



# Technology Assessment: Genetic and Molecular Tests to Identify Tissue of Origin of Cancers of Unknown Primary Site

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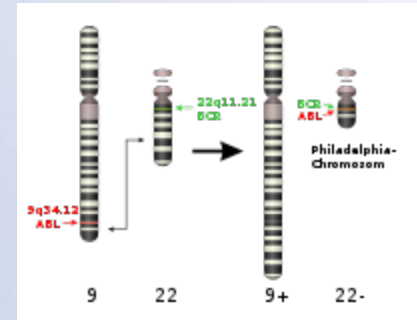
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# Tests to Identify Primary Site

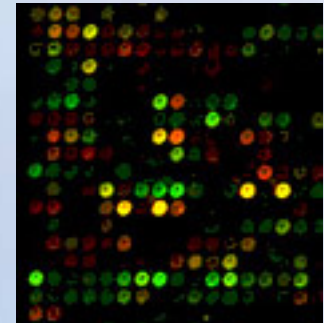
- Light microscopy
  - Tissue type
- Immuno-histochemical staining
  - Cell type
  - Subtype classification
  - Tissue of origin
- Imaging
  - Detect small or latent primary tumors
  - X-ray
  - Computed tomographic (CT) scans
  - Positron emission tomographic (PET) scans
- Molecular and genetic tests

# Molecular and Genetic Tests to Identify Tissue of Origin

- Cytogenetic analysis
  - Rearrangements specific to one cancer type
    - Karyotype
    - Fluorescent in-situ hybridization (FISH)
    - Reverse transcriptase polymerase chain reaction (RT-PCR)
- Gene expression and regulation
  - Pattern identification
  - Test-specific
    - Analytes (mRNA, microRNA)
    - Methodology (RT-PCR, microarray)
    - Panel composition
  - Statistical algorithms
    - Analyze pattern
    - Predict tissue of origin



Credit: Modified by Master Uegly from *Philadelphia Chromosome Translocation*, author A. Obeidat. Obtained via Wikipedia Commons.

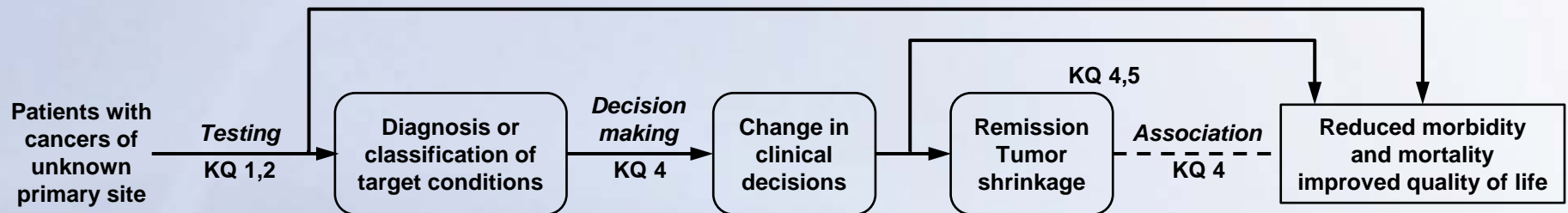


Credit: author Elapied via Wikipedia Commons.

# Key Questions

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. Were valid methods used to develop the statistical algorithms?
  - b. Does the test correctly predict tissue of origin when TOO is known?
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?

# Analytic Framework



# PICOTS

- **Population (KQ 1–4).** Patients of any age with CUPS
- **Population for KQ 5.** Patients 65 and older with CUPS
- **Interventions.** Use of genetic or molecular tests for the identification of tumor TOO in addition to or instead of other methods
- **Comparators for KQ 3.** Standard used in included studies
  - Tumors of known origin
  - IHC staining, PET imaging, or other methods.
- **Comparators for KQ 4.** Treatment regimen or health outcome among patients that did not have genetic or molecular TOO testing.
- **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.** Any geographic location. Inpatients or outpatients

# Methods – Test Identification

- Commercially available tests
  - Mechanism to order the test or a kit to perform the test
  - Identified through Internet search or reference laboratory test directory
- Internet search for tests
  - Search engine: Google
  - Search strategy: "tissue of origin" OR "cancer of unknown" OR "tumors of unknown" AND laboratory test
  - Limits: English, updated in last year



# Methods-Search for Studies

- Sources
  - Pubmed, Cochrane, EMBASE
  - Lexus Nexus, ClinicalTrials.gov, Health Services Research Projects in Progress, European Union Clinical Trials Register
  - Test developers' Web sites
- Included
  - Systematic reviews, controlled trials, observational studies, case series
- Limits
  - Published in English after 1990
- Strategies
  - MeSH Headings
  - Text word
  - By test name

