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Potential clinical utility of gene-expression profiling in identifying tumors of uncertain origin

Aim: To evaluate the potential impact of a gene-expression-based test on the diagnosis of primary tumors in difficult-to-diagnose cases. **Materials & methods:** The Tissue of Origin Test uses 2000 gene measurements to classify the most likely primary tumor. We categorized 284 consecutive samples by pretest diagnosis, then recategorized the samples using test results to identify cases with changes in diagnosis. **Results:** A total of 64% of incoming diagnoses were nonspecific. A leading diagnosis for the primary site was provided for remaining cases, indicating an unresolved differential. Overall, the test predicted a change in the 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 cases. **Conclusion:** A new molecular diagnostic has the potential to change both primary site identification and therapy selection for the majority of patients tested.

KEYWORDS: cancer of uncertain origin ■ gene-expression test ■ tissue of origin

In approximately 3–5% of all new cancer cases, the primary site is difficult to diagnose [1]. These cases manifest in a wide variety of clinical presentations, and pathologists and oncologists often cannot reach consensus on the primary site even after an exhaustive evaluation involving a complete review of clinical history, physician examination, complete blood count, urinalysis, serum chemistries, chest radiograph and computed tomography or MRI [2,3]. Pathological evaluation usually involves an extensive use of immunohistochemistry (IHC) stains. However, IHC stains often lack the range, sensitivity and specificity required for selection of a primary site, particularly if a range of possible sites exists [4–7]. As a result, the success rate of identifying the tissue of origin in patients who present with an unresolved diagnosis can be less than 30% [8].

While rapid advances are being made in characterizing tumors for the presence or absence of targets for drug treatments, the studies that determine the effectiveness of a cancer treatment are uniformly conducted in cohorts of patients with particular tissues of origin whether it is breast, prostate, colorectal or other types of cancer. In addition, the presence of a particular target in a tumor does not necessarily predict activity unless that information is linked to knowledge of the primary site. For example, both breast carcinoma and ovarian cancer are frequently estrogen receptor positive (ER⁺) but antiestrogen therapy is only effective in treatment of ER⁺ breast cancers. Where it is not self evident from clinical, surgical or imaging findings, determination of the tissue

of origin remains a key diagnostic element in the treatment decisions of medical oncologists and radiation therapists.

Gene-expression profiling has been used in recent years to assist in the diagnosis of the primary site in conjunction with standard clinical and pathological evaluations. Several gene-expression molecular diagnostic assays are available and have been validated in clinical trials to identify the primary site in 75–90% of cancers of uncertain primary site [9–14,101]. These tests may be used as a way to support one diagnosis over the other when the histological and clinical evidence points to different primary sites, or if there is simply insufficient evidence to make a diagnosis of the primary site. The Tissue of Origin Test (Pathwork[®] Diagnostics, CA, USA) is a molecular diagnostic test developed as an aid to diagnosis for difficult-to-diagnose malignancies. This study used data collected by a central laboratory (Pathwork Diagnostics Laboratory, CA, USA) on a series of consecutive cases to address the following questions: whether the use of a gene-expression profiling test such as the Tissue of Origin Test results in a change of primary site diagnosis (tissue of origin), and whether the test contributes to the diagnostic precision of cases where the primary site is uncertain. The study also explored whether a change in diagnosis could result in a change in the patient's treatment using the National Comprehensive Cancer Network (NCCN) guidelines for the treatment of tumors of specific primary sites. The purpose of the study was to explore and

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describe the clinical utility of molecular diagnostic tests in the diagnosis and management of this patient population.

Methods & materials

This retrospective observational study utilized de-identified data from a consecutive series of cases submitted to a central laboratory from 1 January to 31 December 2009. While other gene-expression molecular diagnostics are available for determining the primary site of malignancies, this study analyzed consecutive cases that were evaluated during the test's first year of commercial availability.

