Project Name: Technology Assessment on Genetic Testing or Molecular Pathology Testing of Cancers with Unknown Primary Site to Determine Origin
Project ID:CANU0511

Table 1: Invited Peer Reviewer Comments

Reviewer[1]	Section[2]	Reviewer Comments	Author Response[3]
Peer Reviewer 1		I think that the authors of this report did a terrific job. A great deal of work went into the document. I have only a few comments.	We thank the reviewer for the kind comment.
Peer Reviewer 1		One thing that bothered me throughout the analysis was the measure of accuracy. Do these tests use accepted measures of accuracy? Sensitivity and percent correct do not provide sufficient information for us to judge the accuracy of a test and there are many ways to influence these measures. Clearly, the ROC is the metric of choice for assessing these tests and comparing them with other tests. But the reports do not provide the ROC of their test, even though they have the capability to do so. Thus, we are forced to conclude that we cannot judge the accuracy of these tests; we need better reporting by the manufacturers. The burden of proof is on the manufacturers to prove their veracity and utility.	Receiver operating curves (ROCs), are excellent measures of test performance across the range of sensitivity and specificity. The EPC Methods Guide for Medical Test Reviews also lists sensitivity, specificity, and percent correct as other valid measures of accuracy, however. As noted by Florkowski (2008), sensitivity, specificity, predictive values, and likelihood ratios are all ways of expressing test performance. ROC curves compare sensitivity versus specificity in predicting a dichotomous outcome across a range of values. None of these parameters are intrinsic to the test; they are determined by the clinical context in which the test is used.
Peer Reviewer 1		Further, the FDA is not a good guide to the utility of a test because they only require that the test work at some greater than chance level and that it is safe, they do not require that it is significantly better than any other test (i.e., they do not assess comparative effectiveness).	We agree with the reviewer. FDA clearance is reported as a characteristic of the test, but was not considered the evaluation of the validity or utility of the test.
Peer Reviewer 1		Generally speaking, when you visit a land inhabited by test manufacturers, you may wind up acting like one of the natives. In other words, if your evaluation is limited to reports funded partially or wholly by the	We agree with the reviewer. This limitation inherent in evaluation by systematic review: we have no control over what is in the body of evidence.

		manufacturers of the tests, then you have no frame of reference to judge the tests other than that provided by the manufacturers.	
Peer Reviewer 1		Issue that are outside the manufacturers include: Are these tests significantly better than existing tests? For example, IHC does a pretty good job of determining the site of origin of primary and metastatic tumors. (p. 4) So one can ask, do the tests being evaluated significantly increase the accuracy of existing tests? If not, then we may not be interested in using the new tests.	At the time of the initial review, there were no direct comparisons of IHC and molecular tests. In the updated literature search, two articles compared the accuracy of IHC with Pathworks or CancerType ID in identifying tissue of origin in metastatic tumors. In both cases, the molecular test had higher accuracy than IHC. Pathworks accuracy was 90% (9/10) compared to 64% (32/50) by IHC and independent evaluations by 5 pathologists (Kulkarni 2012). CancerTypeID correctly identified the origin of 78% (of 123) of the tumors) compared to 68% correctly identified by IHC.
Peer Reviewer 1		Putting aside the issue of how the reports calculated their accuracies, the claimed accuracies are unexpectedly high. One must look outside the manufacturer's studies to understand this statement. As one who has done many gene expression studies I can say that there is bias related to the gene expression platform and there is a great deal of variance related to patient gene expression (even when normalized). Further, there is a great deal of variability in the raw material, i.e., in the primary tumor gene expression (tumor tissue is heterogeneous even with microdissection), there is even more in metastatic gene expression, and the gene expression between the primary tumor and the metastatic tumor can be very tenuous. Finally, the problem of TOO is itself fraught with bias and variance. Thus, one would expect a relatively low accuracy, e.g., an ROC of around 0.65.	We agree with the reviewer that there is potential for error throughout the sample preparation and testing process. We can only assess the tests by the published literature. Most of the studies do not report ROC, but the proportion of correct TOO assignments in tissues of known origin are consistent across studies.
Peer Reviewer 1		I have a less optimistic view of these tests then that provided by the Technology Assessment. I cannot	As noted in the report, the reported accuracy is measured as the percent correct, not the ROC.

		conclude that these tests have a validated clinical "accuracy," and I certainly do not believe that they have an ROC accuracy of 0.83 – 0.87. In fact, I believe their true accuracies to be much lower. The "evidence" does not allow us to draw any meaningful conclusions at this time. The jury is still out.	The ROC may in fact be lower than the percent correct, since it would also incorporate the specificity of the test.
Peer Reviewer 2		Peer Review Comments Overall, the systematic review does a comprehensive survey of literature in the area of Tissue of Origin tests. Some comments and suggestions as follows:	No response needed.
Peer Reviewer 2		In page 16, a couple of clarifications: (i) the authors state that "…FDA does not regulate laboratory services, only the sale of medical devices…" FDA has clarified on multiple occasions that it does regulate laboratory developed tests but in doing so exercises enforcement discretion. There was a public meeting regarding this recently.[1] [1]http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm212830.htm	We have corrected the text on page 18 regarding FDA approval.
Peer Reviewer 2		(ii) the authors state that TOO tests are for the detection of cancer of unknown primary site, and then go on to say that, "…PathworkDx has been cleared by the FDA…" A review of the labeling of PathworkDx would indicate that the FDA clearance has associated limitations.[2] [2]http://www.pathworkdx.com/tissue_of_origin_test/indications_for_use_and_limitations/	We have included the limitations as stated on the Pathworks Web site on page.
Peer Reviewer 2		In page 18, (i) in Table 6, the authors do not identify colorectal cancer as a tissue of origin identified by CancerTypeID (unlike the other two tests). However, in page 53 of the report, the authors mention a publication by Hainsworth *et al* (2012)	We have added coverage of colorectal cancer by CancerTypeID to Table 6.

		where they mention that CRC was identified by CancerTypeID and this information used to make treatment decision. Based on that information, this table may need to be revised, as also Table 7?	
Peer Reviewer 2		In page 28, (i) In describing the steps for Normalization of gene expression, the authors mention housekeeping or reference genes and go on to say that "…this is an appropriate way to select genes for normalization…" While it may be outside the scope of this report to discuss in detail the potential risks in using reference genes, it will be good to refer to the paper by Lee et al (2002)[3] and Richard Simon's (2003)[4] discussion on normalization methods, both of which ask one to use caution whilst using a gene set to normalize gene expression. [3] Lee, P.D. *et al* (2002) Control genes and variability: absence of ubiquitous reference transcripts in diverse mammalian expression studies. Genome Res. 12:292-297 [4] Richard M. Simon *et al* Design and Analysis of DNA Microarray Investigations (2003) Springer Series on Statistics for Biology and Health - Chapter 6 on Array Normalization	We agree with the reviewers. We have modified the report and added a citation.
Peer Reviewer 2		In page 30, (i) In discussing 'Accuracy' of all the tests, the authors point out that for CancerTypeID, "…Erlander *et al* … do not report 95%CI or provide other details…" However, in Figure 4, it is not clear that 95%CI was not reported as seen from the box and whisker plots.	The box and whisker plots show the 95% CI from the meta analysis.
Peer Reviewer 2		In page 66, For both questions KQ4 and KQ5 followed by the section on Summary of Accuracy, the authors have presented a fair appraisal of the current status of	No response needed.

		research vis-à-vis evidence that TOO results affecting treatment decisions or evidence to support their overall accuracy.	
Peer Reviewer 3	General	After the Executive Summary the citation numbers are not aligned with the numbering in the reference section.	The citations numbers have been corrected.
Peer Reviewer 3	Executive Summary	Check first sentence in “Data Synthesis” section for intended meaning/clarity – “…ability of the tests to correctly identify tests of known origin.”	This sentence has been revised.
Peer Reviewer 3	Executive Summary	Second to last sentence in “Results” – “The evidence that TOO tests affect treatment decisions was also low.” Should this be strength of evidence?	Yes, this should have been the strength of the evidence. The sentence has been revised.
Peer Reviewer 3	Executive Summary	In the “Results” section, it might be helpful to readers if the “Results” section were organized into subsections of analytical validity, clinical validity and clinical utility.	This section was broken into separate paragraphs for analytic validity, clinical validity, and clinical utility.
Peer Reviewer 3	Executive Summary	Table A is very helpful.	We appreciate the kind comment.
Peer Reviewer 3	Executive Summary	Last paragraph in “Summary of Findings” – “As mentioned above, one of the concerns is that all but one of the manuscripts reviewed were funded wholly or partly…” I did not see this mentioned previously in the Executive Summary, however, I may have missed it.	This sentence has been revised.
Peer Reviewer 3	Executive Summary	First sentence in “Future Research” section – should test be pluralized here?	This sentence has been revised.
Peer Reviewer 3	Introduction/Background	Writing is generally very clear and easy to understand in this part of the report.	We appreciate the kind comment.
Peer Reviewer 3	Introduction/Background	Last sentence in “A Brief Overview of Cancer” – “…to know the site of the primary or at least…” Should this be primary tumor, or perhaps primary cancer?	Yes; the sentence has been revised.
Peer Reviewer 3	Introduction/Background	“Computed Tomographic (CT) Scans” section – “For women, mammograms and pelvic CT scans are included in the routine workup.” I assume this means the routine workup for CUP, but may improve clarity to specify.	This sentence has been revised.

Peer Reviewer 3	Introduction/Background	First paragraph of “Meta Analysis” section – The Agency for Health Care Quality and Research Methods Guide…” Should this be Agency for Healthcare Research and Quality?	This revision has been made.
Peer Reviewer 3	Introduction/Background	PICOTS, Outcomes, Health – Does quality of life include potential for reduced morbidity due to fewer adverse side-effects owing to change in treatment decisions?	Reduction in adverse side effects was within the scope of the review, but we did not identify any studies on this topic.
Peer Reviewer 3	Methods	The search strategies appear to be thorough and are well-documented.	We appreciate the kind comment.
Peer Reviewer 3	Methods	I assume that the NIH Genetic Testing Registry was not yet online at the time of the research, and therefore was not searched.	The NIH Genetic Test Registry was not online for the initial search. We searched the GTR during the updated search, but did not find any additional tests.
Peer Reviewer 3	Methods	It may have been advisable to include more than one search engine in the internet search for available tests, however, I do not know of any other such tests that are currently available in the United States.	We also did not identify any additional tests through reviews of peer-review publications or other databases.
Peer Reviewer 3	Methods	I did a quick check of the GAPP Finder database of horizon scanning results on genetic tests http://www.hugenavigator.net/GAPPKB/topicStartPage.do using the search term “unknown primary” and found several matching records. Beyond the tests already included in the report, however, no additional tests in this database appeared to be currently available in the United States.	We thank the reviewer for this additional resource. We searched the database before finalizing the report, but found no additional tests.
Peer Reviewer 3	Methods	Equation 1, describing the univariate fixed-effects model for meta-analysis, does not show up in the report.	This equation can be seen in our copy of the report. Perhaps this is due to a web error?
Peer Reviewer 3	Methods	It is not clear to me why a fixed-effects model for meta-analysis was used instead of random-effects.	The value of accuracy across these studies was very consistent. We did not have a reason to control for covariates such as different populations, different thresholds for positivity etc. The model was estimating the accuracy which

			was assumed to be the same across all studies except for sampling variability. Hence a fixed effect rather than a random effect model
Peer Reviewer 3	Results	Figures 4-6 – vertical axes should probably include a value below the lowest individual study confidence interval lower bound	We have modified the figures.
Peer Reviewer 3	Results	Page 48 – I think this should reference Table 12 instead of 6	We have checked the table numbers and corrected them as needed.
Peer Reviewer 3	Results	Page 49 – I believe that initial heading should be Table 12 instead of 6	We have checked the table numbers and corrected them as needed.
Peer Reviewer 3	Results	Page 59 – Table 8 should be Table 14	We have checked the table numbers and corrected them as needed.
Peer Reviewer 3	Results	Page 61 – Table 9 should be Tabe 15	We have checked the table numbers and corrected them as needed.
Peer Reviewer 3	Discussion/Conclusion	Good overall summary of findings.	We appreciate the kind comment.
Peer Reviewer 3	Discussion/Conclusion	There is no discussion on the findings from cytogenetic analysis.	A discussion of the findings on cytogenetic analysis has been added.
Peer Reviewer 3	Tables	Comments shown in individual sections where tables appear.	No response needed.
Peer Reviewer 3	Figures	Comments shown under individual sections where figures appear.	No response needed.
Peer Reviewer 3	Appendices	Appendix D reference section appears to be off in many cases and should be checked for accuracy.	The citations have been checked and corrected.
Peer Reviewer 3	Appendices	Page E-24 – “Excludes at Full Text Review Stage” should be Excluded…	This change has been made.
Peer Reviewer 3	Appendices	Appendix G reference section also needs to be checked – citation numbers in text exceed number in reference section.	The citations have been checked and corrected.
Peer Reviewer 3	References	Main reference section needs to be aligned/matched with the in text citations. Appendix D and G Reference sections also need to be carefully checked.	The citations have been checked and corrected.

[1] Peer reviewers are not listed in alphabetical order.
[2] If listed, page number, line number, or section refers to the draft report.
[3] If listed, page number, line number, or section refers to the final report.

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Table 2: Public Review Comments

Reviewer Name[1]	Reviewer Affiliation[2]	Section[3]	Reviewer Comments	Author Response[4]
Public Anon1			Having worked in the CUP diagnostic space for 6 years, I know there is a not of confusion as to what each of the CUP test provides. I would suggest that the Caris "Target Now!" test receive comments in addition to the tests already listed. An extremely large lumber of medical oncologists incorrectly believe this test tells where the primary site of origin is for metastatic cancer. Salesreps have told physicians that it does provide the primary site of origin. Originally their requisition indicated that the test was NOT for cancer of unknown primary. That comment has now been removed from their requisition. An email exists from Caris' Medical Director that in fact the "Target Now!" test does NOT provide the primary site of origin for metastatic OR primary cancer.	We have added an explanation of why the Caris Target Now! test was not included in the review (page 18).
Public Anon2			In evaluating the accuracy of any product in determining the origin of a neoplasm, one needs to carefully consider the universe tested--whether the cases are "all comers" or are limited to the types of tumors that the given product is designed to recognize. A test with a high accuracy that can only recognize a limited panel of tumors may be less useful than a test with lower accuracy that can recognize a wider panel of tumors.	We agree with the reviewer, but this is decision for the person ordering the test. It is not a question considered by the systematic review.
Robert C. Babkowski, MD, FACP	Stamford Hospital		As a practicing pathologist who daily evaluates all different types of malignancies from various organ systems, I can not stress enough the importance of ancillary tissue testing in	No response required.