# Methods–Evidence Synthesis

- **Quality Assessment**
  - Methods Guide for Medical Test Reviews
  - Analytic and clinical validity: QUADAS<sup>1</sup> criteria
  - Clinical utility: RTI Question Bank<sup>2</sup>
    - Sample selection
    - Study performance
    - Attrition
    - Detection of outcomes
    - Completeness of reporting
- **Meta-Analysis**
  - Clinical validity: proportion of tumor origins identified accurately
  - Univariate fixed-effects model using MetaSEM package in R

1. Whiting P et al. BMC medical research methodology. 2003 Nov 10;3:25.

2. Viswanathan M, Berkman ND. Development of the RTI Item Bank on Risk of Bias and Precision of Observational Studies. Rockville (MD); 2011.

# Methods-Evidence Synthesis, Continued

- Strength of Evidence
  - KQ 2, KQ 3b, and KQ 4: Evidence Practice Center domains
    - Risk of bias (low, moderate or high)
    - Consistency (consistent, inconsistent)
    - Directness (direct, indirect)
    - Precision of the evidence (low, moderate or high)
  - KQ 3a: Simon criteria for valid algorithm development
    - Normalization
      - Valid if standardization of gene expression levels or expression levels of predetermined set of housekeeping genes
    - Statistical classification method
    - Supervised or unsupervised (preferred supervised classification)
    - Risk of bias in the validation methods
  - Ratings: Low, Moderate, High, Insufficient

Simon R, et al. Journal of the National Cancer Institute. 2003 Jan 1;95(1):14-8

## Methods-Assessing Applicability

- Inclusion of Medicare population in body of evidence
  - Age
  - Race
  - Gender
  - Primary diagnosis

# Search Results

Search Process	Number of Articles
Retrieved	840
Title and abstract review	697
Full text review	150
Included studies: CUP	41
Included studies: Ewing sarcoma	8
Quality Assessments	Good: 33 Fair: 16 Poor: 1

# KQ1-Genetic and Molecular Tests to Identify Tumor Tissue of Origin

	<b>Pathworks TOO</b>	<b>CancerTYPE ID</b>	<b>Mirview mets</b>	<b>Chromosomal Analysis</b>
Analyte	mRNA	mRNA	microRNA	chromosomes
Panel size	TOO-FFPE: 1,550 TOO-FRZ: 2,000 Endometrial: 316	92	Mets: 48 Mets <sup>2</sup> : 62	46
Laboratory methods	Expression microarray analysis	Quantitative real time PCR	Quantitative real time PCR	High resolution G-banded
Statistical Methods	Pairwise comparison, machine learning algorithm	Kohonen neural network (KNN)	Binary decision tree and KNN	NA
No. Sites Identified	FFPE and FRZ: 15 Endometrial: 2	28	Mets: 22 Mets <sup>2</sup> : 24	Not available
Reported Results	Similarity score	Probability of each type	Predicted tumor type from each algorithm	Karyotype

# KQ1-Coverage of Common Tumor Sites

Ten Most Common Primary Sites Identified by Autopsy			
Cancer Type	Pathworks TOO	CancerTYPE ID	miRview
Lung	✓	✓	✓
Pancreas	✓	✓	✓
Liver/bile duct	✓	✓	✓
Kidney/adrenal	✓	✓	✓
Bowel	✓	✓	✓
Genital	✓	✓	✓
Stomach	✓	✓	✓
Bladder/ureter	✓	✓	✓
Breast	✓	✓	✓

Pentheroudakis G, Gelfinopoulos V, Pavlidis N. Switching benchmarks in cancer of unknown primary: from autopsy to microarray. European journal of cancer. 2007 Sep;43(14):2026-

## KQ2-Analytic Validity

	<b>Pathworks TOO</b>	<b>CancerTYPE ID</b>	<b>miRview</b>
Number of studies	4	1	5
Total tumors	640	487	1546
Marker accuracy	Coefficient of reproducibility: 32.48 +/- 3.97	Reproducibility (Ct values): + controls: 1.7% - controls: 1.3%	Interlaboratory concordance: > 0.95%
Assay accuracy and precision	Interlaboratory correlation for SS score: 0.92-0.95	Known tumor type (Mean %CV): 1.6 (range 1.4-1.7) Concordance of prediction: 100%	Not reported