■ Test description

The Pathwork Tissue of Origin Test is a microarray-based assay that uses measurements of expression for 2000 genes to compare a patient's tumor to a panel of 15 known tumor types (bladder, breast, colorectal, gastric, testicular germ cell, kidney, hepatocellular, non-small-cell lung, non-Hodgkin's lymphoma, melanoma, ovarian, pancreatic, prostate, thyroid and sarcoma). The test assesses the patient's tumor gene expression and provides a similarity score for each of the 15 tumor types included in the assay. To order the test, a referring pathologist sends the sample to the central laboratory, where the tumor RNA is isolated and, in a series of amplification and reverse transcription steps, used to generate biotinylated cDNA. The cDNA is hybridized to the microarray platform and after scanning and signal intensity acquisition; a report on the similarity of the tumor sample to the 15 tumor types is generated. Each specimen produces 15 similarity scores, ranging from 0 (very low similarity) to 100 (very high similarity), that sum to 100. The highest similarity score indicates the likely primary site, while a similarity score ≤ 5 rules out the tissue type as the likely primary site with greater than 99% likelihood [10]. If no similarity score is ≥ 20 , the accuracy of the top score is not assured and therefore not reported. The Tissue of Origin Test received US FDA marketing clearance for the testing of formalin-fixed, paraffin-embedded (FFPE) tumor samples in June 2010 [102].

In the clinical validation study of the Tissue of Origin Test using FFPE specimens, samples were obtained from human tumor tissue banks that had a diagnosis of one of the 15 tumor types included in the Tissue of Origin Test and were sent to three independent processing laboratories. A total of 462 qualified specimens were assessed and the likely tissue of origin as identified by the Tissue of Origin Test was

compared with the reference diagnosis for each specimen [10]. The overall agreement with the reference diagnosis was 88.5%, the negative percentage agreement was 99.1%, and an average of 12 tumor types for each specimen could be ruled out with 99.8% accuracy. Intersite reproducibility was also assessed by testing how often different laboratories reported the same result for identical samples. With 149 qualified paired results, the overall concordance was 89.3% [10].

■ Inclusion & exclusion criteria

Cases were eligible for analysis if the following two conditions were satisfied. First, a Tissue of Origin Test was completed and the test report was returned to the pathologist who ordered the test. Cases with samples containing insufficient tissue for gene-expression profiling were not included in this study. All samples were submitted as FFPE blocks or 10 μm sections with accompanying hematoxylin and eosin stained slides of adjacent sections. All samples were qualified as comprised of at least 60% or greater malignant tumor by a board-certified pathologist. Where necessary, blocks or unstained slides were macrodissected to increase the percentage tumor. Samples were processed in accordance with the previously described procedures [10]. Second, a working diagnosis of malignancy and a biopsy site were provided on the test requisition form prior to carrying out the test. Cases with a pretest diagnosis suggestive of non-malignancy (e.g., infections) were excluded because one of the main goals of the study was to determine the extent to which the gene-expression test result would either change or add precision to the pretest diagnosis of the tissue of origin.

■ Determination of pretest diagnosis

Pretest diagnoses were determined using information provided on the test request form by the pathologist ordering the test. The incoming International Statistical Classification of Diseases and Related Health Problems ICD-9 code or codes, text information in the diagnosis field, and text in the biopsy site field of the test request form were used to classify cases as either ones with specific or with nonspecific diagnoses of likely primary site. Based on the reported biopsy sites, cases were also coded as most likely to be a primary or metastatic malignancy.

■ Determination of post-test diagnosis based on test results

For each case, the Tissue of Origin Test result was evaluated with a consideration of both the test's top prediction and the similarity score

associated with it. Based on the clinical validation study of the test, results are predictive of the tissue of origin if the highest similarity score is ≥ 20 and the tissue types with a similarity score ≤ 5 can be ruled out with $>99\%$ likelihood [10]. In light of the validation study, the following approach was used to determine a post-test working diagnosis. Cases with a predicted diagnosis exceeding a similarity score of 20 were considered as having that post-test diagnosis. If no predicted diagnosis reached a similarity score of 20, a rule-in post-test diagnosis was not made and that case was classified as 'no call'. The post-test diagnosis of primary site was then compared with the pretest diagnosis of primary site.

■ Data analysis

The study outcome was defined in terms of change in diagnosis from pre- versus post-test. All study variables were summarized using descriptive statistics. For continuous variables, sample means, medians and ranges were reported. For categorical variables, numbers and frequencies were provided. The proportions of cases with a change of diagnosis were calculated and the 95% CI associated with each proportion was provided using the modified Wald method. All analyses were conducted in Microsoft® Excel (Microsoft Corporation, WA, USA).

Results

■ Description of patients, samples & pretest diagnoses

Of the 300 consecutive cases, 16 cases were excluded because the diagnosis listed on the test requisition form was not a malignancy, leaving a total of 284 cases (95%) for the final analysis sample. Of these, 43% were male and the mean age was 62 years (median: 62; range: 16–89). The tissue specimens for these cases were from biopsies of a wide range of sites with liver (23%) being the most common site followed by lymph node (19%), omentum/peritoneum (10%), and lung (9%) (TABLE 1).

