DOI:10.1093/jnci/djt071 JNCI Journal of the National Cancer Institute Advance Access published May 2, 2013

ARTICLE

Molecular Profiling Diagnosis in Unknown Primary Cancer: Accuracy and Ability to Complement Standard Pathology

F. Anthony Greco, Wayne J. Lennington, David R. Spigel, John D. Hainsworth

Manuscript received September 13, 2012; revised March 22, 2013; accepted April 2, 2013.

Correspondence to: F. Anthony Greco, MD, Director, Sarah Cannon Cancer Center, 250 25th Ave N, Ste 100, Nashville, TN 37203 (e-mail: fgreco@tnonc.com).

Background Molecular tumor profiling (MTP) is a potentially powerful diagnostic tool for identifying the tissue of origin in patients with cancer of unknown primary (CUP). However, validation of the accuracy and clinical value of MTP has been difficult because the anatomic primary site in most patients is never identified.

Methods From March 2008 through January 2010, clinicopathologic data from 171 CUP patients who had MTP (CancerTYPE ID; bioTheranostics, Inc, San Diego, CA) performed on archived material were evaluated. The accuracy of MTP diagnoses was evaluated by comparison with 1) latent primary tumor sites found months/years later; 2) initial single diagnoses by immunohistochemistry (IHC); and 3) additional directed IHC and/or clinicopathologic findings evaluated after MTP diagnoses.

Results A single MTP diagnosis was made in 144 of 149 patients with adequate tumor specimens. Eighteen of 24 patients with latent primaries discovered months to years later had correct diagnoses by MTP (75%), and these diagnoses compared favorably with IHC. Single IHC diagnoses matched MTP diagnoses in 40 of 52 patients (77%). IHC predictions of 2 or more possible primaries compared poorly with MTP diagnoses. However, additional targeted IHC and clinical/histologic evaluation supported the MTP diagnosis in 26 of 35 patients (74%). Clinical features were usually consistent with MTP diagnoses (70%).

Conclusions The diagnostic accuracy of this MTP assay was supported by a high level of agreement with identified latent primaries (75%), single IHC diagnoses (77%), and additional directed IHC and/or clinical/histologic findings (74%) prompted by the MTP diagnoses. MTP complements standard pathologic evaluation in determining the tissue of origin in patients with CUP, particularly when IHC is inconclusive.

J Natl Cancer Inst

Cancer of unknown primary (CUP) comprises a heterogeneous group of patients with metastatic cancer and clinically unidentified primary tumor sites (1). They are ideal candidates for classification of their tissue of origin by gene expression profiling of their biopsies (2,3). Several retrospective studies have suggested that molecular profiling of cancer cells may be useful in identifying the tissue of origin (4–8).

Identification of the tissue of origin by gene expression profiling has recently been reported to improve the survival of CUP patients by allowing more site-specific therapy to be administered (9), rather than the empiric regimens that have been the standard approach for two decades. As therapies for solid tumors improve and become more tumor-specific, the value of an accurate diagnosis of the tissue of origin becomes increasingly important.

Molecular tumor profiling (MTP) assays designed to determine the tissue of origin have been shown as a group to be about 85% accurate in determining the cancer type of known metastatic and primary cancers (10–15). It may seem logical to assume that MTP assays would also be as accurate in CUP, but this may not be true because the biology may be different, primary tumors are not clinically identified, and these cancers may have different genetic aberrations compared with known cancers. Because CUP patients do not have clinically identifiable anatomical primary tumor sites, it remains problematic, without an autopsy, to verify the accuracy of a MTP diagnosis.

Verification of the assay results at autopsy would seem ideal but is not feasible because autopsies are difficult to obtain in this era. However, there are several methods other than an autopsy to assess the accuracy of MTP diagnoses in CUP. Evaluation of CUP patients who subsequently develop clinically detectable primary sites (latent primary sites) months after their initial presentation offers a direct method or gold standard to assess the accuracy of MTP diagnoses. Two other indirect methods involve the comparison of specific MTP diagnoses to other findings: 1) single diagnoses made by immunohistochemistry (IHC) staining and 2) additional directed clinical/histologic findings and IHC staining obtained after the MTP diagnosis was available. In this study, all three methods were used to better define the accuracy of the MTP assay and its role in the diagnostic evaluation of CUP patients.

