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Evaluation of a Gene Expression Microarray-based Assay to Determine Tissue Type of Origin on a Diverse Set of 49 Malignancies

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Abstract

The Tissue of Origin Frozen (TOO-FRZ) assay from Pathwork Diagnostics has been cleared by the Food and Drug Administration as a diagnostic study for malignancies of unknown primary. The goal of this study was to evaluate the performance of TOO-FRZ on a diverse collection of malignancies. We collected a diverse set of 49 malignancies. We classified each case into 1 of 4 groups: common morphology from a tissue type included in the TOO-FRZ assay (n=29), uncommon morphology from a tissue type included in the TOO-FRZ assay (n=10), tumor from a tissue type not included in the TOO-FRZ assay (n=3), and malignancies of unknown primary (n=7). We found strong diagnostic performance for common morphologies from tissue types on the TOO-FRZ [overall accuracy=26 of 29 (90%, 95% CI, 73% to 97%)], with perfect performance in all tissue types except gastric (0 of 2) and pancreatic (1 of 2) tissues. There was a significant decline in performance for uncommon morphologies from tissue types included in the TOO-FRZ assay [6 of 10 (60%) cases with an indeterminate result, 1 of 10 (10%) cases with an incorrect prediction, and 3 of 10 (30%) with a correct prediction] and for tumors from tissue types not included in the assay (incorrect prediction in 2 of 3 cases). For the 7 malignancies of unknown primary in our study set, the TOO-FRZ provided a likely clinically useful result in only 2 of 7 cases. These results provide an insight into the strengths and limitations of this molecular assay for the surgical pathologist, and our findings suggest future directions for research in this area.

Keywords

microarray; tumor of unknown origin; molecular pathology

In the evaluation of tumor specimens, surgical pathologists are responsible for determining the most appropriate diagnostic studies to perform to determine a site of tumor origin. In current practice, the site of origin for diagnostically challenging tumors is determined through a nonstandardized procedure of integrating data from the clinical history of a

patient, and from radiologic findings, surgical findings, and from the tumor's microscopic features and immunohistochemical profile. This procedure is unable to determine a site of origin in 2% to 5% of malignancies.^{2,3}

Gene expression profiling microarrays permit the measurement of ribonucleic acid (RNA) levels from thousands of genes in parallel. This technology has been widely used for molecular profiling of cancer, and several groups have developed microarray-based platforms for determining a tumor's site of origin.^{1,6,10,11,14–16} The Pathwork Tissue of Origin (TOO) assay (Pathwork Diagnostics) is one of the most studied and clinically validated microarray-based platforms for determining the site of origin of carcinomas of unknown primaries. The original Pathwork TOO assay was developed for use with frozen tissue samples and was cleared by the Food and Drug Administration (FDA) for clinical use in 2008. Its utility for identifying the site of origin of poorly differentiated carcinomas has been evaluated in several studies.^{4,9,10,18}

The TOO frozen (TOO-FRZ) assay measures the expression of 1550 genes and applies a proprietary algorithm to determine a similarity score (SS) (ranging from 0 to 100) that indicates the tumor's similarity to each of 15 tumor tissue types included in the assay (Fig. 1). The tissue types included in the assay are as follows: testicular germ cell, soft tissue sarcoma, nonsmall cell lung, ovarian, thyroid, kidney, melanoma, non-Hodgkin lymphoma, hepatocellular, breast, prostate, bladder, colorectal, pancreas, and gastric. This panel includes a wide variety of tissue types, some of which are predominantly associated with a single diagnostic subtype (eg, Colorectal, Hepatocellular), whereas others contain many different diagnostic subtypes (eg, soft tissue sarcoma). The TOO-FRZ assay does not explicitly address diagnostic subtypes within a tissue site of origin, and the algorithm produces an SS to a tissue type but not to a specific morphology within a tissue type (Fig. 2).