Of the 284 included cases, 183 (64%) had a nonspecific pretest diagnosis and 101 cases (36%) had a specific pretest diagnosis (i.e., had a diagnosis for a specific tissue of origin). Of these 101 cases, 85 had a pretest diagnosis that matched one of the 15 tissue types on the Tissue of Origin Test panel. The most common diagnoses were lung (33 cases), colorectal (14 cases), breast (10 cases) and ovarian (10 cases). Among the 16 cases that had a specific pretest diagnosis of a tissue of origin that was not included on the Tissue of Origin Test panel, five were uterine

Table 1. Distribution of biopsy sites.

Biopsy site	Number of samples	Percentage of samples (%)
Liver	65	22.9
Lymph node	53	18.7
Omentum and peritoneum	29	10.2
Lung	26	9.2
Soft tissue	20	7.0
Bone	15	5.3
Neck	12	4.2
Brain	9	3.2
Ovary	9	3.2
Colon and rectum	8	2.8
Pleura	7	2.5
Pelvis	4	1.4
Small bowel	4	1.4
Other	23	8.1
Total	284	100

cervix, two esophageal, and one each adrenal, CNS malignancy, endometrial, gallbladder, squamous cell of head and neck, neuroendocrine, parotid, skin and vagina (TABLE 2).

■ Distribution of post-test diagnoses

Post-test diagnoses are shown in TABLE 2. The post-test diagnoses of tissue of origin covered all 15 tissue types on the test panel except for thyroid carcinoma. The test predicted a specific tissue of origin in 96% of cases. The six most common predictions comprising 78% of all test results were colorectal (15%), breast (15%), ovarian (13%), pancreas (13%), lung (11%) and liver (11%). In 4% of cases, no reliable prediction of a tissue of origin could be made because the similarity score for the top prediction was less than 20.

For the 284 cases included in this analysis, each test provided an average of 10.6 (median: 10; range: 5–14) rule-out tissue types. In the 4% of cases for which no prediction of rule-in tissue of origin could be made because the highest similarity score was less than 20, an average of seven tissue types (range: 5–9) on the panel could be ruled out, thus narrowing the range of differential diagnosis options.

■ Comparison of pre- & post-test diagnoses

The Tissue of Origin Test resulted in a new post-test diagnosis of tissue of origin for 81% of cases (95% CI: 76–85%). Among those without a specific diagnosis prior to the Tissue of Origin Test, 94% received a specific diagnosis post test. Among those with a primary site

Table 2. Comparison of pre- and post-test diagnoses.

Pretest diagnosis	Post-test diagnosis										Total				
	No call	Urinary bladder	Breast	Colorectal	Gastric	Testicular germ cell	Kidney	Liver	Lung	Non-Hodgkin's lymphoma		Melanoma	Ovarian	Pancreas	Prostate
Nonspecific	11	1	30	24	9	1	7	19	15	2	1	22	29	12	183
Breast		5	2						1	1		1			10
Colorectal			10				2	2	1		1				14
Kidney	1					1									2
Liver							6								6
Lung	1	1	6	2	1	4	14				4				33
Ovarian			1								5	3		1	10
Pancreas	1			1		1		1				2			4
Prostate			1			1	1	1				1	1		6
Adrenal							1								1
CNS malignancy			1												1
Uterine cervix			1								3			1	5
Endometrial						1									1
Esophagus			1									1			2
Gallbladder				1											1
Head and neck				1											1
Neuro-endocrine											1				1
Parotid														1	1
Skin			1												1
Vagina											1				1
Total	12	4	42	43	11	2	14	30	32	3	1	38	36	1	284

Numbers in bold suggest no change in diagnosis before and after the Tissue of Origin Test.

indicated prior to the Tissue of Origin Test, 55% received a test result for a different primary site. In 15% of cases, the Tissue of Origin Test confirmed the pretest diagnosis of tissue of origin (95% CI: 12–20%). In 4% of cases (95% CI: 2–7%), the Tissue of Origin Test provided no additional information with respect to a single suspected primary site, however, rule-out tissue types were available for these cases (FIGURE 1) and narrowed the range of differential diagnoses.

In 94% of the 183 cases with a nonspecific pretest diagnosis, the post-test diagnosis was of a specific tissue of origin. The distribution of test results for these cases was similar to the overall distribution of test results: breast (16%), pancreas (16%), colorectal (13%), ovarian (12%), liver (10%) and lung (8%).

