



**Bibliography of Journal Articles,
Poster Sessions, and
Presentations Related to the
Pathwork® Tissue of Origin Test**

Feb 10, 2012

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■ PEER REVIEWED PUBLICATIONS ■

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

BACKGROUND: Gene expression–based assays have been introduced into the clinical arena to assist in the diagnosis of poorly differentiated or undifferentiated tumors. The US Food and Drug Administration has cleared the microarray-based Pathwork Tissue of Origin (TOO) Test (Pathwork Diagnostics, Sunnyvale, CA) for the molecular characterization of such challenging specimens.

OBJECTIVE: We aimed at verifying the analytic and clinical performance of this test on 43 poorly differentiated and undifferentiated tumor samples, including 6 off-panel cases and 7 cancers of unknown primary (CUP).

RESULTS: Our results showed 97% (95% confidence interval, 80.4%-99.8%) agreement between the Pathwork TOO Test result and the complete diagnosis, which included clinical correlations and immunohistochemical staining, after the original diagnosis.

CONCLUSIONS: We concluded that for off-panel and CUP samples, the tissue type and the cell type may be confounded by the Pathwork TOO Test and that careful clinicopathologic assessment is needed when interpreting results from this helpful ancillary tool for pathologists.

POTENTIAL CLINICAL UTILITY OF GENE-EXPRESSION PROFILING IN IDENTIFYING TUMORS OF UNCERTAIN ORIGIN

M Laouri, M Halks-Miller, WE Henner, JS Nystrom. Personalized Medicine, (2011) 8 (6), 615-622

AIM: To evaluate potential impact of a gene-expression based test on diagnosis of primary tumors in difficult-to-diagnose cases.

MATERIALS AND METHODS: The Tissue of Origin Test uses 2000 gene measurement classify the most likely primary tumor. We categorized 284 consecutive samples by pretest diagnosis, then recategorized using test results to identify cases with changes in diagnosis.

RESULTS: A total of 64% of incoming diagnoses were nonspecific. A leading diagnosis for primary site was provided for remaining cases, indicating an unresolved differential. Overall the test predicted a change in most likely primary site, either a change from nonspecific to specific site or a change from one specific primary site to another in 81% of the cases and confirmed the suspected primary site for 15% of the cases.

CONCLUSION: A new molecular diagnostic has the potential to change both primary site identification and therapy selection for the majority of patients tested.

GENE-EXPRESSION ASSAYS AND PERSONALIZED CANCER CARE: TISSUE-OF-ORIGIN TEST FOR CANCER OF UNKNOWN PRIMARY ORIGIN

H Takei, FA Monzon. Personalized Medicine (2011) 8(4), 429–436

EXECUTIVE SUMMARY:

Cancer of unknown primary origin: evolving understanding of the disease

- Cancer of unknown primary (CUP) origin is not considered to be a single entity, but rather a clinical syndrome representing a challenging heterogeneous collection of malignancies that share a unique clinical behavior.
- Recently, several favorable subsets of CUP (approximately 20% of all patients) that respond to systemic platinum-based chemotherapy or managed by locoregional treatment have been recognized.
- Categorizing CUP patients into favorable and unfavorable groups based mainly on clinical information is important for the current treatment guidelines. This classification bases therapies on the most likely tissue of origin for a metastatic tumor based on the clinical presentation.

CUP origin diagnosis: current common laboratory medicine approach

- A detailed pathologic examination is crucial for CUP cases and typically consists of routine histologic examination and immunohistochemistry (IHC) analysis.
- IHC marker stains against commonly used cytokeratin subtypes (e.g., cytokeratin 7 and 20) and relatively specific tumor antigens are of great use in defining the tumor lineage as well as predicting the primary site of tumor.
- A meta-analysis study revealed IHC investigation provided the correct primary sites of metastatic tumors in 65.6% of cases.

Molecular assays to predict the site of tissue of origin

- Gene-expression assays to identify the primary site and classify histologic subtypes in CUP patients have been recently developed.
- Three different molecular gene-expression assays are currently commercially available for tissue of origin determination using formalin-fixed paraffin-embedded tissue in the USA.
- The CancerTYPE ID® test measures 92 mRNA transcripts and is reported to distinguish among 30 tumor types and 54 histological subtypes.
- The Pathwork Tissue of Origin® test is an US FDA-cleared microarray-based assay that measure ~2000 mRNA transcripts and is reported to distinguish 15 known characterized tissue types.
- The miRview® mets test measures the expression level of 48 miRNA by quantitative reverse-transcriptase-PCR and is reported to classify 25 different tumor types, corresponding to 17 distinct tissues and organs.
- Overall accuracy of these assays in identifying the source of poorly differentiated lesions from known primary cancers has been reported to be 75–89%, although validation data for some of these tests is not publicly available.

CUP: evolving management/therapy

- The current recommended treatment of most patients with CUP is empiric, broad-spectrum combination chemotherapy, and the first-line regimens produce overall

response rates of 30–40% with median survival of 7–11 months.

- The growing use of new cytotoxic regimens and targeted agents that are known to be efficacious in anatomically defined tumors could bring substantial benefits to some of CUP patients if the primary site was accurately identified.

Future perspective of personalized cancer care

- The premise of the value of molecular tests for tissue of origin, is that an appropriate use of gene-expression tests may eliminate unnecessary diagnostic tests for CUP patients and allow therapeutic selection that is tailored to the ‘genomic taxonomy’ of the tumors.
- It is possible that the treatment of CUP patients tailored to the molecular signatures will have a significant positive impact on outcomes.

IDENTIFICATION OF TISSUE OF ORIGIN IN BODY FLUID SPECIMENS WITH A GENE EXPRESSION MICROARRAY ASSAY

GA Stancel, D Coffey, K Alvarez, M Halks-Miller, A Lal, D Mody, T Koen, T Fairley, FA Monzon. Cancer Cytopathology, Jun 29, 2011

BACKGROUND: Body fluid specimens may be the first and only pathologic specimen for clinical evaluation in metastatic cancer cases. The challenge of identifying the tissue of origin in metastatic cancer has led to the emergence of molecular-based assays, such as the microarray-based Pathwork Tissue of Origin gene expression test. The ability to use body fluid specimens in this test would be valuable in providing diagnoses to cancer patients without clearly identifiable primary sites. In this study, we evaluated the Tissue of Origin Test for use with malignant effusion specimens.

METHODS: Twenty-seven metastasis-positive body fluid specimens from different body sites, including pleural, ascites, pericardial, and pelvic wash fluids, were obtained from patients with known diagnoses. Nine specimens from non-malignant body fluids were included as controls. RNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue and gene expression analysis was performed with the Tissue of Origin Test.

RESULTS: Seventeen of 27 metastasis-positive samples were non-necrotic with $\geq 60\%$ tumor and yielded sufficient RNA. Of these samples, 94.1% (16/17) were in agreement with the available diagnosis. Of the 9 negative control samples evaluated, 7 (77.7%) showed microarray expression profiles most similar to lymphoma, which is consistent with the predominance of inflammatory cells in these specimens.

CONCLUSIONS: Our findings show that FFPE cell blocks from cytologic body fluid specimens yield adequate diagnostic material for the Pathwork test and can be used in the workup of patients with unknown primary tumors.

MOLECULAR TUMOR PROFILING IN THE DIAGNOSIS OF PATIENTS WITH CARCINOMA OF UNKNOWN PRIMARY SITE: RETROSPECTIVE EVALUATION OF GENE MICROARRAY ASSAY

JD Hainsworth, R Pillai, WD Henner, M Halks-Miller, C Lane, FA Greco. J Mol Biomark Diagn 2011, 2:2

BACKGROUND: Molecular tumor profiling has potential importance in identifying the tissue of origin in patients with cancer of unknown primary (CUP). We retrospectively performed the Tissue of Origin test, an FDA-cleared commercially available gene microarray assay, on biopsy specimens from patients with CUP. Assay results were correlated with clinical and pathologic features, and with previous results using the Veridex 10-gene CUP assay, a molecular RT-PCR assay designed to detect 6 primary sites.

METHODS: Archival formalin-fixed, paraffin-embedded biopsy specimens from 48 patients with CUP were tested. The assay results were reported without incorporation of clinicopathologic information except biopsy site and patient gender. The assay results were correlated with clinicopathologic information, treatment results, and with results of the previously performed Veridex assay.

RESULTS: The Tissue of Origin test was successfully performed in 45 tumor specimens. In 43 of 45 assays (96%), a specific tissue of origin was predicted. The most commonly identified tissues of origin included: lung (11), pancreas (6), sarcoma (6), ovary (5), and colon (4). Most diagnoses were compatible with the clinical features, IHC staining, and response to treatment. The finding of 6 sarcomas was unusual in this patient population and was suggested by routine pathology in only 1 patient. The Tissue of Origin test provided predictions in a higher percentage of patients than did the Veridex CUP assay (96% versus 53%). However, concordance between assay results was relatively low.

CONCLUSIONS: The Tissue of Origin test provided predictions of the primary site in 96% of patients with CUP. Predictions were generally consistent with clinicopathologic features. Agreement between the Tissue of Origin test and the Veridex CUP assay was relatively low, possibly related to the limited number of genes assessed by the Veridex CUP assay. Additional trials are necessary to confirm the value of these assays in patient management.

GENE EXPRESSION PROFILING FROM FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUE FOR TUMOR DIAGNOSIS

JP Grenert, A Smith, W Ruan, R Pillai, AH Wu. Clinica Chimica Acta 412 (2011) 1462–1464

BACKGROUND: Molecular profiling assays have emerged as a promising tool in tumor diagnosis. Recent advances have allowed the use of formalin-fixed, paraffin-embedded (FFPE) tissues in such assays involving the use of microarrays. The Pathwork Tissue of Origin (TOO) Test was developed for use with FFPE tissue to aid tumor diagnosis. We

sought to determine the performance of the TOO test on routine specimens from an independent laboratory.

METHODS: Forty-five blinded, archived clinical specimens from the UCSF Department of Pathology were tested. Total RNA was processed to prepare labeled cDNA for hybridization to Pathchip microarrays. Hybridization data was analyzed with a 2000-gene classification model to quantify similarity between RNA expression of the study specimens and the 15 tissues on the test panel.