Previous studies have evaluated the performance of the TOO-FRZ assay for classifying the tissue type of origin of poorly differentiated tumors from known tissue types included in the TOO-FRZ platform.^{4,9,18} A single study conducted recently evaluated the performance of the assay in malignancies of unknown primary and found that the assay gave a helpful diagnosis in most cases from this small study.¹⁰

The goal of the current study was to evaluate the utility of the Pathwork TOO-FRZ assay on a diverse set of solid tumors encountered in clinical practice at a single center. The study set was predominately comprised of cases with a confirmed site of origin, based on clinical, pathologic, and, in some cases, immunohistochemical data. A primary goal of our work was to assess the performance of the TOO-FRZ assay on tumors of known origin, in the hope that our analysis would suggest the strengths and weaknesses of the assay for identifying the true site of origin of malignancies of unknown primary that originate from sites included in our study set. In contrast to previous studies, which evaluated the TOO-FRZ assay predominantly on common morphologies from tissue types included in the TOO-FRZ assay, our study sought to assess the performance of the TOO-FRZ assay on common morphologies, and on rare morphologies and tissue types not included in the TOO-FRZ assay. In addition, we did assess TOO-FRZ performance on a small set of malignancies of unknown primary.

MATERIALS AND METHODS

Clinical Samples

This study included a total of 49 fresh frozen samples acquired from the Stanford University Department of Pathology. Institutional review board approval was obtained for samples included in the study. The specimens acquired for our study were classified into 4 groups before performing the TOO-FRZ assay (Fig. 1): common morphology from a tissue type included in the TOO-FRZ platform (n=29); uncommon morphology from a tissue type in the TOO-FRZ platform (n=10); tissue type and morphology not included in the TOO-FRZ platform (n=3); and malignancy of unknown primary (n=7). Of the malignancies of known primary, the study included both metastatic tumors (n=29) and primary tumors (n=13).

There were morphologies for each TOO-FRZ tissue type that were explicitly either “included” in or “excluded” from the assay (Supplemental Table 1 in Ref 9). This list of morphologies was not exhaustive and many morphologic types exist in the TOO-FRZ tissue types that are neither explicitly included nor excluded. In our study, if an uncommon morphologic type arose from one of the 15 tissue types included in the TOO-FRZ assay report, we classified the tumor as being of “uncommon morphology from a tissue type on the TOO-FRZ platform.” Our study did not include in this category any excluded morphologies from the table by Monzon et al.⁹ In Table 2 of our study, we indicated whether the rare morphology was explicitly included in the TOO-FRZ assay.

Samples were required to contain at least 60% tumor and to be <20% necrotic. This study was performed entirely on frozen archival samples; however, we note that Pathwork has subsequently implemented a form of the TOO assay that can be performed on formalin-fixed paraffin-embedded tissues (TOO-FFPE) (<http://www.pathworkdx.com/TissueOfOriginTest/LaboratoryService/>).¹² TOO-FFPE was cleared by the FDA in 2010.

Gene Expression Microarray Assay

Gene expression profiling was performed following the Pathwork Diagnostics protocol as previously described.⁴ In brief, total RNA was extracted from tumors using the RNeasy mini kit (Qiagen, Valencia, CA). Adequate concentration and purity of total RNA were assessed by spectrophotometry and gel analysis. Complementary DNA was generated from the total RNA, followed by in vitro transcription synthesis and labeling of complementary RNA, and fragmentation and hybridization on the proprietary Pathwork Pathchip. Microarrays were processed and analyzed on the Affymetrix 3000DX GeneChip fluidics station and scanner, which generated gene expression data. The data were immediately submitted for analysis to Pathwork Diagnostics and reports were obtained.

Fluorescence intensity data were transformed into gene expression values, which were standardized against 121 endogenous messenger RNA (mRNA) markers known to be stable in their expression patterns. After data verification, the Pathwork TOO-FRZ Test algorithm was performed, which compares the expression of 1550 genes from each tumor with the gene profiles of each of the 15 tissues included in the TOO-FRZ panel. The results generated by the algorithm are expressed in an SS, ranging from 0 (low) to 100 (high) (Fig. 2).