In the 101 cases with a pretest diagnosis of a specific tissue of origin, the test confirmed the diagnosis in 44% of the cases. In the remaining 55% of cases, the test predicted a new, unsuspected tissue of origin, and did not predict a diagnosis in one case. The most common new post-test diagnoses were ovarian (n = 20%), colorectal (16%), breast (13%) and renal cell (11%). This category also included one diagnosis of testicular germ cell and one of non-Hodgkin's lymphoma.

Discussion

The cases described in this series represent a small minority of cases of metastatic tumor for which neither the clinical history, IHC nor imaging studies could provide the treating physician with a definitive diagnosis of the tissue of origin. With the advent of tumor targeted therapies, the ability to identify the tissue of origin has great value in selecting an optimal treatment course, which may increase survival or, in some cases, even cure the disease. While the median survival rate for patients with an unidentified primary site ranges from 6 to 10 months, many patients with metastatic cancers such as testicular germ cell carcinomas or non-Hodgkin's lymphomas can be cured and patients receiving tissue-specific therapies for other tumor types (e.g., ovarian, prostate, breast, renal or colon cancer) may have survival measured in years even after the discovery of metastatic disease [101]. In addition, removing uncertainty around the tissue of origin can validate the use of specific treatments such as trastuzumab for breast cancer; erlotinib for lung cancer; sorafenib and sunitinib for renal cell carcinoma; and bevacizumab for renal cell carcinoma, breast cancer and colorectal cancer.

However, as the majority of cancers of unknown or uncertain primary site are poorly differentiated or undifferentiated carcinomas, the

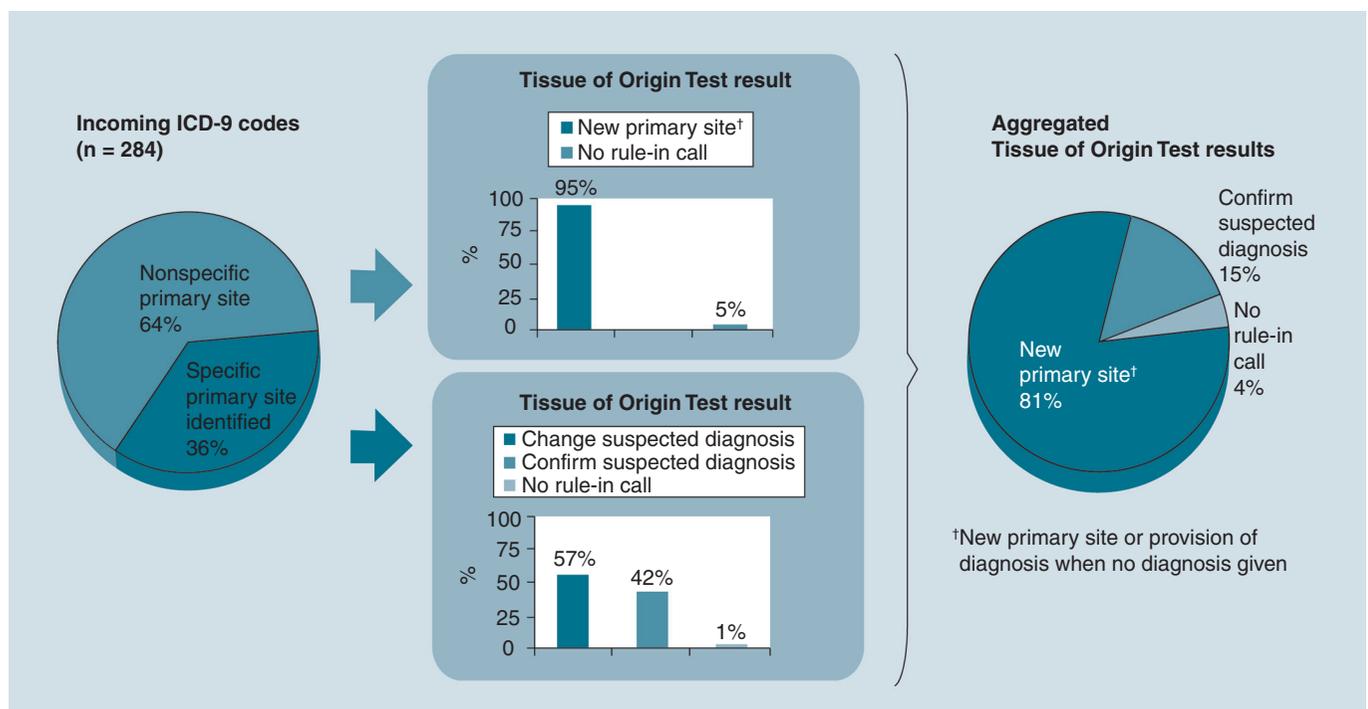


Figure 1. Change in diagnosis based on Tissue of Origin Test results. Tissue of Origin Test results are categorized by incoming diagnosis (nonspecific vs specific tissue diagnosis specified) and by test result (new primary site vs confirmation of suspected primary site).

