	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 3b – 3f. Accuracy of the TOO test in classifying the origin and type of tumors of known primary site: CancerTypeID	3	Three studies with relatively small sample sizes have compared ability of tests to identify origin of tumor in tissues of known origin. All report accuracy of inclusion. Accuracy of exclusion is reported only in one MS.	Moderate
KQ 3b – 3f. Accuracy of the TOO test in classifying the origin and type of tumors of known primary site: MiReview	3	Two independent studies with over a hundred specimens each tested the ability of the miReview to identify site of origin in tissues of known origin. Accuracy of inclusion and exclusion are both reported.	Moderate
KQ 3b – 3f. Accuracy of the TOO test in classifying the origin and type of tumors of known primary site: PathworkDx	7	Seven studies including a prospective blinded study with over 500 specimens report of 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

Table 16. Overview of study outcomes (continued)

Key Question	Number of Studies	Conclusion	Strength of Evidence
KQ 4. Percent of cases with for which a TOO test identified a TOO	11	All studies reviewed found the test provided useful guidance in diagnosis. TOO tests indicate a TOO almost all cases.	Moderate
KQ 4:Percent of cases with for which a TOO test identified a TOO call	2	Only two studies, one for PathworkDx and one for CancerTypeID had independent confirmation of the site of origin identified by the TOO test. When independent confirmation of the TOO test was available, 75% of the calls were confirmed.	Low
KQ 4: Percent of CUP cases where test was considered clinically useful by physician or researcher	4	All studies found test clinically useful in a proportion of case. 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	4	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 to usual treatment for CUPS cases	1	Small samples, insufficient studies to draw conclusions on any of the tests.	Insufficient
KQ 4: Change in Survival	2	Small samples, insufficient studies to draw conclusions on any of the tests.	Insufficient
KQ 4: Change in Disease Progression	1	Small samples, insufficient studies to draw conclusions on any of the tests.	Insufficient

KQ 1 TOO Tests

Four genetic or molecular tests are available to inform diagnosis of the TOO in cancers with unknown primary site. Three tests, CancerTypeID, PathworkDx, and miRview are microarray tests of gene or microRNA expression levels in tumor cells. The fourth test is karyotype of G-banded chromosomes, which can identify cancer types that are associated with specific chromosomal rearrangements.

KQ 2 Analytic Validity

Six articles[24-27, 30, 32] in total reported on four studies of 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, so we were unable to assess the consistency of measures of analytic validity across studies. We graded the strength of the evidence for the analytic validity of the tests as insufficient (Appendix F). Only one article[24] reported on the analytic validity of the CancerTypeID TOO test. Although three studies, reported in two articles and a poster, examined the analytic validity of the miRview test, no two studies report the same measures of analytic validity. One study[25242929] reports on analytic reproducibility between testing platforms and sample times. A second reports on inter-laboratory reproducibility, and a third[26] reports the quality control measures for the assay. One study, reported in two[30, 32, 57] articles, examined the analytic validity of the PathworkDx TOO test. The papers reported on different aspects of the same study. One study[57] reported on inter-laboratory reproducibility and quality control measures. A second reported on the effect of sample storage time on the study results.[30]

KQ 3a Statistical Validity:

One good study,[34] described the development of the algorithm used in CancerTypeID. The normalizations, 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., [25 24 29 29] graded good, describe the development of the algorithm for miReview. All aspects of the development of the algorithm are well described and the published evidence on the quality of the algorithm used in miReview is high.

Dumur et al.[32] and Pillai et al.,[30] graded good, both described the normalization process used in the PathworkDx TOO. The dimension reduction algorithm and the classification are considered proprietary and not described in enough detail in order to determine the appropriateness of the algorithm. The published evidence on the statistical validity of the algorithm is insufficient.

KQ 3b –3f: Accuracy of the TOO test in classifying the origin and tissue of the tumor

Three studies[24, 34, 42] with relatively small sample sizes have compared ability of tests to identify origin of tumor in tissues of known origin. Based on 3 good studies, with similar estimates of accuracy and fair precision, and the meta-analytic summary estimate (82% [78%, 86%]) the evidence is high that CancerTypeID correctly identifies tumor type in known tissue nearly 80 percent of the time.

Two independent studies[26, 27] with over a hundred specimens and 2 studies with smaller sample sizes[26, 36] each tested the ability of the miReview origin of tumor in tissues of known origin. Based on 3 good studies and 1 fair study, with 4 very similar estimates of accuracy and 2 of exclusion and the combined meta-analytic summary estimate, the evidence is high that miRview correctly identifies tumor source in known tissue 85 percent of the time, 95% CI (83%, 88%).

Seven studies[30, 32, 38-41] including a prospective blinded study with over 500 specimens report on the ability of the test to identify the origin of tumor in tissues of known origin. Based on 7 good studies and 1 fair study with very similar estimates of accuracy and 2 similar estimates of specificity, and the meta-analytic summary estimate the evidence is high that the PathworkDx test correctly identifies tumor source 87 percent of the time, 95% CI (86%, 89%) .

KQ 4 - Diagnosis

Eleven studies[19, 24, 28, 36, 37, 42-44, 46, 48, 51] reported on the success of genomic TOO tests in diagnosing CUP patients. 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.[58] Therefore, a test may be useful in the evaluation of CUP cases even if it provides a diagnosis in a minority of cases. We graded the evidence that genomic tests can be clinically useful in the diagnosis of CUP patients as moderate. Eleven studies[19, 24, 28, 36, 37, 42-44, 46, 48] fair, reported on the use of a genomic or molecular test in the diagnosis of CUP. The tests predicted a TOO in 25 percent (G-banded chromosomes)[19] to 99 percent (CancerTypeID)[24] of CUP cases. A confirmatory diagnosis was available in six[24, 28, 36, 42, 44, 46] studies; the predicted diagnosis was confirmed in 48 percent[24] to 84 percent[28] of cases. In four studies,[24, 37, 43, 44] the study author[24, 37, 44] or the treating physician[43] judged whether the test was clinically useful. The test was found to be useful in 29 percent[37] to 84 percent[24] of cases.

Limited evidence is available on the usefulness of the CancerTypeID or miRview tests for CUP diagnosis. One good[42] and one fair[24] study indicate that CancerTypeID predicts a TOO in almost all cases (90 to 99%), and shows that a moderate to large proportion (48 to 83%) of these predictions can be confirmed. Erlander et al.[24] judged the TOO test results to be clinically useful in 84 percent of their clinical series. One good[28] and one fair[36] study suggest the miRview test will predict a TOO in 88 percent to 94 percent of CUP cases, and that the prediction is confirmed in 80 percent to 84 percent of cases. No evidence on clinicians' judgment of the clinical usefulness of the test was available.