Methods

Patient Selection and Study Population

A total of 171 patients divided into two groups was selected. The first group, which contained 151 patients, was prospectively seen from March 2008 through January 2010 at the Sarah Cannon Cancer Center and clinics of Tennessee Oncology. The second group was comprised of 20 patients recognized retrospectively from a group of 501 patients seen between 2001 and 2008 who had latent primary tumor sites discovered after their initial diagnosis. All patients had either excisional/incisional biopsies or core needle biopsies (fine needle aspirations excluded).

The definition of CUP included no anatomical primary site detected after an evaluation consisting of complete history; physical examination; complete blood count; chemistry profile; prostate specific antigen (PSA) in men; urinalysis; computed tomography scans of chest, abdomen, and pelvis; mammography in women; and appropriate additional targeted evaluation of any specific signs or symptoms. Patients with latent primary tumor sites discovered were included if the anatomical primary site was identified 8 weeks or later after the patient's initial evaluation failed to detect a primary site. Patients within favorable subsets, as previously described (1), were excluded from this study. An institutional review board found official review unnecessary because all patient information was deidentified and no extra study procedures were performed.

Assay Methods

Patients had a standard pathologic evaluation of their biopsy specimen, including histologic examination and IHC stains. These biopsies were initially evaluated by several pathologists because many patients were referred after their pathologic "diagnosis." In most tumors in the prospective patient series, IHC staining was done with well-recognized antibodies for the detection of CK7, CK20, TTF-1, CDX-2, and several other proteins in a formalin-fixed Ventana assay platform. Stains were usually selected based on the histology and the clinical setting. Classic staining profiles were required for diagnoses of a single tissue of origin (16). One pathologist (W. J. Lennington) reviewed the pathologic data on the 151 prospectively evaluated patients and helped to decide which additional IHC stains to obtain.

The MTP assay (CancerTYPE ID; bioTheranostics, San Diego, CA) was performed on biopsies, as previously described (12). The CancerTYPE ID assay is a 92-gene reverse-transcription polymerase chain reaction assay developed to predict the tissue of origin in CUP patients (10,12). In the validating studies, the assay correctly identified the tissue of origin in 85% of patients with tumors of known primary. The first version of the assay, which was used in this study, was capable of identifying 26 different tumor types (12).

If the MTP assay diagnosis prediction did not match any possible diagnoses made by pathologic examination, additional IHC (if tissue available) and directed clinical/histologic evaluation were done. The results of the MTP assay were not generally used to plan therapy for these patients because there were no data to support an improved outcome from this approach during the study period.

Statistical Analysis

The primary purpose of this study was to estimate the accuracy of the MTP assay in tissue of origin diagnosis. Sample sizes and efficacy/accuracy were determined by single-stage designs (17) with type 1 errors of 5% and powers of 80% to 90%. The direct method (considered a gold standard) compared the MTP diagnoses with the actual latent primary tumor sites found. An accuracy of ≥60% determined solely by the assay with a ≤30% inaccuracy rate was the target endpoint considered to be relatively accurate and clinically useful. This would require at least nine of 17 correct MTP predictions and no more than five incorrect predictions (type 1 error = 5%; power of 80%).

Two indirect methods were also employed to estimate the accuracy of the assay. The degree of agreement of the MTP assay with the single IHC diagnoses is an important estimate of the accuracy of the MTP diagnoses. We decided that ≥50% agreement and ≤30% disagreement were necessary to consider the molecular assay sufficiently accurate, and this would require 53 patients (type 1 error = 5%; power of 90%). A second method involved patients whose MTP assay diagnoses did not agree with any of the suspected diagnoses made by IHC. In these patients, additional directed IHC and/or clinicopathologic review were obtained in an attempt to support or refute the MTP diagnosis. We decided that additional data supporting the MTP diagnosis in ≥60% with ≤30% having no supporting data would substantiate the relative accuracy of the molecular diagnoses, and this would require 34 patients (type I error = 5%; power of 90%).

Results

Patient Characteristics and MTP Assay Results

The characteristics of the 171 patients are presented in Table 1. Female patients were slightly more common than male patients, and adenocarcinoma represented the most common histologic diagnosis. The majority of the patients had multiple metastatic sites.

The MTP assay diagnoses are listed in Table 2. In 22 patients (12.9%), there was insufficient tumor to do the assay. In five additional patients (3%), the assay was successful but was not diagnostic of a single tissue of origin (unclassifiable). In 144 of 149 patients with adequate tumor specimens, a single diagnosis was rendered (96%). Twenty-three tumor types were predicted.