Data Analysis

For interpretation of the TOO-FRZ assay, we followed the Pathwork TOO-FRZ recommendations for interpretation, which state that if a single tissue type achieves an SS ≥ 30 then that tissue type is determined as the tissue type of origin; a SS between 5 and 30 indicates indeterminate results, and a SS ≤ 5 rules out that tissue type as the site of origin. The Pathwork interpretive recommendations state that if, in the same report, 2 sites receive a score ≥ 30 , then one of those results indicate the likely tissue of origin. Given the ambiguity in this result, we interpreted these cases as indeterminate. Contamination with cells from the site of tumor location is not uncommon and this should be taken into account during interpretation.

The primary outcome measure of this study was to assess the ability of the TOO-FRZ assay to provide accurate, clinically informative diagnostic information for a diverse collection of malignancies, with distinct assay output evaluation criteria for the 4 types of specimens (Fig. 1). For the samples from common morphologies from tissue types included in the TOO-FRZ assay, our primary objective was to confirm the utility of the TOO-FRZ for classifying the case correctly. For samples from tissue types not included in the TOO-FRZ assay, our primary objective was to assess the ability of the TOO-FRZ to not provide support for the 15 tissue types in the TOO-FRZ panel (ie, for each of the 15 tissue types to achieve an SS <30). For malignancies of unknown primary, our primary objective was to assess the ability of the TOO-FRZ assay to provide a potentially clinically useful result. To test the statistical significance of observed differences in the proportions of cases that received an accurate prediction from the different groups of tumors, we performed the Fisher exact test.

RESULTS

Common Morphologies From Tissue Types included in the TOO-FRZ Panel

The TOO-FRZ showed a strong capability for classifying common morphologies from tissue types included in the TOO-FRZ (Table 1), correctly classifying 26 of 29 (90%, 95% CI, 73%–97%) of these cases. Of the 12 common morphologies from tissue types included in the TOO-FRZ, the TOO-FRZ performed perfectly in all but gastric adenocarcinomas and pancreatic ductal adenocarcinomas. Of the 2 gastric adenocarcinomas, 1 produced a clinically indeterminate result (with no tissue type achieving an SS ≥ 30) and 1 gastric adenocarcinoma produced an incorrect result. For the latter, 2 specimens metastatic to different sites from a single primary gastric adenocarcinoma were tested separately, and each produced an incorrect prediction: a sample metastatic to the ovary was misclassified as being of ovarian origin and a sample metastatic to the omentum was misclassified as being of pancreatic origin. One of 2 cases of pancreatic ductal adenocarcinoma was misclassified as being of gastric origin. These results are similar to those reported in the study by Monzon et al,⁹ which evaluated the TOO-FRZ on 547 specimens and reported a test sensitivity of 87.8% (95% CI, 84.7%–90.4%) for achieving positive agreement with reference diagnosis.

Uncommon Morphologies From Tissue Types Included in the TOO-FRZ Panel

A potential limitation of the TOO-FRZ assay is that it does not explicitly address the topic of diagnostic subtypes within a tissue site of origin. We evaluated the performance of the TOO-

FRZ on relatively uncommon morphologies from tissue sites included in the TOO-FRZ assay. Overall, the TOO-FRZ assay correctly classified only 3 of 10 (30%, 95% CI 10% to 61%) rare tumor subtypes included in the assay, gave indeterminate results in 6 of 10 (60%, 95% CI 31%–83%) cases, and an incorrect result in 1 of 10 (10%, 95%CI <0.01% to 43%) cases (Table 2). The 6 cases that received “indeterminate” results were as follows: 1 of 2 ovarian clear cell carcinomas, embryonal sarcoma of the liver, synovial sarcoma, and gastrointestinal stromal tumor, and 2 ovarian mucinous tumors of low malignant potential (LMP). In 4 of the 6 cases, no diagnostic site on the TOO-FRZ assay received an SS ≥ 30 . In addition to these 4 cases, 2 of 2 ovarian mucinous tumors of LMP received an “indeterminate” result. For both cases of ovarian mucinous LMP, the site “ovary” received an SS ≥ 30 ; however, in both cases an additional site also received an SS ≥ 30 (gastric and pancreas) (Fig. 2). It is not surprising that mucinous tumors arising from the ovary would receive high SS to gastric and pancreatic tissue types, as the morphologic and immunophenotypic profiles of ovarian mucinous tumors is highly similar to those of gastric and pancreatobiliary tumors (Fig. 2). One of 10 uncommon morphologies from tissue types included in the TOO-FRZ received an incorrect classification. This tumor was a primary pulmonary adenocarcinoma with enteric differentiation. The TOO-FRZ incorrectly classified this tumor as being of colorectal origin. Although the morphologic and immunophenotypic features of this tumor were highly suggestive of colorectal origin, the clinical and radiographic findings strongly favored a primary pulmonary adenocarcinoma with enteric differentiation, which is a rare but established diagnostic entity.⁷