# KQ3–Statistical Validity of Algorithm Development

	<b>Pathworks TOO</b>	<b>CancerTYPE ID</b>	<b>miRview</b>
Normalization	Total expression	Housekeeping genes	Total expression
Dimension Reduction	Not enough detail to assess	Clustering with GLM to assess predictive value	Logistic regression to assess predictive value
Classification Rule Supervision	Supervised Not enough detail to assess	Supervised	Supervised
Internal Validation	Yes	Yes	Yes
External Validation	Yes	Yes	Yes
Criteria Met?	Mostly - classification and dimension reduction not evaluable	Yes	Yes

## KQ3b-Clinical Validity

	<b>Pathworks TOO</b>	<b>CancerTYPE ID</b>	<b>miRview</b>
Number of Studies	9	6	4
Total tumors	1,243	1,478	1,198
Comparison Standards	Cancers of known origin / IHC	Final diagnosis	Known origin
Percent Accuracy (range)	74-97	82-95	85-88
Percent Indeterminate (range)	5-18	NR	NR
Meta-analysis estimate of accuracy	0.88 95% CI: 86-89%	0.85 95% CI: 83-86%	0.85 95% CI: 83-87%

## KQ4-Clinical Utility–Diagnosis

Outcome	Number of Studies	Summary of Results
TOO Predicted	17	57 – 100% > 90% in 9 studies
TOO Confirmed	9	48 – 88%
Test Changed or Resolved Diagnosis	5	44 – 81%
Test Reported to be Clinically Useful	1	66 – 67 %

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

## KQ4-Clinical Utility for Treatment Decisions

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

## KQ4-Clinical Utility for Improving Outcomes

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

## KQ5-Applicability to Medicare Patients

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

# Summary of Strength of Evidence

<b>Analytic Validity</b>	<b>Number of Studies</b>	<b>Risk of Bias</b>	<b>Consistency</b>	<b>Precision</b>	<b>Strength of Evidence</b>
CancerTYPE ID	1	Low	Unknown	NA	Insufficient
miRview	4	Low	Unknown	NR	Insufficient
Pathworks TOO	3	Low	Moderate	High	High
<b>Clinical Validity</b>	<b>Number of Studies</b>	<b>Risk of Bias</b>	<b>Consistency</b>	<b>Precision</b>	<b>Strength of Evidence</b>
CancerTYPE ID	7	Low	Consistent	Moderate; 95%CI 76-86%	High
miRview	5	Low	Consistent	High; 95% CI 83-88%	High
Pathworks TOO	10	Low	Consistent	High; 95% CI 86-89%	High

# Summary of Strength of Evidence

Outcome	Number of Studies	Risk of Bias	Consistency	Precision	Strength of Evidence
<b>TOO Predicted</b>	17	Moderate	Consistent	G-Band:25% Microarray: Moderate N> 40: High	Moderate
<b>TOO Confirmed</b>	5	Moderate	Consistent	Moderate	Low
<b>Test Useful</b>	6	High	Consistent	Low	Low
<b>Treatment Change</b>	5	Moderate	Consistent	Moderate	Insufficient
<b>Treatment Response</b>	4	Moderate	Consistent	Low	Insufficient
<b>Survival</b>	5	High	Consistent	Moderate	Low
<b>Disease Progression</b>	1	Low	Unknown	NR	Insufficient



# Strengths and Limitations of the Body of Evidence

## ■ Limitations

- Difficulty in determining true primary site in CUPS makes it difficult to know accuracy in actual clinical use.
- No well controlled studies of effect on treatment decisions or on health outcomes.
- Test manufacturers were involved in the conduct or funding of almost all studies.

## ■ Strengths

- Multiple well-designed studies that tested the accuracy of tissue of origin tests by testing tumors of known primary site.
- Use of creative study designs to determine the accuracy of prediction in true CUPS case
- Recent studies directly compare diagnostic success of molecular TOO tests with that of IHC.

# Strengths and Limitations of the Review

- Strengths
  - Systematic review and assessment
    - Evidence-based practice center methodology
    - ACCE framework
  - Rigorous search captured published studies, conference abstracts, and early publications studies.
  - Meta-analysis of accuracy of identification of tumors with known primary (clinical validity)
- Limitations
  - Manufacturers update and improve tests.
  - Rapidly evolving literature

## Conclusions

- Molecular and genetic tissue of origin tests are moderately accurate when tested on tumors of known primary site
- The accuracy of prediction on CUPS cases is still unclear.
- Additional and more rigorous studies of clinical utility are needed.
- Studies conducted and funded independently of the test manufacturers are needed.