RESULTS: 44/45 (98%) specimens were successfully processed. 37 cases met study inclusion criteria. Of these, 35 (95%) gave results which were in agreement with the reference diagnosis. In no case was the reference diagnosis ruled out.

CONCLUSIONS: The Tissue of Origin Test gave a high agreement with the reference diagnosis when archived clinical specimens from UCSF were assessed. Molecular profiling assays are highly accurate, and can be a useful tool in cancer diagnosis.

UNIQUE PATTERNS OF METASTASES IN COMMON AND RARE TYPES OF MALIGNANCY

S Leong, E Nakakura, R Pollock, M Choti, D Morton, WD Henner, A Lal, R Pillai, O Clark, B Cady. J Surg Oncol 2011; 103:607--614

This review on the unique patterns of metastases by common and rare types of cancer addresses regional lymphatic metastases but also demonstrates general principles by consideration of vital organ metastases. These general features of successfully treated metastases are relationships to basic biological behavior as illustrated by disease-free interval, organ-specific behavior, oligo-metastatic presentation, genetic control of the metastatic pattern, careful selection of patients for surgical resection, and the necessity of complete resection of the few patients eligible for long-term survival because lymph node metastases themselves do not destroy a vital organ function, and therefore have no causal relationship to overall survival. When a cancer cell spreads to a regional lymph node, does it also simultaneously spread to the systemic site or sites? Alternatively, does the cancer spread to the regional lymph node first and then it subsequently spreads to the distant site(s) after an incubation period of growth in the lymph node? Of course, if the cancer is in its incubation stage in the lymph node, then removal of the lymph node in the majority of cases with cancer cells may be curative. The data from the sentinel lymph node era, particularly in melanoma and breast cancer, is consistent with the spectrum theory of cancer progression to the sentinel lymph node in the majority of cases prior to distant metastasis. Perhaps, different subsets of cancer may be better defined with relevant biomarkers so that mechanisms of metastasis can be more accurately defined on a molecular and genomic level.

VALIDATION AND REPRODUCIBILITY OF A MICROARRAY-BASED GENE EXPRESSION TEST FOR IDENTIFYING THE PRIMARY SITE OF TUMORS IN FORMALIN-FIXED PARAFFIN-EMBEDDED SPECIMENS

R Pillai, R Deeter, CT Rigl, JS Nystrom, M Halks Miller, L Buturovic, WD Henner. J Molec Diagn, 13 (2011) pp. 48-56

Tumors whose primary site is challenging to diagnose represent a considerable proportion of new cancer cases. We present validation study results for a gene expression-based diagnostic test (the Pathwork Tissue of Origin Test) that aids in determining the tissue of origin using formalin-fixed, paraffin-embedded (FFPE) specimens. Microarray data files were generated for 462 metastatic, poorly differentiated, or undifferentiated FFPE tumor specimens, all of which had a reference diagnosis. The reference diagnoses were masked, and the microarray data files were analyzed using a 2000-gene classification model. The algorithm quantifies the similarity between RNA expression patterns of the study specimens and the 15 tissues on the test panel. Among the 462 specimens, overall agreement with the reference diagnosis was 89% (95% CI, 85% to 91%). In addition to the positive test results (ie, rule-ins), an average of 12 tissues for each specimen could be ruled out with >99% probability. The large size of this study increases confidence in the test results. A multisite reproducibility study showed 89.3% concordance between laboratories. The Tissue of Origin Test makes the benefits of microarray-based gene expression tests for tumor diagnosis available for use with the most common type of histology specimen (ie, FFPE).

GENE EXPRESSION PROFILES HELP IDENTIFY THE TISSUE OF ORIGIN FOR METASTATIC BRAIN CANCERS

A Wu, J Drees, H Wang, S VandenBerg, A Lal, WD Henner, R Pillai. Diagn Pathol. 2010, 5:26

BACKGROUND: Metastatic brain cancers are the most common intracranial tumor and occur in about 15% of all cancer patients. In up to 10% of these patients, the primary tumor tissue remains unknown, even after a time consuming and costly workup. The Pathwork® Tissue of Origin Test (Pathwork Diagnostics, Redwood City, CA, USA) is a gene expression test to aid in the diagnosis of metastatic, poorly differentiated and undifferentiated tumors. It measures the expression pattern of 1,550 genes in these tumors and compares it to the expression pattern of a panel of 15 known tumor types. The purpose of this study was to evaluate the performance of the Tissue of Origin Test in the diagnosis of primary sites for metastatic brain cancer patients.

METHODS: Fifteen fresh-frozen metastatic brain tumor specimens of known origins met specimen requirements. These specimens were entered into the study and processed using the Tissue of Origin Test. Results were compared to the known primary site and the agreement between the two results was assessed.

RESULTS: Fourteen of the fifteen specimens produced microarray data files that passed all quality metrics. One originated from a tissue type that was off-panel. Among the remaining 13 cases, the Tissue of Origin Test accurately predicted the available diagnosis in 12/13 (92.3%) cases.

DISCUSSION: This study demonstrates the accuracy of the Tissue of Origin Test when applied to predict the tissue of origin of metastatic brain tumors. This test could be a very useful tool for pathologists as they classify metastatic brain cancers.

DIAGNOSIS OF METASTATIC NEOPLASMS: MOLECULAR APPROACHES FOR IDENTIFICATION OF TISSUE OF ORIGIN

FA Monzon, TJ Koen. Arch Pathol Lab Med 2010 Feb; 134 (2):216-24

CONTEXT: Tumors of uncertain or unknown origin are estimated to constitute 3% to 5% of all metastatic cancer cases. Patients with these types of tumors show worse outcomes when compared to patients in which a primary tumor is identified. New molecular tests that identify molecular signatures of a tissue of origin have become available.

OBJECTIVE: To review the literature on existing molecular approaches to the diagnosis of metastatic tumors of uncertain origin and discuss the current status and future developments in this area.

DATA SOURCES: Published peer-reviewed literature, available information from medical organizations (National Comprehensive Cancer Network), and other publicly available information from tissue-of-origin test providers and/or manufacturers.

CONCLUSIONS: Molecular tests for tissue-of-origin determination in metastatic tumors are available and have the potential to significantly impact patient management. However, available validation data indicate that not all tests have shown adequate performance characteristics for clinical use. Pathologists and oncologists should carefully evaluate claims for accuracy and clinical utility for tissue-of-origin tests before using test results in patient management. The personalized medicine revolution includes the use of molecular tools for identification/confirmation of the site of origin for metastatic tumors, and in the future, this strategy might also be used to determine specific therapeutic approaches.

IDENTIFICATION OF TISSUE OF ORIGIN IN CARCINOMA OF UNKNOWN PRIMARY WITH A MICROARRAY-BASED GENE EXPRESSION TEST

FA Monzon, F Medeiros, M Lyons-Weiler, WD Henner. Diagn Pathol. 2010, 5:3

BACKGROUND: Carcinomas of unknown primary (CUP) represent approximately 3%-5% of malignant neoplasms. Identifying the tissue of origin (TOO) in these tumors allows for more specific treatment and improves outcomes. However, primary classification remains a challenge in many cases. We evaluated the ability of a microarray-based gene expression test to identify the TOO in tumor specimens from 21 patients with a diagnosis of CUP. Methods:

The Pathwork® TOO Test was used to measure gene expression patterns for 1550 genes; these were compared for similarity to patterns from 15 known tissue types. Results: The TOO Test yielded a clear single positive call for the primary site in 16 of 21 (76%) specimens and was indeterminate in 5 (24%). The positive results were consistent with clinicopathologic suggestions in 10 of the 16 cases (62%). In the remaining six cases the positive results were considered plausible based on clinical information. Positive calls included colorectal (5), breast (4), ovarian (3), lung (2), and pancreas (2). The TOO Test ruled out an average of 11 primary tissues in each CUP specimen.

CONCLUSION: The Pathwork TOO Test reduced diagnostic uncertainty in all CUP cases and could be a valuable addition or alternative to current diagnostic methods for classifying uncertain primary cancers.

DIAGNOSIS OF UNCERTAIN PRIMARY TUMORS WITH THE PATHWORK® TISSUE OF ORIGIN TEST

FA Monzon, CI Dumur. Expert Review of Molecular Diagnostics, January 2010, Vol. 10, No. 1, Pages 17-25

Clinical workup of metastatic malignancies of unknown origin is an arduous and expensive process, which is reported to be unsuccessful in up to 30% of cases. Global gene expression-based molecular testing may offer accurate classification of metastatic tumors in which a primary site has not been identified. Recently, the US FDA cleared the Pathwork® Tissue of Origin test, which is a gene expression microarray-based test that quantifies the molecular similarity of tumor specimens to 15 known tissue types. A blinded, multicenter validation on poorly differentiated and undifferentiated tumors showed 87.8% sensitivity and 99.4% specificity in frozen tissue samples. The availability of ancillary gene expression-based molecular tests for tissue of origin determination represents a milestone in cancer patient management as part of the personalized medicine revolution.

DETERMINING TISSUE OF ORIGIN FOR METASTATIC CANCERS; META-ANALYSIS AND LITERATURE REVIEW OF IMMUNOHISTOCHEMISTRY PERFORMANCE

GG Anderson, L Weiss. Applied Immunohistochemistry & Molecular Morphology: January 2010 – Volume 18 – Issue 1 – pp 3-8

BACKGROUND: Pathologists use various panels of immunohistochemical (IHC) stains to identify the site of tissue of origin for metastatic tumors, particularly poorly or undifferentiated cancers of unknown or uncertain origin. Although clinicians believe that immunostains contribute greatly to determining the probable primary site among 3 or more possibilities, objective evidence has not been convincingly presented. This meta-analysis reviews the objective evidence supporting this practice and summarizes the performance

reported in 5 studies published between 1993 and 2007. Methods: A literature search was conducted to identify IHC performance studies published since 1990 that were masked, included more than 3 tissues types, and used more than 50 specimens. The 5 studies found in this search were separated into 2 subgroups for analysis: those, which included only metastatic tumors (n=368 specimens) and the blended studies, which combined primary tumors and metastases (n=289 specimens). Results: The meta-analysis found that IHCs provided the correct tissue identification for 82.3% (95% confidence interval=77.4%-86.3%) of the blended primary and metastatic samples and 65.6% (95% confidence interval=60.1%-70.7%) of metastatic cancers. This difference is both clinically and statistically significant. CONCLUSIONS: This literature review confirms that there is still an unmet medical need in identification of the primary site of metastatic tumors. It establishes minimum performance requirements for any new diagnostic test intended to aid the pathologist and oncologist in tissue of origin determination.