Six studies[37, 43-46, 51] provide moderate evidence that the PathworkDx TOO test can predict a TOO in 57 percent to 96 percent of cases. Two studies[44, 46] indicate that the prediction will be confirmed in 63 percent to 75 percent of CUP cases. Three good studies[43, 44] and one fair[37] study provide moderate evidence that the results of the PathworkDx TOO test helped identify the TOO in 29 to 76 percent of CUP cases.

KQ 4 – Treatment

There is low evidence that TOO results affect treatment decisions and treatment response. Gutierrez et al.[43] found that PathworkDx results changed treatment recommendations in 65 percent of patients. Hainsworth et al.[53] found patients who were predicted to have colorectal cancer based on their CancerTypeID results who received treatment for colorectal cancer were more likely to respond than those who received empirical treatment for CUP.

KQ 5 – Relevance of TOO Tests and Evidence to Medicare Population

Identifying the TOO of cancer of unknown primary site is clearly relevant to the Medicare population: Approximately 53,000 Medicare eligible individuals are expected to be diagnosed with CUP annually.[11] Although we could not find any information on the primary site of CUP in Medicare patients, the TOO test panels include the most common origin sites of CUP cases overall.[22] At least one study of each test included patients 65 years or older of both genders, the core Medicare population.

Summary of Accuracy

Most of the studies that examined the accuracy of the tests compared to a known primary site were rated good. All three studies for CancerTypeID, three out of four studies for miReview and six out of seven studies for PathworkDx were rated good. 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 82 percent for CancerTypeID, 85 percent for miReview 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 have variously used the following gold standards: (a) the discovery of a latent tumor post TOO test that confirmed the TOO result[36]; (b) improved response to tissue-specific treatment regimens[53] or (c) consistency between the clinicopathological presentation and as evidence of accuracy of the test.[35] 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, since 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 the 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[37] of the published studies were either conducted by or funded by the test 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.,[37] 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 cannot rule out the possibility of bias towards publication of positive studies.

The evidence was insufficient to answer KQs on analytic validity or the effect of the tests on treatment or outcomes. No more than one study provided evidence on any given measure of analytic validity for any TOO test. In addition, the published information[30, 32] on the development of the statistical algorithm used in the PathworkDx text was too limited to assess whether the development process met the Simon[3] criteria.

The greatest gap was in the data on clinical utility, however. Although six studies reported on the clinical use of PathworkDx as a component of the diagnosis of CUP cases, only two studies reported similar information for the CancerTypeID or miRview TOO tests. The studies published to date have reported the use of TOO tests in addition to the current recommended work up to diagnosis tumor site, rather than on the comparative effectiveness of such tests to the current recommended work up. Only one paper[53] on the effect of TOO tests on treatment outcomes has been published. Three additional studies were only available as posters[51, 54] or an abstract.[43] The data presented suggest that TOO tests affect treatment decisions, but additional evidence is needed to be conclusive. Information on health outcomes was limited to two studies[45, 53] that used the CancerTypeID test. Both studies lacked an appropriate comparison group. No information was available on the effect of testing by miRview or PathworkDx on health outcomes. The clinical utility of these tests is still uncertain.

Summary of Findings

We assessed four tests in this review: cytogenetic analysis, CancerTypeID, miReview and PathworkDx TOO. Of the three tests, CancerTypeID has the broadest panel with the ability to detect 29 different tumor sites. miReview 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. The effect of this change in therapy on outcomes is limited to two papers that used CancerTypeID as the TOO test. One focused on CRC and the other on all CUP cases. There is no study with a sufficient sample size that compares outcomes between patients who received tissue-specific therapy and those who did not.

As mentioned, 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.

Future Research

Most studies included in the current were funded wholly or partly by the manufacturers of the tests. The most urgent need in the literature is to have the test be evaluated by research groups that have no evident conflict of interest. Publication bias cannot be ruled out.

Since tissue-specific therapy would be 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 diagnosis from a TOO test into empiric therapy or tissue-specific therapy. A prospective trial such as the one reported by Monzon et al.[39] maybe the best design available in this case.

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 cost effectiveness 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 CancerType ID 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**.

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**

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 537 unique citations. Fifteen of these were protocol references with 522 unique citations remaining for title and abstract review.

Appendix C. Evidence Tables

Table 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
CancerTYPE ID Erlander, Ma 2011 NS Multinational, US	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
CancerTYPE ID Greco 2010 2000-2007 US Only	NR	Multiple age groups	25 - 50: 4 50-64: 8 65+: 8	11	28	Breast, primary peritoneal, ovary, colon, non-small cell lung cancer, gastric, melanoma, pancreas
CancerTYPE ID Hainsworth, Schnabel et al. 2011 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

Table 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
CancerTYPE ID Hainsworth 2010 [Disease] NS NS	NR	Multiple age groups	Median: 64 Range: 26-85	33	110	Cancer of unknown primary site
CancerTYPE ID Hainsworth 2010 [Survival] NS NS	NR	Multiple age groups	Median: 64 Range: 26-85	33	110	Cancer of unknown primary site
CancerTYPE ID Ma, Patel, et al., 2006 NS US 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, Menigioma, 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

Table 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
G-banded karyotype (supplemented by FISH and comparative genomic hybiridization Pantou 2003 2001 Multinational, not US	NR	Multiple age groups	< 25 (1) 25-50 (4) 50-64 (4) 65-older (7) Mean age: 59.2	3	34	cancer of unknown primary
miRview Chajut 2011 Multinational, US	NR	NR	NR	NR	179	Adrenocortical Carcinoma, Pheochromocytoma, Squamous Cell Carcinoma (Anus or Skin), Cholangioca. or Adenoca. of Extrahepatic Biliary Tract, Urothelial Carcinoma, Astrocytic tumor, Oligodendroglioma, Adenocarcinoma of the Breast, Squamous Cell Carcinoma (Uterine Cervix), Colorectal adenocarcinoma, Carcinoid (Gastrointestinal Tract), Gastrointestinal Stromal Tumor (GIST), Renal Cell Carcinoma (chromophobe), Renal Cell Carcinoma (clear cell), Renal Cell Carcinoma (papillary), Hepatocellular Carcinoma, Lung, large cell or adenocarcinoma, Lung (small cell carcinoma), Carcinoid (Lung), Squamous Cell Carcinoma (Lung, Head&Neck, or Esophagus), Pleural Mesothelioma,

Table 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 Chajut 2011 Multinational, US (continued)					Training set,1282; Validation set, 509	Lymphoma, Ovarian Primitive Germ Cell Tumor, Pancreatic Adenocarcinoma, Pancreatic Adenocarcinoma, Prostatic Adenocarcinoma, Ewing Sarcoma, Chondrosarcoma, Malignant Fibrous Histiocytoma (MFH) or Fibrosarcoma, Osteosarcoma, Rhabdomyosarcoma, Synovial Sarcoma, Liposarcoma, Melanoma, Gastric or Esophageal adenocarcinoma, Non-Seminomatous Testicular Germ Cell Tumor, Seminomatous Testicular Germ Cell Tumor, Thymoma/Thymic Carcinoma, Thyroid Carcinoma (follicular), Thyroid Carcinoma (papillary), Thyroid medullary
miRview Mueller 2011 2008 Germany	NR	NR	NR	NR	102	Billary tract, Breast, Head & Neck, Kidney, Liver, Lung, Melanocyte, Ovary , Stomach or esophagus, Thyroid, Colon
miRview Rosenfeld 2008 NS Multinational, not US	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