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			working up malignancies, especially those cancers which present as metastatic disease. Nowhere in medicine are there higher stakes then the patient who presents in distress to a hospital emergency room, riddled with cancer, where after imaging studies and biopsy of tumor from a metastatic site, we as physicians are unclear as to what the cancer is. All cancer treatment is based on the fundamental question of "what kind of cancer we are dealing with?". Life prolonging treatment can not be given if a Pathologist can not classify the malignancy. Medical Oncologists, Radiation Oncologists, Surgeons, and of course the patient, all expect as accurate a cancer diagnosis and tumor classification as can possibly be given – because its that diagnosis which determines all medical interventions that follow. No one wants, and no human deserves, treatment decisions based on a "Pathologists best guess". Most of the time, I can determine the origin of such metastatic, poorly differentiated cancers and classify such malignancies by using histologic (under the microscope) analysis, and through immunohistochemitry (antibody based tissue based testing). When these tools fail to help me classify the malignancy – I need "another tool in my toolchest". Ancillary molecular pathology tissue testing is crucial at that point. Molecular tests are that tool.	
Robert C. Babkowski, MD, FACP	Stamford Hospital		There are currently 3 options for me to further work up cancer cases such as described above. I can use ancillary molecular pathology tests offered by Pathworks, Biotheranostics, or Rosetta Genomics. Each has utility in helping me determine what the origin of that metastatic cancer is.	No response required.
Robert C. Babkowski, MD, FACP	Stamford Hospital		These tests should only be used/ordered by a Pathologist who is working up the case, can correlate radiologic and clinical findings, and who has exhausted traditional diagnostic tools of histology and immunohistochemistry. It is the	No response required.

Reviewer Name[1]	Reviewer Affiliation[2]	Section[3]	Reviewer Comments	Author Response[4]
			metastatic, poorly differentiated carcinomas which deserve this kind of additional scrutiny.	
Robert C. Babkowski, MD, FACP	Stamford Hospital		I am partial to the Rosetta Genomics test because microRNAs represent a distinct and novel analyte class, separate from protein product, mRNA, or DNA . MicroRNAs are highly preserved in specific tissue types, and in poorly differentiated tumors, microRNAs are conserved and more representative of lineage then mRNA or expressed proteins.	No response required.
Robert C. Babkowski, MD, FACP	Stamford Hospital		This testing has made a difference. It helped me as a Pathologist categorize these horrible cancers more accurately, and directed my Oncology colleagues to choose the appropriate treatment options. In the end – it gave patients struck with these cancers the most precious gift of time.	No response required.
Elena Brachtel, M.D.		General	Molecular cancer classifiers use validated gene expression algorithms. Molecular cancer classifiers can determine cancer types and subtypes, tissue of origin (TOO) and aid in the diagnosis of carcinomas of unknown primary (CUP), or confirm suspected diagnoses. The term ?TOO? is most often used in this draft but might be too narrow.	Tissue of origin tests may provide additional information as well as tissue of origin. However, the scope of this technology assessment was limited to the ability of these tests to identify the tissue of origin in cancers of unknown primary.
Elena Brachtel, M.D.		Results	As a senior author of the recently published validation study on CancerTypeID (Kerr SE, et al., Clin Cancer Res 2012:18;3952?60), I wish to draw attention to this and other recent publications that were not represented in the current draft of the Technology Assessment. Our study used a cohort of 790 clinical test samples from three major research hospitals (Mayo Clinic, MGH, UCLA) with rigorous diagnostic adjudication by the pathologist investigators. Only cases that represented validated histopathological diagnoses supported by ancillary clinical information were selected for molecular testing.	We updated the search while the draft report was out for peer review. We have included this publication as well as other published or e-published by November 7, 2012.

Reviewer Name[1]	Reviewer Affiliation[2]	Section[3]	Reviewer Comments	Author Response[4]
Mitchell Burken	Novitas Solutions, Inc.	General	It was encouraging to see that this TA gave similar results to a more informal review of the literature by my company, as well as another Medicare MAC contractor, as we two contractors made recent coverage determinations on this specialized lab testing. Having this confirmatory review is a good "quality control" type check on the Medical Director efforts regarding our assessment of the evidence.	No response needed.
Mitchell Burken	Novitas Solutions, Inc.	General	However, I would add that for this particular type of lab testing, I believe that the final KQ's on health outcomes are not as relevant. In contrast to a predictive type lab test, which must have test results and outcomes closely intertwined, tumor of unknown origin testing is really directed toward a more precise diagnostic "label" such that more specific therapy might be entertained.	The review was designed to evaluate the evidence that the tests are accurate **and** clinically useful. Clinical utility implies that that diagnostic accuracy of the test makes a difference in the treatment prescribed and ultimately in patient outcome.
Tina Edmonston, MD, FCAP	Cooper University Hospital		This is a very thorough and rigorous analysis of CUP testing using molecular tumor profiling assays that 3 commercial labs provide. I have a few comments that I would like to add in general and to some specific points.	We appreciate the kind comment.
Tina Edmonston, MD, FCAP	Cooper University Hospital	KQ1 Table and discussion	The three diagnostic assays are summarily described as microarray assays. It would be more accurate to describe the technology and targets as summarized below, as each of these platforms has technical advantages and disadvantages. Cancer Type ID-quantitative real time PCR-target is mRNA; PathworkDx-expression microarray-target is mRNA; miRview mets-quantitative real time PCR-target is microRNA; (the assay development phase of miRview mets included micoarrays for microRNA). This assay has actually been replaced by a second generation assay on a microRNA microarray platform that recognizes a larger tumor spectrum than the one described for miRview mets. This current assay is marketed under the name of miRview mets2.	We have modified the description of laboratory method in Table 1 to better describe the methods used by these tests. We modified the summary description to molecular tests rather than microarray tests.

Reviewer Name[1]	Reviewer Affiliation[2]	Section[3]	Reviewer Comments	Author Response[4]
Tina Edmonston, MD, FCAP	Cooper University Hospital		I may have overlooked the explanation for the "RNS" abbreviation but it seems that "RNA" is more commonly used.	This error has been corrected.
Tina Edmonston, MD, FCAP	Cooper University Hospital	Introduction/ Background	The limitation of light microscopy (without IHC) is that without IHC it is unlikely that a primary can be identified, even though histologic subtypes can be identified and certain subtypes are typical for certain primaries. E.g. Adenocarcinoma could have a primary in the lung, in the breast, in the prostate, in the GI tract, or be a GYN malignancy.	No response required.
Tina Edmonston, MD, FCAP	Cooper University Hospital	Introduction/ Background	IHC: Cytokeratins are usually abbreviated as "CK", e.g. CK7, CK20 and are typically expressed in Carcinomas, whereas other immunohistochemical markers characterize sarcomas, melanomas and hematologic malignancies.	We have modified the abbreviations in the text.
Tina Edmonston, MD, FCAP	Cooper University Hospital		Regarding Mueller et al 2011. (please note that I am a co-author in the study) Rosetta provided the tests for free and paid for materials and shipping of the specimens. There were no other payments made to the Institution or to the German Researchers. The German Researchers who were involved in the study selected the cases and provided their interpretations of the cases in this retrospective study before the miRview mets test was performed. The risk for a biased study should therefore be considered low, especially as the nature of the molecular test which uses predefined algorithms doesn't allow any "interpretation".	This study was rated as fair because the section entitled classification example seems to indicate that the final diagnosis was revisited when the test result indicated a different tumor origin than that previously diagnosed. If, as the comment indicates, the final case diagnosis was independent of the test results, the quality of the study would be good. It is hard to reconcile this with the section of the article entitled 'Classification Example' however. We have therefore left the study rating as 'Fair'.
Tina Edmonston,	Cooper University		Table 6 should be corrected to include all tumors that were represented in the miRview mets test.	Table 6 has been updated to show the tumors reported

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MD, FCAP	Hospital			as identified by the MiRview mets[2] test in Meiri et al., 2012 (Meiri E, Mueller WC, Rosenwald S, et al. A second-generation microRNA-based assay for diagnosing tumor tissue origin. Oncologist. 2012;17(6):801-12. PMID: 22618571).
Tina Edmonston, MD, FCAP	Cooper University Hospital		As you discuss in Final Discussion and Future Research, it is challenging to come up with a study format for prospective trials that allows the comparison between outcomes for patients with a tissue-specific diagnosis and tissue-specific therapy vs. general CUP diagnosis and treatment	No response required.
Tina Edmonston, MD, FCAP	Cooper University Hospital		In addition to your arguments you can consider the fact that the number of different tumors that are recognized by the different assays is large and would lead to different tissue-based treatments, will make it almost impossible to get statistically strong outcome data that demonstrate a difference in outcome.	We agree it is challenging, but the challenge can be addressed through statistical techniques for small samples, study designs that evaluate the impact of the test in the overall population for which it is designed (see Greco abstract), or examining the impact in one type of diagnosis (see Hainsworth).
Tina Edmonston, MD, FCAP	Cooper University Hospital		Therefore the benefit of molecular tumor profiling will see seen on a case-by –case basis when individual cases of CUP that receive a molecular profile-based diagnosis (and if indicated additional IHC or imaging for confirmation) can then receive a tissue-based more specific therapy and improved outcomes become evident on a case-by –case basis.	Case series are not a good alternative to well designed and controlled studies. Without a control group, it is not possible to determine whether using the test improves outcomes in the population of patients for

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				which it is designed.
Tina Edmonston, MD, FCAP	Cooper University Hospital		As a matter of disclosure, I am a former full-time employee at Rosetta Genomics, but my employment terminated in May 2011. I do not have stock options or any other financial interest in Rosetta Genomics and I have not received any payments or other benefits from Rosetta Genomics since termination of my employment.	No response needed.
Frank A. Greco, MD	Sarah Cannon Cancer Center and Research Institute	General	Since these data were compiled an additional large prospective study in CUP patients has been presented in abstract form at the American Society of Clinical Oncology (ASCO) meeting in June 2012 at Chicago.(see Greco et al J Clin Oncol 2012;30: Abstract 10530) The manuscript is now in press in Journal of Clinical Oncology. These data are from a prospective study of treatment in 252 CUP patients based on the tissue of origin diagnoses as determined by the RT-PCR assay (bioTheranostics;CancerTYPE ID). Outcome was measured by patient survival. The median survival of 194 patients who received the site-specific chemotherapy based on molecular assay tissue of origin diagnoses was prolonged (12.5 months) compared to 9 months as determined from multiple (>1000 patients)previously treated with empiric regimens. Furthermore when the assay predicted tumor types that are known to be more responsive to site-specific chemotherapy (breast,ovary, lung ,others) the median survival was significantly improved compared to predictions of more resistant (melanoma, biliary tract, pancreas, liver, others) tumors (13.4 months versus 7.6 months; p=0.04). These outcome data provide strong evidence of the usefulness of this molecular assay in providing more appropriate site directed therapy for CUP patients and is associated with improved outcome. This large study was done by the Sarah Cannon Research Consortium/Cooperative Group (14 different sites participated) which is not affiliated with bioTheranostics. The study was supported in part by grants from Genentech and	The JCO article was added to the updated search.

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			bioTheranostics. These data are important for CUP patients since outcomes are improved and the molecular assay provides a tissue of origin diagnosis in the majority of patients.Treatment based on these diagnoses provide improvements in survival compared to "broad spectrum" or "shot gun" empiric regimens which previously were the standard treatment for these patients.Molecular assays are important in some patients who are not easily classified by Hennerstandard pathology (including IHC) and help direct more appropriate therapy.	
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	General	Because of its rigorous methodology and unbiased literature based assessment, the AHRQ Tech Assessment program is highly influential with key purchasers of health care technology, especially the third party payers in the United States. The future of companies developing and commercializing innovative molecular diagnostic tests will be impacted by the report, along with their ability to provide important services to physicians and their patients. For this reason, the AHRQ has a responsibility to make a fair and accurate assessment. To that end we have provided detailed feedback correcting factual errors as well as input on some of the more philosophical questions surrounding a test like this for which a gold standard is, by definition, challenging to identify. Our comments primarily relate to the Pathwork TOO test but they also are generalizable in regard to the conclusions of the AHRQ report.	We appreciate your input.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	General	In terms of the factual matters, we would especially like to focus the authors on the feedback related to analytical and clinical validity. The field is moving quickly and there are there a number of crucial references which were omitted from their analysis.	We updated the search while the report was out for public comment. We have included all articles published or e-published by 11/7.
Raji Pillai, PhD, Ljubomir	Pathwork Diagnostics	General	Furthermore, it is important to keep in mind that both the frozen and FFPE versions of the Pathwork Tissue of Origin	The EPC review is independent of that done by

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Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.			Test have been cleared by the FDA which, for a diagnostic test, is responsible for determining its analytical and clinical validity via an exceptionally rigorous process.	the FDA. We do not have access to material provided to the FDA, so we could only evaluate the published evidence.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	General	In that light, it is disturbing to see the validity of the Tissue of Origin Test described as ?insufficient? and we hope that the information provided in our responses will rectify this misunderstanding of the available data.	We could only evaluate the published evidence on analytic and clinical validity.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	General	In order to fairly assess the degree to which these tests demonstrate clinical utility, it is imperative that the definition of clinical utility for this type of test be articulated.	Clinical utility is defined in the list of key questions. See Key Question 4, page 8.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	General	We agree with the authors? statement that a randomized controlled trial, which is the most reliable way to determine improvement in patient survival, would not be feasible in the US.	We agree a randomized controlled trial would be difficult to conduct.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	General	In contrast to tests that determine response to therapy (e.g. KRAS), these ?tumor classification? tests do not directly influence patient survival. Therefore, indirect measures of the tests impact on survival are appropriate.	The question that we asked is whether or not the test influenced treatment decisions and whether the changed treatment decisions changed outcomes. The primary selling point for these tests is that knowing the site of

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				the primary will allow the oncologist to provide site specific therapy which in turn will improve patient outcome. So patient outcome, i.e. survival, is a valid way to assess the utility of these tests, if the data is available..
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	General	Last but not least, these new molecular diagnostic tests are adjuncts to immunohistochemistry (IHC) for tumor diagnosis. IHC (along with most diagnostic tests) have never been subjected to the standard of demonstration of improvement in patient survival.	The claimed benefit of these tests is that they inform site specific therapy, which is said to improve patient survival compared to empiric therapy. The only way to evaluate that claim is to look at changes in treatment and the effect of the treatment changes on patient outcome.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	General	For these reasons, we believe the proper standard of clinical utility for these tumor classification tests are: (i) Demonstrated impact of the test on physician behavior ? change in diagnosis and treatment and (ii) Evidence that the test performs favorably compared to the current standard of care ? IHC (Comparative Effectiveness).	The claimed benefit of these tests is that they inform site specific therapy, which is said to improve patient survival compared to empiric therapy. The only way to evaluate that claim is to look at changes in treatment and the effect of the treatment changes on patient outcome.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker,	Pathwork Diagnostics	General	In the detailed comments, we provide recently published evidence to demonstrate that the Tissue of Origin Test does meet this standard of clinical utility.	