Tumors From Tissue Types not Included in the TOO-FRZ Panel

Our study included 2 cases of uterine endometrial adenocarcinoma (Table 3). Both cases were erroneously diagnosed: 1 case was classified as being of ovarian origin and 1 as soft tissue sarcoma. In the first case, the gross description of the specimen clearly demonstrated that the tumor mass arose from within the uterus and did not involve the ovaries; therefore, the true site of origin was not in question. It is problematic that the TOO-FRZ assay is unable to differentiate between ovarian and uterine origin (uterine is not included in the TOO-FRZ panel), as this is a differential diagnosis that is not uncommonly encountered in clinical practice. For clinical management, it is important to differentiate metastatic tumors of the ovary and uterus from each other and from synchronous primaries occurring at these sites.¹³

The case of endometrial adenocarcinoma classified as soft tissue sarcoma is instructive, because it demonstrates the importance of integrating multiple data types to predict a site of origin, rather than relying on a single result in isolation. The tumor in this case was heterogeneous, containing both poorly differentiated high-grade areas suggestive of sarcoma and regions of well-differentiated grade 1 endometrial adenocarcinoma as well. It is likely that a portion of the high-grade sarcoma-like region of tumor was submitted for molecular analysis by TOO, resulting in the predicted site of “soft tissue sarcoma.” This case demonstrated the importance of representative sampling for both morphologic and molecular analyses of tumors. Our study included 1 case of pulmonary carcinoid. This tumor received an indeterminate result, based on the fact that the SS was <30 for all 15 tissue types on the TOO-FRZ panel.

Overall, the assay accurately provided no support for the 15 TOO-FRZ tissue types for the pulmonary carcinoid, but the assay did make inaccurate predictions for 2 cases of endometrial adenocarcinoma. We note that Pathwork states on their website that a limitation of the TOO assay is that it “should not be used to distinguish tumor types in the database from tumor types not in the database” (<http://www.pathworkdx.com/TissueOfOriginTest/IVDKit/>). Our results support this recommendation, and suggest that the TOO assay should not be used when a tumor not included in the TOO panel is in the pretest differential diagnosis. However, this recommendation will be difficult to apply in clinical practice, as it will frequently be difficult to determine whether a tumor type from a site not included on the TOO is in the differential diagnosis of a tumor of uncertain origin.

Malignancies of Unknown Primary

Our study included 7 cases of malignancies of unknown primary (Table 4). Three of these cases received indeterminate diagnoses (all predicted sites received an SS <30), and consequently the test was of limited clinical use in these cases because the clinical utility of the test in these cases would be limited to tissues ruled out. One case from a male patient was a poorly differentiated malignancy with an immunohistochemical profile consistent with malignant mesothelioma (CKMix⁺, calretinin⁺, CK7⁻, CK20⁻). This tumor tissue was predicted to be of ovarian origin by the TOO, which was clearly not possible given the patient's sex; however, we note that the surface of the ovary is lined by mesothelial cells, which suggests a potential cause of the high SS for the case of suspected mesothelioma. In this case, no other site received an SS ≥ 30. One of the malignancies of unknown primary cases was from a patient with a papillary serous carcinoma involving both the endometrium and the ovaries. It was noted in the gross and microscopic descriptions that the carcinoma extensively involved the uterus with only surface ovarian involvement, which favored a uterine primary site. The TOO-FRZ assay in this case predicted an ovarian primary. This result is of limited clinical use, as the site “uterus” is not included in the TOO-FRZ assay, and the other pathologic data favored a uterine primary site. The last 2 cases were both metastatic and poorly differentiated carcinomas (one was of squamous differentiation, and the other was an adenocarcinoma). The pre-TOO test differential diagnosis for both malignancies included nonsmall cell lung carcinoma; however, the definitive diagnosis was not certain. Both tumors were predicted by the TOO to originate from nonsmall cell lung cancer. The TOO result in these 2 cases was probably clinically useful.