MULTICENTER VALIDATION OF A 1,550-GENE EXPRESSION PROFILE FOR IDENTIFICATION OF TUMOR TISSUE OF ORIGIN

FA Monzon, M Lyons-Weiler, LJ Buturovic, CT Rigl, WD Henner, C Sciulli, CI Dumur, F Medeiros, GG Anderson. J Clin Oncol. 2009;27:1-13

PURPOSE: Malignancies found in unexpected locations or with poorly differentiated morphologies can pose a significant challenge for tissue of origin determination. Current histologic and imaging techniques fail to yield definitive identification of the tissue of origin in a significant number of cases. The aim of this study was to validate a predefined 1,550-gene expression profile for this purpose.

METHODS: Four institutions processed 547 frozen specimens representing 15 tissues of origin using oligonucleotide microarrays. Half of the specimens were metastatic tumors, with the remainder being poorly differentiated and undifferentiated primary cancers chosen to resemble those that present as a clinical challenge. Results: In this blinded multicenter validation study the 1,500-gene expression profile was highly informative in tissue determination. The study found overall sensitivity (positive percent agreement with reference diagnosis) of 87.8% (95% CI, 84.7% to 90.4%) and overall specificity (negative percent agreement with reference diagnosis) of 99.4% (95% CI, 98.3% to 99.9%). Performance within the subgroup of metastatic tumors (n = 258) was found to be slightly lower than that of the poorly differentiated and undifferentiated primary tumor subgroup, 84.5% and 90.7%, respectively ($P = .04$). Differences between individual laboratories were not statistically significant. Conclusion: This study represents the first adequately sized, multicenter validation of a gene-expression profile for tissue origin determination restricted to poorly differentiated and undifferentiated primary cancers and metastatic tumors. These results indicate that this profile should be a valuable addition or alternative to currently available diagnostic methods for the evaluation of uncertain primary cancers.

INTERLABORATORY PERFORMANCE OF A MICROARRAY-BASED GENE EXPRESSION TEST TO DETERMINE TISSUE OF ORIGIN IN POORLY DIFFERENTIATED AND UNDIFFERENTIATED CANCERS

CI Dumur, M Lyons-Weiler, C Sciulli, CT Garrett, I Schrijver, TK Holley, J Rodriguez-Paris, JR Pollack, JL Zehnder, M Price, JM Hagenkord, CT Rigl, LJ Buturovic, GG Anderson and FA Monzon. J Molec Diagn. 2008, Vol. 10, No. 1

Clinical workup of metastatic malignancies of unknown origin is often arduous and expensive and is reported to be unsuccessful in 30 to 60% of cases. Accurate classification of uncertain primary cancers may improve with microarray-based gene expression testing. We evaluated the analytical performance characteristics of the Pathwork tissue of origin test, which uses expression signals from 1668 probe sets in a gene expression **microarray**, to quantify the similarity of tumor specimens to 15 known tissues of origin. Sixty archived tissue specimens from poorly and undifferentiated tumors (metastatic and primary) were analyzed at four laboratories representing a wide range of preanalytical conditions (eg, personnel, reagents, instrumentation, and protocols). Cross-laboratory comparisons showed highly reproducible results between laboratories, with correlation coefficients between 0.95 to 0.97 for measurements of similarity scores, and an average 93.8% overall concordance between laboratories in terms of final tissue calls. Bland-Altman plots (mean coefficients of reproducibility of 32.48 ± 3.97) and κ statistics ($\kappa > 0.86$) also indicated a high level of agreement between laboratories. We conclude that the Pathwork tissue of origin test is a robust assay that produces consistent results in diverse laboratory conditions reflecting the preanalytical variations found in the everyday clinical practice of molecular diagnostics laboratories.

■ OTHER PEER-REVIEWED PUBLICATIONS OF INTEREST ■

CARCINOMA OF UNKNOWN PRIMARY WITH A COLON-CANCER PROFILE— CHANGING PARADIGM AND EMERGING DEFINITIONS

GR Varadhachary, MN Raber, A Matamoros, JL Abbruzzese; Lancet Oncol 2008; 9: 596–99

Carcinoma of unknown primary (CUP), which accounts for about 3–5% of all new cancers, is a challenging heterogeneous entity with an unmet research need. Traditionally, CUP has been managed with broad-spectrum chemotherapy, but with the increasing availability of sophisticated diagnostic techniques and the emergence of new treatments that have been shown to be effective in specific cancers the one-treatment-fits-all approach to CUP might eventually no longer be valid. CUP in association with a colon-cancer profile (CCP-CUP) is an example of an emerging, specific CUP subset that seems to benefit from a tailored approach. CCP-CUP is identified by CK20 and CDX2-positive and CK7-negative immunohistochemistry and a clinical course consistent with that of patients known to have metastatic colon cancer. Our findings suggest that patients with CCP-CUP derive substantial benefit from the use of specific treatments developed for colon cancer and larger clinical trials are warranted to more definitely test this finding. In the era of molecular profiling, we expect that additional work with CCP-CUP and other CUP subsets will provide attractive tailored treatment alternatives, with efficacies that exceed the current one-treatment-fits-all approach.

ANALYSIS OF A DIAGNOSTIC STRATEGY FOR PATIENTS WITH SUSPECTED TUMORS OF UNKNOWN ORIGIN

JL Abbruzzese, MC Abbruzzese, R Lenzi, KR Hess, MN Raber; J Clin Oncol 1995; 13:2094-2103

PURPOSE: Diagnostic strategies designed to identify the underlying primary malignancies in patients with unknown primary tumors (UPTs) have relied on retrospective analyses. We analyzed 879 consecutive patients referred with suspected UPTs to determine the yield and cost of a limited diagnostic evaluation, assess the contribution of specific studies to diagnosis, and analyze the survival patterns of patients in whom the primary tumor was diagnosed.

PATIENTS AND METHODS: Data from patients with a suspected UPT were entered into a computerized data base, and the patients underwent a predefined limited diagnostic evaluation. Primary malignancies were diagnosed by pathologic review alone or by pathologic criteria plus a physical or radiographic finding. Survival was measured from diagnosis, estimated using the Kaplan-Meier method, and compared using the Cox-Mantel log-rank test.

RESULTS: A primary tumor was found in 179 of 879 patients (20%). The survival duration

of patients in whom the primary tumor was diagnosed was superior to that of patients in whom the primary tumor remained unknown. Specific patient subsets contributed most to the improved

survival duration of the group in which the primary tumor was found, including lymphoma patients diagnosed solely by pathologic criteria and female patients with primary breast or ovarian cancer. The cost of diagnosis was mostly due to the extensive use of computed tomography. Except for ovarian cancer, computed tomography rarely identified treatable primary tumors.

CONCLUSION: The limited diagnostic evaluation used in this study identified patients with treatable malignancies and increased the survival duration of a population of suspected UPT patients. Primary malignancies with the best survival can be diagnosed through careful pathologic review and focused evaluations for breast and ovarian cancer in women.

■ POSTERS, ABSTRACTS, AND PRESENTATIONS ■

COST-EFFECTIVENESS OF GENE-EXPRESSION PROFILING FOR TUMOR-SITE ORIGIN

J Hornberger, I Degtjar, H Gutierrez, A Shewade, WD Henner, S Becker, S Raab AACR-IASLC Joint Conference on Molecular Origins of Lung Cancer: Biology, Therapy, and Personalized Medicine, January 10, 2012, San Diego, CA

BACKGROUND: Gene-expression profiling (GEP) reliably supplements traditional clinicopathological information on the tissue of origin (TOO) in metastatic or poorly differentiated cancer. A cost-effectiveness analysis of GEP TOO testing versus usual care was conducted from a third-party payer perspective in the United States.

METHODS: A retrospective, observational study examined treatment changes in patients whose physicians had received the GEP TOO test results to help diagnose the tissue-site of their patient's malignancy and to guide appropriate therapy. Changes in planned chemotherapy, surgery, radiation therapy, added blood tests, imaging investigations, and referral to hospice care before and after the GEP TOO test results were recorded. The effect of changes in chemotherapy on survival were based on randomized controlled trials informing appropriate use of chemotherapy cited in National Comprehensive Cancer Network (NCCN) and Up-to-Date guidelines. Drug and administration costs were based on average doses reported in NCCN guidelines. Centers for Medicare and Medicaid Services (CMS) fee schedules were used to obtain other unit costs. Quality-of-life weights were obtained from literature sources. Changes in overall survival, costs, and cost per quality-adjusted life year (QALY) gained were estimated using bootstrap methods.

RESULTS: One hundred and seven patients participated in the study. Use of chemotherapy regimens consistent with guidelines for the final tumor-site diagnosis increased significantly from 42% to 65% (net difference 23%; $p < 0.001$). Overall survival was projected to increase from 15.9 months to 19.5 months (mean difference 3.6 months, 95%CI: 2.0, 5.1). The average estimated increase in survival adjusted for quality of life was 2.7 months (95%CI: 1.4, 3.9), and average third-party payer costs per patient increased by \$10,360 (95% CI: \$5,668, \$15,053). The cost per QALY gained was \$46,858 (95% CI: \$17,995, \$75,718).

CONCLUSIONS: GEP TOO testing significantly altered clinical practice patterns for treating metastatic cancer of uncertain primary. It is projected to increase overall survival, QALYs, and costs, resulting in an expected cost per QALY of less than \$50,000.