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MD and W. David Henner MD, PhD.				
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	Executive Summary - Results	1. The authors erroneously state that the data on analytic performance of the Pathwork Tissue of Origin Test is insufficient to assess the analytic validity of the test. It is important to note that the Pathwork Tissue of Origin Test is the only gene expression profiling test for tumor classification which is cleared by the FDA which rigorously evaluates the analytical and clinical validity of a diagnostic test. Our detailed response is in the Results section, response to KQ2a.	We based our assessment on the published evidence on analytic validity, which is limited.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		2. The authors state that assessing the validity of the Pathwork Tissue of Origin Test algorithm is difficult. We disagree with this as the algorithm has been tested extensively on multiple independent sets of specimens, which by definition proves its validity.	Our assessment of the evidence is based criteria established by Simon et al. and the published evidence. The publications on the Pathwork Tissue of Origin Test algorithm do not provide sufficient detail on the statistical algorithm used, the methods of dimension reduction or classification to determine if the Simon criteria were met. The ability of the algorithm to correctly identify a known tissue of origin is assessed separately.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner	Pathwork Diagnostics		3. The authors judge the strength of the results as low that the tests accurately determine the tissue of origin. We disagree with this conclusion: the authors reference a paper that includes both known reference specimens AND unknown specimens. The test performance can only be calculated using known reference specimens. We recommend either	The ability of a test to accurately diagnosis CUPS cases must be evaluated using tumors from CUPS cases or similar tumors. Primary tumors and

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MD, PhD.			excluding the citations that have unknown specimens or excluding the subset of unknown specimens from those citations. Furthermore, two additional published papers were not included in the analysis. Our detailed response is in the Results section, response to KQ3b.	metastatic tumors of known origin may have different or more consistent gene expression profiles.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		4. We disagree with the author?s statement that the evidence that the Pathwork Tissue of Origin Test affects treatment decisions is low, and in our detailed response to KQ4a, point the authors to two publications.	We have added recent publications to the table, but based on the evidence available, we believe our original assessment is correct, and note that the peer reviewers agreed.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		5. On outcomes, as stated in our general comments, we believe that given the infeasibility of randomized controlled outcome trials in the US, indirect measures of the tests impact on survival are appropriate, namely, impact on physician behavior, and comparative effectiveness, i.e. evidence that the test performs favorably compared to the current standard of care. In our detailed response to KQ4b and in the Discussion/Conclusion section, we point the authors to publications that report such data.	The claimed benefit of these tests is that they inform site specific therapy, which is said to improve patient survival compared to empiric therapy. The only way to evaluate that claim is to look at changes in treatment and the effect of the treatment changes on patient outcome.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		In Summary of Findings, We disagree with the authors assessment that the literature on the effect of the test on treatment decisions is very limited and that there is low evidence that the test alters the treatment course from empiric therapy usually used in CUP to tissue-specific therapy. As detailed in responses to KQ4a, two recent peer-reviewed publications show that the Pathwork Tissue of Origin test changes treatment decisions in the majority of cases. It guides patient management resulting in clinical outcomes more favorable than historical outcomes.	The literature on the effect of the test on treatment decisions is limited even with the recent publications.
Raji Pillai, PhD, Ljubomir	Pathwork Diagnostics		In Future Research, The report states that studies funded by companies lead to publication bias. While we understand the	The concern about publication bias is that

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Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.			concern, this is impractical because neither academics nor government granting agencies have the budget to perform these initial studies. We have put appropriate controls in place to assure objectivity. Test manufacturers work to counter bias by supporting independent external validation studies, as well as working through the peer review process which is rigorous and more often than not biased against industry-sponsored research.	manufacturers may maintain control of publication, which can result in a failure to publish studies with unfavorable results.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	Introduction/ Background	Traditional methods of identifying the tissue of origin (TOO) for CUP have had limited success,14 Pathwork comment: A recently published direct comparison supports this statement. COMPARISON OF HISTOPATHOLOGY TO GENE EXPRESSION PROFILING FOR THE DIAGNOSIS OF METASTATIC CANCER. A Kulkarni, R Pillai, Buturovic, Becker, and Henner, A Ezekiel, WD Henner, C Handorf. Diagnostic Pathology 2012, 7:110	We updated the literature search during the peer review process. The article by Kulkarni et al. is included in the updated review.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		p4. 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 Pathwork comment: In addition, a recently published direct comparison supports these findings. COMPARISON OF HISTOPATHOLOGY TO GENE EXPRESSION PROFILING FOR THE DIAGNOSIS OF METASTATIC CANCER. A Kulkarni, R Pillai, Buturovic, Becker, and Henner, A Ezekiel, WD Henner, C Handorf. Diagnostic Pathology 2012, 7:110	We updated the literature search during the peer review process. The article by Kulkarni et al. is included in the updated review.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W.	Pathwork Diagnostics	Methods	p11-12. Literature search and strategies. Pathwork comment: The overall approach seems thorough and impressive. However, the date until which articles were retrieved is not provided. Several crucial references	The date of the final search has been added to the methods section.

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David Henner MD, PhD.			regarding the Pathwork Tissue of Origin Test have either been missed entirely, or are referred to as posters when they are now published, and are listed below.	
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	Methods	CLINICAL VERIFICATION OF THE PERFORMANCE OF THE PATHWORK TISSUE OF ORIGIN TEST. CI Dumur, CE Fuller, TL Blevins, JC Schaum, DS Wilkinson, CT Garrett, CN Powers. Am J Clin Pathol 2011;136:924-933	This article was added during the updated search.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	Methods	A GENE EXPRESSION PROFILE TEST FOR THE DIFFERENTIAL DIAGNOSIS OF OVARIAN VERSUS ENDOMETRIAL CANCERS. A Lal, R Panos, M Marjanovic, M Walker, E Fuentes, DS Kapp, WD Henner, L Buturovic, and M Halks Miller. Oncotarget, Vol. 3, No. 2, February 2012	This article was added during the updated search.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	Methods	GENE-EXPRESSION MICROARRAY-BASED ASSAY TO DETERMINE TUMOR SITE OF ORIGIN IN A SERIES OF METASTATIC TUMORS TO THE OVARY AND PERITONEAL CARCINOMATOSIS OF SUSPECTED GYNECOLOGICAL ORIGIN. A Azueta, O Maiques, A Velasco, M Santacana , J Pallares, A Novell , X Gonzalez-Tallada, A Mozos, J Prat, R Pillai, Buturovic, Becker, and Henner, M Mata, X Matias-Guiu. Human Pathology, 2012 Aug 30. [Epub ahead of print	This article was added during the updated search.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	Methods	MOLECULAR TUMOR PROFILING IN THE DIAGNOSIS OF PATIENTS WITH CARCINOMA OF UNKNOWN PRIMARY SITE: RETROSPECTIVE EVALUATION OF GENE MICROARRAY ASSAY. JD Hainsworth, R Pillai, Buturovic, Becker, and Henner, WD Henner, M Halks?Miller, C Lane, FA Greco. Journal of Biomarkers and Diagnosis, June 27, 2011	This article was added during the updated search.
Raji Pillai, PhD,	Pathwork	Methods	CLINICAL UTILITY OF GENE-EXPRESSION PROFILING	This article was added

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Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Diagnostics		FOR TUMOR-SITE ORIGIN IN PATIENTS WITH METASTATIC OR POORLY DIFFERENTIATED CANCER: IMPACT ON DIAGNOSIS, TREATMENT, AND SURVIVAL. JS Nystrom, J Hornberger, G Varadhachary, R Hornberger, H Gutierrez, WD Henner, S Becker, M Amin, M Walker. Oncotarget, Advance Publications 2012, published June 9 2012	during the updated search.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	Methods	POTENTIAL CLINICAL UTILITY OF GENE EXPRESSION PROFILING IDENTIFYING TUMORS OF UNCERTAIN ORIGIN. M Laouri, M Halks?Miller, WD Henner, JS Nystrom. Personalized Medicine (2011) 8 (6), 615-622	This article was added during the updated search.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	Methods	CANCER OF UNKNOWN PRIMARY: FROM IMMUNOHISTOCHEMISTRY TO GENE EXPRESSION PROFILING. WM Chiang, M Kapadia, NV Laver, JS Nystrom. Published ahead of print on Sep 10, 2012: http://jco.ascopubs.org/cgi/doi/10.1200/JCO.2011.41.1827	This article was added during the updated search.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	Methods	COMPARISON OF HISTOPATHOLOGY TO GENE EXPRESSION PROFILING FOR THE DIAGNOSIS OF METASTATIC CANCER. A Kulkarni, R Pillai, Buturovic, Becker, and Henner, A Ezekiel, WD Henner, C Handorf. Diagnostic Pathology 2012, 7:110	This article was added during the updated search.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	Methods	CANCER OF UNCERTAIN ORIGIN: COMPARATIVE EFFECTIVENESS OF IMMUNOHISTOCHEMISTRY AND GENE EXPRESSION PROFILING IN DIAGNOSING THE PRIMARY SITE IN METASTATIC CANCER. Handorf et al, JMD November 2012, AMP abstract in press.	This was not published by the last search date.

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Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	Methods	Cost effectiveness (the AHRQ report refers to a poster?this is now published) COST-EFFECTIVENESS OF GENE-EXPRESSION PROFILING FOR TUMOR-SITE ORIGIN. J Hornberger, I Degtiar, H Gutierrez, A Shewade, WD Henner, S Becker, G Varadhachary, S Raab. Value in Health, in press	This article was added during the updated search.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	Results	1. KQ 1. What genetic or molecular TOO tests are available for clinical use in the United States and what are their characteristics? (p16.) Pathwork comment. The authors do not seem clear on the fact that there are two published tests: the Tissue of Origin Test with frozen specimens (1550 genes) and the Tissue of Origin with FFPE specimens (2000 genes).	We have listed the two versions of the Pathworks TOO test in KQ1.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		2. KQ 2. What is the evidence on the analytic validity of the TOO tests? Pathwork comment: The report erroneously states that analytic validity of the Pathwork Dx is insufficient.	The report states that the evidence regarding the analytic validity of the Pathworks DX is insufficient to determine its analytic validity. We stand by this assessment based on the criteria described in the methods of the report.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		a. The detailed analysis on Pathwork Dx on Page 25 covers the data in Dumur et al JMD 2008 quite thoroughly, and assesses the analytical validity as high.	It assessed the quality of the paper as high and the analytic validity as described in this paper as high.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker,	Pathwork Diagnostics		The authors have not included in their assessment similar data provided in Pillai, Buturovic, Becker, and Henner et al JMD 2011, summarized below.	This paper was included in the review.

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MD and W. David Henner MD, PhD.			i. Reproducibility was measured as concordance of test results for 60 of the 462 samples processed at 3 different laboratories. ii. Inter-site reliability was 89.3% and statistics (? >0.85) also indicated a high level of agreement between laboratories.	
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		b. However, in Table 16 (Summary of evidence page 63), the authors deem the strength of evidence for analytic validity of PathworkDx as insufficient. The Strength of Evidence table (Appendix F Table 1 page F-1) does not support the assessment of insufficient.	For the most part, the studies by Dr. Pillai and Dr. Dumur report different measures of analytic validity, so we were unable to assess consistency in the measures between the two studies. For this reason, we judged the evidence to be insufficient to evaluate the analytic validity of any of the tests.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		c. The Tissue of Origin Test has been shown to produce consistently reliable results in Dumur et al JMD 2008 and in Pillai, Buturovic, Becker, and Henner et al JMD 2011.	These studies are included in the body of evidence.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		Furthermore, it is important to note that the Pathwork Tissue of Origin Test is the only gene expression profiling test for tumor classification which is cleared by the FDA which rigorously evaluates the analytical and clinical validity of a diagnostic test.	Our assessment is based only on the published evidence, not the FDA clearance. FDA clearance is noted in KQ5.
Raji Pillai, PhD, Ljubomir Buturovic, PhD,	Pathwork Diagnostics		3. 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	The report states that the evidence in the published literature is insufficient to

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Shawn Becker, MD and W. David Henner MD, PhD.			Simon et al. (2003)? Pathwork Comment: The report erroneously states that the Simon criteria were not followed for the Pathwork Dx test.	make an informed decision on whether the Simon criteria were followed.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		a. In an independent review of molecular tests by Monzon and Koen, Arch Pathol Lab Med 2010, the authors refer to the Simon criteria in their assessment of current molecular test for tumors of uncertain origin, using two publications: Simon et al JNCI 2003, and Simon JCO 2005. (quote from Monzon and Koen 2010) The translation of multigene expression assays into diagnostic or prognostic classification tests has well identified requirements23 and pitfalls that should be addressed in validation studies.24 Classification algorithms usually perform best when used to classify samples used in the classifier development (overfitting to the training set); thus, validation with a large and independent sample set is paramount for establishing true performance of a given classifier. In this regard, Simon25 outlined key steps that should be taken into account when developing and validating therapeutically relevant genomic classifiers: (1) ensuring that the classifier addresses a specific and important clinical decision, (2) ensuring that the classifier shows sufficient accuracy in internal validation to assess further development, (3) translation to a platform for broad clinical application, (4) demonstration of reproducibility, and (5) independent validation of the prespecified classifier. It has also been recommended that validation studies show (1) adequate sample size for validation, to statistically demonstrate that classifications are accurate; (2) validation in all classes for which it was created, with enough specimens for each class; and (3) inclusion of indeterminate results in reported performance. 24,25 Clearly, genomic classifiers for tissue-of-origin determination do address a clinically important	The published literature on the Pathwork Dx test does not include enough detail to confirm the Simon criteria for valid algorithm development were met. Specifically, the publications do not provide details on the statistical algorithm used, the methods of dimension reduction or classification are not described. The only information provided is that ranking was used for dimension reduction and that a machine learning algorithm was used.