Table 1. Patient characteristics (n = 171)

Characteristic	No. of Patients (%)
Median age, y (range)	59 (24–85)
Sex	
Male	80 (47)
Female	91 (53)
Histologic diagnosis	
Adenocarcinoma	63 (37)
Poorly differentiated adenocarcinoma	33 (19)
Poorly differentiated carcinoma	44 (26)
Squamous cell carcinoma	9 (5)
Neuroendocrine carcinoma	
Well differentiated	2 (1)
Poorly differentiated	8 (5)
Poorly differentiated neoplasm/uncertain lineage	12 (7)
Number of metastatic sites	
1	70 (40)
2	59 (34)
≥3	42 (26)

Table 2. Molecular profile assay diagnosis (n = 171)

Site	No. (%)
Insufficient tumor	22 (12.9)
Unclassifiable	5 (3.0)
Colorectal	26 (15.2)
Lung/adeno, large cell	18 (10.5)
Lung/small cell	6 (3.5)
Lung/squamous cell	1 (0.6)
Breast	15 (8.8)
Hepatocellular	10 (5.8)
Ovary	9 (5.2)
Pancreas	9 (5.2)
Kidney	7 (4.0)
Bladder	7 (4.0)
Gallbladder	6 (3.5)
Skin/squamous	5 (3.0)
Melanoma	5 (3.0)
Sarcoma	4 (2.3)
Endometrium	3 (1.7)
Testicle	3 (1.7)
Thyroid	2 (1.2)
Stomach	2 (1.2)
Mesothelioma	2 (1.2)
Prostate	1 (0.6)
Brain	1 (0.6)
Lymphoma	1 (0.6)
Uterine cervix	1 (0.6)

The clinical features were generally consistent with the MTP assay diagnoses. The metastatic sites were noteworthy in several molecularly diagnosed patients. In those diagnosed with ovarian or breast carcinoma, nine of nine and 13 of 15 were female patients, respectively. Typical/expected metastatic sites were seen in colorectal (90% of patients had liver and/or peritoneal metastasis), non–small cell lung (88% had mediastinal or hilar lymph nodes, liver, bone, or multiple lung metastasis), ovary (88% had peritoneal, abdominal/retroperitoneal, or pleural metastasis), pancreatic (88% had liver or peritoneal metastasis), and hepatocellular carcinoma (90% had liver lesions).

Agreement of MTP Assay Diagnoses With Latent Primary Tumor Sites Found Months to Years Later

Retrospective review of 501 patients seen from 2000 to 2008 identified 38 (7.6%) patients with a latent primary tumor site found later during life (median = 12.25 months; range = 2.25–78.5 months after initial diagnosis). Twenty of these 38 patients had adequate initial biopsies and were tested with the MTP assay between March 2008 and January 2009. Data on these 20 patients were previously published (5) Four additional patients with latent primaries were identified during their subsequent follow-up visits among the prospective series of 151 patients (2.6%) seen between March 2008 and January 2010 (Figure 1). In 18 of these 24 (75%) patients, the MTP assay diagnoses matched the latent primary tumor sites (Table 3). Correct assay diagnoses included breast cancer in five patients, ovarian/primary peritoneal cancer in four patients, non–small cell lung cancer in three patients, colorectal/intestinal cancer in two patients, melanoma in two patients, stomach cancer in one patient, and skin squamous cancer in one patient. Four of the 24 (16.5%) MTP diagnoses proved to be inaccurate (1 testicular cancer, 1 colorectal cancer, 1 perivascular epithelioid tumor cancer, and 1 sarcoma), and two of 24 (8.5%) biopsies were unclassifiable (2 non–small cell lung cancer).

A comparison of the use of clinical features, histology of the biopsies, and IHC with the single MTP diagnoses is of interest. A single tissue of origin was diagnosed by IHC in seven of 24 patients (30%) compared with 22 of 24 (92%) by the MTP assay. The single MTP diagnoses were correct in 75% (n = 18 of 24 patients) of patients compared with 25% (6 of 24 patients) of patients with a single diagnosis using the clinicopathologic features alone (see Tables 3 and 4).

Agreement of MTP Diagnoses With Single Tissues of Origin Diagnoses by IHC

Agreements between MTP diagnoses, IHC staining diagnoses, and clinical features are summarized in Figure 2. A single diagnosis of the tissue of origin was made by IHC staining in 59 of the 171 patients (34%). In these patients, a median number of six IHC stains was obtained. Seven patients did not have any remaining biopsy to perform the assay. Fifty-two of these patients had a successful MTP assay, and in 40 (77%) of these patients, the diagnoses matched the IHC diagnoses (Table 5).