Table 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 Rosenwald 2010 NS Multinational, US	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 2008-2010 US 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
Pathwork Beck 2011 NS US only	NR	NR	NR	NR	49	Bladder, breast, colorectal, gastric, hepatocellular, melanoma, liver, synovial sarcoma, sarcoma, ovarian, pancreatic, prostate, renal, thyroid
Pathwork Dumar 2008 NS US only	NR	NR	NR	NR	60	breast, Colorectal, Non-small-cell lung, non-Hodgkins's lymphoma, lymphoma, pancreas, bladder, gastric, germ cell, hepatocellular, kidney, melanoma, ovarian, prostrate, soft tissue, sarcoma, thyroid
Pathwork Grenert, Smith 2011 2000-2007 US only	NR	Multiple age groups	Median: 55 Range: 22 -74	20	45	Bladder, Breast, Colorectal, Gastric, Testicular gem cell, Kidney, Hepatocellular, Non-small cell lung, Non-Hodgkin's Lymphoma, Melanoma, Ovarian, Pancreas, Prostate, Sarcoma, Thyroid

Table 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 Gutierrez 2011 NS US only	NR	NR	NR	63 patients	66 physicians, 111 patients	NR
Pathwork Hainsworth 2011 2005 US only	NR	Multiple age groups	Range: 21-86 Median: 56	21	48	Cancer of unknown primary
Pathwork Laouri 2010 2009 NS	NR	Multiple age groups	Median: 62 Range: 16-89	161	284	Liver, Lymph Node, Omentum and Peritoneum, Lung, Soft Tissue, Bone, Neck, Brain, Ovary, Colon and Rectum, Pleura, Pelvis, Small Bowl, Other
Pathwork Medeiros 2008 NS US only	NR	NR	NR	NR	11	Cancer of unknown primary
Pathwork Monzon 2010 2006 US only	NR	Multiple age groups	40-49: 1 50-59: 4 60-69: 6 70-79: 7 80-89: 3	15	21	Cancer of unknown primary

Table 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 Monzon 2009 NS Multinational, US	NR	Multiple age groups	< 50 (142 50-59 (133) 60-69 (139) >= 70 (132)	290	547	Colorectal, Pancreatic, Non-small Cell Lung, Breast, Gastric, Kidney, Hepatocellular, Oavry, Sarcoma, Non-Hodgkin's Lymphoma, Thyroid, Prostate, Melanoma, Bladder, Testicular Germ Cell
Pathwork Pillai 2010 NS US only		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 non-small cell lung cancer, as well as non-Hodgkin's lymphoma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, thyroid cancer, and sarcoma.
Pathwork Stancel 2011 NS US only	NR	NR	NR	NR	27	Lung, Lymphoma, Colon, Pancreas, Breast, Ovarian, Gastric
Pathwork Wu 2010 NS US only	NR	Multiple age groups	Range: 21-56 Median: 41	3	15	Lung, Breast, Melanoma, Lymphoma, Sarcoma, Colon, Head & Neck, Gastric, Kidney

Table 2. KQ2. 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
CancerTYPE ID Erlander, Ma 2011	Sensitivity NR Specificity NR	NR 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
miRview Chajut	Sensitivity Interlaboratory concordance: > 0.95% in 160 samples Specificity NR	NR NR	NR NR	NR	NR	NR	NR

Table 2. KQ2. 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 Rosenfeld 2008	Sensitivity NR Specificity NR	NR NR	NR NR	NR	NR	Array platform validated by RT-PCR. miRNSS maintianed expression distributions and diagnositic roles.	NR
miRview Varadhachary 2011	Sensitivity NR Specificity NR	NR NR	NR NR	NR	87/104 samples passed tumor cotent criteria. 74/87 passed all QA criteria.	Controls: No sample; no RNS; external positives. Quality parameters for RNS amplicfication.	NR
Pathwork Dumar 2008	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 RNS quantity and quality produced sufficient cRNS for hybirdization. 31/227 samples required >1 labeling reaction. Data verification algorithm addresses RNS quality, inadequate amplification, insufficient quanity of labeled RNS, idadequate hybirdization time or temperature. 218/227 gene expression data files passed verification. All 9 failed files showed evidence of RNS 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 2. KQ2. 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 Pillai 2010	Sensitivity Overall interlaboratory concordance: 133/149 Specificity NR	NR NR	NR NR	NR	Percent 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.	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 speciments outside 95% limit of agreement Overall Signal >= 10	No change in test performance by age of specimen

Table 3. KQ3a. Evidence of accuracy of tissue-of-origin tests in classifying the origin and type of tumor and adherence of statistical methods to guidelines [3]

Tissue of Origin Test , Author, Year	Total Expression	Selected Housekeeping	Hypothesis Tests	Ranking	Clustering (e.g., PCA)	Classification	Internal	External
CancerTYPE ID Ma, Patel, et al., 2006	NR	Raw Cy5/Cy3 ratios per array were normalized using nonlinear local regressionn, 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	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
miRview Rosenwald 2010	Expression of each microRNS - average expression of all microRNSs of the sample + scaling constant (the average expression over the entire sample set)	NR	NR	Decesion tree algorithm used that finally selected 48 miRNSs through feature selction	NR	Unsupervised and Supervised Classifier that combines binary decision tree and K-nearest neighbors (KNN) trained on 649 patients	NR	Test performance assessed using independent set of 204 validation samples
Pathwork Dumur 2008	NR	All raw expression based on aggregate expression levels for 121 mRNS markers stably expressed across cell types	NR	Classification model uses 1550 markers chosen by gene ranking	NR	NR	NR	NR

Table 4. KQ3b-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 standards

Tissue of Origin Test , Author, Year	Sample Size	Gold standard (Comparison Test)	Sensitivity	Specificity
CancerTYPE ID Erlander, Ma 2011	187	Known origin	0.83 (numbers not reported)	0.99
CancerTYPE ID Ma, Patel, et al., 2006	Validation: 119 FFPE	Known origin	"Accuracy" Overall (95% CI): 82% (74% - 89%) Unclassifiable - 8 (6.7%)	NR
G-banded karyotype (supplemented by FISH and comparative genomic hybiridization Pantou 2003	20	NR	5/20 identified cases 2/20 no mitoses 1/20 normal karyotype	NR
miRview Chajut	509; 489 processed successfully	Known origin	418/489	> 99% (numbers not shown)
miRview Mueller 2011	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	83 (blinded test set)	Known origin	Combined accuracy using DecisionTree & KNN = 86%	Combined value not available Decision Tree=99% KNN=NR
miRview Rosenwald 2010	204 validation samples; 7 metastesis 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%
Pathwork Beck 2011	42 (39 on panel)	known origin	29/39 Indeterminate: 7/39 Incorrect: 3/39	NR
Pathwork Dumar 2008	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
Pathwork Dumur, Blevins et al., 2008 abstract	20	Pathology report or IHC	Agreed: 14/17 Indeterminate: 1/17 Discordant with pathology: 2	NR