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			question that impacts treatment decisions for patients with uncertain primary cancers; thus, the first requirement is fulfilled for all these tests. In the following paragraphs, we review the publicly available evidence for each of the commercially available tests and evaluate the above parameters (aside from clinical utility) in these molecular tests for the determination of tissue of origin.	
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		b. For the Pathwork Tissue of Origin Test, the authors conclude that the published evidence indicated that the test meets the criteria for successful translation outlined in the two Simon papers, as was also judged by the FDA.	As noted above, the published literature lacks critical details on the development of the statistical algorithm. No additional publications were cited in the Monzon review.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		4. KQ 3b-3f. What is the evidence on the accuracy of the TOO test in classifying the origin and type of the tumor? Pathwork Comment: The calculation of the Pathwork Tissue of Origin Test performance at 87% is inaccurate.	The calculation reflects the accuracy of Pathworks Dx TOO as shown in the published literature. It should be noted that the review by Monzon and Koen includes a similar estimates of test accuracy, 86.7% - 87.8%
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		a. The authors reference a paper (Beck 2011) that includes both known reference specimens AND unknown specimens. The test performance can only be calculated using known reference specimens. We recommend either excluding the citations that have unknown specimens or excluding the subset of unknown specimens from those citations.	Findings for known reference specimens were considered evidence on KQ3b, clinical validity. Findings for unknown specimens were considered evidence on KQ4, diagnosis.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker,	Pathwork Diagnostics		b. Table 11 (page 45-46) lists the studies used to compute the 87% for the Pathwork Tissue of Origin Test. Dumur et al AJCP 2011, and Azueta et al Hum Pathol 2012 also addressed this question, and known reference	Dumur 2011 and Azueta 2012 were added during the updated search. The meta-analysis has also been

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MD and W. David Henner MD, PhD.			specimens from these papers must be included in computing this metric. Alternatively, Beck AJSP 2011, Dumur AJCP 2011, and Azueta et al Hum Pathol 2012 could all be excluded.	updated.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		c. p30. KQ 3b-3f. Key Question 3 has a part a and a part b. KQ3 c-f are not articulated.	This has been addressed in the final report. KQb-f were collapsed into a single question.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		5. KQ 4. How successful are TOO tests in identifying the TOO in CUP patients? Pathwork Comment: This section is missing references and has incorrect accuracy data.	The citations have been checked and updated.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		a. Recently published clinical utility data have not been included., i.e. Hainsworth et al Journal of Biomarkers and Diagnosis, 2011 The study was led by investigators from Sarah Cannon Research Institute / Tennessee Oncology. Archived biopsy specimens from 48 patients with carcinoma of unknown primary [CUP] were tested. Results were correlated with clinical features, results of routine pathologic evaluation, previous results of the Veridex 10?gene CUP assay, and response to treatment. The Tissue of Origin Test provided predictions of the primary site in 96% of patients with CUP, which was substantially higher than the prediction rate by the Veridex CUP assay (53%).	The paper is included in the updated search.

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			In general, predictions were consistent with clinical features, histology, and response to empiric therapy.	
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		b. P66. The report erroneously states that the Pathwork Tissue of Origin Test was accurate in 29 to 76 percent of CUP cases. We are unable to find a reference where 29% is reported.	Beck et al. reported that the TOO test results were compatible with the clinicopathologic characteristics in 2 of 7 (29%) malignancies of unknown primary.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		6. KQ 4a. What is the evidence that genetic TOO tests change treatment decisions? Pathwork comment: We disagree with the authors? assessment, because two recent two-peer reviewed publications show that the Pathwork Tissue of Origin test changes treatment decisions. Please include the following references. Laouri et al Personalized Medicine 2011 The study design was a consecutive case series of 284 Tissue of Origin cases for which information regarding pre-test diagnosis and ICD-9 codes, biopsy site, pathology report and Tissue of Origin Test result were available. Overall, it was demonstrated that the test had a significant impact on patient management by allowing for a markedly increased rate of specific tissue diagnosis In 81% of cases the test either assigned a new primary site or established a primary site in those lacking a primary site diagnosis In 15% of cases the test confirmed a suspected diagnosis In 4% of cases a call could not be made	Laouri et al. and Nystrom et al. have been added to the updated search. However, the body of evidence is still extremely limited.
Raji Pillai, PhD, Ljubomir	Pathwork Diagnostics		Nystrom et al Oncotarget 2012 This IRB-approved registry study assessed the impact of the	Laouri et al. and Nystrom et al. have been added to the

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Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.			Tissue of Origin Test on tissue site working diagnosis, subsequent management decisions, and survival when the test was used as an adjuvant to clinicoopathological evaluation in 107 patients. The study was sponsored by Pathwork and conducted by independent investigators associated with Tufts Medical Center, Stanford University Medical Center, University of Texas MD Anderson Cancer Center, Cedars Sinai Medical Center and Cedar Associates. Data collected from treating physician compared their pre-test and post-test (Tissue of Origin) working diagnoses of primary site and pre-test and post-test plans for patient management. After Tissue of Origin testing there was a 50% change in tissue-site diagnosis (95% CI [43%, 58%]). In 65% of cases (95% CI [57%, 72%]) physicians reported a change in cancer-specific management after receiving the Tissue of Origin Test results. Following the Tissue of Origin test, the percentage of guideline consistent chemotherapy regimens increased from 42% to 65% and recommendations for non-guideline consistent regimens declined from 28% to 13%.	updated search. However, the body of evidence is still extremely limited.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		7. KQ 4b. What is the evidence that the genetic TOO tests change outcome? p58. Pathwork Comment: The report erroneously states that there is no published evidence on the test changing outcomes in patients with CUP. Nystrom et al Oncotarget 2012 This IRB-approved registry study assessed the impact of the Tissue of Origin Test on tissue site working diagnosis, subsequent management decisions, and survival when the test was used as an adjuvant to clinicoopathological evaluation in 107 patients. The study was sponsored by Pathwork and conducted by independent investigators associated with Tufts Medical Center, Stanford University Medical Center, University of Texas MD Anderson Cancer	Nystrom et al. has been added to the updated search. However, the body of evidence is still extremely limited. None of the studies of treatment outcomes or survival have assessed or controlled for differences in the patient population.

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			Center, Cedars Sinai Medical Center and Cedar Associates. Data collected from treating physician compared their pre-test and post-test (Tissue of Origin) working diagnoses of primary site and pre-test and post-test plans for patient management. At last follow-up, 69 patients of the 107 patients in the cohort had died. The overall median survival was 14.0 months . Median survival in this cohort compares favorably with survival of 9 months for cancer of unknown primary (CUP) as reported in historical control series.	
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		8. KQ 5. Is the TOO test relevant to the Medicare population? P61-62. Pathwork comment: The report erroneously states? None of the studies provided information on the race or ethnicity of the cases.? Race/ethnicity was provided in Pillai, Buturovic, Becker, and Henner et al JMD 2011.	This information has been added to the report. We regret we overlooked it in our first review.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	Discussion/ Conclusion	1. Ethical considerations would preclude a randomized controlled trial?, p68 a. We support this conclusion of the paper and agree there are significant barriers to doing a randomized trial b. Very high number of cases required because of the large number of tumor types and heterogeneity of clinical situations encountered c. Accrual would be challenging as some US investigators have expressed unwillingness to enroll patients in a trial where information about GEP is withheld from control patients d. Difficult to gain patient consent and perhaps to gain IRB approval e. Importantly, use of IHC has never been shown to improve outcomes and so GEP should not be held to this	This study was incorrectly attributed. The reference and the citation have been corrected.

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			standard, either. (NCCN guidelines on Occult Primary, Aug, 2012: ??outcomes data are not currently available to recommend routine use of molecular profiling in the workup of occult primary tumors; likewise, no such data exist to endorse the automatic or indiscriminate use of immunohistochemistry. Until more robust outcomes and comparative effectiveness data are available, pathologists and oncologists must collaborate on the judicious use of these modalities on a case by case basis, with the best possible individualized patient outcome in mind.?) f. A prospective trial such as the one reported by Monzon et al 39 may be the best design available in this case.? p68. Please include the citation to which the sentence above refers.	
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		We agree with the authors? conclusion that a randomized controlled outcome trial would not be feasible in the US. Given that, other standards for ?clinical utility? must be used for a tumor classification test. Since diagnostic tests influence patient survival through patient management decisions, appropriate measures of GEP?s clinical utility are: (i) Demonstrated impact of the test on physician behavior ? change in diagnosis and treatment. (See response to KQ4a below) and (ii) Evidence that the test performs favorably compared to the current standard of care ? IHC (Comparative Effectiveness) Although immunohistochemistry (IHC) is considered the standard of care for the initial attempt to identify the site of tissue of origin for tumors, the limitations of IHC have become increasingly clear. Pathwork Diagnostics has two publications demonstrating superiority to IHC in direct comparisons. Kulkarni et al Diagnostic Pathology 2012 The study compared the performance of the Tissue of Origen Test vs	The claimed benefit of these tests is that they inform site specific therapy, which is said to improve patient survival compared to empiric therapy. The only way to evaluate that claim is to look at changes in treatment and the effect of the treatment changes on patient outcome. The Handorf reference was not available at the time of the updated search.

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			IHC and was sized as a pilot investigation. Ten-archived formalin-fixed paraffin embedded metastatic tumor samples for which the primary site had been clinically determined were sent for blinded analysis by 5 different pathologists utilizing IHC. Those results were compared to the Tissue of Origin Test. The Tissue of Origin Test determined the correct diagnosis in 90% of cases (9 of 10) The five pathologists reached the correct diagnosis in an average of 64% of cases (32 of 50 case reviews- the 10 specimen were sent to 5 pathologists) The pathologists ordered an average of 8.8 IHC stains/slides per case The average number of slides needed for the Tissue of Origin Test was 3. The Tissue of Origin test was more accurate and used less tissue than IHC Handorf et al, JMD November 2012, AMP abstract in press.Cancer of Uncertain Origin: Comparative Effectiveness of Immunohistochemistry and Gene Expression Profiling in Diagnosing the Primary Site in Metastatic Cancer Charles R. Handorf1, MD, PhD, Anand Kulkarni1, MD, Ashley M. Ezekiel1, James P. Grenert2, MD PhD, Oliver S. Kim3, MD, William M. Rogers4, MD, Lawrence M. Weiss5, MD, Catherine I. Dumur6, PhD, Michael O. Idowu6, MD, George E. Sandusky7, DVM, PhD, Federico A. Monzon8, MD, Meredith Halks-Miller9, MD, Andrea Pingitore9, MD, Eloisa Fuentes9, MD, Rebecca Panos9, Jing Shi9, MD, PhD, Michael Walker9, PhD, Raji Pillai, Buturovic, Becker, and Henner9 PhD, W. David Henner9, MD PhD. 1University Of Tennessee Health Science Center, Memphis, TN, 2University of California San Francisco, San Francisco, CA, 3Advocate Good Shepherd Hospital, Barrington, IL, 4El Camino Hospital, Mountain View, CA,	

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			5Clarient Inc., Aliso Viejo, CA, 6Virginia Commonwealth University, Richmond, VA, 7Indiana University, Indianapolis, IN, 8Baylor College of Medicine, Houston, TX, 9Pathwork Diagnostics, Redwood City, CA INTRODUCTION Determining the origin of metastatic cancer with confidence can be challenging. Pathologists commonly use a battery of immunohistochemical (IHC) stains to determine the primary site. Gene expression profiling (GEP ? Pathwork Tissue of Origin Test), which relies on mRNA expression of 2000 genes to predict the primary site from a panel of 15 tumor tissue types, is an alternative method for evaluating these cases. We directly compared the accuracy of IHC and GEP in identifying the origin of 160 blinded metastatic specimens. METHODS Sample eligibility criteria included 1) formalin-fixed paraffin-embedded surgical biopsy specimens from the 15 tumor tissue types on the GEP test panel, containing ?60% tumor and adequate size to provide at least 25 sections, 2) an identifiable primary site based on clinical records e.g. imaging and surgical reports. Samples where the primary site diagnosis relied on IHC were excluded. The four evaluating pathologists (EPs) and the team performing GEP were given patient sex and gross sample description and were blinded to the primary site. Study design allowed the EP to diagnose a primary site in a maximum of 3 steps: after evaluation of H&E images (initial diagnosis), first batch of stains (intermediate diagnosis) and second batch of stains (final diagnosis). A panel of 84 IHC and histochemical stains were available to the EPs. Slides were digitized for EP analysis. Conditional logistic regression was used to compare the results from the two methods.	

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			RESULTS 157 samples were evaluated by both IHC and GEP. The GEP test results were more accurate at identifying the primary site for metastatic tumors (89%) than IHC (83%) (p=0.013). Histology and IHC-based diagnoses increased from 64% accuracy (average) following H&E alone to 81% after one IHC round and to 83% after two rounds. For GEP, the average number of sections used per case was 3.8 (median 4, range 1-8) vs. 8.4 stains (median 8.0, range 0-20) per EP per case. In poorly differentiated and undifferentiated cases, GEP was 94% accurate compared to 79% for IHC (p=0.016). CONCLUSION In this study of comparative effectiveness of IHC and GEP, the accuracy of primary site diagnosis using GEP was superior to accuracy achieved with IHC-based diagnosis. These results suggest that GEP can be a useful tool for determining the site of origin for metastatic tumors.	
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		2. P68. Future Research. ?A prospective trial such as the one reported by Monzon et al.39 maybe the best design available in this case.? Pathwork comment. Due to misnumbering, unclear to which paper the authors refer.	This study was incorrectly attributed. The reference and the citation have been corrected.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	Tables	p17. Table 5 incorrectly lists 1550 genes for the Pathwork Dx FFPE test. The FFPE test uses 2000 genes as published in Pillai, Buturovic, Becker, and Henner et al JMD 2011.	We have noted the two versions of the Pathworks Dx TOO in the table.
Raji Pillai, PhD,	Pathwork		p18. Table 6 list Endometrium and Head and Neck as not	Table 6 refers to the

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Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Diagnostics		offered by PathworkDx. Pathwork comment: Both are part of the Tissue of Origin Testing Service and are included in the Tissue of Origin Endometrial Test and the Tissue of Origin Head and Neck Test. (Lal et al Oncotarget 2012; Lal et al AACR poster http://cancerres.aacrjournals.org/cgi/content/short/72/8_MeetingAbstracts/1724?rss=1;)	primary test for CUPS patients only, not auxiliary tests. We have included the Endometrial Test in KQ1 and the Lal article in the review.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		p23. Table 7, ?Dumar 2008, ref 29?. Comment: The superscripted reference numbers do not point to the intended references. The authors should include Pillai, Buturovic, Becker, and Henner et al JMD 2011 as reproducibility for the FFPE test was reported there.	The references have been checked and corrected as necessary.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		p27. Table 8, ?Dumur 2008, ref 33?. Comment: The superscripted reference numbers do not point to the intended references.	The references have been checked and corrected as necessary.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics		p44. Table 11. The authors reference a paper (Beck 2011) that includes both known reference specimens AND unknown specimens. The test performance can only be calculated using known reference specimens. We recommend either excluding the citations that have unknown specimens or excluding the subset of unknown specimens from those citations.	Findings for known reference specimens were considered evidence on KQ3b, clinical validity. Findings for unknown specimens were considered evidence on KQ4, diagnosis.
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W.	Pathwork Diagnostics		p50. Table 12, numbering and correctness of references Comment: The superscripted reference numbers do not point to the intended references. Gutierrez refers to an abstract that has now been published	The citations have been checked and corrected. The updated search includes the referenced citations.