UTILITY OF GENE-EXPRESSION PROFILING FOR REPORTING DIFFICULT-TO-DIAGNOSE CANCERS

M Pollen, G Wellman, M Lowery-Nordberg. Association for Molecular Pathologists Annual Meeting, November 17-19, 2011, Grapevine, TX

INTRODUCTION: Studies of primary and metastatic lesions of poorly differentiated malignancies find approximately 80% of lesions are identified with routine stains and immunohistochemistry (IHC). When examining only metastatic carcinomas of unknown primary, this figure drops to 65%. Limited clinical information further decreases this value. Inexperienced pathologists will arrive at erroneous conclusions more frequently. When the primary tumor site is ambiguous among three or more possibilities, there are no standard algorithms for IHC panels. Information from these stains is often indeterminate. The appropriate use of targeted chemotherapy requires knowledge of tumor type. These expensive therapies have side-effects and are only effective for select patients. A determination of primary tumor is requisite for their use. Pathwork Diagnostics® offers a Tissue-of-Origin®(TOO) test as an adjunct to IHC to reduce ambiguity of stain interpretation and provide greater accuracy in determination of cancers of unknown origin.

METHODS: This is a retrospective study of cases referred for TOO from April 1, 2009 through the present. Cases included: are considered difficult-to-diagnose carcinomas, have histopathology reports, have three or more possible sites of origin. Original diagnosis, TOO results, and difference, if any, between working and final diagnoses are compared. TOO relies on mRNA expression in 15 organs. 2,000 mRNA sequences compile the expression profiles. Tumors are given a similarity score, ranging from 0-100, to a particular tissue based on mRNA sequences. The scores sum to 100 across tissue types. A high score confidently assesses origin. Scores under 20 are non-contributory.

RESULTS: Nineteen cases of difficult-to-diagnose carcinomas were examined for original working diagnoses, gene-expression profiling and difference in final opinion based on gene-expression results. Of the nineteen cases, 12(63%) revealed a different site of origin from the pathologist-favored site. Of these, 2 did not yield high enough similarity scores to change the position of the pathologists. In 2 cases, a clinically assumed primary lesion turned out to be metastatic. 2(10%) cases were not able to arrive at a similarity score above 20, the lowest meaningful score. The remaining 5(26%) cases had results that concurred with the original interpretation.

CONCLUSION: The Pathwork Diagnostic® Tissue-of-Origin® test is a valuable adjunct to immunohistochemistry in determining site of origin for lesions that are poorly differentiated or show staining and morphology patterns that are inconsistent with expectation. Given the current clinical need for origin determination, the Tissue-of-Origin® can provide increased diagnostic acumen for challenging specimens.

A GENE EXPRESSION PROFILE TEST FOR THE DIFFERENTIAL DIAGNOSIS OF OVARIAN VERSUS ENDOMETRIAL CANCERS

A Lal, R Panos, M Marjanovic, M Walker, E Fuentes, WD Henner, L Buturovic, M Halks-Miller. Cancer Cytogenomics Microarray Consortium Summer Meeting Abstract Presentation, Aug 8, 2011, Chicago, IL

BACKGROUND: Carcinomas that are either metastatic or involve both the ovary and endometrium can present a diagnostic dilemma. For example, endometrial cancers metastatic to the ovary can often mimic ovarian primaries on gross and microscopic examination. We have developed a gene expression profile test (Pathwork Tissue of Origin Endometrial Test) that distinguishes ovarian and endometrial cancers in formalin-fixed, paraffin-embedded (FFPE) specimens.

METHODS: The Test was developed using a 316-gene classification model and was validated in a blinded study using a pre-specified algorithm and microarray data files for 75 metastatic, poorly differentiated or undifferentiated FFPE tumor specimens that had either a known ovarian or endometrial diagnosis.

RESULTS: Measures of test performance include overall agreement with the available diagnosis of 94.7% (95% CI, 87% to 99%), an area under the ROC curve (AUC) of 0.997 and a diagnostics odds ratio (DOR) of 406 for both ovarian and endometrial cancers. Ovarian cancers (N=30) gave an agreement with reference diagnosis of 96.7% and endometrial cancers (N=45) gave an agreement of 93.3%. In a precision study, concordance in test results for adjacent FFPE sections from the same specimen processed in the same run was 100% (95% CI, 92% to 100%). Reproducibility in test results between two laboratories had a concordance of 96.6% (95% CI, 82% to 100%).

CONCLUSIONS: The Tissue of Origin Endometrial Test can aid in resolving this important differential diagnostic question in gynecologic oncology.

CLINICAL UTILITY OF GENE-EXPRESSION PROFILING FOR TUMOR SITE ORIGIN

HR Gutierrez, JC Hornberger, GR Varadhachary, RJ Hornberger, WD Henner, S Becker, M Walker, M Amin, JS Nystrom. J Clin Oncol 29: 2011 (suppl; abstr e21093)

BACKGROUND: An important step for validating a novel diagnostic is the assessment of its real-world clinical utility. Gene expression profiling (Pathwork Tissue of Origin Test) is recently available to aid in identifying the tumor tissue of origin for metastatic cancers whose primary site is not initially classifiable. The effect of the test on diagnosis and cancer-specific management was assessed in a survey of physicians who ordered the test.

METHODS: The study was an IRB-approved registry, in which data were collected

from participating physicians who ordered the Tissue of Origin Test. To minimize recall bias, a detailed interview was conducted using a web-based questionnaire and a follow-up, confirmatory telephone interview with the enrolled physicians. Data were collected on patient demographics, clinical presentation, diagnostic and imaging procedures, pathology report, immunohistochemistry tests, and cancer-specific management. The physicians were queried regarding their working diagnoses and treatment recommendations prior to and after availability of the Tissue of Origin Test result.

RESULTS: Sixty six physicians were enrolled, providing reports on 111 patients (57% women). Patients underwent extensive evaluation, including imaging tests (mean, 3.2) and immunohistochemistry tests (mean, 9.9) prior to gene-expression profiling. The primary diagnosis site changed in 54% (95% CI [46%, 62%]; $p < 0.0001$) of patients after Tissue of Origin testing, and treatment recommendations changed for 65% (95% CI [57%, 72%]; $p < 0.0001$) of patients. Chemotherapy regimens changed in 61 patients (55%, 95% CI [47%, 63%]; $p < 0.0001$). In 67% of cases, physicians agreed or strongly agreed that the test results were clinically useful.

CONCLUSIONS: Patients with difficult to diagnose metastatic cancer undergo extensive clinicopathological testing. The addition of Tissue of Origin Test changed a substantial proportion of primary diagnosis sites and treatment recommendations.

PERFORMANCE OF A GENE EXPRESSION MICROARRAY ASSAY TO DETERMINE TISSUE OF ORIGIN IN CYTOLOGY BODY FLUID SPECIMENS

FA Monzon, GA Stancel, K Alvarez, T Fairley, D Mody, D Coffey. Poster presented at USCAP, March 2011, San Antonio, TX

BACKGROUND: Identification of the tissue of origin is a common challenge for cytology specimens. Cytologic body fluids are routinely obtained in the diagnostic workup of cases with a metastatic tumor of uncertain origin. The Pathwork Tissue of Origin (TOO) test was recently cleared by the FDA as an in vitro diagnostic device for formalin-fixed paraffin-embedded (FFPE) tissue. We evaluated the performance of this assay in body fluid specimens, including a comparison between specimens preserved with thrombin and Cellient™ cell block methodologies.

DESIGN: 37 body fluid specimens (29 metastases-positive and 8 metastases-negative) were tested; 8 of these had both thrombin (T) and Cellient (C) cell blocks (Total $n=45$). RNA was extracted from 10 micron sections and gene expression assays were performed according to a standardized protocol (Pathwork Diagnostics, Redwood City, CA). A Tissue of Origin report was generated for each sample, and compared with that of the reference diagnosis. In addition, results between the thrombin and Cellient cell block methods were compared.

RESULTS: 7 samples were excluded due to an estimated tumor content of $<60\%$ after Pathologist's review. From the remaining 38 samples, 95% achieved successful labeling/amplification. Only 2 samples failed array data quality verification. Therefore, 34 of 38 specimens (89%) successfully yielded test results. All metastases-negative cases but one, showed an expression profile that was most similar to lymphoma, in

agreement with the predominant presence of inflammatory cells. TOO results for 16/20 specimens with malignant cells (80%, T/C duplicates not counted) were concordant with the reference diagnosis. Upon review of clinical history, one discordant case originally reported as breast was confirmed as an ovarian metastasis, improving the agreement with reference diagnosis to 85%. Thrombin and Cellient block results were concordant in all cases.

CONCLUSIONS: Our results show that it is possible to obtain gene expression profiles from FFPE body fluid specimens using the FFPE version of the Pathwork Tissue of Origin test, when samples meet the >60% tumor content criteria. These results demonstrate that gene expression profiling in body fluid cytology specimens has similar performance characteristics to those obtained in FFPE tissue samples. Pathologist interpretation is important for fluid specimens since negative samples will yield a lymphoma result that is reflective of an inflammatory infiltrate.

EFFECT OF A GENE EXPRESSION-BASED TISSUE OF ORIGIN TEST'S IMPACT ON PATIENT MANAGEMENT FOR DIFFICULT-TO-DIAGNOSE PRIMARY CANCERS

J. C. Hornberger, M. Amin, G. R. Varadhachary, W. D. Henner, J. S. Nystrom. Poster presented at ASCO GI, January 22, 2011, San Francisco, CA

BACKGROUND: An important step for a novel test is assessing its clinical utility and real-world effect on diagnosis and patient management. This report describes the results of a survey of physicians who have ordered a gene expression profile assay for identification of tumor tissue of origin (Pathwork Tissue of Origin Test) for patients with difficult-to-diagnose primary cancers.

METHODS: The IRB-approved registry collects data from participating physicians who have ordered the Tissue of Origin test for their patients. To minimize recall bias, a detailed interview is conducted using both a web-based questionnaire and a confirmatory telephone interview with the physicians. Chart survey includes collection of data on patient demographics, diagnostic procedures including imaging and pathology including immunohistochemistry as well as therapy. The physicians are queried regarding their working diagnoses and treatment recommendations prior to and after Tissue of Origin Test result availability.

RESULTS: Enrollment in the registry is ongoing. Fifty-nine patients (34 women) have been studied and 48 participating physicians have completed the interview. Survey results suggest that most patients have undergone extensive evaluation including multiple imaging tests (median, 6) and exhaustive immunohistochemistry (median, 10) prior to gene expression analysis. For these 59 pts, based on clinicopathologic evaluation, physicians reported working diagnoses for 46% of patients prior to test results and 83% after test results. Treatment recommendations were changed for 53% of patients. The majority (70%) of treatment changes were for chemotherapy. Physicians

(n=26) reported that the Tissue of Origin test decreased the diagnosis associated anxiety in 62% of patients.