Overall Results

We observed variable performance in the 4 different groups of tumors analyzed in our study (Fig. 3). The proportion of cases correctly classified from common morphologies from tissue types included in the TOO-FRZ assay (26 of 29; 90%, 95% CI, 73% to 97%) was significantly higher than the proportion of cases correctly classified from rare diagnostic subtypes included in the TOO-FRZ assay (3 of 10; 30%, 95% CI, 10% to 61%) ($P<0.001$). Overall, we note strong performance of the TOO-FRZ assay on common morphologies from tissue types included in the TOO-FRZ panel and relatively poor performance on uncommon morphologies from sites included in the TOO-FRZ assay and on the small set of tumors in our study from tissue types not included in the TOO-FRZ. In addition, for the malignancies

of unknown primary included in our study set, the TOO assay was only able to provide a potentially clinically useful result in 2 of 7 cases.

DISCUSSION

Several multiplex molecular assays have been developed to assist in the determination of the tissue of origin for malignancies. These include a quantitative reverse transcription-polymerase chain reaction mRNA assay to measure 92 mRNA transcripts (bioTheranostics, CancerTYPE ID), a quantitative reverse transcription-polymerase chain reaction assay to measure 48 miRNA mature transcripts (Prometheus, ProOnc TumorSource), and a microarray-based assay to measure mRNA transcripts in frozen tissues (1550 mRNA transcripts) or in FFPE tissue samples (2000 mRNA transcripts) (Pathwork Diagnostics, Tissue of Origin assay). Both assays are FDA cleared and, although similar, the 2 assays use different algorithms and evaluate a different set of transcripts. The TOO-FRZ assay has been evaluated in several previous studies for performance on poorly differentiated malignancies. A recent study evaluated its performance in a small set of carcinomas of unknown primary.¹⁰ The primary goal of this study was to evaluate the performance of the TOO-FRZ assay in a diverse collection of tumors as encountered in pathology practice, including the following: malignancies of a common morphology from tissue types included in the TOO-FRZ assay, malignancies of an uncommon morphology from tissue types included in the TOO-FRZ assay, malignancies from tissue types not included in the platform, and malignancies of unknown primary.

For common morphologies from tissue types included in the TOO-FRZ assay, the assay showed perfect performance in all evaluated samples, with the exception of gastric and pancreatic adenocarcinoma samples (Table 1, Fig. 3). These findings support data from the literature reporting excellent performance in poorly differentiated carcinomas from sites included in the TOO-FRZ assay.⁹ We observed a significant decline in performance in rare morphologies from tissue types included in the TOO-FRZ and in tissue types not included in the TOO-FRZ.

Several factors likely account for this decline in performance observed in rare subtypes from sites included in the TOO-FRZ assay. First, the performance of all classification algorithms is highly dependent on the set of cases used to train the algorithm.⁵ In a previous study, Pathwork Diagnostics stated that they trained their algorithm on a relatively large number of tumors (2039) representing 60 different morphologies⁹; however, the exact set of samples used to train the algorithm is not presented, and it is likely that few cases of the rare diagnostic subtypes used in the current study were present in the TOO-FRZ training set, resulting in the decreased performance in these diagnostic subtypes tested in this study. A second, related cause of the decline in performance is the intrinsic degree of variability within tumors from different tissue types. For example, the set of tumors arising from the colorectum consists almost exclusively of adenocarcinomas, with a relatively small set of subtypes (perhaps 5)⁸; consequently, this site would likely not require a large number of tumors to create an algorithm to accurately identify tumors arising from it. In contrast, soft tissue sarcoma is an extremely heterogeneous tumor category that includes hundreds of diverse histologic subtypes¹⁷ and would likely require a very large and varied training set to

build an algorithm to accurately classify diverse sarcomas as arising from the same “tissue of origin.”