Table 4. KQ3b-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 standards (continued)

Tissue of Origin Test , Author, Year	Sample Size	Gold standard (Comparison Test)	Sensitivity	Specificity
Pathwork Grenert, Smith 2011	37	95% (81.8 - 99.3)	99.60%	NR
Pathwork Monzon 2009	547	Known origin	480/547 (87.8%) Non-agreement: 39/547 Indeterminate: 28/547	99.40%
Pathwork Pillai 2010	462	Known origin	Overall agreement: 409/462. Sensitivity: 88.5	99.1
Pathwork Stancel 2011	20 Passed QA: 19	Known origin	15/19 (78.9%)	NR
Pathwork Wu 2010	15 Results, 1 off-panel specimen: 14 Sample size = 13	Known origin	12/13	NR

Table 5. KQ3b-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	Site	Sample size	Sensitivity	Specificity
CancerTYPE ID	1. Adrenal	1. 2	1. 1.00	1. 1.00
	2. Brain	2. 5	2. 1.00	2. 1.00
Erlander, Ma 2011	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

Table 5. KQ3b-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	Site	Sample size	Sensitivity	Specificity
CancerTYPE ID	1.Adrenal	1. 1	FFPE Accuracy	NR
	2. Brain	2. 3	1. 1.00	
Ma, Patel, et al., 2006	3. Breast	3. 1	2. 1.00	
	4. Carcinoid–intestine	4. 2	3. 1.00	
	5. Cervix–adeno	5. 2	4. 1.00	
	6. Cervix–squamous	6. 3	5. 0.50	
	7. Endometrium	7. 3	6. 0.67	
	8. Gallbladder	8. 0	7. 0.67	
	9. Germ–cell	9. 9	8. -	
	10. GIST	10. 3	9. 0.78	
	11. Kidney	11. 4	10. 1.00	
	12. Leiomyosarcoma	12. 3	11. 1.00	
	13. Liver	13. 2	12. 0.33	
	14. Lung–adeno–large cell	14. 3	13. 1.00	
	15. Lung–small	15. 5	14. 0.00	
	16. Lung–squamous	16. 3	15. 0.40	
	17. Lymphoma	17. 10	16. 1.00	
	18. Meningioma	18. 3	17. 1.00	
	19. Mesothelioma	19. 5	18. 1.00	
	20. Osteosarcoma	20. 2	19. 0.80	
	21. Ovary	21. 5	20. 1.00	
	22. Pancreas	22. 3	21. 1.00	
	23. Prostate	23. 7	22. 1.00	
	24. Skin–basal cell	24. 4	23. 1.00	
	25. Skin–melanoma	25. 4	24. 0.75	
	26. Skin–squamous	26. 3	25. 0.75	
	27. Small and large bowel	27. 6	26. 1.00	
	28. Soft-tissue	28. 8	27. 0.83	
	29. Stomach–adeno	29. 3	28. 0.88	
	30.Thyroid–follicular–papillary	30. 3	29. 0.00	
	31.Thyroid–medullary	31. 0	30. 1.00	
	32.Urinary bladder	32. 6	31. -	
	33. Overall	33. 481	32. 1.00 33.119	

Table 5. KQ3b-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	Site	Sample size	Sensitivity	Specificity
G-banded karyotype (supplemented by FISH and comparative genomic hybiridization Pantou 2003	NR	NR	NR	NR
miRview Chajut	NR	NR	NR	NR
miRview Mueller 2011	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 5. KQ3b-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	Site	Sample size	Sensitivity	Specificity
miRview	1. Bladder	1. 2	Decision Tree	Decision Tree
	2. Brain	2. 5	1. 0	1. 100
Rosenfeld 2008	3. Breast	3. 5	2. 100	2. 100
	4. Colon	4. 5	3. 60	3. 97
	5. Endometrium	5. 3	4. 40	4.99
	6. Head & neck	6. 8	5. 0	5.99
	7. Kidney	7. 5	6. 100	6.99
	8. Liver	8. 2	7. 100	7.99
	9. Lung	9. 5	8. 100	8.99
	10. Lung pleura	10. 2	9. 80	9.95
	11. Lymph node	11. 5	10. 50	10. 99
	12. Melanocytes	12. 5	11. 60	11. 100
	13. Meninges	13. 3	12. 60	12. 97
	14. Ovary	14. 4	13. 100	13. 99
	15. Pancreas	15. 2	14. 75	14. 97
	16. Prostate	16. 2	15. 50	15. 100
	17. Sarcoma	17. 5	16. 100	16. 100
	18. Stomach	18. 7	17. 40	17. 99
	19. Stromal	19. 2	18. 71	18. 96
	20. Testis	20. 1	19. 100	19. 100
	21. Thymus	21. 2	20. 100	20. 100
	22. Thyroid	22. 3	21. 100	21. 98
			22. 100	22. 100

Table 5. KQ3b-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	Site	Sample size	Sensitivity	Specificity
miRview	Validation	Validation only	(1 or 2 predictions)	(1 or 2 predictions)
Rosenwald 2010	1. Biliary tract	1. 7	1. 66.7%	1. 94%
	2. Brain	2. 11	2. 100%	2. 100%
	3. Breast	3. 38	3. 66.7%	3. 93.6%
	4. Colon	4. 9	4. 88.9%	4. 94.4%
	5. Esophagus	5. 1	5. 100%	5. 98.4%
	6. Head and neck	6. 3	6. 100	6. 92.4%
	7. Kidney	7. 10	7. 87.5%	7. 99.4%
	8. Liver	8. 8	8. 100%	8. 99.4%
	9. Lung	9. 26	9. 91.3%	9. 84.9%
	10. Melanoma	10. 7	10. 85.7%	10. 97.8%
	11. Ovary	11. 13	11. 84.6%	11. 100%
	12. Pancreas	12. 6	12. 50%	12. 97.8%
	13. Prostate	13. 20	13. 89.5%	13. 99.4%
	14. Stomach or esophagus	14. 7	14. 40%	14. 98.9%
	15. Testis	15. 8	15. 100%	15. 100%
	16. Thymus	16. 6	16. 83.3%	16. 97.8%
	17. Thyroid	17. 24	17. 100%	17. 98.2%
Pathwork Beck 2011	NR	NR	1. 100% 2. 0% 3. 0%	1. 100% 2. 0% 3. 0%
Pathwork Dumar 2008	NR	NR	NR	NR
Pathwork Dumur, Blevins et al., 2008 abstract	NR	NR	NR	NR