Subsequent Additional Clinical/Histologic Findings and IHC Staining to Support or Refute MTP Diagnoses in Patients With Uncertain IHC Diagnoses

In 112 patients (66%), IHC could not diagnose a single tissue of origin. Ninety-seven of these patients had adequate biopsy remaining to perform the MTP assay. IHC predictions of two or more possible primaries compared poorly with MTP diagnoses. Forty-seven patients had two possible primary sites suggested by IHC; in 20 of these patients (42%), the MTP diagnoses matched one of the IHC diagnoses. When three or more possible primary sites were suggested by IHC (50 patients), the MTP assay diagnosis corresponded with one possible IHC diagnosis in 23 (46%) of the patients.

In the 54 patients in whom the MTP assay diagnoses did not agree with any possibilities suggested by IHC, 41 patients (75%) had clinical features consistent with the MTP assay diagnoses. Thirty-five of these 54 patients (64%) had remaining biopsy tissue available and subsequently had additional targeted IHC staining and clinical/histologic evaluation performed to substantiate or refute the MTP diagnoses. In 26 of these 35 patients (74%), these additional findings supported the accuracy of the MTP diagnoses (Table 6). Clinical features were usually consistent with MTP diagnoses (70%).

Discussion

Molecular tumor profiling is a potentially powerful diagnostic tool for identifying the tissue of origin in patients with CUP. However, validation of the accuracy and clinical value of MTP has been difficult because the anatomic primary site in most patients is never identified. Although a number of small series and anecdotal case reports have provided circumstantial evidence to support the value of MTP (ie, clinical and pathologic features consistent with the diagnosis), MTP is not yet considered a standard part of the diagnostic evaluation for CUP patients.

