

# A Gene Expression-Based Assay for Identifying Tissue of Origin: Application to Metastatic Lesions in the Brain

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## ABSTRACT

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.

## BACKGROUND

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.

Even with guideline-directed use of immunohistochemistry (IHC), electron microscopy, and advanced imaging procedures (e.g., computed tomography, magnetic resonance imaging, fluorodeoxyglucose positron emission tomography), the primary tumor is ultimately identified in only about 20 to 25% of living patients with metastatic tumors for which the primary site is not apparent after the initial workup. (Pavlidis 2006)

Recently, newer diagnostic techniques have been evaluated to improve classification of clinicopathologically ambiguous tumors. Thus, gene expression microarrays, which capture data from tens of thousands of expressed genes in a single test, have the potential to allow a more accurate classification of tumors of unknown primary, including those with high histologic grade.

The Pathwork™ Tissue of Origin Test is an in vitro diagnostic test for evaluating the tissue of origin (TOO) in poorly differentiated or undifferentiated tumors. This microarray-based gene expression test quantifies the similarity of tumor specimens to 15 known TOOs by means of a proprietary machine learning algorithm trained on 2039 well-characterized tumor specimens, acquired from 14 laboratories. The tumor types included in the Pathwork TOO Test are: bladder, breast, colorectal, gastric, germ cell, hepatocellular carcinoma, kidney, non-small-cell lung, non-Hodgkin's lymphoma, melanoma, ovarian, pancreatic, prostate, soft tissue sarcoma, and thyroid.

Previous studies have shown performance of the TOO test with specimens derived from metastatic deposits in liver, lung, soft tissue and lymph node (Table 1; derived from Monzon 2007). The current study was designed as a pilot study to determine the performance of the TOO test with specimens derived from biopsy or resection of lesions from the central nervous system.

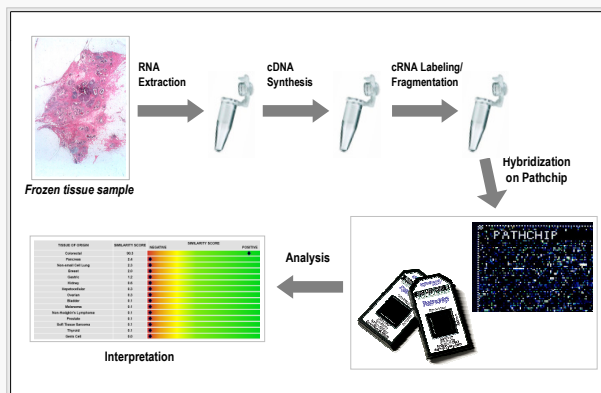
## PERFORMANCE OF THE TOO TEST

Biopsy Site	# Specimens*	% Agreement	% Non-agreement	% Indeterminate
Liver	24	79.2	8.3	12.5
Lung	29	82.8	13.8	3.4
Lymph Node	52	78.8	11.5	9.6
Other	129	91.5	4.7	3.9
Overall (all metastases)	234	86.3	7.7	6.0
95% CI		[81.2, 90.5]	[4.6, 11.9]	[3.3, 9.8]

\*Specimens have reference diagnoses that correspond to one of the fifteen tissues on the Tissue of Origin Test panel.

## MATERIALS AND METHODS

### Figure 1. Work Flow for the Tissue of Origin Test



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 an Affymetrix 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 (see above).

## RESULTS

All eight of the specimens produced TOO Test results making a call for one of the 15 tissues on the TOO Test panel (SS > 30) (Table 2). None of the tests were indeterminate. Of the 6 specimens with primaries on the test panel five of the six produced a TOO Test result in agreement with the known primary. One of six produced a strong TOO test result for ovarian carcinoma in a woman with presumed metastatic non-small cell carcinoma of the lung. Two of the eight samples were from primary sites that are "off-panel" for the TOO test. These produced results of non-small cell lung carcinoma and gastric for the squamous cell carcinoma of the head and neck and gastroesophageal primaries, respectively.

Reference Diagnosis	TOO Test Result	TOO Test Top Score	Sample Description
Non-Hodgkin's Lymphoma	Non-Hodgkin's Lymphoma	90.4	Malignant lymphoma, large B-cell type
Non-Hodgkin's Lymphoma	Non-Hodgkin's Lymphoma	87.7	Diffuse large B-cell lymphoma
Colorectal Carcinoma	Colorectal Carcinoma	83.4	Metastatic adenocarcinoma
Non-Small Cell Lung Carcinoma	Non-Small Cell Lung Carcinoma	91.0	Metastatic carcinoma
Breast Carcinoma	Breast Carcinoma	90.5	Metastatic adenocarcinoma c/w breast primary
Head and Neck Carcinoma	Non-Small Cell Lung Carcinoma	77.3	Metastatic squamous cell carcinoma from laryngeal primary
Gastroesophageal carcinoma	Gastric Carcinoma	35.0	Metastatic adenocarcinoma, TTF-1 negative, h/o esophageal cancer
Non-Small Cell Lung Carcinoma	Ovarian Carcinoma	89.4	Metastatic carcinoma c/w lung primary

Test is not cleared by FDA for sale in the U.S. For Investigational Use Only.

## Figure 2. Examples of TOO Test Reports in Agreement with Reference Diagnoses



Figure 2. Examples of Pathwork TOO Test Reports in Agreement with the known tissue of origin. A) Non-Hodgkin's Lymphoma B) Non-Hodgkin's Lymphoma C) Colorectal Carcinoma and D) Non-Small Cell Lung Carcinoma.

## Figure 3. An example of a discordant result

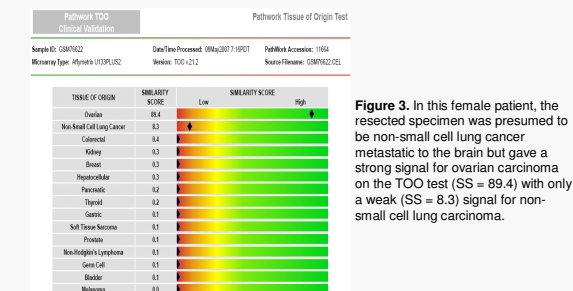


Figure 3. In this female patient, the resected specimen was presumed to be non-small cell lung cancer metastatic to the brain but gave a strong signal for ovarian carcinoma on the TOO test (SS = 89.4) with only a weak (SS = 8.3) signal for non-small cell lung carcinoma.

## CONCLUSIONS

- 1) The TOO test was able to identify a tissue of origin in all eight brain biopsy samples.
- 2) For 5 of 6 specimens where the primary site was one of the 15 tissues on the TOO test panel, the TOO test was able to correctly identify the primary site.
- 3) For the 2 specimens where the primary site was "off-panel", the TOO test result identified a tissue of origin with a very similar biology / histology to the known primary (e.g. NSCLC for squamous cell of head and neck and gastric for gastroesophageal adenocarcinoma)
- 4) The Pathwork TOO test should present useful information to aid in the diagnosis and appropriate treatment of patients presenting with new brain metastases and with no apparent primary site at the time of diagnosis.

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