Table 5. KQ3b-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	Site	Sample size	Sensitivity	Specificity
Pathwork	1. Bladder 3	1. 3	1. 3	NR
	2. Breast 2	2. 2	2. 2	
Grenert, Smith 2011	3. Colorectal 3	3. 3	3. 3	
	4. Gastric 2	4. 2	4. 2	
	5.Testicular gem cell 3	5. 3	5. 2	
	6. Kidney 4	6. 4	6. 4	
	7. Hepatocellular 3	7. 3	7. 3	
	8. Non-small cell lung 2	8. 2	8. 2	
	9. Non-Hodgkin's Lymphoma 3	9. 3	9. 3	
	10. Melanoma 2	10. 2	10. 2	
	11. Ovarian 3	11. 3	11. 2	
	12. Pancreas 2	12. 2	12. 2	
	13. Prostate 1	13. 1	13. 1	
	14 Sarcoma 3	14. 3	14. 3	
	15 Thyroid 1	15. 1	15. 1	
Pathwork	1. Bladder	1. 28	1. 22/28	1. 519/519
	2. Breast	2. 68	2. 64/68	2. 471/479
Monzon 2009	3. Colorectal	3. 56	3. 52/56	3. 487/491
	4. Gastric	4. 25	4. 18/25	4. 519/522
	5. Germ Cell	5. 30	5. 22/30	5. 517/517
	6. Hepatocellular	6. 25	6. 23/25	6. 521/522
	7. Kidney	7. 39	7. 37/39	7. 507/508
	8. Melanoma	8. 26	8. 21/26	8. 520/521
	9. Non-Hodgkin’s lymphoma	9. 33	9. 31/33	9. 511/514
	10. Non–small cell lung	10. 31	10. 27/31	10. 509/516
	11. Ovarian	11. 69	11. 64/69	11. 473/478
	12. Pancreas	12. 25	12. 18/25	12. 521/522
	13. Prostate	13. 26	13. 23/26	13. 521/521
	14. Soft tissue sarcoma	14. 31	14. 26/31	14. 513/516
	15. Thyroid	15. 35	15. 32/35	15. 510/512

Table 5. KQ3b-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	Site	Sample size	Sensitivity	Specificity
Pathwork Pillai 2010	1. Bladder 2. Breast 3. Colorectal 4. Gastric 5. Hepatocellular 6. Kidney 7. Melanoma 8. Non-Hodgkin's lymphoma 9. Non-small 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
Pathwork Stancel 2011	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
Pathwork Wu 2010	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

Table 6. KQ4. 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
CancerTYPE ID	Erlander, Ma 2011	296 (142)	4	252
	Greco 2010	18 (15)	2	NR
G-banded karyotype (supplemented by FISH and comparative genomic hybiridization	Pantou 2003	5/20 identified cases 2/20 no mitoses 1/20 normal karyotype	NR	NR
miRview	Mueller 2011	50 (40) 10 were discordant 4 origin never diagnosed	NR	NR
	Varadhachary 2011	74 (62)	NR	IHC not helpful: 9 TOO prediction: 9 TOO consistent with clinicopathological: 7
Pathwork	Beck 2011	4 (2 clearly incorrect)	3	2
	Gutierrez 2011 (abstract)	Changed diagnosis, % (95% CI): 54% (46-62) p <0.0001	NS	67%
	Hainsworth 2011 (J Mol Biomark Diagn)	43	2	NR
	Medeiros 2008 (abstract)	8 (73%) TOO results 6/8 consistent with clincopathologic characteristics	3	NR
	Monzon 2010	16 (10) 6 plausible	5	16
	Laouri 2010	Non-specific dx: 172 Specific Dx: 100 (New 57,;Confirm 43)	No Specific Dx: 11 Specific Dx: 1	NR

Table 7. KQ4a. 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
CancerTYPE ID Hainsworth 2010	110	Received assay directed treatment: 66 Evaluated: 51	Diagnosed with CUP after standard diagnostic evaluation; Metastatic carinoma; Exclusion: treatable subsets of CUP No previous systematic treatment Biopsy material available for assay ECOG performance status 0-2 No uncontrolled brain metasteses	NS	Pancreatic: 11 Colorectal: 8 Urinary Bladder: 8 Non-small cell lung: 5 Ovarian: 4 Carcinoid - interstine: 4 Breast: 3 GallBladder: 3 Liver: 3 Renal cell: 3 Skin (squmous): 3 Other: 7 No specific diagnosis: 5	Assay directed treatment	Objective treatment response: 21	44 (40%)	66 (60%)	51% response for assay directed therapy
CancerTYPE ID Hainsworth, 2011	125	42 (34%)	NS	NS	Identified by TOO test with ≥ 80% probability as colorectal adenocarinoma	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 7. KQ4a. 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
Pathwork Gutierrez 2011	111	111	NR	SS	NR	NR	NR	NR	Changed treatment recommendations: 65% (95% CI: 57 - 75) Changed chemotherapy regimen: 61 (55%, 95% CI: 47 - 63)	P< 0.0001
Pathwork Laouri 2010	284 Non-specific dx: 183 Specific Dx: 101	284	NR	SS	TOO Predicted: 221 (78%) Colorectal (15%) Breast (15%) Ovary (13%) Pancreas (13%) Non-small 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 8. KQ4b. 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
CancerType ID Hainsworth, 2011	125	42 (34%)	NS	NS	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
CancerType ID Hainsworth 2010 [Disease]	66	51 evaluated	NR	Diagnosed with CUP after standard diagnostic evaluation	NR	Pancreatic: 11 Colorectal: 8 Urinary Bladder: 8 Non-small cell lung: 5 Ovarian: 4 Carcinoid - interstine: 4 Breast: 3 GallBladder: 3 Liver: 3 Renal cell: 3 Skin (squmous): 3 Other: 7 No specific diagnosis: 5	Stable disease TOO: 21/51 Control: None	NR	NR
CancerType ID Hainsworth 2010 [Survival]	66	51 evaluated	NR	Diagnosed with CUP after standard diagnostic evaluation	NS	Pancreatic: 11 Colorectal: 8 Urinary Bladder: 8 Non-small cell lung: 5 Ovarian: 4 Carcinoid - interstine: 4 Breast: 3 GallBladder: 3 Liver: 3 Renal cell: 3 Skin (squmous): 3 Other: 7 No specific diagnosis: 5	Survival TOO: 12.9 months Control: None	NR	NR