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David Henner MD, PhD.			as a journal article(Nystrom et al Oncotarget 2012). Medeiros 2008 refers to an abstract that has now been published as Monzon et al Diag Pathology, 2010. Laouri 2010. Refers to an abstract that has been published as Laouri et al Personalized Medicine 2011.	
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD..	Pathwork Diagnostics	Figures	p40, Fig 6, forest plot. Comment. The figure shows 4 Dumur 2008 data sources. There are only three in the reference section Dumur JMD publication on reproducibility 2008 Dumur AMP abstract on reproducibility 2008 Dumur AMP abstract on tumor utilization 2008.	The figure has been corrected
Raji Pillai, PhD, Ljubomir Buturovic, PhD, Shawn Becker, MD and W. David Henner MD, PhD.	Pathwork Diagnostics	References	References are misnumbered and superscripted numbers do not point to the intended citation. It is hard to assess correctness of what is reported because of the misnumbering. Also, it is unclear what paper reports 29% of tests as clinically useful. To our knowledge 29% has never been reported for the Pathwork Tissue of Origin Test.	The citations have been checked and corrected. The article reporting 29% (2 of 7) of tests as clinically useful was Beck et al.
Sarah E. Kerr, MD	Mayo Clinic	Methods	I applaud the authors of this review for their draft attempt at summarizing the current evidence in regards to molecular-based cancer classifiers, an important topic of interest to physicians and payors when many treatments are tied to specific cancer types and current pathology methods sometimes fall short of definitive tumor classification. As molecular oncology testing is a rapidly advancing field, I wanted to draw attention to an article published more recently than the time period of the draft review that I think is important to include in the technological assessment of CancerTYPE ID: Kerr et al. Multisite Validation Study To Determine Performance Characteristics of a 92?gene Molecular Cancer Classifier. Clin Cancer Res. 2012;18:3952?60. This was a rigorously conducted double blinded evaluation of the CTID classifier using a large cohort of well-adjudicated tumors from a wide variety of	This publication is included in the updated search.

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			clinicopathologic contexts and sample types.	
Wolf C Mueller	Department of Neuropathology at the Institute of Pathology, Heidelberg University, Heidelberg, Germany	General	The assessment draft focuses on three different tests available in the U.S. for the identification of tissue of origin (TOO) in patients with cancer of unknown primary (CUP).	No response needed.
Wolf C Mueller	Department of Neuropathology at the Institute of Pathology, Heidelberg University, Heidelberg, Germany	General	In the assessment the authors compare the performance of our recently published miReview test1 to that of CancerType and PathworkDx. They come to the conclusion that the clinical accuracy of all the three tests is similar, ranging from 83 percent to 87 percent. However, they claim the evidence that the tests contribute to identifying a TOO to be moderate, and that there is not sufficient evidence to assess the effect of the tests on treatment decision and outcomes. A major point of criticism of the currently available studies, according to the assessment draft, is that most of the validation studies were funded wholly or partially by the manufacturers of the tests, thereby not fully excluding publication bias. The authors see an urgent need in the literature for the tests to be evaluated by research groups that have no evident conflict of interest. Secondly, given the difficulty of assessing the accuracy of the TOO in CUP cases, the authors outline a possible future research focus that should focus on the benefits from the test to the patient in terms of effect on treatment decisions and resulting outcomes, based on the tests results. In the authors opinion, these studies should help assess cost effectiveness of the TOO tests in the near future.	No response needed.
Wolf C Mueller	Department of Neuropathology at the Institute of	General	1st addressing the issue of a possible publication bias: The authors criticize that the currently available validation studies were funded wholly or partially by the manufacturers	The concern about publication bias is that manufacturers may

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	Pathology, Heidelberg University, Heidelberg, Germany		of these tests. They see a possible publication bias. This does not hold for the microRNA-based test that was validated at our Department of Neuropathology at the Institute of Pathology at Heidelberg, University, Germany. In the project with Rosetta Genomics we aimed at minimizing any publication bias upfront and designed the study accordingly. While it is true, that the company performed the test, the data validation of the test itself was performed mainly at our department with the companies employees completely blinded to the clinical data of the patients. Thereby we completely excluded any bias in data acquisition and data interpretation. Our department is completely independent from Rosetta Genomics. None of our employees were directly funded by Rosetta nor were any of our employees on Rosetta Genomics payroll, thereby excluding any conflict of interest of the authors coming from our Neuropathology Department. We respectfully request that the authors acknowledge that there was no bias in the studies performed by our institution.	maintain control of publication, which can result in a failure to publish studies with unfavorable results. Authors that are employed by the test manufacturer may be reluctant to sign off on papers reporting unfavorable results.
Wolf C Mueller	Department of Neuropathology at the Institute of Pathology, Heidelberg University, Heidelberg, Germany	General	2nd addressing the issue of future research focus in an effort to assess the cost effectiveness of the TOO tests. We fully agree that at this point it is way too early to draw any conclusions yet regarding all clinical issues related to the care of patients with CUP in dependence of the implementation of our microRNA based assay. However, in our opinion the authors fall short of fully appreciating the initial aim of these studies. Our study was designed to show that with our test we can identify TOO with high sensitivity and specificity. It is our conviction that these validation and feasibility studies have to come first, before one can think of initiating any further studies that address clinical issues i.e. outcome of patients when treated according to the test results. Based on the data of our first two microRNA based assays we feel confident that we have enough arguments to initiate studies that address the authors` points of criticism ?	The purpose of our review was to assess the current evidence for the validity and utility of these tests. Our objective was a comprehensive, neutral review. Therefore, we respectfully decline to add the requested sentence to the report.

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			but we feel that the authors of the technology assessment should appreciate what we already achieved rather than to point out currently missing evidence for cost effectiveness of the TOO tests, or the power to positively influence patient care ? data that at this time of test development can hardly be expected. Rather, they should encourage clinicians to use these tests in an effort to address all issues that they raise ? independent test validation and investigation of the potential of the tests to individualize patient treatment based on the test results. We therefore respectfully request that the following sentence be added in the Future Research section of the assessment on page 63; However, the absence of data showing improved outcomes in CUP patients should not discourage clinicians from using these tests in patients where knowing the TOO could result in delivery of specific treatments proven to be of benefit.	
Wolf C Mueller	Department of Neuropathology at the Institute of Pathology, Heidelberg University, Heidelberg, Germany	General	We would also like to comment on this currently available draft of the technology assessment focussed on the description and presentation of the results of our recently published data on the identification of TOO in patients with CUP implementing microRNA expression patterns 1-2.	No response needed.
Wolf C Mueller	Department of Neuropathology at the Institute of Pathology, Heidelberg University, Heidelberg, Germany	General	First and foremost, we are very surprised that the authors unfortunately missed our more comprehensive recent study implementing a second generation microRNA-based assay for diagnosing tumor tissue origin 2. The first generation microRNA assay was considered a first proof of principle study to investigate and hopefully proof the potential power of microRNA- profiling in diagnosing tumor tissue origin in patients with CUP and especially brain metastases as a first sign of disease. For this reason and the limited number of primary tumors regularly metastasizing to the brain, it was felt that a relatively small number of potential primary tumor	The study by Meiri et al is included in the updated search.

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			entities should suffice. The fact that the authors found that this pilot study already had a similar power as the other two assays highlights the power of our microRNA- based test. However, to fully appreciate the power, if the authors wish to perform an assessment on the second generation assay, then they will have to include the data of our second generation microRNA-based assay that cover the majority of all known cancer types that was developed and trained on 1,282 samples, and that was validated on a high number of different tissues obtained from different institutions around the world. In our opinion, the assessment in its current form is focussed on the first-generation miRview mets assay and not really on the second-generation miRview mets2 assay. 1. Mueller WC, Spector Y, Edmonston TB, St Cyr B, Jaeger D, Lass U, Aharonov R, Rosenwald S, Chajut A: Accurate classification of metastatic brain tumors using a novel microRNA-based test. Oncologist 2011, 16: 165-174. 2. Meiri E, Mueller WC, Rosenwald S, Zepeniuk M, Klinke E, Edmonston TB, Werner M, Lass U, Barshack I, Feinmesser M, Huszar M, Fogt F, Ashkenazi K, Sanden M, Goren E, Dromi N, Zion O, Burnstein I, Chajut A, Spector Y, Aharonov R: A second-generation microRNA-based assay for diagnosing tumor tissue origin. Oncologist 2012, 17: 801-812.	
Wolf C Mueller	Department of Neuropathology at the Institute of Pathology, Heidelberg University, Heidelberg, Germany	References	In our opinion, the assessment in its current form is focussed on the first-generation miRview mets assay (Mueller WC et al.) and not really on the second-generation miRview mets2 assay (Meiri E and Mueller WC et al.). We respectfully request that the authors also acknowledge our 2nd generation miRview assay in their technology assessment. 1. Mueller WC, Spector Y, Edmonston TB, St Cyr B, Jaeger D, Lass U, Aharonov R, Rosenwald S, Chajut A: Accurate classification of metastatic brain tumors using a novel microRNA-based test. Oncologist 2011, 16: 165-174.	The study by Meiri et al was included in the updated search. The information about the miRview test has been updated to reflect the newer version of the test.

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			2. Meiri E, Mueller WC, Rosenwald S, Zepeniuk M, Klinke E, Edmonston TB, Werner M, Lass U, Barshack I, Feinmesser M, Huszar M, Fogt F, Ashkenazi K, Sanden M, Goren E, Dromi N, Zion O, Burnstein I, Chajut A, Spector Y, Aharonov R: A second-generation microRNA-based assay for diagnosing tumor tissue origin. Oncologist 2012, 17: 801-812.	
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	THESE COMMENTS ARE A REVISION FROM THOSE PROVIDED ON SEP 21, 2012 TO INCLUDE A PUBLICATION STATUS UPDATE We have read with interest the new draft Technology Assessment?Technology Assessment on Genetic Testing or Molecular Pathology Testing of Cancers with Unknown Primary Site to Determine Origin?made available on September 7, 2012. As developers of one of the tests reviewed in this Technology Assessment (CancerTYPE ID?), we appreciate the opportunity to review and comment. We agree with the authors that Cancers of Unknown Primary Site represent an important clinical unmet need, and molecular classification tests represent a new technology with a rapidly growing and evolving base of clinical evidence. Thus, this Technology Assessment is timely and should be of high interest to physicians and payers.	No response needed.
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	As the authors have detailed, CancerTYPE ID is a real-time RT-PCR assay that uses the collective expression of 92-genes to predict tumor type of an unknown specimen. However, there are a number of important features of CancerTYPE ID that we felt could be more accurately represented in the final Technology Assessment. In addition, a number of clinical studies that have been published or presented recently are not included in this draft version. We wanted to make the authors aware of these data and ask that they be reviewed and included before the Assessment is issued in its final	No response needed.

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			format. In addition, in light of the completion of these seminal studies, we believe the authors should reconsider conclusions on the clinical evidence supporting molecular cancer classification, particularly CancerTYPE ID, and its clinical utility in patient management. In the text below, these issues are discussed in detail; in addition, we have listed the key issues in summary tabular format at the end of the document.	
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	I. Clarification of number of tumor types classified by CancerTYPE IDOne of the key features of CancerTYPE ID is its ability to provide tumor classification of both site of origin and tumor subtype. The current marketed version of CancerTYPE ID classifies 50 distinct tumor types.(1) However, in the draft Technology Assessment, the number of tumors classified by CancerTYPE ID is listed as 29 on Page ES-3 and as 27 in several other places (eg, Page 16, Table 5, Table 6). The distinction between identifying site of tumor origin vs tumor type and subtype has very important clinical utility for treating physicians. For example: Lung Cancer: While all originating from lung, lung adenocarcinoma, lung squamous cell carcinoma, and small cell carcinomas of the lung each have ifferent optimal treatment regimens. Several therapies (eg, Avastin and Alimta) are approved for lung adenocarcinoma, but are not indicated for lung squamous cell carcinoma. Furthermore, Avastin is contraindicated in patients with lung squamous cell carcinoma due to risk of pulmonary hemorrhage Neuroendocrine tumors: Pancreatic islet cell carcinomas have several targeted therapies approved for use (eg, Afinitor, Sutent) that are not indicated in other Neuroendocrine tumor types. Ovarian Cancer: First line treatment for many ovarian cancers typically involves a combination of paclitaxel and carboplatin; however, evidence suggests that ovarian	Tissue of origin tests may provide additional information as well as tissue of origin. However, the scope of this technology assessment was limited to the ability of these tests to identify the tissue of origin in cancers of unknown primary. We have corrected the number of tumor sites identified. We did not address subtypes in this review.

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			mucinous tumors are more responsive to fluorouracil-based chemotherapy. We request that the number of tumor types and subtypes classified by CancerTYPE ID is clarified in the Technology Assessment, including notation of both 28 tumor types and 50 tumor subtypes.	
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	II. Recently published or presented clinical studies A number of clinical studies of CancerTYPE ID have recently been completed and are either published /In Press or have recently been presented at major Oncology or Pathology scientific congresses. Significantly, the first prospective outcomes trial in which treatment decisions for patients diagnosed with cancer of unknown primary were directed by a molecular classifier (CancerTYPE ID) has been published (http://jco.ascopubs.org/content/early/2012/10/01/JCO.2012.43.3755.abstract(2) These studies fundamentally enhance the evidence-base for molecular classification in general and for CancerTYPE ID specifically, and should be discussed and integrated into the Technology Assessment before it is issued as a final version.	We updated the literature search during the peer review process. The status of the citations is noted below.
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	1. Multi-Institutional Clinical Validation (Kerr et al, 2012, Clin Cancer Res)(1)This was a blinded, multi-institutional clinical validation of CancerTYPE ID led by external investigators from three centers of excellence (MGH, Mayo Clinic, UCLA) and included 790 independently-adjudicated samples?the largest validation study of a molecular classification test completed. In addition, unlike validation studies of other molecular tests reviewed in this Technology Assessment, rigorous adjudication between the investigational sites was performed to establish diagnosis in all cases entered in the trial. This aspect is important as validation studies using samples obtained from tumor banks rely on corresponding	This article was included in the updated search.