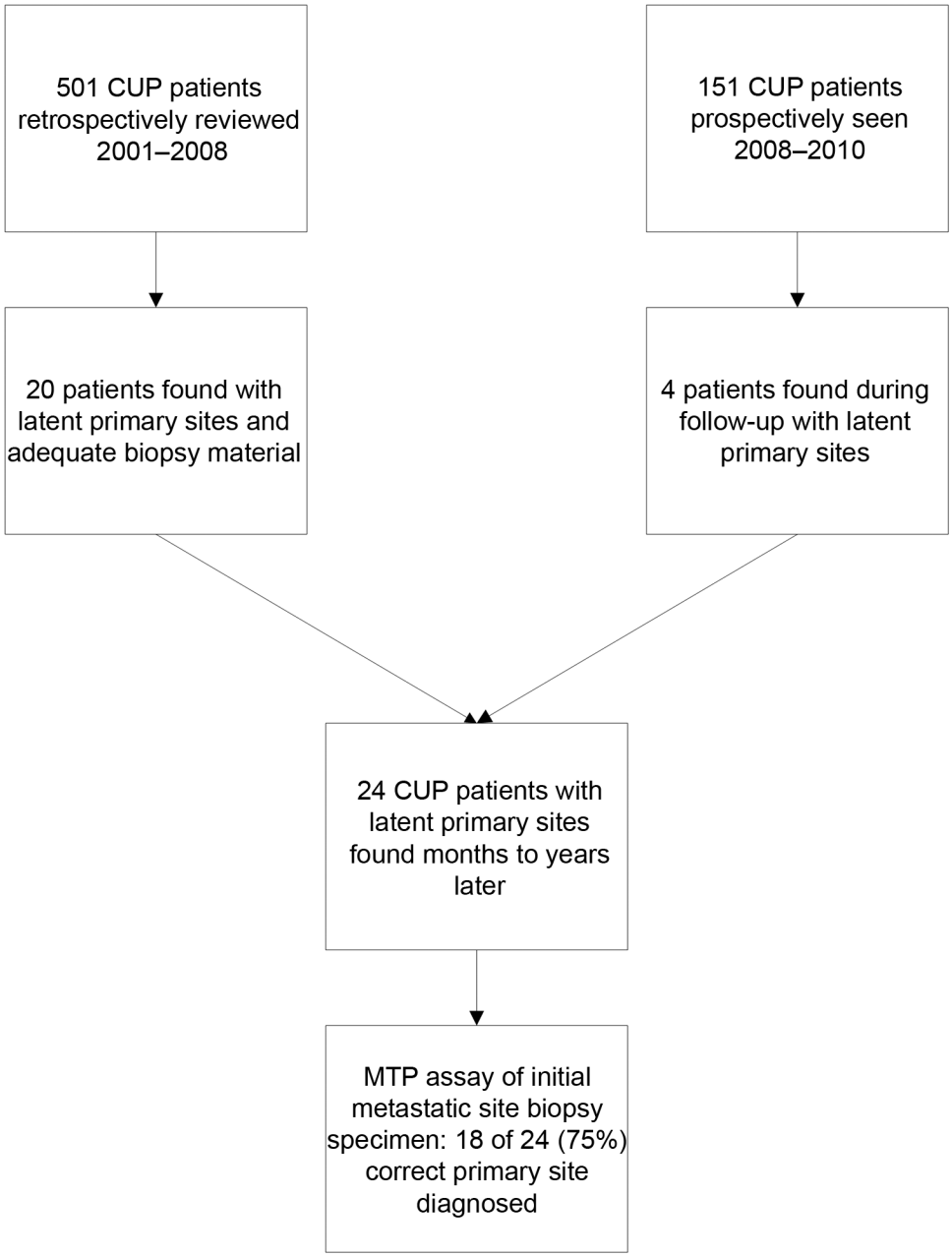

Figure 1. Latent primary tumors in cancer of unknown primary (CUP): patient population. MTP = molecular tumor profiling.

In this study, several methods were used to evaluate the accuracy of MTP diagnoses in a large group of CUP patients. The collaboration with a pathologist allowed a detailed comparison of diagnoses obtained with MTP vs standard pathologic methods, which has not been a focus of previous clinical reports. Although two of the three methods used in this study provide indirect evidence, all support the accuracy and value of MTP in this setting.

The accuracy of MTP was directly assessed in the 24 CUP patients who eventually had their anatomic primary sites identified, thus providing a gold standard for comparison. In this group, the MTP diagnoses matched the documented anatomic primary site in 75% (n = 18 of 24) of the patients. Autopsy studies in the past have revealed that the majority of CUP patients (approximately 75%) have a detectable, usually very small primary site (18), but discovery of latent primary sites during life in patients with CUP is rare, as evidenced by the large number of patients (>650) required to review to find 24. The overall accuracy of the MTP diagnoses met the expectations of this study.

The MTP assay compared favorably with the IHC markers. The correct IHC diagnosis was documented in 25% (n = 6 of 24) of patients, compared with 75% (n = 18 of 24) of patients with an MTP diagnosis in those patients with a reference latent primary recognized months to years later. The number of observations is small, and a less-than-ideal panel of IHC stains was used in this predominantly retrospective group of patients, but the MTP assay more often provided a single correct diagnosis of the primary tumor site.

Table 3. Comparison of immunohistochemistry staining, suspected primary site, molecular assay diagnosis, and latent primary tumor site found*