Appendix D. Quality Assessment Ratings of Studies

Title (Year)	KQ2 and KQ3	KQ4
Barr (1995)[59]	G	NA
Beck (2011)[37]	G	F
Chajut (2011)[27]	G	NA
Dumur (2008)[32]	G	NA
Dumur (2008) Assessing the impact of tumor devitalization time (poster)[60]	F	NA
Dumur 2008. Clinical verification of the Pathwork TOO Test (abstract)[29]	G	NA
Erlander (2011)[24]	G	NA
Gamberi (2011)[61]	F	NA
Greco (2010)[42]	G	NA
Grenert (2011)[38]	G	NA
Gutierrez[43]	NA	G
Hainsworth, Pillai et al. (2011)[48]	NA	G
Hainsworth, Schnabel et al. [55](2011)	NA	F
Hainsworth Spigel et al. (2010) (poster)[54]	NA	G
Lae (2002)[62]	G	NA
Laouri[50]	NA	F
Lewis (2007)[63]	G	NA
Ma (2006)	G	NA
Medeiros (2008) (abstract)[49]	NA	F
Mhawech-Fauceglia (2006)[64]	G	NA
Monzon (2009)[39]	G	NA
Monzon (2010)[44]	G	NA
Mueller (2011)[36]	F	F
Pantou (2003) [19]	NA	G
Patel (2005)[65]	G	NA
Pillai (2010)[66]	G	NA
Rosenfeld (2008) [25]	G	NA
Rosenwald (2010)[26]	G	NA
Stancel (2011) [40]	G	G
Varadhachary (2011)[28]	G	G
Wu (2010)[41]	G	NA
Yamaguchi (2005)[67]	F	NA

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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 as Background

Excluded at Abstract Stage

1. . CDKN2A mutations and MC1R variants in Italian patients with single or multiple primary melanoma. Pigment Cell & Melanoma Research. 2008 2008/12//;21(6):700+. Academic OneFile. Exclusion Code: EXC1.
2. . Abstracts from the 2008 Annual Meeting of the American Epilepsy Society. Epilepsia. 2008 2008/10//;49(s7):1+. Academic OneFile Exclusion Code: EXC1.
3. . CLINICAL VIGNETTES. Am J Gastroenterol. 2008 2008/09//;103(s1):S206. Academic OneFile Exclusion Code: EXC2.
4. . COLORECTAL CANCER PREVENTION. Am J Gastroenterol. 2008 2008/09//;103 (s1):S537. Academic OneFile Exclusion Code: EXC1.
5. . INFLAMMATORY BOWEL DISEASE. Am J Gastroenterol. 2008 2008/09//;103(s1):S408. Academic OneFile Exclusion Code: EXC1.
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7. Mueller WC, Spector Y, Edmonston TB, et al. Accurate classification of metastatic brain tumors using a novel microRNA-based test. Oncologist. 2011;16(2):165-74. PMID: 21273512. Exclusion Code: EXC4

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Appendix F. Strength of Evidence Tables

Appendix Table 1. Strength of evidence for the analytic validity of tests to identify the tissue-of-origin for cancers of unknown primary? (KQ 2)

Test	Reported Measures of Analytic Validity	Number of Studies	Risk of Bias	Directness	Consistency	Precision	Strength of Evidence
CancerTYPE ID	High assay reproducibility; CV in repeat assays of same samples	1	Low	Direct	Unknown	NA	Insufficient
miRview	Interlaboratory concordance between markers; Validation of microarray against RT-PCR; Study QC results	3	Low	Direct	Unknown	NR	Insufficient
Pathworks	Interlaboratory correlation: pre- and post standardization expression levels; Coefficient of reproducibility; Inter-laboratory correlation	1	Low	Direct	Unknown	High	Insufficient

NA – Not applicable. NR- Not reported. CV- coefficient of variation

Appendix Table 2. Strength of evidence for the statistical validity of the development of the algorithms used by the tissue-of-origin for cancers of unknown primary? (KQ 3a) Assessed using Simon[3] criteria for classification algorithms for microarray tests.

Test	Number of Studies	Normalization	Statistical Methods	Supervision	Risk of Bias in Validation	Strength of Evidence
CancerTYPE ID	1	Valid	Valid	Valid	Low	High
miRview	2	Valid	Valid	Valid	Low	High
Pathwork DX	1	Valid	NR	NR	NR	Low

Appendix Table 3. Strength of evidence that tissue of origin test accurately classify the tissue-of-origin of cancers of known primary site (KQ 3b, clinical validity

Test	Number of Studies	Risk of Bias	Directness	Consistency	Precision	Strength of Evidence
CancerTYPE ID	3	Moderate	Direct	Consistent	Moderate Meta-analysis 95% CI: 78-86%	Moderate
miRview	2	Moderate	Direct	Consistent across studies	Moderate: Meta- Analysis 95% CI of accuracy: 83-88%	Moderate
Pathwork DX	7	Low	Direct	Consistent	High Meta-analysis 95% CI: 86-89%	High

Appendix Table 4. Strength of evidence that tissue of origin tests aid diagnosis or treatment decisions or improve health outcomes in cases of cancers of unknown primary? (KQ 4, clinical utility)

Measure of clinical utility	Number of Studies	Risk of Bias	Directness	Consistency	Precision	Strength of Evidence
Percent of cases with for which a tissue of origin the test identified a tissue of origin	11	Moderate	Direct	Consistent	G-banded chromosomes: 25% Microarray tests: Moderate 6 studies with > 40 samples: High	Moderate
Percent of CUP cases for which the TOO identified by the test was independently confirmed	2	Moderate	Direct	Consistent	Moderate	Low
Percent of CUP cases where test was considered clinically useful by physician or researcher	4	High	Indirect	Consistent	Low	Low
Change in treatment decisions	4	Moderate	Indirect	Consistent	Moderate	Low
Treatment Response: Tissue specific treatment based on TOO test Directed compared to usual treatment for CUPS cases	1	Moderate	Indirect	Unknown	Low	Insufficient
Change in survival	2	Moderate	Indirect	Consistent	Moderate	Insufficient
Change in disease progression	1	Low	Indirect	Unknown	NR	Insufficient

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 family, and non-Hodgkins lymphomas are the prototypic SRCTs.[68, 69] The recently described desmoplastic SRCT and rhabdoid tumors are SRCTs. The tumors that make up this group are listed in Table 1.[69, 70]

Table 1. Small Round Cell Tumors

Ewing sarcoma
Peripheral neuroectodermal tumor
Rhabdomyosarcoma
Synovial sarcoma
Non-Hodgkins lymphoma
Retinoblastoma
Neuroblastoma
Hepatoblastoma
Nephroblastoma

The most common SRCTs are the Ewing sarcoma family. Ewing sarcoma is a highly malignant tumor that commonly occurs in bone and soft tissues of children and adolescents, but is occasionally seen in adults.[71] It is the second most common pediatric bone tumor, accounting for 30 percent of all primary bone tumors. The Ewing sarcoma family of tumors shares common immunohistology and molecular genetics and is considered to be one single group.[72] The pathognomonic genetic marker of the Ewing sarcoma 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).[73, 74] Molecular identification of the specific type of translocation, as well as tumor type, has prognostic significance for SRCTs. Patients with localized Ewing sarcoma disease and tumor expressing type 1 EWS-FLI1 fusion transcript have longer disease-free survival than those with other fusion transcript types.[75]