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			pathology reports which may contain inaccurate diagnoses, leading to over- or underestimation of test performance. The increased rigor provided by peer adjudication allows a more precise characterization of test performance. The results demonstrated an overall sensitivity of 87% at the main type level and 82% at the subtype level. Furthermore, the accuracy of CancerTYPE ID was assessed in clinically relevant subsets including metastatic, poorly-differentiated tumors, and limited biopsy specimens. Consistent with requirements for clinical applicability, CancerTYPE ID demonstrated a high level of accuracy and stable performance in metastatic disease vs primary tumors (p=0.157), high-grade tumors vs low-grade tumors (p=0.577), and cases with limited tissue vs excision biopsies (p=0.161).	
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	2. Prospective Clinical Trial of Assay-directed Therapy in Patients with Cancer of Unknown Primary (Greco et al, 2012, J Clin Oncol)(2(http://jco.ascopubs.org/content/early/2012/10/01/JCO.2012.43.3755.abstract) This was a prospective clinical trial led by the Sarah Canon Research Institute. In this study, patients diagnosed with Cancer of Unknown Primary (CUP) were treated with site-specific chemotherapy regimens based on CancerTYPE ID predictions. In total, 289 patients diagnosed with CUP were enrolled. The study met is primary endpoint (>30% improvement in OS compared to previous trials of empiric CUP therapy at SCRI; 12.5mo vs 9.1mo). Furthermore, the median overall survival of patients treated with assay-directed therapy (12.5mo) was greater than patients treated with empiric CUP regimens in this trial (4.7mo; p=0.02). The investigators noted that while a randomized two-arm trial would have been ideal, given the strong published evidence that CancerTYPE ID provides an accuracy prediction for tumor type, a randomized trial that assigned patients to	The Hainsworth 2012 article reporting these results were included in the updated search.

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			empiric therapy would have been unethical; thus, this trial design represents the definitive study design for assessing whether molecular classification can improve patient outcomes in patients with CUP.	
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	3. CancerTYPE ID vs Immunohistochemistry (IHC) in Diagnosis of Primary Site of High-grade Metastatic Cancers (Weiss et al, 2012, ASCO)(3) This was a blinded study led by investigators at the City of Hope National Medical Center comparing standard of care IHC + morphology vs CancerTYPE ID in diagnosing primary site of origin in 122 difficult to diagnose, cancer cases composed of high grade , primarily metastatic tumors. CancerTYPE ID demonstrated a 10% increase in overall accuracy compared to IHC/morphology (P = 0.019.	This article was included in the updated search.
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	We request that studies 1 and 3 be integrated into the discussion and level of evidence summary for KQ 3b-3f, and that study 2 be integrated into the discussion and level of evidence summary for KQ 4.	These studies have been included in the updated review.
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	III. Level of evidence standards Third, we feel that it is important to comment on the level of evidence standards discussed in the draft Technology Assessment. A number of different frameworks for levels of evidence have been proposed for molecular diagnostic tests, and no unified framework has been established to date. However, based on standards discussed in various guidelines documents, the CancerTYPE ID clinical study program has been designed to meet the highest standards of rigor, and to lead the field of molecular classification. For example, the CancerTYPE ID validation and performance characterization studies(1,3) were led by external investigators from centers of excellence including UCLA, Mayo Clinic, MGH, and City of Hope. These were blinded studies, with unblinding and data analyses performed by the	As noted in the report, we used the EPC framework to evaluate the strength of the evidence.

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			study investigators and/or a third party. Finally, a prospective trial which directly investigates the impact of the CancerTYPE ID assay on patient outcomes has been completed and accepted for publication, providing level 1 evidence for the test. Previous studies assessing effects of molecular classification assays on patient outcome have been retrospective in design, and include the limitations of retrospective investigations.	
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	IV. Additional specific comments: 1. KQ 4 ? Treatment: Low evidence that test results affect treatment response The authors noted that evidence that tumor of origin tests change outcome is limited and that there is not sufficient evidence to assess the effect of the tests on treatment outcomes. However, we believe that with the results of the prospective trial described above now available, there is sufficient evidence for CancerTYPE ID. The results of the prospective trial led the investigators to note that ?the body of evidence is sufficient to support the use of molecular tumor profiling in the standard management of patients with CUP.? We believe that the summary statement regarding KQ 4 (ie, low evidence that test results affect treatment response) should be reconsidered and amended for CancerTYPE ID, given that the definitive clinical trial to address this question has now been completed.	We reviewed the additional literature and re-evaluated the strength of the evidence in light of these new studies. However, the body of evidence addressing these questions is still low, and few studies, including the referenced trial, have an appropriate comparison group.
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	2. KQ 4 ? Treatment: Impact on treatment decisions The authors note that there is low evidence that molecular classification affects treatment decisions. However, in addition to the publication that was discussed (Hainsworth et al, 2011. Clinical Colorectal Cancer), there is now significant additional evidence of impact on treatment decisions. First, the completed prospective trial demonstrates improved patients outcomes in patients treated based on CancerTYPE	We reviewed the additional literature and re-evaluated the strength of the evidence in light of these new studies. However, the body of evidence addressing these questions is still small, and few studies,

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			ID predictions. In addition, in a publication not cited in the draft Technology Assessment, Greco described the change in treatment recommendations in a series of patients with CUP that had site of origin predicted by CancerTYPE ID.(7)	including the referenced trial, have an appropriate comparison group. The Greco study cited is included in the updated report.
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	3. Page 67: Potential for bias in published literature The authors noted a lack of studies published by independent investigators. However, we feel that it is important to note that while the CancerTYPE ID studies described above were funded by bioTheranostics, the studies were all led by independent external investigators from centers of excellence, and validation studies were performed in a blinded manner with unblinding and data analyses completed by the investigators or third parties. \ Furthermore, several recent clinical studies have assessed CancerTYPE ID have been published by independent investigators that were not funded by bioTheranostics.(4,5,6) We suggest that these studies should be discussed and included in the final Technology Assessment.	The concern about publication bias is that manufacturers may maintain control of publication, which can result in a failure to publish studies with unfavorable results. Authors that are employed by the test manufacturer may be reluctant to sign off on papers reporting unfavorable results. The updated report includes the new studies cited.
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	4. Table 5: The following modifications in a revised version are requested: FDA submission status--Regulatory application for CancerTYPE ID has been submitted to the FDA. Laboratory method?CancerTYPE ID is a gene expression-based test that is analyzed on an RT-PCR, not a microarray, platform. The number of tumors in reference database?the number of tumors in the reference database is listed as 578. This was the number first described in 2006; however, the reference database has been expanded in subsequent versions of the test, and currently includes 2094 tumor samples (range 26-228.(1)	We have updated the information regarding FDA submission and corrected the laboratory methods and the number of tumors in the reference database.
Catherine A.	Senior Director,	General	In summary, the authors of the draft Technology Assessment	We thank the authors for

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Schnabel	Medical & Scientific Affairs, bioTheranostics Inc.		characterize the literature on molecular classification tests as ?in its infancy.? We believe that this characterization is in part due to the lag time of publishing the results of clinical trials. When viewed collectively, we believe that there is a strong and consistent evidence base supporting the analytical validity, clinical validity, and clinical utility of molecular classification testing for identification of tumor types. In particular, the CancerTYPE ID clinical trial program has now matured to include investigation of over 4,500 tumor samples, and includes more published clinical data than a number of biomarkers that are included in nationally-recognized clinical practice guidelines (eg, NCCN). Analytical validity data have been presented in 2 manuscripts;(9,10) clinical validity has been assessed in 5 independent clinical studies, including a 790-sample, independently-adjudicated, blinded study;(1,3,8,10,11) and clinical utility for patient treatment and outcomes have been presented in 3 studies, including a newly-completed 3-year prospective clinical trial which assessed the effect of CancerTYPE ID-directed therapy in patients with CUP.(2,7,12) We commend the authors on reviewing and compiling the data presented in the draft assessment and hope that the information provided above is helpful to the authors in finalizing the Technology Assessment.We appreciate the consideration of our comments.	this information.
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	Summary Table of Comments Section: KQ 1 Topic: Clarification of number of tumor types classified by CancerTYPE ID Comment: In the draft Technology Assessment, the number of tumors classified by CancerTYPE ID is listed as 29 on Page ES-3 and as 27 in several other places (eg, Page 16, Table 5, Table 6). However, the current marketed version of	Tissue of origin tests may provide additional information as well as tissue of origin. However, the scope of this technology assessment was limited to the ability of these tests to identify the tissue of origin in cancers of unknown primary. We have corrected

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			CancerTYPE ID classifies 50 distinct tumor types. In addition, it is important to note that CancerTYPE ID classification is not limited to ?tissue of origin?; rather, CancerTYPE ID identifies both tumor type and subtype, a distinction with high clinical importance. We request that the number of tumor types and subtypes classified by CancerTYPE ID is clarified in the Technology Assessment, including notation of both tumor sites of origin and subtypes.	the number of tumor sites identified. We did not address subtypes in this review.
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	Section: KQ 3b-3f, KQ 4 Topic: Recently published and presented clinical studies Comment: A number of clinical studies of CancerTYPE ID have recently been completed and are either In Press or have been presented at major Oncology or Pathology scientific congresses. We believe that these studies fundamentally enhance the evidence-base for molecular classification in general and for CancerTYPE ID specifically, and should be discussed and integrated into the Technology Assessment before it is issued as a final version.	We have included all studies published or e-published by 11/7/2012.
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	1. Multi-Institutional Clinical Validation (Kerr et al, 2012, Clin Cancer Res(1) 2. Prospective Clinical Trial of Assay-directed Therapy in Patients with Cancer of Unknown Primary (Greco et al, 2012, J Clin Oncol)(2) 3. CancerTYPE ID vs Immunohistochemistry (IHC) in Diagnosis of Primary Site of High-grade Metastatic Cancers (Weiss et al, 2012, ASCO)(3)	These studies are included in the updated review.
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	Topic: Level of evidence standards Comment: The CancerTYPE ID clinical study program was designed to meet the highest standards of rigor. For example, the CancerTYPE ID validation and performance characterization studies were led by external investigators from centers of excellence. The studies were blinded, and unblinding and data analyses were performed by the study investigators and/or a third party. Finally, CancerTYPE ID has now been investigated in a prospective clinical trial directly assessing the impact of the assay on patient	No response needed.

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			outcomes.	
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	Section: KQ 4 Topic: Low evidence that test results affect treatment response Comment: The authors noted that evidence that there is not sufficient evidence to assess the effect of the tests on patient outcomes. However, with the results of the prospective trial described above now available, we believe that there is sufficient evidence for CancerTYPE ID. We suggest that the summary statement regarding KQ 4 should be reconsidered and amended for CancerTYPE ID, given that the definitive clinical trial to address this question has now been completed	We reviewed the additional literature and re-evaluated the strength of the evidence in light of these new studies. However, the body of evidence addressing these questions is still small.
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	General	Section: KQ 4 Topic: Impact on treatment decisions Comment: The authors note that there is low evidence that molecular classification affects treatment decisions. However, there are now multiple studies that have presented data addressing this question.(2,7,12) We believe that this conclusion should be amended given the newly-available clinical study data.	We reviewed the additional literature and re-evaluated the strength of the evidence in light of these new studies. However, the body of evidence addressing these questions is still small.
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	Summary	Topic: Potential for bias in the published literature Comment:The authors noted a lack of studies published by independent investigators. However, it is important to note that while the pivotal CancerTYPE ID studies were funded by bioTheranostics, the studies were all led by independent external investigators from academic centers of excellence, and validation studies were performed in a blinded manner with unblinding and data analyses completed by the investigators or third parties. Furthermore, several recent clinical studies have assessed CancerTYPE ID have been published by independent investigators that were not funded by bioTheranostics.(4,5,6) We suggest that these studies	We have included all studies published or e-published by 11/7/2012.

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			should be discussed and included in the final Technology Assessment, and that the information provided regarding study design and independent studies should be considered in the assessment of potential publication bias.	
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	Summary	Section: KQ1 Topic: Reference database for CancerTYPE ID Comment: In the description of CancerTYPE ID (Table 5), the number of tumors in the reference database is listed as 578. This was the number described in the initial test publication in 2006; however, the reference database has been expanded in subsequent versions of the test, and currently includes 2094 tumor samples (range 26-228).(1)	We have updated this information.
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	Summary	Section: KQ3b-3f Topic: Additional published validation study in Chinese population Comment: In addition to the other new published studies described, CancerTYPE ID has also been validated in a set of tumors from a Chinese population.(8) We suggest that this publication is also included in the validation section of the Technology Assessment.	This study is included in the updated review.
Catherine A. Schnabel	Senior Director, Medical & Scientific Affairs, bioTheranostics Inc.	Summary	References: (1) Kerr et al. Multisite Validation Study To Determine Performance Characteristics of a 92-gene Molecular Cancer Classifier. Clin Cancer Res. 2012;18:3952-60. (2) Greco et al. Molecular gene expression profiling to predict the tissue of origin and direct site-specific therapy in patients (pts) with carcinoma of unknown primary site (CUP): Results of a prospective Sarah Cannon Research Institute (SCRI) trial. J Clin Oncol. Published online before print October 1, 2012, doi: 10.1200/JCO.2012.43.3755. (3) Weiss et al. Blinded comparator study of	These studies are included in the updated review.

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			immunohistochemistry (IHC) versus a 92-gene cancer classifier in the diagnosis of primary site in metastatic tumors. J Clin Oncol. 2012, 30:(suppl; abstr e21019). (4) Greco et al. Carcinoma of Unknown Primary Site: Outcomes in Patients with a Colorectal Molecular Profile Treated with Site Specific Chemotherapy. Journal of Cancer Therapy, 2012, 3, 37-43. (5) Thompson et al. Molecular tumor profiling (MTP) in cancer of unknown primary site (CUP): A complement to standard pathologic diagnosis. J Clin Oncol 29: 2011 (suppl; abstr 10560). (6) Greco et al. Carcinoma of unknown primary site (CUP): Outcomes in patients with a colorectal molecular profile treated with site-specific chemotherapy. J Clin Oncol 29: 2011 (suppl; abstr 3563). (7) Greco FA. Evolving understanding and current management of patients with cancer of unknown primary site. Commun Oncol 2010, 7:183-188 (8) Wu et al. 92-Gene molecular profiling in identification of cancer origin: a retrospective study in Chinese population and performance within different subgroups. PLoS One. 2012;7(6):e39320. Epub 2012 Jun 22. (9) Ma et al. Molecular classification of human cancers using a 92-gene real-time quantitative polymerase chain reaction assay. Arch Pathol Lab Med. 2006 Apr;130(4):465-73. (10) Erlander et al. Performance and clinical evaluation of the 92-gene real-time PCR assay for tumor classification. J Mol Diagn. 2011 Sep;13(5):493-503.	