CONCLUSIONS: This ongoing registry study confirms that in patients with difficult-to-diagnose primary cancers, extensive baseline diagnostics is not uncommon and less than half of the patients have an established working diagnosis. Survey results suggest that the Tissue of Origin Test led to a change in working diagnosis and changed treatment recommendations in over half the patients.

GENE EXPRESSION PROFILING FROM FFPE TISSUE FOR TUMOR DIAGNOSIS

JP Grenert, R Pillai, AHB Wu. Poster presented at AMP, November 17 – 20, 2010, San Jose, CA

BACKGROUND: Molecular profiling assays have emerged as a promising tool in tumor diagnosis. Advances in recent years have allowed the development of such assays using formalin-fixed paraffin embedded (FFPE) tissue, an RNA source that has long presented a challenge for microarray analysis. The Pathwork Tissue of Origin (TOO) Test was developed by Pathwork Diagnostics for use with FFPE tissues as an aid for tumor diagnosis.

METHODS: Forty-five specimens were selected from archived clinical specimens from the UCSF Department of Pathology and blinded. Total RNA was isolated and processed to prepare labeled cDNA for hybridization to Pathchip microarrays. Data verification was performed on the resulting microarray data files prior to analysis with a 2000-gene classification model (Pathwork Tissue of Origin Test algorithm). The algorithm quantified the similarity between RNA expression patterns of the study specimens and the 15 tissues on the test panel.

RESULTS: Forty-four of 45 (98%) of specimens were successfully processed. 37 cases were cancers represented on the TOO Test panel and met study inclusion criteria. Of these, 35 (95%) gave results which were in agreement with the UCSF reference diagnosis. For the two non-agreements, the TOO Test did not rule out the reference diagnosis as a possibility.

CONCLUSIONS: The Tissue of Origin Test gave a high agreement with the reference diagnosis when archived clinical specimens from UCSF were assessed. Molecular profiling assays are highly accurate, and can be a useful tool in cancer diagnosis.

MOLECULAR TUMOR PROFILING IN THE DIAGNOSIS OF PATIENTS WITH CARCINOMA OF UNKNOWN PRIMARY (CUP): RETROSPECTIVE EVALUATION OF THE TISSUE OF ORIGIN TEST (PATHWORK DIAGNOSTICS)

J Hainsworth, D Henner, R Pillai, F Anthony Greco. Poster presented at ASCO-NCI-EORTC, October 18 – 20, 2010, Hollywood, FL

BACKGROUND: For most patients with carcinoma of unknown primary site (CUP), standard pathologic evaluation with histologic examination and IHC staining cannot define the tissue of origin. Most of these patients receive a trial of empiric chemotherapy, often with a taxane/platinum regimen. Reliable identification of the tissue of origin would probably improve the treatment of CUP patients by allowing site-specific therapy to be administered.

METHODS: In this study, we retrospectively performed the Tissue of Origin Test on biopsy specimens from 48 patients with CUP. Assay results were correlated with clinical features, results of routine pathologic examination, and response to treatment. In addition, results were compared to those given by the Veridex 10-gene CUP assay which had been previously performed on all of these tumor specimens (JCO 26: 4442, 2008).

RESULTS: The Tissue of Origin Test was able to predict the site of origin in 43 of 45 tissue biopsies (96%) from patients with CUP.

- In general, predictions were consistent with clinical features, histology, and response to empiric therapy. The 5 patients identified as “ovarian” had atypical distribution of metastases. The number of sarcomas (6) was unusual; sarcoma was considered as a possibility by histologic exam in only 1 case.
- The Tissue of Origin Test was able to predict a primary site in a higher percentage of biopsies than the Veridex 10-Gene Assay (96% vs 53%). The substantial discrepancy between the assay results may be related to the limited number of genes assessed by the Veridex assay.
- Molecular tumor profiling appears to make a valuable contribution to the diagnosis of patients with CUP. Further studies are necessary to confirm the value of these assays in patients management.

IMPACT OF A GENE EXPRESSION-BASED TISSUE OF ORIGIN TEST ON DIAGNOSIS AND RECOMMENDATIONS FOR FIRST-LINE CHEMOTHERAPY

M Laouri, JS Nystrom, M Halks-Miller, WD Henner. Poster presented at ASCO, June 4 - 8, 2010, Chicago, IL

BACKGROUND: The Pathwork Tissue of Origin Test for FFPE is a microarray –based gene expression test that aids in the diagnosis of poorly differentiated and metastatic tumors. An accurate diagnosis increases the likelihood that appropriate treatment is

administered. This study evaluates the impact of the Tissue of Origin Test results on the diagnosis of the primary site and the impact on recommended first-line chemotherapy based on the NCCN Guidelines.

METHODS: A consecutive series of 284 clinical samples with Tissue of Origin Test results were categorized according to the clinician's pre-test diagnosis (e.g., ICD-9 codes) and the biopsy site. The test result was compared to the original submitted diagnosis to identify changes in the primary site, and potential changes in recommended therapy were determined using the NCC Guidelines.

RESULTS: 85% of study cases were metastatic malignancies. 64% of study cases had non-specific diagnoses of primary site, and for the remaining 36%, the submitting clinician submitted a specific diagnosis for the primary site, most likely indicating an unresolved differential. The Tissue of Origin Test resulted in either a change from non-specific to a specific site or a change from one primary site to another in 81% of cases. For 15% of cases, the Tissue of Origin Test confirmed the submitted diagnosis. Overall, based on test prediction and the NCCN Guidelines, first-line chemotherapy treatment would change for 63% of the cases.

CONCLUSIONS: The Tissue of Origin Test is clinically useful for cases in which the location, histology or IHC results make it difficult to diagnose the primary site reliably. For the majority of patients with unidentified primary sites, the Tissue of Origin Test results are likely to change recommended first-line chemotherapy.

EVALUATION OF A GENE EXPRESSION MICROARRAY ASSAY TO DETERMINE TISSUE OF ORIGIN IN BODY FLUID SPECIMENS

GA Stancel, D Mody, K Alvarez, T Fairley, FA Monzon. Poster presented at USCAP, March 20 - 26, 2010, Washington, DC

BACKGROUND: Identification of the tissue of origin is a common challenge for cytology specimens since tumors with uncertain origin represent 5-10% of all new cancer cases. Cytologic body fluids are routinely obtained in the diagnostic workup of these cases. The Pathwork Tissue of Origin test was cleared by the FDA as an in vitro diagnostic device for frozen tissue, and a version of the test has been developed that works with formalin-fixed paraffin embedded (FFPE) tissue, but is not cleared by FDA. Here, we present initial results for the evaluation of this assay in body fluid specimens using both the thrombin and Cellient™ cell block methodologies.

DESIGN: We retrieved 8 tumor-positive and 7 negative body FFPE fluid specimens processed with both thrombin and Cellient™ (Hologic, Bedford, MA) cell block methods (30 total samples). Two negative body fluids and two non-cytology tumor samples were used as controls. RNA was extracted from the FFPE samples and assays were performed according to a standardized amplification protocol and hybridized to Pathchip™ microarrays (Pathwork Diagnostics, Redwood City, CA). A Tissue of Origin report was generated for each sample, and compared with that of the primary tumor site. In addition, results between the thrombin and Cellient cell block methods are compared.

RESULTS: All 30 samples provided sufficient RNA of adequate quantity and quality

for the Tissue of Origin assay. To test the FFPE Tissue of Origin assay in our laboratory, we analyzed RNA from 2 primary tumors (one frozen, one FFPE), which correlated with the gene expression pattern of the original primary tumor (kidney and lymphoma). Complete data on 2 samples (1 thrombin, 1 Cellient) negative for tumor showed good performance with the Pathchip array, passing all quality thresholds and producing reliable gene expression profiles consistent with inflammatory and mesothelial cells.

CONCLUSIONS: Our preliminary results show that it is possible to obtain gene expression profiles from FFPE body fluid cytology specimens using the FFPE version of the Pathwork Tissue of Origin test. The remainder of our samples will be analyzed to determine the ability to detect tissue of origin and the results from the thrombin and Cellient methods will be compared. These preliminary results suggest that gene expression profiling could be a useful approach for body fluid cytology specimens.

MICROARRAY-BASED GENE EXPRESSION ASSAY FOR IDENTIFICATION OF PRIMARY SITE USING FORMALIN-FIXED PARAFFIN-EMBEDDED (FFPE) TISSUE

R Pillai, R Deeter, L Buturovic, JS Nystrom, M Halks-Miller, D Henner. Poster presented at ASCO GI, January 22 - 24, 2010, Orlando, FL

BACKGROUND: Previous studies utilizing frozen tissues have validated the use of microarray measurements of gene expression patterns to identify challenging tumors, including poorly differentiated, undifferentiated, and metastatic cancers. However applying this technology to the embedded (FFPE) specimens requires a new approach. Here we report the validation and reproducibility of the Pathwork® Tissue of Origin Test for FFPE specimens.

METHODS: 462 poorly differentiated and metastatic FFPE human tumor specimens with available diagnoses representing the 15 different tissue of origin sites on the Origin-FFPE panel were blinded and processed at three independent laboratories. A pre-specified classification model using 2000 genes was applied to each data file to yield Similarity Scores corresponding to the 15 tissues on the test panel. Results were un-blinded and compared with the reference diagnosis for primary site. Reproducibility was measured as the concordance of test results for 60 samples processed at three independent laboratories.

RESULTS: For the 462 data files 409 results (88.5%, 95% CI 85.3, 91.3) were in agreement with the reference diagnosis (“rule-ins”). An average of 12 out of 15 specimens for each tissue could be ruled out with >99% probability. Performance with metastatic and poorly differentiated primary specimens was equivalent. Inter-site reproducibility (concordance) was 89.3% at three independent laboratories.

CONCLUSIONS: The performance of the FFPE-Origin Test has now been validated in multi-site blinded studies and can utilize the most common type of histology specimen, FFPE.