The annual incidence of Ewing sarcoma for 1973 and 2004 in the United States was 2.93 cases /1,000,000.[76] Ewing sarcoma 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%).[77] The presence of metastases significantly affects long-term survival of patients with Ewing sarcoma. Although multidisciplinary care has improved the survival rate of patients with localized Ewing sarcoma 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.[78]

Table 2. Ewing Sarcoma 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	Ewing sarcoma 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	Ewing sarcoma Primitive neurodec-todermal tumor Clear cell sarcoma	NA	Signal pattern	NA
Ewing Sarcoma 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 cm3	Reverse Transcription Polymerase Chain Reaction/ Fluorescence Monitoring	Ewing sarcoma	Not found	Not found	Not found

Other mesenchymal tumors with specific translocations that should be considered in the differential diagnosis of Ewing sarcoma 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 rabdomyosarcoma and can be distinguished from other tumors by the detection of a PAX-FKHR gene translocation.[63] Alveolar rhabdomyosarcomas containing a PAX7-FKHR translocation are usually less invasive and have a better prognosis than those with the PAX3-FKHR gene translocation.[79]

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)).[63] Ladanyi et al. have reported that cases involving the SYT-SSX1 gene fusion have a worse prognosis.[80]

The diagnostic distinctions among the SRCTs are becoming increasingly important as specific, successful treatments are developed for each tumor. However, differential diagnosis had 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 majority of the tumor types is an important advantage in the differentiation of the SRCTs and a base for molecular testing.[59] 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 to diagnosis, RT-PCR enables the detection of circulating metastatic tumor cells in blood.[77] The growing needs for efficient genetic identification have led to development and application of commercially available molecular assays for detection of the chromosomal translocations and fusions in clinical samples of the SRCTs. The commercially available assays are listed in the Table 2.

Methods

As discussed in Chapter 2,[1] we designed the literature searches to identify papers on tissue of origin 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 Ewing sarcoma 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 diagnosis of small round cells tumors differed from those for Cancer of Unknown Primary Site (CUPS) tissue of origin test. A systematic review of genetic testing for diagnosis of small round cells tumors would have somewhat different key questions and required different search strategies. After discussion with AHRQ, we decided to focus resources on the CUPS tissue of origin (TOO) test.

We report here on 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 3. KQ3. Evidence of analytic validity of tissue-of-origin tests for Ewing sarcoma

Tissue of Origin 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
Ewing sarcoma (FISH and RT-PCR) Yamaguchi, 2012 NS Multinational, not U.S.	Age: 10–24: 7 25–49: 13 50–64: 6 65+: 2 NR: 9 Female: 13 N: 37	Ewing sarcoma /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: Ewing sarcoma/ 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
Ewing sarcoma (FISH) Mhawech-Fauceglia, 2006 NS U.S. only	Age: NS Female: NS N: 58	Ewing sarcoma	50%	100%	NR	NR
Ewing sarcoma (RT-PCR) Barr, 1995 1988–1993 U.S. only	Age: NS Female: NS N: 79	Alveolar rhabdomyosarcoma, embryonal rhabdomyosarcoma, Ewing sarcoma, 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 to FISH: 19/19	Technical success RT-PCR: 79/80 FISH: 31/41 Karotyping: 29/74	NR

Table 3. KQ3. Evidence of analytic validity of tissue-of-origin tests for Ewing sarcoma (continued)

Tissue of Origin 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
Ewing sarcoma (RT-PCR) Bridge, 2006 2004 U.S. only	Age: NS Female: NS N: 66	Ewing sarcoma/primitive neuroectodermal tumors undifferentiated round cell sarcomas, small cell carcinomas, neuroblastomas, alveolar rhabdomyosarcomas, fibrosarcomas, malignant teratoma	Ewing sarcoma/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
Ewing sarcoma (RT-PCR) Delattre, 1994 1993 NR	Age: Range: 1 to 48 Median: 13 Female: NR N: 114	Osseous Ewing Sarcoma, 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
Ewing sarcoma (RT-PCR) Gamberi, 2011 2006–2009 Multinational, not U.S.	Age: NS Female: 73 N: 222	Ewing sarcoma family tumors	Interpretable RT-PCR or FISH: 188/222 RT-PCR +: 144 FISH+: 24	NR	Tissue quality standards: Inadequate tissue ≤ 1000 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 tumor types of tumors, and a water blank

Table 3. KQ3. Evidence of analytic validity of tissue-of-origin tests for Ewing sarcoma (continued)

Tissue of Origin 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
Ewing sarcoma (RT-PCR) Lae, 2002 1992–2000 U.S. only	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 Ewing' sarcoma, primitive neuroectodermal tumor, rhabdomyosacoma, intraabdominal small round cell tumor, intraabdominal carcinoma.	28/29 (Southern blot confirmation) No molecular analysis: 2	1/1	NR	
Ewing sarcoma (RT-PCR) Lewis, 2007 NS U.S. only	Age: Range: 1 - 26 0–9: 9 10–24: 39 25–49: 2 Female: 15/50 N: 69 (2 cases had multiple tumors)	Ewing sarcoma Controls: Ewing sarcoma, 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
Ewing sarcoma (RT-PCR) Patel et al., 2005 NS U.S. only	Age: NS Female: NS 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

Abbreviations: FISH = fluorescent in situ hybridization; RT-PCR = reverse transcriptase polymerase chain reaction

Tumor-specific rearrangements were first identified and tested for using G-banded karotypes.[80] Over the last two decades, however, fluorescent in situ hybridization (FISH) or reverse transcriptase polymerase chain reaction (RT-PCR) have become the techniques 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 ability of 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 Ewing sarcoma as a laboratory test rather than a kit. ARUP Laboratories offer both a FISH and a RT-PCR test for Ewing sarcoma diagnosis.

Analytic Validity

We identified nine studies[59, 61-65, 67, 71, 80] that examined the analytic validity of genetic tests (RT_PCR, FISH, or karotype) for the diagnosis of Ewing sarcoma (Table 3). Five studies[59, 62-65] were rated good, and four were rated fair.[61, 67, 71, 80] All studies that performed RT-PCR used non-commercial probes for the analysis. Three good studies[61, 64, 65] and two fair studies[67, 80] reported on experience using the commercially available FISH break apart probe produced by Vysis, Inc. Mhawech-Faucegalia et al.[64] compared the Vysis FISH probe to 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 SRCT. Neither test was clearly better. Patel et al.[65] found that RT-PCR analysis detected fusion transcripts in all 8 of their samples, but the FISH probe detected translocations in only 7 (88%) samples. Gamberi et al.[61] 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.[80] 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, due primarily to the lower sensitivity of the RT-PCR assay.[80] 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.[67] also compared RT-PCR to the Vysis Inc. FISH probe. FISH detected translocations in 14 of 16 ES/PNET cases. RT-PCR results were only available for 7 cases; a transcript was detected for 5. Each method detected 1 translocation that was not detected by the other method. Fusion transcripts were detected by both methods in all 6 cases of DSRCT and in 5 of 6 cases of clear cell sarcoma.