Although previous evaluations of TOO-FRZ performance have focused exclusively on malignancies from sites included in the TOO-FRZ assay,^{4,9} our study included 3 tumors from tissue types not included in the TOO-FRZ assay. For these 3 samples, we sought to evaluate whether the TOO-FRZ assay would correctly rule out the 15 sites of tissue of origin included in the assay. In 2 of the 3 cases, the TOO-FRZ provided an incorrect classification (Table 3, Fig. 3), suggesting that the assay may yield unreliable results in tumors from sites not included in the TOO-FRZ test report. We note that these 2 cases were uterine cancers, which represent the fourth most common cancer in women, behind only breast, lung, and colorectal cancer (<http://apps.nccd.cdc.gov/uscs/toptencancers.aspx>). These findings suggest that the addition of a uterine site might be a useful modification to the TOO-FRZ assay.

Our study also included a set of 7 malignancies of unknown primary (Table 4). In only 2 of these 7 cases did the TOO-FRZ assay provide a potentially clinically useful diagnosis. In one of the cases, the TOO-FRZ prediction was incorrect because of the prediction of an ovarian site of origin in a male patient. In another case, the TOO-FRZ prediction of an ovarian primary was unlikely to be correct because of the gross and microscopic impression that the tumor was in fact originating from the uterus. In the remaining 3 cases, the assay produced an indeterminate result, which was of limited clinical utility, because, although no site was positively identified, the assay may help rule out the sites that received low SSs.

These findings suggest new strategies for molecular assay design for adjuvant studies in surgical pathology. The majority of the incorrect classifications involved TOO-FRZ predictions of sites of origin that had a very low pretest probability based on clinical and/or pathologic characteristics of the case (eg, ovarian site in a male patient). This observation suggests the value and need of incorporating previously derived clinical and pathologic information into a classification algorithm to avoid predictions that are obviously inconsistent with the pretest pathologic and clinical data.

In the coming years, adjustments to existing assays and development of new genomic assays for cancer diagnostics are likely to continue. There is currently some uncertainty as to which physicians (oncologists or pathologists) will be primarily responsible for ordering and interpreting these studies. Our findings strongly support the importance of ensuring that pathologists are the primary physicians responsible for interpretation of the TOO-FRZ test, and that standard pathologic analyses of morphology and immunohistochemistry are integrated with the TOO-FRZ findings to increase the likelihood of an accurate diagnosis. If the TOO-FRZ results are interpreted in isolation, the results may be misleading. Pathologic and clinical data might one day be systematically incorporated into a molecular-based diagnostic algorithm; however, implementation of this proposal will require additional research, development, and validation. Currently, the best solution is to ensure communication of oncologists with pathologists in the clinical interpretation of the TOO-FRZ assay and in other molecular studies for cancer diagnosis.

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FIGURE 1. Overview of experimental design. The left portion of the figure summarizes the 4 different categories of malignancies evaluated in this study. The middle panel lists the different diagnostic sites included in the TOO-FRZ assay. The right panel lists the different evaluation criteria used for the 4 different categories of malignancies.

FIGURE 2.

Example of a TOO-FRZ result from a tumor originating from a rare morphology from a tissue type included in the TOO-FRZ assay. This tumor specimen was removed from the ovary. Hematoxylin and eosin-stained sections (left panel) showed an epithelial neoplasm with mucinous features. Immunohistochemical stains showed strong positivity for CK7 (middle panel) and CK20 (right panel), with scattered expression of CDX2, consistent with mucinous differentiation. The clinical history, operative findings, and gross examination were most consistent with an ovarian primary; however, the possibility of a metastasis from the gastric or pancreatobiliary tract could not be entirely excluded. The tumor was diagnosed as “Favor ovarian mucinous tumor of LMP, intestinal type.” The TOO-FRZ assay showed an SS 30 for both gastric and ovarian tissue types (bottom panel).

FIGURE 3.

Summary of results from 3 groups of tumors of known primary. The numbers of cases that received correct, incorrect, or indeterminate results from the 3 groups of malignancies of known primary (common morphologies from TOO-FRZ tissue types, uncommon morphologies from TOO-FRZ tissue types, tissue types not on TOO-FRZ) are represented in the bar plot. The y axis indicates the number of cases.