ICD: International Statistical Classification of Diseases and Related Health Problems.

initial diagnostic evaluation, including history and physical examination, blood and imaging studies, and tumor pathological evaluation (e.g., IHC marker studies), does not usually define the tissue of origin. Although more precise IHC stains have been developed, no single IHC or standard IHC panels can determine tissue origin and in many cases, the IHC approach is not standardized. It is estimated that even after an exhaustive work-up with IHC, the success rate of identifying the origin of metastatic tumors is only 67% overall and it drops to less than 30% for patients presenting with metastatic cancer [7,8].

Gene-expression profiling is an emerging diagnostic tool for identifying tumors. The frequency with which the Tissue of Origin Test generates a match between the RNA expression pattern of the clinical sample and specific tumor types on the test panel is largely consistent with the known distribution of tissue of origin for occult primary malignancies as reported in autopsy series. Both autopsy studies and the Tissue of Origin Test in patients with undiagnosed primaries find colorectal cancer, non-small-cell lung cancer, pancreatic cancer and hepatobiliary cancer most frequently [15,16,102]. Interestingly, ovarian, breast and renal cancers are becoming increasingly recognized as the tissue of origin. The Tissue of Origin Test appears to identify primary sites previously infrequently recognized at necropsy, which historically fails to discover a primary site over 25% of the time. With more treatable primary cancers now being identified, the potential for improved palliation with specifically directed primary therapy appears possible. Finally, it is not surprising that non-Hodgkin's lymphoma, melanoma, thyroid, testicular germ cell and prostate carcinomas comprise so few identified primary sites with the Tissue of Origin Test (combined <3%) given the relatively effective IHC markers available to identify these tumor types.

The potential impact of a change in diagnosis of primary site on treatment selection was assessed using the NCCN guidelines. It was assumed that in the absence of the Tissue of Origin Test, treatment would be based on the pretest diagnosis of tissue of origin as stated on the test requisition form. Following the test, treatment would be selected on the basis of post-test diagnosis of tissue of origin, namely, the Tissue of Origin Test's top prediction of the tissue of origin. In both scenarios, treatment selection would follow the NCCN guidelines for treatment of advanced metastatic disease of patients with a good performance status.

Patients with a nonspecific pretest diagnosis were recommended for chemotherapy with either taxane and platinum combination or gemcitabine plus platinum combination. For patients with squamous cell differentiation, 5-fluorouracil was added [101]. While these empiric therapies may be a reasonable approach to treatment of occult primary tumors, they would rarely be the standard of care once a tissue of origin is identified.

Using the pretest diagnosis, the majority of all cases would have been treated with empiric chemotherapy for cancers of unknown primary site. Yet, using the post-test diagnoses of tissue of origin, treatment with taxane and platinum would only be indicated for approximately 20% of cases because 81% of cases had a post-test diagnosis of a specific tissue of origin.

Only for the cases where the pretest diagnosis was non-small-cell lung cancer and the post-test diagnosis was ovarian cancer might the recommended chemotherapy regimens be the same despite the change in diagnosis. However, patients designated as 'new' lung primary cancers are now candidates for EGF receptor mutational analysis and potential treatment with anti-EGF receptor agents.

Although the clinician's diagnosis and treatment decision presented above reflect a hypothetical scenario (i.e., a final working diagnosis was made exclusively on the basis of the gene-expression profiling test and the post-test treatment decision was exclusively driven by the gene-based diagnosis) that may differ in actual clinical practice, this study demonstrated the potential clinical utility of a gene-profiling molecular diagnostic test when integrated into a standard diagnostic process in conjunction with current clinical and pathological practice.