One good study[59] and one fair study[71] compared RT-PCR, karyotype, and FISH, although not all tumors were tested with all three tests. In one study,[59] 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,[71] 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[59] and 6[71] additional cases. In the Delattre study,[71] FISH identified one translocation not identified by RT-PCR. Barr et al. 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 to FISH, the sensitivity of RT-PCR was 92 percent (11/12) and specificity was 100 percent.

Lae et al. 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.[63] 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 due to poor sample quality. Extraction or PCR failure was not associated with sample age.

Clinical Validity

Nine studies[59, 61-65, 67, 71, 80] examined the clinical validity of genetic tests (RT_PCR, FISH, or karotype) for the diagnosis of Ewing sarcoma (Table 4). Five studies[59, 62-65] were rated good, and four were rated fair.[61, 67, 71, 80]

Four studies,[61, 64, 67, 80] two good[61, 64] and two fair,[67, 80]reported on the sensitivity and specificity of FISH to diagnose ES or other SCRT by the detection of specific chromosomal translocations. The sensitivity ranged from 50 percent (19 of 38)[64] to 91 percent.[80] The two studies rated good reported lower sensitivity of FISH—50 percent[64] and 66 percent[61]—than the two studies rated fair—88 percent [67] and 91 percent.[80] In all four studies,[61, 64, 67, 80] specificity was 100 percent (15/15).

Eight studies[59, 61-63, 65, 67, 71, 80] reported on the ability of RT-PCR to diagnosis ES and other SCRTs by detecting fusion transcripts associated with specific tumors. Four studies[59, 62, 63, 65] were rated good, and four were rated fair.[61, 67, 71, 80] In 7 of the 8 studies, the sensitivity of RT-PCR ranged from 70 percent[65] to 93 percent.[62] The exception reported RT-PCR sensitivity to be 54 percent.[80] As previously noted, the reporting for this study[80] 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[80] to 100 percent.[61, 63, 65, 71] The quality of the study was not related to the reported sensitivity or specificity.

Table 4. KQ 3b - 3f. Evidence of accuracy of the tissue-of-origin test in Ewing Sarcoma

Tissue of origin test, Author, year Study Dates, Region	Sample Characteristics	Cancer/tumor types	Sample size	Gold standard (comparison test)	Sensitivity	Specificity
Ewing sarcoma (FISH) Mhawech-Fauceglia, 2006 NS U.S. only	Age: NS Female: NS N: 58	Ewing sarcoma	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)
Ewing sarcoma (FISH) Yamaguchi, 2012 NS Multinational, not U.S.	Age: 10–24: 7 25–49: 13 50–64: 6 65+: 2 Female: 13 N: 28	Ewing sarcoma/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
Ewing sarcoma (RT-PCR) Barr, 1995 1988–1993 U.S. only	Age: NS Female: NS N: 79	Alveolar rhabdomyosarcoma, embryonal rhabdomyosarcoma, Ewing sarcoma, desmoplastic small round cell tumor, undifferentiated small round cell tumor, other.	Expected positive: 1. Alveolar rhabdomyosarcoma=21 2. Ewing sarcoma=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 4. KQ 3b - 3f. Evidence of accuracy of the tissue-of-origin test in Ewing Sarcoma (continued)

Tissue of origin test, Author, year Study Dates, Region	Sample Characteristics	Cancer/tumor types	Sample size	Gold standard (comparison test)	Sensitivity	Specificity
Ewing sarcoma (RT-PCR) Bridge, 2006 2004 U.S. only	Age: NS Female: NS N: 66	Ewing sarcoma/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%
Ewing sarcoma (RT-PCR) Delattre, 1994 1993 NR	Age: Range: 1 to 48 Median: 13 Female: NR N: 114	Osseous Ewing sarcoma, atypical ES, peripheral primitive neuroectodermal, not ES tumors, lacking hallmarks of specific disease	114 (as stated in article)	Clinicopathological assessment	83/87	Non-ES: 0/12 Undifferen-tiated: 9/15
Ewing sarcoma (RT-PCR) Gamberi, 2011 2006–2009 Multinational, not U.S.	Age: NS Female: 73 N: 222	Ewing sarcoma family tumors	188	IHC	144/156	SRCT: 0/4 Non EFT/SRCT: 0/28
Ewing sarcoma (RT-PCR) Lae, 2002 1992–2000 U.S. only	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 Ewing sarcoma, primitive neuroectodermal tumor, rhabdomyosacoma, intraabdominal small round cell tumor, intraabdominal carcinoma.	32	Clinico-pathological assessment	RT-PCR = 28/30 (93%) Southern blot hybridization = 29/30 (97%)	NR

Table 4. KQ 3b - 3f. Evidence of accuracy of the tissue-of-origin test in Ewing Sarcoma (continued)

Tissue of origin test, Author, year Study Dates, Region	Sample Characteristics	Cancer/tumor types	Sample size	Gold standard (comparison test)	Sensitivity	Specificity
Ewing sarcoma (RT-PCR) Lewis, 2007 NS U.S. only	Age: Range: 1–26 0–9: 9 10–24: 39 25–49: 2 Female: 15/50 N: 69 (2 cases had multiple tumors)	Ewing sarcoma Controls: Ewing sarcoma, neuroblastoma, leiomyosarcoma, desmoplastic sarcoma, synovial sarcoma, rhabdomyosarcoma	ES: 53 non ES: 11	IHC	41/50 (82%)	0/11
Ewing sarcoma (RT-PCR) Patel et al., 2005 NS U.S. only	Age: NS Female: NS N: 42	Clear cell sarcoma, malignant melanoma	Clear cell sarcoma N = 10 Malignant melanoma N = 32	Known origin	7/10 (70%)	0/32 (100%)
Ewing sarcoma (RT-PCR) Yamaguchi, 2012 NS Multinational, not U.S.	Age: 10–24: 7 25–49: 13 50–64: 6 65+: 2 Female: 13 N: 37	Ewing sarcoma/primitive neuroectodermal tumour (ES/PNET), desmoplastic small round cell tumour (DSRCT), clear cell sarcoma (CCS) Negative controls: Poorly differentiated synovial sarcoma, alveolar rhabdomyosarcoma, neuroblastoma	37	Clinico-pathological assessment	RT-PCR: EWS/PNET: 5/7 DSRCT: 6/6 CCS: 5/5	6/9

Clinical Utility

One study[61] 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 not complete or systematic. The literature reviewed here was identified ancillary to our systematic review of genetic or molecular tests for cancers of unknown primary 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[59, 71] compared molecular analysis to G-banded karyotype, but both demonstrated that the molecular techniques have much higher success and sensitivity. Neither FISH or 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.,[61] would provide the best sensitivity in clinical practice.

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