GENE EXPRESSION PROFILES HELP IDENTIFY THE TISSUE OF ORIGIN FOR METASTATIC BRAIN CANCERS

*AHB Wu, JC Drees, HP Wang, SB VandenBerg, A Lal, WD Henner, R Pillai.
Poster presented at AMP, November 19 – 22, 2009, Kissimmee, FL*

BACKGROUND: Metastatic brain cancers are the most common intracranial tumor and occur in about 15% of all cancer patients. In up to 10% of these patients, the primary tumor tissue remains unknown, even after a time consuming and costly workup. The Pathwork® Tissue of Origin Test (Pathwork Diagnostics, Redwood City, CA) is a gene expression test to aid in the diagnosis of metastatic, poorly differentiated and undifferentiated tumors. This test uses microarray technology to measure the gene expression pattern, comprising more than 1,500 genes, in metastatic, poorly differentiated and undifferentiated tumors and compares it to expression patterns of a panel of 15 known tumor types, representing 90 percent of all solid tumors and 58 morphologies overall. The purpose of this study is to demonstrate the utility of measuring genome-wide expression profiles in the diagnosis of the tissue of origin for metastatic brain cancer patients. Accurate identification of primary tumors could lead to improved clinical management, and consequently, better prognosis for these patients.

METHODS: Fifteen fresh-frozen metastatic brain tumor specimens of known origins met specimen requirements and were entered into the study. These specimens were processed using the Pathwork Tissue of Origin Test. Results were compared to the known tissue of origin (“Available Diagnosis”) and the agreement between the two diagnoses was assessed.

RESULTS: Fourteen of the fifteen specimens resulted in qualified data files. One had originated from a tissue type that was off-panel. Among the remaining 13 cases, the Pathwork Tissue of Origin Test accurately predicted the available diagnosis in 12/13 (92.3%) cases.

CONCLUSIONS: This study demonstrates the accuracy of the Pathwork Tissue of Origin Test when applied to predict the tissue of origin of metastatic brain tumors. This test could be a very useful tool for pathologists as they classify metastatic brain cancers.

A MICROARRAY-BASED GENE EXPRESSION TEST AS AN AID TO TUMOR DIAGNOSIS USING FORMALIN-FIXED PARAFFIN-EMBEDDED (FFPE) SPECIMENS

R Pillai, R Deeter, CT Rigel, M Halks-Miller, L Buturovic, D Henner; Pathwork Diagnostics. Poster presented at CAP, October 11 - 14, 2009, Washington, D.C.

CONTEXT: Tumors that are poorly differentiated, undifferentiated, or metastatic represent 5-10% of all new cancer cases. The Pathwork® Tissue of Origin Test is the first FDA-

cleared gene expression test to aid in the diagnosis of tumors using frozen specimens. Here we report on the validation of a version of the test that works with formalin-fixed paraffin-embedded (FFPE) specimens.

DESIGN: Poorly differentiated and metastatic FFPE human tumor specimens with available diagnoses representing the 15 different tissue of origin sites on the Tissue of Origin Test panel were blinded and processed at three independent labs to generate microarray data files. A pre-specified classification model using >1500 genes was applied to each data file to yield Similarity Scores corresponding to the 15 tissues on the test panel. Results were un-blinded and compared with the available diagnoses.

RESULTS: Of 549 specimens processed to data files, 462 (84%) yielded qualified data files. Based on the top Similarity Score, the overall agreement with available diagnoses was 89%. Metastatic and poorly differentiated primary specimens showed similar performance. In addition to the positive test results (“rule0ins”), as an average of 12 out of 15 tissues for each specimen could be ruled out with >99% probability.

CONCLUSIONS: The large size of this study allows an accurate estimate of the confidence of test results for both ruling in and ruling out tissues as likely sites of origin. The Tissue of Origin Test makes the potential benefits of microarray-based gene expression tests for tumor diagnosis available for use with the most common type of histology specimen, FFPE.

USE OF A MICROARRAY-BASED 1550-GENE EXPRESSION PROFILE IN THE DIAGNOSIS OF CARCINOMA OF UNKNOWN PRIMARY (CUP)

FA Monzon, F Medeiros, WD Henner. Poster presented at ASCO 2009 Gastrointestinal Cancers Symposium; January 15-17, 2009; San Francisco CA

BACKGROUND: Carcinoma of unknown primary (CUP) represents about 3-5% of all cancers. These tumors often originate or metastasize to the gastrointestinal (GI) tract. Imaging tests, serum tumor markers and immunohistochemistry (IHC) may offer evidence to identify the tissue of origin; however, these tests are not highly sensitive or specific. The Pathwork® Tissue of Origin Test (“Origin Test”) measures the similarity of a specimen’s gene expression pattern for 1550 genes to the gene expression pattern of the same genes in a database of 15 tissue types, representing a more robust method for identifying the probable origin of CUP.

METHODS: Twenty-one fresh-frozen specimens identified as CUP by institutional standards were collected from the University of Pittsburgh Medical Center (N=10) and the Mayo Clinic (N=11). Each patient had a full clinical, imaging and pathologic work-up, including standard histologic and IHC examination prior to the diagnosis of CUP.

Specimens were processed and hybridized to the Pathchip® microarray. The resulting .CEL file was analyzed by the Pathwork Tissue of Origin Test algorithm. Reports were sent in a blinded fashion to the investigators and then compared to clinicopathologic findings.

RESULTS: The Tissue of Origin Test made a positive call for primary site in 16 of 21 (76%) of specimens and was indeterminate in 5 (24%). The Origin Test indicated a likely

GI (7 [5, colorectal; 2 pancreas]), breast (4), lung (2) or ovarian (3) primary origin. In 6 of 7 positive GI calls (87%; 5 colorectal, 1 pancreas), the Origin Test provided results consistent with the clinical differential. Overall, the Origin Test ruled-out an average 11 tissues of origin including in the indeterminate cases. In all cases, the Origin Test reduced diagnostic uncertainty.

CONCLUSIONS: The Pathwork Tissue of Origin Test shows promise in aiding in the diagnosis of the tumor of origin in cases cases previously classified as CUP. Further studies confirming the use of the Origin Test in CUP are ongoing.

VALIDATION OF A MICROARRAY-BASED GENE EXPRESSION TEST FOR TUMORS WITH UNCERTAIN ORIGINS USING FORMALIN-FIXED PARAFFIN-EMBEDDED (FFPE) SPECIMENS

R Pillai, R Deeter, CT Rigl, M Halks-Miller, WD Henner, L Buturovic. J Clin Oncol 27, 2009 (suppl; abstract e22015)

BACKGROUND: Microarray-based gene expression has been validated as an aid in the diagnosis of tumors with uncertain origins when the specimen is frozen tissue. Microarray use has been largely limited to RNA derived from frozen specimens. This study evaluated performance of a microarray-based test in identifying the tumor type in FFPE specimens. METHODS: FFPE human tumor specimens (n=405) representing the 15 tissue of origin sites on the Pathwork® Tissue of Origin Test panel were blinded and evenly distributed between two independent processing labs. All specimens consisted of a 10-µm-paraffin curl containing at least 60% viable tumor and were either metastatic or poorly differentiated primaries. Each specimen was processed through RNA extraction, amplification, labeling, hybridization to a Pathchip® microarray, and was scanned to generate a qualified data file. A pre-specified classification algorithm utilizing more than 1500 genes was applied to each data file to yield Similarity Scores corresponding to the 15 tissues on the test panel. Results were then unblinded and compared to the available diagnoses. Results: Of the 405 specimens, 352 yielded qualified data files (87%). Based on the top Similarity Score, the overall agreement with available diagnoses was 89% (95% CI, 85%-92%) and for each specimen an average of 12 out of 15 tissues could be ruled out with > 99% probability. Results for all tissue types were highly informative with diagnostic odds ratios ranging from 178 to 28509. Performance was similar for metastatic (n=150; 91% agreement) and poorly differentiated primary specimens (n=202; 87% agreement).

CONCLUSIONS: The large size of this study allows an accurate estimate of the confidence of test predictions for both ruling in and ruling out tissues as likely sites of primary origin. The Pathwork Tissue of Origin Test makes the potential benefits of microarray-based gene expression tests for tumors with uncertain origins available for use with the most common type of histology specimen, FFPE.

THE EVALUATION OF GENOMIC APPLICATIONS IN PRACTICE AND PREVENTION (EGAPP) INITIATIVE: METHODS OF THE EGAPP WORKING GROUP

SM Teutsch, MD, MPH, L Bradley, PhD, G Palomaki, BS, JE Haddow, MD, M Piper, PhD, N Calonge, MD, MPH, WD Dotson, PhD, MP Douglas, MS, and AO Berg, MD, MPH, Chair, on behalf of the EGAPP Working Group; Genetics in Medicine, Volume 11, Number 1, January 2009

The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Initiative, established by the National Office of Public Health Genomics at the Centers for Disease Control and Prevention, supports the development and implementation of a rigorous, evidence-based process for evaluating genetic tests and other genomic applications for clinical and public health practice in the United States. An independent, non-federal EGAPP Working Group (EWG), a multidisciplinary expert panel selects topics, oversees the systematic review of evidence, and makes recommendations based on that evidence. This article describes the EGAPP processes and details the specific methods and approaches used by the EWG.

A META-ANALYSIS OF THE USE OF IHC TESTING IN METASTATIC DISEASE: THE NEED FOR INTEGRATION OF NEW TECHNOLOGIES

GG Anderson, LM Weiss. Poster presented at the ASCO-NCI-EORTC Annual Meeting on Molecular Markers in Cancer; Oct 30 - Nov 1, 2008; Hollywood, FL

BACKGROUND: Histologists use various panels of immunohistochemical (IHC) stains to identify the site of tissue of origin for metastatic tumors, particularly poorly or undifferentiated cancers of unknown or uncertain origin. While clinicians believe that immunostains contribute greatly to determining the probable primary site among three or more possibilities, objective evidence has not been convincingly presented. This meta-analysis reviews the objective evidence supporting this practice and summarizes the performance reported in five studies published between 1993 and 2007.

METHODS: A literature search was conducted to identify all studies published since 1990 designed to quantify IHC performance in tissue determination. The literature search produced hundreds of IHC methodology articles, but only five met the pre-defined criteria; appropriately masked, including more than three tissue types and more than 50 specimens. Data from the five studies were separated into two subgroups for analysis: metastatic tumors only (n = 368 specimens), and the blended studies which combined primary tumors and metastases (n = 298 specimens).