The Tissue of Origin Test and other gene-profiling molecular tests are intended for use in the context of the patient's clinical history and other diagnostic tests and the results are not intended to be stand alone diagnoses of a metastatic tumor's primary site. This study analyzed a consecutive case series submitted to Pathwork from the community. The pretest diagnosis and biopsy site information submitted on the test requisition form were used as an approximation of the clinician's pretest diagnostic impression. The nonstandardization of these fields in the test requisition form precludes an assessment of the accuracy or certainty of these diagnoses. However, the fact that a test was requested is a strong indication that a specific origin could not be determined or multiple differential diagnoses could not be ruled

out. In this study, approximately two thirds of the submitted cases had a nonspecific working diagnosis and the remaining one third of cases had a suspected primary tissue type where differential diagnoses may exist. However, without the detailed clinical history, imaging and pathology reports, one could not determine how frequently differential diagnoses existed and what the specific diagnoses were. The current study is a very simplified model of how the Tissue of Origin Test is likely to impact clinical diagnoses and treatment decisions. Clinical decision-making in real practice is multifactorial. A decision on diagnosis will take into account a wide variety of clinical, imaging and pathologic features of the case. Treatment decisions are not limited only to first-line chemotherapy as modeled here but also include decisions on subsequent chemotherapy, radiation therapy, surgery and hospice. Such differences could lead to either an overestimate or underestimate of the impact of this test on diagnosis and treatment decisions in clinical practice. Rather than being the 'final call' of the diagnosis, the prediction of the Tissue of Origin Test should be integrated with other information such as the clinical history, imaging data, histology and IHC data to arrive at a final diagnostic consensus of tissue origin. In addition, optimal treatment should be made in light of prognostic factors, in particular performance status, presence of liver metastases, lactate dehydrogenase levels and serum albumin, and treatment plans should be made jointly between physicians, patients, and their relatives or caregivers. Therefore, there may have been cases where a change in diagnosis did not necessarily lead to a change in treatment [103]. Most of the cases submitted for the Tissue of Origin Test are metastatic, and therefore, clinicians will be selecting therapy for advanced cancer. However, not all of these patients necessarily have stage IV disease. For example, a metastatic axillary mass where the differential is between breast cancer and lung cancer could be stage II for the former and stage IV for the latter. However, for most of the cases submitted, selection of therapy will likely be for metastatic or advanced disease.

Although current literature suggests appropriate primary tumor treatment benefits patients, studies have not demonstrated whether tissue of origin identification in cancer with unresolved tumors improves treatment outcomes.

Prospective or retrospective registries, including clinical and laboratory findings, imaging, pathological reports, treatment and long-term survival data, are warranted. A retrospective observational study of physicians ordering the Tissue of Origin Test is currently underway to understand the percentage change in physician-reported diagnosis of tissue site origin before and after receiving the Tissue of Origin Test results, the percentage change between post-test treatment and planned treatment prior to ordering the Tissue of Origin Test, and the response to cancer-specific therapy and patient outcomes [17]. The preliminary results suggested that after receiving the Tissue of Origin Test, diagnosis of tissue site of origin and first-line cancer specific management both changed in the majority of patients [17].

This study indicates that the predictions of the Tissue of Origin Test or other similar gene profiling molecular tests, if incorporated into the clinical practice, have the potential to result in a change in diagnosis for approximately four-fifths of the cases and a revision in treatment plan for the majority of patients according to the first-line treatment guided by the NCCN practice guidelines. The correct understanding of the tissue of origin is not only important for selection of first-line treatment, but it is also needed for selection of second-line chemotherapy, decisions regarding debulking surgery or surgery for resection of metastases, optimal management of symptoms, recommendations regarding entry into hospice care, and making recommendations to family members as to familial risk of cancer and the need for genetic testing. Furthermore, follow on studies are needed to provide additional evidence of clinical utility through observation of treatment outcomes directed by the this and other gene-expression-based tests for determination of primary site.

Conclusion

In summary, the results of this analysis indicate that the Tissue of Origin Test, as used in clinical practice, may likely impact the diagnosis of primary site for the preponderance of cases. The changes in the diagnosis of primary site based on the Tissue of Origin Test results could lead to a major change in the first-line chemotherapy for the majority of patients when treatment choice is guided by the primary site.

Executive summary

- The Tissue of Origin Test is commercially available and is being integrated into clinical practice.
- This new molecular diagnostic test has the potential to change both primary site identification and therapy selection for the majority of patients tested selected according to the widely-used National Comprehensive Cancer Network guidelines for site-specific therapy.

Financial & competing interests disclosure

Quorum Consulting, employer of Marianne Laouri, consults with Pathwork Diagnostics. Meredith Halks-Miller and W David Henner are employees of and stockholders in Pathwork Diagnostics. J Scott Nystrom is a consultant for Pathwork Diagnostics. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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