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			(11) Greco et al. Molecular profiling in unknown primary cancer: accuracy of tissue of origin prediction. Oncologist. 2010;15(5):500-6. (12) Hainsworth et al. A retrospective study of treatment outcomes in patients with carcinoma of unknown primary site and a colorectal cancer molecular profile. Clin Colorectal Cancer. 2012 Jun;11(2):112-8.	
George Pentheroudakis	Assistant Professor of Oncology, Medical School, University of Ioannina, Greece	Executive Summary	1. The correct name of the test is miRview mets	We apologize for the autocorrection error.
George Pentheroudakis	Assistant Professor of Oncology, Medical School, University of Ioannina, Greece	Executive Summary	2. An essential comment on the true nature of CUP is that there is no certainty that a CUP biologically assigned to a primary tissue of origin behaves similar to a typical metastatic tumor of that primary. In other words, CUP may be defined by a CUP-specific, prometastatic signature in parallel to its primary tissue-specific signature. This makes important the validation of any molecular platform not only to exhibit accuracy in identification of primary in metastases from known primary (as in most validation series) but mostly to correctly assign primaries in CUP cases. We have recently presented in ASCO 2012 our experience with the miRview test, which assigned a primary in 85 CUP cases with 92% accuracy (92% agreement with a final physican diagnosis which was based not only on clinicopathologic data at baseline but also on management and outcome data. The paper has been recently submitted in a peer reviewed journal. ASCO 2012 Meeting (1-5 June 2012, Chicago, US) - Abstract #93860. Microrna-based classification of	This abstract is included in the updated review. The paper was not available by the last search update.

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			metastases of unknown primary shows 92% accuracy. Nicholas Pavlidis, George Pentheroudakis, Brianna St. Cyr, Yael Spector, Eti Meiri, George Fountzilas, Anna Gousia, Hila Benjamin, Vassiliki Malamou-Mitsi, Vassiliki Kotoula, Aikaterini Stoyianni, Dimitrios Krikelis, Mats Olot Sanden, Karin Ashkenazi, Hellenic Cooperative Oncology Group, Athens, Greece; Ioannina University Hospital,, Ioannina, Greece; Hellenic Cooperative Oncology Group , Athens, Greece; Rosetta Genomics Inc., Philadelphia, PA; Rosetta Genomics Ltd., Rehovot, Israel; Hellenic Cooperative Oncology Group, Athens, Greece; Ioannina University Hospital, Ioannina, Greece; Rosetta Genomics Ltd, Rehovot, Israel	
George Pentheroudakis	Assistant Professor of Oncology, Medical School, University of Ioannina, Greece	Executive Summary	3. In view of the availability of targeted therapies that improve survival of patients with metastatic tumors, all of them being administered in a primary tissue of origin-dependent context, assignment of a primary in a CUP patient by means of molecular profiling would make him eligible for effective targeted therapies. This would increase chances for disease control and improvement of patient survival. We agree that no randomised controlled trial showed improvement of survival in CUP patients with the administration of primary-specific modern therapy. However, such a trial would necessitate large sample size as it would involve survival comparisons among several patient subgroups of moderate size. Consequently, it is difficult to take place and if it does, it will probably be underpowered.	No response needed.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	General	Upon review of the Rosetta Genomics product description, summary of findings, quality control measures, clinical data and associated references, it is clear that this technology assessment was performed almost entirely on an early version of our TOO assay called miRview? mets. While a single poster on our currently, commercially available version of the assay, miRview? mets2 (Chajut et al. Development	We have updated the review to note the two versions of the test. We maintained the Chajut abstract and also included the new article and abstract on the mets[2] test. Our

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			and validation of a second generation microRNA-based assay for diagnosing tumor tissue origin. AACR; 2011), is included in the list of references, it only appears to somewhat confuse the technology assessment, and does not add to the otherwise comprehensive review of the earlier miRview? mets assay. In fact, we note that in ?Table A. Overview of study outcomes (page ES-4)?, that the poster does not appear to have been used as a reference to answer either KQ2 or KQ3b. It is also important to understand that the miRview? mets assay was developed on an RT-PCR platform, and was the basis for the development of the current, and more advanced, microarray-based miRview? mets2 assay. It is our opinion that it is really not possible, in the format of the current technical assessment, to appropriately modify the entire document to include a full technical assessment of both the early version miRview? mets assay and the current miRview? mets2 assay, especially as there is an additional publication, an additional poster, and unpublished data on file for the miRview? mets2 assay. For the purposes of clarity to readers, we ask the authors to remove the poster reference (Chajut et al.), make all the necessary parallel changes (which we discuss in our additional comments below), and to acknowledge that this technical assessment was performed on the early version of our TOO assay called miRview? mets, and not our currently, commercially available TOO assay called miRview? mets2. We have performed a detailed analysis of all data relating to the miRview mets assay relating to accuracy, sensitivity, specificity, concordance and TOO prediction in this technical assessment, and we have concluded that the removal of the Chajut reference does not change any of these performance parameters. We therefore ask that these performance parameters remain unchanged, unless specified in the comments below.	review does not include unpublished data.
Mats Sanden	Rosetta	General	We congratulate the authors on their thorough review of	No response needed.

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MD. DDS FCAP	Genomics Inc.		miRview? mets, and we respectfully offer further comments and corrections within.	
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	General	Structured Abstracts: The miRview test is mentioned 3 times in this section (First page, Results: lines 2, 7 and 12) and the correct spelling and name is miRview mets and not miReview.	We apologize for the autocorrection spelling error. We have corrected the spelling and the name of the test.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Executive Summary	Page ES-2 and ES-3, Results: The miRview mets assay is mentioned 2 times in this section (line 2, and 12) and the correct spelling and name is miRview mets and not miReview. The assay is further mentioned 2 times in the Summary of Findings Page ES-3 (line 1 and 3) and misspelled, and named, in both places.	We apologize for the autocorrection spelling error. We have corrected the spelling and the name of the test.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Executive Summary	Page ES-3, Line 15, It is stated: ?There is low evidence that the test alters the treatment course from empiric therapy usually used in CUP to tissue-specific therapy?. Current work-up for CUP generally includes physical examination, imaging studies, blood work, and pathologic assessment including H&E and IHC. None of these activities can generally identify the primary tissue alone, but are rather used together, along with the treating physicians best medical judgment, to determine appropriate therapy for their patients. Molecular TOO tests, including miRview mets, are meant to be adjunctive to standard clinico-pathologic work-up. In clinical studies with CUP patients miRview mets has demonstrated high accuracy (80-84% concordance with reference diagnoses). In cases where the primary site cannot be determined, or there is a differential diagnosis, molecular CUP assays can help the treating physician to reach a final diagnosis, confirm initial suspicion regarding the primary site, or direct additional pathology testing. Thus, molecular CUP assays help to address the clinical unmet needs of diagnosing the primary tissue of origin, or in helping to direct additional diagnostic work-up. Once a	This is a well stated summary of the theory behind the expected benefit of these tests. This review identified and assessed the evidence that these test actually change treatment and the effect patient outcome through these changes. We respectfully decline to add the requested acknowledgments.

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			treating physician is able to reach a final diagnosis of the tissue of origin they can use their medical judgment, supported by substantial literature on oncology drugs and drug indications for use, to select the most appropriate therapy, or to determine if patients may be eligible for approved clinical trials. If the selected therapy is a targeted therapy, it is not unreasonable to expect that at least some patients will experience increased survival - and that the added value of performing additional outcomes studies in these patients is uncertain. We therefore ask the authors to acknowledge (1) that molecular TOO assays do help meet the unmet needs for better tools to diagnose CUP or in helping to direct additional diagnostic work-up and (2) that even though the effect of these assays on decision making needs further study, clinicians should not be discouraged from ordering molecular TOO assays if they believe that the test result will increase the likelihood of administering targeted therapy with demonstrated benefit beyond that of empiric therapy for CUP.	
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Executive Summary	Page ES-3, Line 21, It is stated: ?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.? All three studies referenced in this technical assessment for the miRview? mets test were well designed and IRB approved. Two of these miRview mets studies, both rated good in this technical assessment, are published in scientific journals and were both subject to peer-review by the independent editorial boards of the journals. To suggest that data in these studies may have been tainted in some way implicates all study investigators, including those at some of the worlds top cancer institutions. We do not believe this is appropriate and, for the sake of fair balance, we ask the authors to modify the verbiage cited above Page ES3, Line 21), as follows:	The concern about publication bias is that manufacturers may maintain control of publication, which can result in a failure to publish studies with unfavorable results. Authors that are employed by the test manufacturer may be reluctant to sign off on papers reporting unfavorable results. We respectfully decline to add the requested acknowledgments.

Reviewer Name[1]	Reviewer Affiliation[2]	Section[3]	Reviewer Comments	Author Response[4]
			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. However, we also acknowledge that there is no evidence that there was any publication bias in the available literature.	
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Executive Summary	In addition, with respect to the study by Mueller et al. (page G-16 reference 27), neither the authors at the institution, nor the institution itself, received any funding from Rosetta Genomics. We therefore request that the following paragraph is inserted on page ES-3 just before the section titled ?Future Research? "Furthermore, for the Mueller et al. study27, we acknowledge that neither the Ruprecht-Karls University Heidelberg, nor the authors then employed by the Ruprecht-Karls University Heidelberg, received any funding from Rosetta Genomics."	This may be true regarding the authors at Ruprecht-Karls University Heidelberg. However, this study includes multiple authors employed by and with ownership interests in Rosetta Genomics. . We respectfully decline to add the requested acknowledgment.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Introduction/ Background	Page 6, Line 37, Objectives of the Review, Meta Analysis: The miRview mets assay is mentioned in this section and the correct spelling, and name, is miRview mets and not miReview.	We apologize for the autocorrection spelling error.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Results	Page 16: Tissue of Origin (TOO) Tests for Cancer of unknown Primary site (CUP): Page 16, line 26; Text says: predicts 42 tumor types, which should be ?predicts 25 tumor types. (Reference # 25 Rosenwald S, Gilad S, Benjamin S, et al. Validation of a microRNA-based qRT-PCR test for accurate identification of tumor tissue origin. Mod Pathol. 2010Jun; 23(6):814-23. PMID: 20348879.)	This error has been corrected.
Mats Sanden MD. DDS	Rosetta Genomics Inc.	Results	Page 19, miRview: Line1; Two papers25, 26 should be Two papers24, 25? (according to the list of references on page	We have corrected the citation numbers. We

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FCAP			69-71). The poster (Chajut, 2011) with reference # 27 (which should be reference # 26 Development and validation of a second generation microRNA-based assay for diagnosing tumor tissue origin. AACR; 2011) should be taken out, as discussed in our comments in the General section. Line 6; ?11 years25? should be ?11 years24? (Rosenfeld N, Aharonov R, Meiri E, et al. MicroRNAs accurately identify cancer tissue origin. Nat Biotechnol. 2008 Apr; 26(4):462-9. PMID: 18362881).	apologize for the error.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Results	Page 28, miRview, Normalization, Line 1: The reference number for Rosenfeld et al. is 25 and should be reference # 24; (Rosenfeld N, Aharonov R, Meiri E, et al. MicroRNAs accurately identify cancer tissue origin. Nat Biotechnol. 2008 Apr;26(4):462-9. PMID: 18362881.)	We have corrected the citation numbers. We apologize for the error.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Results	Page 29, miRview, Validation, Line 1: The reference number for Rosenfeld et al. is 25 and should be reference # 24.(Rosenfeld N, Aharonov R, Meiri E, et al MicroRNAs accurately identify cancer tissue origin. Nat Biotechnol. 2008 Apr;26(4):462-9. PMID: 18362881.)	We have corrected the citation numbers. We apologize for the error.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Results	Page 30, MiRview, Accuracy, Line 2: KNN25 should be KNN24. Rosenfeld et al.25 should be Rosenfeld et al.24(Rosenfeld N, Aharonov R, Meiri E, et al . MicroRNAs accurately identify cancer tissue origin. Nat Biotechnol. 2008Apr; 26(4):462-9. PMID: 18362881)	We have corrected the citation numbers. We apologize for the error.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Results	Page 30, Line 4: Rosenwald et al.26 should be ?Rosenwald et al.25? (Rosenwald S, Gilad S, Benjamin S, et al. Validation of a microRNA-based qRT-PCR test for accurate identification of tumor tissue origin. Mod Pathol. 2010 Jun; 23(6):814-23. PMID: 203488759)	We have corrected the citation numbers. We apologize for the error.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Results	Page 30, Line 8: Mueller et al.36 should be Mueller et al.35 (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).	We have corrected the citation numbers. We apologize for the error
Mats Sanden	Rosetta	Results	Page 31: The following text refers to the Pathwork	We have moved this text to

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MD. DDS FCAP	Genomics Inc.		Diagnostics test but is presently under the heading of miRview, Accuracy and should be removed: 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)?.	the appropriate section.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Results	Page 52, line 1: One good study28 should be One good study27? (Varadhachary GR, Spector Y, Abbruzzese JL, et al. Prospective Gene Signature Study Using microRNA to Identify the Tissue of Origin in Patients with Carcinoma of Unknown Primary. Clin Cancer Res. 2011 Jun 15; 17(12): 4063-70. PMID: BIOSIS: PREV201100444558.	We have corrected the citation numbers. We apologize for the error
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Results	Page 52, Line 9: One study36 should be One study35 (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)	We have corrected the citation numbers. We apologize for the error.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Discussion/ Conclusion	Page 63, Summary of evidence, Table 16: Row KQ 3b-3f: MiReview should be correctly spelled and named as miRview mets. The text says Two independent studies should be Three independent studies	We have corrected the spelling. The summary of evidence table has been corrected and updated to include studies identified in the updated search.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Discussion/ Conclusion	Page 65, KQ 3b ?3f: Accuracy of the TOO test in classifying the origin and tissue of the tumor: The paragraph referring to the miRview mets assay (line 6) is confusing as incorrect reference numbers are used. We ask that the paragraph be corrected as follows: Two independent studies24, 25 with over one hundred specimens, and a third study with a smaller sample size35, each tested the ability of the miRview mets assay to identify	We have corrected the citation numbers

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			the tumor origin in tissues of known origin. Based on 2 good studies and one fair study, with 3 very similar estimates of accuracy and 2 of exclusion, and the combined meta-analytic summary estimate, the evidence is high that miRview mets correctly identifies tumor source in known tissue 85% of the time, 95% CI (83%, 88%)."	
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Discussion/ Conclusion	Page 67, Gaps and Issues in the Literature on TOO Tests, Line 6: It is stated: 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. As explained in ?Executive Summary: Summary of Findings?, please modify the language (Page 67, Line 6), as follows: ?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. However, we also acknowledge that there is no evidence that there was any publication bias in the available literature.?	The concern about publication bias is that manufacturers may maintain control of publication, which can result in a failure to publish studies with unfavorable results. Authors that are employed by the test manufacturer may be reluctant to sign off on papers reporting unfavorable results. We respectfully decline to add the requested acknowledgments.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Discussion/ Conclusion	Page 67, Line 23: It is stated: 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. As explained in Executive Summary: Summary of Findings, We ask the authors to acknowledge that molecular TOO assays do help meet the unmet need of better tools to diagnose CUP or in helping to direct additional diagnostic work-up. Specifically, we ask the authors to modify the language (Page 67, Line 23), as follows: ?No information was available on the effect of testing by miRview mets or PathworkDx on health outcomes. The clinical utility of these tests is still uncertain. However, we do	This is a well stated summary of the theory behind the expected benefit of these tests. This review identified and assessed the evidence that these tests actually change treatment and affect patient outcome through these changes. We respectfully decline to add the requested acknowledgments.