TABLE 1

Common Morphologies From Tissue Types Included in TOO-FRZ Assay

Tumor Type	N	Primary; Metastatic	TOO Prediction Correct	Incorrect Prediction Site
Bladder transitional cell carcinoma	1	0; 1	1 of 1	
Breast invasive ductal carcinoma	1	0; 1	1 of 1	
Colonic adenocarcinoma	5	1; 4	5 of 5	
Gastric adenocarcinoma	2	1; 1	0 of 2	Indeterminate (n=1); Incorrect (n=1) (For incorrect case, both ovary and pancreas with SS 30)
Hepatocellular carcinoma	1	0; 1	1 of 1	
High grade sarcoma	2	2; 0	2 of 2	
Melanoma	2	0; 2	2 of 2	
Ovarian serous carcinoma	4	2; 2	4 of 4	
Pancreatic ductal adenocarcinoma	2	0; 2	1 of 2	Gastric
Prostatic adenocarcinoma	2	0; 2	2 of 2	
Renal cell carcinoma	6	1; 5	6 of 6	
Thyroid papillary carcinoma	1	0; 1	1 of 1	
Total	29	7; 22	26/29 correct (90%) 1/29 indeterminate (3%) 2/29 incorrect (7%)	

TABLE 2

Uncommon Morphologies From Tissue Types Included in TOO-FRZ Assay

Tumor Type	Primary (P) or Met (M)	TOO Prediction	Correct Incorrect Prediction Site
Gastrointestinal stromal tumor *	M	Indeterminate	No tissue site with confident prediction
Ovarian clear cell carcinoma *	P	Indeterminate	No tissue site with confident prediction
Ovarian clear cell carcinoma *	P	Correct	
Thyroid medullary carcinoma *	M	Correct	
Liver embryonal sarcoma	M	Indeterminate	No tissue site with confident prediction
Ovarian granulosa cell tumor	M	Correct	
Ovarian mucinous LMP	P	Indeterminate	Indeterminate; Both Ovary and Gastric with SS 30
Ovarian mucinous LMP	P	Indeterminate	Indeterminate; Both Ovary and Gastric with SS 30
Primary lung adenocarcinoma with enteric differentiation	P	Incorrect	Colorectal
Synovial sarcoma	M	Indeterminate	No tissue site with confident prediction
Total	P=5; M=5	3/10 correct (30%) 6/10 indeterminate (60%) 1/10 incorrect (10%)	

The other rare morphologies come from tissue types included in the TOO-FRZ assay, but it was not stated in the TOO-FRZ assay literature whether these specific morphologies were included or excluded on the assay panel.

* Morphology was explicitly included in the TOO-FRZ panel.⁹

TABLE 3

Tissue Type/morphology From Sites Not Included on TOO-FRZ Assay

Tumor Type	Primary (P) or Met (M)	TOO Prediction	Incorrect Prediction Site
Endometrial adenocarcinoma	M	Incorrect	Ovary
Endometrial adenocarcinoma	P	Incorrect	Softtissue sarcoma
Lung carcinoid	M	Indeterminate	No tissue site with confident prediction
Total	P=1; M=2	2/3 incorrect (66.7%) 1/3 indeterminate (33.3%)	

TABLE 4**Malignancies of Unknown Primary**

Pre-test Differential Diagnosis	TOO Result	Diagnostically Useful Result
Poorly differentiated carcinoma, possible sites include: lung, prostate, renal, and skin	Indeterminate	Limited
Favor malignant mesothelioma	Ovary	Limited
Adenocarcinoma, possible sites include: breast, lung, thyroid, ovarian, and endometrial	Lung	Yes
Carcinoma, possible sites include: renal, gastric, pancreatic, and bladder	Indeterminate	Limited
Neuroendocrine carcinoma from lung or thyroid	Indeterminate	Limited
Poorly differentiated squamous cell carcinoma, possible sites include: anogenital, lung, and bladder sites	Lung	Yes
Favor uterine papillary serous carcinoma	Ovary	Indeterminate
Total		4/7 (57%) limited 2/7 (29%) yes 1/7 (14%) indeterminate