RESULTS: The meta-analysis found that IHCs provided the correct tissue identification for 82.3% (95% CI = 77.4 to 86.3%) of the blended primary and metastatic samples and 65.6% (95% CI = 60.1 to 70.7%) of metastatic cancers. This difference is both clinically and statistically significant.

CONCLUSIONS: This meta-analysis confirms the clinical need for improved diagnostic methods in tissue of origin determination. It establishes minimum performance requirements for any new diagnostic test intended to aid the pathologist and oncologist in tissue of origin determination. Finally, it underscores the importance of well-designed validation studies that are statistically sound, adequately sized, and appropriately masked. It is only through such validation that new, genomic diagnostic tests can establish an appropriate place in the practice of evidence-based medicine.

GENE EXPRESSION MICROARRAY-BASED DIAGNOSTIC TEST MAY IDENTIFY PRIMARY TUMOR SITE IN PATIENTS WITH CARCINOMA OF UNKNOWN PRIMARY (CUP)

F Medeiros, et al. Poster presented at the Association for Molecular Pathology; October 29 - November 2, 2008; Grapevine, TX

BACKGROUND: Patients with cancer of unknown primary (CUP) have metastatic disease for which the tumor of origin cannot be identified. CUP accounts for about 3% of all malignancies; representing one of the 10 most frequent cancer diagnoses worldwide. Correct identification of a patient's tissue of origin is vital for choosing the appropriate therapy. The workup of such tumors is often laborious and expensive; many malignancies remaining unclassified as to site of origin. The gene expression-based Tissue of Origin Test was validated using metastatic tumors for which the primary site was known (*Monzon 2007, Dumur 2008*). Agreement with pathologist-issued diagnoses was 89.5% (95% CI 86.4 – 92.1%, n = 477). The purpose of this study was to determine the diagnostic performance of this test using CUP specimens from the Mayo Clinic frozen tissue bank.

METHODS: Eleven fresh-frozen tumor specimens derived from patients with CUP were processed with the Pathwork Tissue of Origin Test (The "Origin Test") (Pathwork Diagnostics, Sunnyvale, CA). The Origin Test uses expression signals from more than 1,500 genes to measure the similarity of tumor specimens to 15 tissues of origin. Each patient previously underwent a full clinical and imaging workup; tissue specimens were previously thoroughly evaluated by immunohistochemistry, with no determination of site of origin. Origin Test results were compared with clinical, radiologic, and pathologic findings.

RESULTS: Of the 11 CUP specimens, 8 (73%) were positive for one of the 15 tissues on the panel and 3 (27%) were indeterminate. In 6 of the positive calls (4 colorectal, 2 ovarian), the result was consistent with the clinicopathologic differential. In 2 cases, the Origin Test result was unexpected but strongly indicated breast and lung primaries, respectively (1 each). An average of 12 tissues per specimen could be eliminated as potential tissue of origin with high probability. The use of the Origin Test in the differential diagnoses will be shown in CUP cases with associated clinical, biochemical, and immunohistochemistry data.

CONCLUSIONS: The Pathwork Tissue of Origin Test indicated a probable origin of metastatic carcinomas in 73% of the specimens tested. We conclude that the test has the potential to aid in the diagnosis of cancer patients presenting with metastatic carcinomas of unknown primary (CUP).

ASSESSING THE IMPACT OF TUMOR DEVITALIZATION TIME ON GENE EXPRESSION-BASED TISSUE OF ORIGIN TESTING

Cl Dumur, A Ladd, A Ferreira-Gonzalez, CT Rigl, RA Deeter, DS Wilkinson, CN Powers, CT Garrett. Poster presented at the Association for Molecular Pathology; October 29 - November 2, 2008; Grapevine, TX

BACKGROUND: The utilization of genome-wide gene expression microarray technology in tumor stratification has proven a powerful tool to identify gene expression signatures associated with cancer prognosis as well as tissue of origin of undifferentiated metastases from unknown primary sites. Standardized protocols and guidelines for handling of tumor tissues post operatively have yet to be defined. We assessed the effect of tissue devitalization time on ovarian tumor gene expression profiling, particularly in terms of tissue of origin testing, using high-density oligonucleotide microarrays.

Materials and Methods: Residual tissue from two surgical pathology specimens, corresponding to an immature teratoma of the ovary and a serous papillary ovarian tumor, was divided into 5 samples per specimen. One of the samples, for each tumor, was immediately snap-frozen in liquid nitrogen. The remaining four samples per tumor were kept at room temperature for 15, 30, 60, and 120 minutes, at which time the tissue was snap-frozen in liquid nitrogen, and stored at -80°C until RNA extraction. Tissue of origin assessment was performed using the Pathwork® Tissue of Origin (TOO) test. For each sample, standardized expression (SE), similarity scores (SS), and physician guided conclusion (PGC) were analyzed. For the continuous variables, SE and SS, linear correlation analysis was used for each devitalization time comparison to generate Pearson's r coefficients.

RESULTS: Very good correlations of the SE values were observed between each devitalization sample and the corresponding snap-frozen sample, with Pearson's r coefficients greater than 0.93 and 0.95 ($P < 0.0001$) for the immature teratoma and the serous papillary ovarian tumor, respectively. Likewise, Pearson's r coefficients corresponding to SS correlations were greater than 0.88 ($P < 0.0001$) for the immature teratoma specimen and equal to 1.00 ($P < 0.0001$) for the serous papillary tumor. For each ovarian tumor, PGC results were identical for all devitalized and snap-frozen samples and were in agreement with the original diagnosis.

CONCLUSIONS: Our study suggests that with proper sample handling and rigorous QC procedures for RNA extraction and microarray analysis, tumor classification based on global gene expression data will not be adversely affected if devitalization times are kept within a 120-minute window.

OVARIAN MUCINOUS NEOPLASMS: CAN GENE EXPRESSION MICROARRAY-BASED TECHNOLOGY DISTINGUISH PRIMARY VERSUS METASTATIC TUMORS?

F Medeiros, KC Halling, CP Kolbert, F Rakhshan Rohakhtar, TM Kane Lindgren, MJ Wilson, CM Rendler, MP Goetz, DA Bell. Poster presented at the International Congress of the International Academy of Pathology; October 12 - 17, 2008; Athens, Greece

BACKGROUND: It has been estimated that metastatic tumors to the ovary account for 7% of all ovarian tumors and the majority of these represent spread from primary lesions in the gastrointestinal tract. The histopathologic distinction between primary ovarian malignancy and metastasis is usually challenging, particularly for mucinous lesions.

Immunohistochemical studies have shown considerable overlap for all currently available markers, including CK7, CK20 and CDX2. A correct diagnosis is critical for staging, therapeutic, and prognostic reasons. This study aims to determine the utility of a gene expression microarray-based test to differentiate primary versus metastatic mucinous tumors of the ovary.

DESIGN: The study comprised 24 ovarian mucinous tumors, including 10 primary ovarian mucinous adenocarcinomas, 7 metastatic adenocarcinomas from gastrointestinal primaries and 7 ovarian borderline mucinous tumors. RNA was extracted from fresh-frozen tumor specimens and processed with the Pathwork® Tissue of Origin (TOO) Test (Pathwork Diagnostics, Sunnyvale, CA). The TOO Test has been validated to detect tissue from 15 different primary sites including ovarian, pancreas, gastric and colorectal. Each patient underwent a full clinical and imaging assessment. Detailed histopathologic evaluation and immunohistochemistry for CK20, CK7 and CDX2 were performed in all cases using formalin-fixed, paraffin-embedded sections. TOO Test results were blindly compared to clinical, histopathologic and immunohistochemical findings in order to evaluate the potential of the test to correctly indicate the site of tumor origin, whether ovarian or gastrointestinal.

RESULTS: The combination of clinical, histopathologic and immunohistochemical features confidently indicated ovarian origin in 10 cases and gastrointestinal origin in 7 cases. There were 7 cases in which the tumor origin was uncertain. Clinically these 7 cases were considered likely to be primary ovarian mucinous adenocarcinomas. However, they had unusual histopathologic features or extensive extra-ovarian disease at diagnosis also suggesting a non-ovarian primary. The TOO test made a tissue of origin determination for 87% of the specimens (21/24) that was either ovarian, pancreas, colorectal, or gastric. The TOO test assigned an ovarian origin to 5 of 10 cases that were ovarian primaries and assigned gastrointestinal origin to 5 of the 7 cases that were gastrointestinal primaries. The discordant cases were most commonly primary ovarian mucinous adenocarcinoma or borderline tumors that were interpreted by the TOO Test as pancreatic tumors metastatic to the ovary. The TOO test indicated a pancreatic or gastric origin for 6 of 7 cases with uncertain primary site based on clinicopathologic features.

CONCLUSIONS: The Pathwork TOO Test tissue calls were concordant with the clinicopathologic findings for the majority of the cases that were considered to be mucinous tumors metastatic to the ovary or primary ovarian neoplasms. All uncertain primary cases

were determined by the TOO Test to be of gastric or pancreatic origin. Considering the lack of reliable criteria to differentiate primary versus metastatic mucinous ovarian tumors and the great impact of this distinction, the TOO test may be of significant clinical utility. However, additional studies that correlate treatment outcomes with current clinicopathologic classification vs. TOO classifications are needed to clarify the clinical utility of the TOO assay.

A GENE EXPRESSION-BASED ASSAY FOR IDENTIFYING TISSUE OF ORIGIN: APPLICATION TO METASTATIC LESIONS IN THE BRAIN

S Cheshier, S Chang, LJ Buturovic, CT Rigl, WD Henner, GG Anderson. Poster presented at the annual meeting of the Society for Neuro-Oncology, November 15 - 18, 2007; Dallas, TX; Abstract GE-15

Patients with a metastatic lesion to brain as the first presentation of malignant disease can present difficult diagnostic and management problems, particularly when the primary is not readily apparent from review of the standard stained slides nor from imaging studies designed to identify a primary tumor mass. A gene expression-based test to identify the tissue of origin (primary) for such metastatic lesions could be quite useful in targeting the diagnostic workup to allow treating physicians to more rapidly and efficiently reach a conclusion as to the primary site. The Pathwork™ Tissue of Origin Test (TOO) is a microarray-based test that is designed to quantify the molecular similarity between a tumor biopsy samples and a panel of fifteen common tissue types. An objective probability-based score is provided for each potential tissue of origin based on the expression profiles of > 1,600 genes. The performance and reproducibility of this test for specimens derived from biopsy sites other than brain have been reported previously.