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			acknowledge that molecular TOO assays are useful tools that help meet the unmet need for better tools to diagnose CUP or in helping to direct additional diagnostic work-up and, even though the effect of these assays on decision making needs further study, clinicians should not be discouraged from ordering molecular TOO assays if they believe that the test result will increase the likelihood of administering targeted therapy with demonstrated benefit beyond that of empiric therapy for CUP.?	
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page ES-4: In Table A. Overview of study outcomes; the miRview mets assay is misspelled, and named, for KQ2, KQ3a and KQ3b. KQ 3b-3f, miRview: Text says Two independent studies, which should be ?Three independent studies	We have corrected
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page 17, Table 5: Row; miRview, column; Number of tumor types: States 22 and should be 25 (Reference # 25 Rosenwald S, Gilad S, Benjamin S, et al. Validation of a microRNA-based qRT-PCR test for accurate identification of tumor tissue origin. Mod Pathol. 2010Jun; 23(6):814-23. PMID: 20348879)	We have corrected the spelling and the name. The summary of evidence table has been corrected and updated to include studies identified in the updated search.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page 18, Table 6: The following tumor types are missing from the miRview column; Cholangiocarcinoma (included in Biliary), Endometrium, Esophagus, Gastric, Hepatocellular. Please divide the generic diagnosis of Neuroendocrine into the following specific diagnoses: Lung Carcinoid, Lung Small Cell, and Thyroid Medullary Carcinoma.	Table 6 was updated based on the paper by Meiri et al.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page 21, Table 7; the poster by Chajut et al. 2011 indicates the number of samples was 179 instead of the correct number of 509. However, this poster should be removed from this assessment, as discussed in our comments in General section.	We have corrected the sample size for the Chajut abstract. As noted above, we include studies using MiRview mets and MiRview mets^2 in the review.
Mats Sanden	Rosetta	Tables	The following reference should be included in Table 7:	Rosenwald 2010 has been

Reviewer Name[1]	Reviewer Affiliation[2]	Section[3]	Reviewer Comments	Author Response[4]
MD. DDS FCAP	Genomics Inc.		Rosenwald 2010 (reference # 25; Rosenwald S, Gilad S, Benjamin S, et al. Validation of a microRNA-based qRT-PCR test for accurate identification of tumor tissue origin. Mod Pathol. 2010Jun; 23(6):814-23. PMID: 20348879) and Mueller 2011 (reference # 35; 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)	added to table 7 (it had been inadvertently deleted). The Mueller article does not add any information on analytic validity that is not already represented in the table.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page 22, Table 7; the reference number for the Varadhachary 2011 study is reference # 28 and should be reference # 27 (Varadhachary GR, Spector Y, Abbruzzese JL, et al. Prospective Gene Signature Study Using microRNA to Identify the Tissue of Origin in Patients with Carcinoma of Unknown Primary. Clin Cancer Res. 2011 Jun 15; 17(12):4063-70. PMID: BIOSIS: PREV201100444558).	The citation numbers have been corrected.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page 26, Table 8; The reference number for the first study (Rosenfeld 2008) is reference # 25 and should be reference # 24 (Rosenfeld N, Aharonov R, Meiri E, et al. MicroRNAs accurately identify cancer tissue origin. Nat Biotechnol. 2008 Apr; 26(4):462-9. PMID: 18362881) and the reference number for the second study (Rosenwald 2010) is reference #26 and should be reference # 25 (Rosenwald S, Gilad S, Benjamin S, et al. Validation of a microRNA-based qRT-PCR test for accurate identification of tumor tissue origin. Mod Pathol. 2010 Jun;23(6):814-23. PMID: 203488759). In addition, Internal validation for Rosenwald 2010 says NR but should be; Internal: Leave one-out cross validation within the training set?	The citation numbers have been corrected. The internal validation has been corrected
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page 33, Table 9: the first study (poster) by Chajut et al. 2011 should be taken out, as discussed in our comments in the General section.	As noted above, we include studies using MiRview mets and MiRview mets^2 in the review.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page 34. Table 9: The reference number for the first study (Rosenfeld 2008) is 25 and should be reference # 24 (Rosenfeld N, Aharonov R, Meiri E, et al. MicroRNAs	The citation numbers have been corrected.

Reviewer Name[1]	Reviewer Affiliation[2]	Section[3]	Reviewer Comments	Author Response[4]
			accurately identify cancer tissue origin. Nat Biotechnol. 2008Apr; 26(4):462-9. PMID: 18362881) and the reference number for the second study (Rosenwald 2010) is 26 and should be reference # 25 (Rosenwald S, Gilad S, Benjamin S, et al. Validation of a microRNA-based qRT-PCR test for accurate identification of tumor tissue origin. Mod Pathol. 2010 Jun; 23(6):814-23. PMID: 203488759)	
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page 43, Table 11: For the Rosenfeld 2008 study the reference number should be reference # 24 (Rosenfeld N, Aharonov R, Meiri E, et al . MicroRNAs accurately identify cancer tissue origin. Nat Biotechnol. 2008Apr; 26(4):462-9. PMID: 18362881)	The citation numbers have been corrected.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page 43, Table 11: Only the results from the miRview mets decision tree are presented for sensitivity and specificity, however in the article; Rosenfeld N, Aharonov R, Meiri E, et al. MicroRNAs accurately identify cancer tissue origin. Nat Biotechnol. 2008Apr; 26(4): 462-9. PMID: 18362881, in table 3 on page 467 there are also unified results from both the decision tree and the KNN classifier presented under the heading of ?Union?. We respectfully ask the authors to present the results of the miRview mets assay as ?Union sensitivity? of the decision tree and the KNN, as specified in the table below, instead of as currently presented: Sample Size (Decision Tree and KNN) Union Specificity Union Sensitivity 1 Bladder 1. 2 1. 0% 1. 100% 2 Brain 2. 5 2. 100% 2. 100% 3 Breast 3. 5 3. 60% 3. 97% 4 Colon 4. 5 4. 60% 4. 99% 5 Endometrium 5. 3 5. 67% 5. 99% 6 Head and Neck 6. 8 6. 100% 6. 99% 7 Kidney 7. 5 7. 100% 7. 99% 8 Liver 8. 2 8. 100% 8. 99%	Using the union of two results overestimates the sensitivity, since it allows two chances to be correct. The effect on specificity depends on how the union is calculated. We included only one classifier so results would be comparable to the other tests. We chose the decision tree algorithm because it is an unsupervised algorithm.

Reviewer Name[1]	Reviewer Affiliation[2]	Section[3]	Reviewer Comments	Author Response[4]
			9 Lung 9. 5 9. 100% 9. 99% 10 Lung 10. 2 10. 50% 10 .99% 11 Lung Pleura 11. 5 11. 80% 11. 100% 12 Lymph-node 12. 5 12. 80% 12. 97% 13 Melanocytes 13. 3 13. 100% 13. 99% 14 Meninges 14. 4 14. 100% 14. 97% 15 Ovary 15. 2 15. 100% 15. 100% 16 Prostate 16. 2 16. 100% 16. 100% 17 Sarcoma 17. 5 17. 80% 17. 99% 18 Stomach 18. 7 18. 86% 18. 96% 19 Stromal (GIST) 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%	
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page 44, Table 11: The reference number for the Rosenwald 2010 study is 26 and should be reference # 25 (Rosenwald S, Gilad S, Benjamin S, et al. Validation of a microRNA-based qRT-PCR test for accurate identification of tumor tissue origin. Mod Pathol. 2010 Jun; 23(6):814-23. PMID: 203488759) Page C-4, Table 1.	The citation numbers have been corrected.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	The Mueller et al. 2011 study (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) should be included in this table.	This article is included; the table heading was incorrect. We have corrected the table heading and flow.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page C-6-7, the first study by Chajut et al. 2011 should be taken out, as discussed in our comments in General section.	As noted above, we include studies using MiRview mets and MiRview mets^2 in the review.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page C-7: The sample size in the Rosenfeld 2008 study was 83 and not 80 as shown here.	This has been corrected.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page C-11, Table 2: the study by Chajut et al. 2011 should be taken out, as discussed in our comments in the General section.	As noted above, we include studies using MiRview mets and MiRview mets^2 in the

Reviewer Name[1]	Reviewer Affiliation[2]	Section[3]	Reviewer Comments	Author Response[4]
				review.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page C-14, Table 2.: Rosenfeld 2008, under the column ?External? it says; ?Blinded test set? it should say ?Blinded test set of 83 samples was used?	This correction has been made.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page C-15, Table 4: the study by Chajut et al. 2011 should be taken out, as discussed in our comments in the General section	As noted above, we include studies using MiRview mets and MiRview mets^2 in the review.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	In addition, the following study should be included in this table: Varadhachary et al. 2011 study (reference # 27 Varadhachary GR, Spector Y, Abbruzzese JL, et al. Prospective Gene Signature Study Using microRNA to identify the Tissue of Origin in Patients with Carcinoma of Unknown Primary. Clin Cancer Res. 2011 Jun 15; 17(12):4063-70. PMID: BIOSIS: PREV201100444558) rated good in Table 6, page 49.	This study included CUPS cases and is included in KQ4.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page 17, Table 5: Row; miRview, column; Number of tumor types: States reference is 22 and should be 25 (Reference # 25 Rosenwald S, Gilad S, Benjamin S, et al. Validation of a microRNA-based qRT-PCR test for accurate identification of tumor tissue origin. Mod Pathol. 2010Jun; 23(6):814-23. PMID: 20348879)	The citation numbers have been corrected.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page 17, Table 5: Row; miRview, column; Number of tumor references: is presently stated as 336 (1-49) and should be 1282 (5-140)	It is unclear where in the table this comment references, as none of the columns in table 5 are named number of tumor headings, and none of the miRview rows in this table have a sample size of 336.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Tables	Page F-2, Table 3: states miRview has 2 studies. It should be 3 studies (Rosenfeld 2008 (Rosenfeld N, Aharonov R, Meiri E, et al. MicroRNAs accurately identify cancer tissue origin. Nat Biotechnol. 2008 Apr; 26(4): 462-9. PMID: 18362881), Rosenwald 2010 (Rosenwald S, Gilad S, Benjamin S, et al.	This correction has been made.

Reviewer Name[1]	Reviewer Affiliation[2]	Section[3]	Reviewer Comments	Author Response[4]
			Validation of a microRNA-based qRT-PCR test for accurate identification of tumor tissue origin. Mod Pathol. 2010 Jun; 23(6): 814-23. PMID: 203488759) and Mueller 2011; 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).	
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Figures	Page 39, Fig 5: the results from the study by Chajut 2011 should be taken out, as discussed in our comments in the General section.	As noted above, we include studies using MiRview mets and MiRview mets^2 in the review.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Appendices	Page D1, Appendix D: Chajut (2011) should be taken out, as discussed in our comments in the General section.	As noted above, we include studies using MiRview mets and MiRview mets^2 in the review.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Appendices	Mueller (2011) has reference # 36 should be reference # 40; 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.	The citation numbers have been corrected.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	Appendices	Varadhachary (2011) has reference # 28 should be #30; Varadhachary GR, Spector Y, Abbruzzese JL, et al. Prospective Gene Signature Study Using microRNA to Identify the Tissue of Origin in Patients with Carcinoma of Unknown Primary. Clin Cancer Res. 2011 Jun 15; 17(12):4063-70. PMID: BIOSIS: PREV201100444558.	The citation numbers have been corrected.
Mats Sanden MD. DDS FCAP	Rosetta Genomics Inc.	References	Page G-15: Please remove the Chajut reference, #8, as discussed in our comments in the general section. Also please note that the author?s name, Chajut, was missing from this reference. Normally, removing a reference would require re-numbering all the subsequent references, however such renumbering could potentially introduce errors so we respectfully suggest that you keep #8 in the reference list and call it: ?No reference cited for this number?.	As noted above, we include studies using MiRview mets and MiRview mets^2 in the review.
Lawrence Weiss, MD	Clarient Pathology	General	Disclaimer: I am a diagnostic pathologist with extensive experience in immunohistochmistry who has received funding	We updated the search during the peer review

Reviewer Name[1]	Reviewer Affiliation[2]	Section[3]	Reviewer Comments	Author Response[4]
	Services, Inc.		from both Biotheranostics and Pathworks. I do not disagree with with the assessment, which speaks to the state of the art at the time of the analysis. I will only point out that I am aware of a number of recently submitted manuscripts that may potentially add depth to the discussion. I would urge some delay in publishing the final assessment to attempt to incorporate the findings in these studies. As noted in the draft assessment, the field is not yet mature.	process and have all identified studies published or e-published prior to 11/7/2012.

[1] Names are alphabetized by last name. Those who did not disclose name are labeled "Anonymous Reviewer 1," "Anonymous Reviewer 2," etc.
[2] Affiliation is labeled "NA" for those who did not disclose affiliation.
[3] If listed, page number, line number, or section refers to the draft report.
[4] If listed, page number, line number, or section refers to the final report.