METHODS: Eight tumor specimens derived from biopsy or resection of brain lesions where the true primary tissue of origin was known (reference diagnosis) were processed to RNA, labeled and hybridized to a Pathchip™ microarray. The resulting hybridization patterns were analyzed according to the Pathwork TOO algorithm to yield Similarity Scores (SS) for the specimen to each of the 15 tissues of origin on the Pathwork TOO panel. For each specimen all SS total to 100 and an SS of ≥ 30 (indicating a > 95% likelihood of a match) was a positive call.

RESULTS: For brain-derived metastatic lesions, the Pathwork TOO Test was able to make a call with a SS ≥ 30 for all 8 samples. Five of the TOO calls (2 lymphoma, 1 breast, 1 colorectal, 1 lung) were exact matches to the clinical impression for the primary (reference diagnosis). One of the TOO calls was a non-match in which the clinical impression (reference diagnosis) was non-small cell lung cancer on the basis of a lung mass. However, the TOO call was for ovarian tissue and was associated with a very high SS score (SS = 89.4). For two of the samples the clinical impression of the primary can not be matched to the TOO call because the primary was not one of the 15 tissue types on the TOO panel.

DISCUSSION: This study demonstrates the feasibility of using the Pathwork TOO Test to identify the primary tissue of origin for samples derived from biopsy or resection of tumors

metastatic to brain. Where the tissue of origin was one of the 15 tissue types on the TOO panel, the results of the TOO test matched the clinical impression in 5/6 (83.3%). For samples where the TOO Test provides a strong call but is discordant from the clinical impression, it may be useful in targeting further histologic and imaging studies that might confirm either the initial clinical impression or the TOO result. In those cases for which the clinical impression was a primary from a tissue site not included in the TOO panel, the TOO call may still provide some potentially useful indications regarding either the location (gastric/esophageal) or biology (squamous non-small cell lung cancer/squamous cell head and neck) of the primary, and help in eliminating from the differential diagnosis tissue types for which SS is less than 5.

VALIDATION OF A GENE EXPRESSION-BASED TISSUE OF ORIGIN TEST APPLIED TO POORLY DIFFERENTIATED AND UNDIFFERENTIATED CANCERS

FA Monzon, CI Dumur, M Lyons-Weiler, CM Sciulli, CT Garrett, JM Hagenkord, LJ Buturovic, R Deeter, SH Becker, CT Rigl, GG Anderson. Poster presented at the annual meeting for the Association for Molecular Pathology; November 7 - 10, 2007; Los Angeles, CA

BACKGROUND: The Pathwork™ Tissue of Origin (TOO) Test uses the expression of 1550 distinct genes to quantify the molecular similarity between a tissue biopsy sample and fifteen tissues of origin of clinical relevance. Previous studies described the proprietary microarray (Pathchip™), prediction algorithm and performance across laboratories. Here we describe the results of the clinical validation studies evaluating the performance of the Pathwork TOO Test in a clinical setting.

METHODS: Fresh frozen tissue biopsy samples for 477 poorly differentiated and undifferentiated primary and metastatic human tumors were obtained from the Health Sciences Tissue Bank at the University of Pittsburgh and commercial tissue repositories. All samples were of known origin (reference diagnosis) as established by a pathologist. Tissue samples were processed in three laboratories with a recommended protocol. Data from the gene expression assay was analyzed with the Pathwork TOO algorithm and a Tissue of Origin report was generated for each sample. The TOO results were compared against the reference diagnoses to establish the performance characteristics of the Pathwork TOO Test.

RESULTS: The test revealed overall agreement of 89% with the reference diagnoses for all 15 tissue sites and >92% agreement in at least 8 tissues of origin. If the quantitative result (Similarity Score) exceeded the recommended threshold, the probability that the indicated tissue is present was 95% across all tissue types. If the Similarity Score was less than 5, the probability that the indicated tissue is absent was 98% across all tissue types.

CONCLUSIONS: The Pathwork Tissue of Origin (TOO) Test successfully identified the origin of primary tumors in 89% of samples tested and provided >92% agreement for at least 8 tissues of origin. This test has the potential to be an effective aid in the diagnosis of cancer patients presenting with poorly differentiated and undifferentiated tumors.

ANALYTICAL PERFORMANCE OF A MICROARRAY-BASED EXPRESSION TEST TO DETERMINE TISSUE OF ORIGIN IN UNCERTAIN PRIMARY CANCERS

CI Dumur, M Lyons-Weiler, CT Garrett, TK Holley, I Schrijver, J Rodriguez-Paris, JR Pollack, JL Zehnder, CM Sciulli, JM Hagenkord, M Price, CT Rigl, LJ Buturovic, GG Anderson, FA Monzon. Poster presented at the annual meeting of the Association for Molecular Pathology; November 7 - 10, 2007; Los Angeles, CA

BACKGROUND: Clinical workup of metastatic cancers is often arduous and expensive and unsuccessful in 30 to 60% of cases. Accurate classification of the tumor of origin may improve with microarray-based gene expression testing. We evaluated the analytical performance characteristics of the Pathwork™ Tissue of Origin (TOO) test, which uses expression signals from 1,550 probe sets in a gene expression microarray, to quantify the similarity of tumor specimens to 15 tissues of known origin.

MATERIALS AND METHODS: Sixty archived tissue specimens from poorly and undifferentiated tumors (metastatic and primary) were analyzed at four laboratory sites representing a wide range of preanalytical conditions (such as personnel, reagents, instrumentation, and protocols). Reproducibility was analyzed by cross-wise comparisons of all 4 sites for 3 categories of results: Standardized Expression values (SE), which are assay signals; Similarity Scores (SS), which are quantitative results; and Physician Guided Conclusions (PGC), which are clinical calls. For the continuous variables, SE and SS, linear regression analysis was used for cross-lab comparisons to generate correlation coefficients. Raw expression values from all replicates were also standardized using the Affymetrix MAS5 algorithm. Bland-Altman plots (difference between the SS values for the reference diagnosis TOO) from two sites versus the average of the SS values for those sites) were compared to all the other site combinations in order to test for possible systematic bias between laboratories. The inter-laboratory agreement for the categorical variable PGC was evaluated by use of the kappa statistic.

RESULTS: Cross-laboratory comparisons showed highly reproducible results between laboratories with correlation coefficients ranging from 0.95 to 0.97 for measurements of SS, which were significantly improved (Wilcoxon onesided paired test, $p = 0.01563$) from those obtained with MAS5 normalized values (0.65 to 0.82). Moreover, an average of 93.8% overall concordance between laboratories in terms of final TOO calls was obtained. Bland-Altman plots (mean coefficients of reproducibility of 32.48 ± 3.97) and kappa statistics ($k > 0.86$) also indicated a high level of agreement between laboratories.

CONCLUSIONS: We conclude that the Pathwork™ TOO test is a robust assay that can produce consistent results in diverse laboratory conditions reflecting the preanalytical variations found in the everyday clinical practice of molecular diagnostics laboratories.

ON THE OPTIMAL NUMBER OF GENE EXPRESSION MARKERS FOR TISSUE OF ORIGIN CANCER DIAGNOSTICS

LJ Buturovic. Poster presented at the annual meeting for the American Association for Cancer Research; September 17 - 20, 2007; Atlanta, GA; B4

Multiplex genomic tests based on gene expression combine multiple markers using computer algorithms to generate clinically useful information. The number of markers used in the gene expression tests has implications for the choice of gene expression platform. A large number of markers may favor microarrays, smaller numbers may favor other platforms such as quantitative PCR. Theory [1] provides lower bound on the number of markers (at least $c-1$, where c is the number of test categories). Also, it suggests that the required number of markers increases with the number of categories. We undertook to determine the optimal number of markers for a particular diagnostic problem: cancer classification. An example of a test designed to solve the problem is the Pathwork Tissue of Origin Test (TOO).

OBJECTIVES: The Pathwork Tissue of Origin Test is designed to identify the tissue of origin of metastatic and poorly differentiated cancers by comparing the expression signature of the test biopsy sample with expression patterns of 15 common cancer types. TOO uses a machine-learning algorithm (model) to identify the tissue of origin. The models considered here take expression signatures over the selected markers as input, and produce predicted tissue identity as output. The goal was to determine the minimum number of markers required for the optimal performance of the TOO test.

METHODS: Tissue of Origin models were built using 2034 training samples (gene expression profiles) and varying the number of markers. The data originated from 14 laboratories and 15 cancer types, on Affymetrix GeneChip Human Genome U133A and U133 Plus 2.0 platforms. For a given number of markers, d , we chose the best d markers, and built the optimal model using gene expression profiles of the best markers. d varied from 50 to 10,000 in the steps of 50, with an additional model with 22,000 markers. For each model, 10-fold cross-validation was used to estimate the TOO performance. The performance criterion was percent agreement between predicted Tissue of Origin and clinical truth (or, alternatively, the prediction error rate). The combination of machine learning algorithm and cross-validation was designed to reduce overfitting.

RESULTS: We achieved 5.4% error rate with 1550 markers. The performance gain over the optimal TOO 100-marker classifier (7.2%) was clinically and statistically significant (two sample chi-square = 4.7, $P = .03$). Observed slight performance decrease with increasing number of markers used in the test. The trend was maintained when all (approx. 22,000) markers on the U133A chip were used in the model. The same pattern was observed when the models were applied on independent test set of 477 specimens.

CONCLUSIONS: Cancer classification diagnostic tests based on gene expression may require relatively high number of markers – over a thousand – to approach clinically useful performance. This conclusion favors DNA microarrays over quantitative PCR for cancer classification problems. Pathwork is actively investigating whether this finding holds for other types of diagnostic problems (drug resistance, prognosis).



Learn more about the Pathwork Tissue of Origin Test. Call 1.877.808.0006 or visit www.pathworkdx.com.