Patient No.	Age, y/Sex	Biopsy site/ histology	Immunohistochemistry stains on initial diagnostic biopsy	Primary site suspected	Molecular assay diagnosis on initial diagnostic biopsy	Latent primary tumor site found†
1	59/F	Axilla/PDC	CK 7$^+$, CK20$^-$, ER$^-$, PR$^-$, TTF-1$^-$, Her-2/neu$^-$	Breast	Breast	Breast
2	65/F	Axilla/PDA	EMA$^+$, S100$^-$, Her-2neu$^+$, CK7$^+$, ER$^-$, PR$^-$, Melan A$^-$, HMB45$^-$	Breast, Lung	Breast	Breast
3	51/F	Bone/PDC	CK AE1/3$^+$, ER$^-$, Mammaglobin$^-$	Lung	Breast	Breast
4	64/F	Supraclavicular/PDA	CK7$^+$, CK20$^-$, ER$^-$, PR$^-$, TTF-1$^-$	Lung, pancreas, castric	Breast	Breast
5	85/F	Chest Wall/PDA	CK7$^+$, ER$^-$, PR$^-$, TTF-1$^-$, Ca125$^+$	Lung, breast	Ovary‡	Primary peritoneal
6	69/F	Inguinal node/adeno	CK7$^+$, CK20$^-$, CDX-2$^-$, Ca125$^+$	Lung, breast, ovary	Ovary	Primary peritoneal
7	87/F	Abdominal mass/PDA	CK7$^+$, ER$^+$	Lung, ovary, breast	Ovary	Primary peritoneal
8	63/F	Paratracheal node/PDC	CK7$^+$, TTF-1$^-$	Lung, pancreas	Ovary	Ovary
9	49/M	Liver/PDA	CK7$^-$, CK20$^+$, CDX-2$^+$	Colorectal	Intestinal§	Colon
10	47/F	Mesenteric node/PDA	CK7$^-$, CK20$^+$, CDX-2$^+$	Colorectal	Intestinal	Colon
11	42/F	Brain/PDA	CK7$^+$, CK20$^-$, TTF-1$^+$	NSCLC	NSCLC	NSCLC
12	67/M	Subcutaneous mass/ squamous	Not done (squamous cell)	Lung, head/neck	NSCLC	NSCLC
13	59/M	Brain/PDA	CK7$^+$, CK20$^-$, TTF-1$^-$	NSCLC	NSCLC	NSCLC
14	74/M	Bone/adeno	CK7$^+$, Vimentin$^+$, TTF-1$^-$	Lung, renal, pancreas	Gastric	Gastric
15	76/M	Axilla/PDC	CK AE1/3$^-$, HMB45$^-$, S100$^-$, CK7$^-$	Unknown	Melanoma	Melanoma
16	60/M	Small intestine/PDC	CK7$^+$, CK20$^-$, TTF-1$^-$, HMB45$^-$, S100$^-$	Lung, pancreas	Indeterminate	NSCLC
17	38/M	Mediastinum/PDA	CK7$^+$, TTF-1$^-$, PLAP$^-$, CK20$^-$, Chromogranin$^-$	Lung, pancreas	Indeterminate	NSCLC
18	61/M	Supraclavicular/PDC	CK7$^+$, CK20$^-$, TTF-1$^-$	Lung, pancreas	Testes	Pancreas
19	52/M	Retroperitoneum/PDC	CK7$^+$, CK20$^+$	Colorectal, pancreas	Intestinal	Gastric
20	62/M	Chest wall mass/PDC	CK7$^+$, CK20$^-$, Chromogranin$^-$	Lung, pancreas, castric	Sarcoma	NSCLC
21	53/F	Peritoneal Nodule/ adeno	CK7$^+$, CK20$^-$, ER$^+$, PR$^+$, Her-2/ neu$^-$, WT1$^-$, TTF-1$^-$, calretinin-,GCDFP-15$^-$ CDX-2$^+$, Mammaglobin$^-$	Breast	Breast	Breast
22	60/M	Retroperitoneum/ melanoma	Melan A+, HBM45+, CKAE1/AE3$^-$, CK7$^-$,CK20$^-$, S100$^-$, CD34$^-$, inhibin$^-$, chromogranin$^-$, synaptophysin$^-$	Melanoma, sarcoma	Leiomyosarcoma	Perivascular epitheloid cell neoplasm
23	56/F	Inguinal node/ squamous	Not done (squamous)	Anus, uterine cervix	Skin/squamous	Skin/squamous
24	79/F	Inguinal node/spindle cell neoplasm (type not certain)	CK7$^-$, CD10$^-$, CK20$^-$, CKAE1/AE3$^-$, S100$^+$, Mart 1$^+$	Sarcoma/melanoma	Melanoma	Skin/melanoma

* adeno = adenocarcinoma; CK = cytokeratin; EMA = epithelial membrane antigen; ER = estrogen receptor; F = female; HER = human epidermal growth factor receptor; HMB = human melanoma black; M = male; NSCLC = non–small cell lung cancer; PDA = poorly differentiated adenocarcinoma; PDC = poorly differentiated carcinoma; PLAP = placental alkaline phosphatase; PR = progesterone receptor; TTF = thyroid transcription factor.

† Eighteen of 24 latent primary sites were biopsied, and six were documented by medical imaging (mean = 53 weeks; range = 9–314 weeks).

‡ Ovary or primary peritoneal (indistinguishable by assay).

§ Colon or small intestine (indistinguishable by assay).

Table 4. Latent primary tumors in cancer of unknown primary: accuracy of diagnosis of the primary site comparing clinical features and immunohistochemistry (IHC) staining with or without molecular tumor profiling (MTP) assay

Method	Single primary site diagnosed	Percentage of total	Diagnosis (No.)	Percentage of total correct
Clinical features, IHC	7 of 24	30	Breast (2) Lung (3) Colorectal (2)	25
Clinical features, IHC, MTP	22 of 24	92	Breast (5) Lung (3) Intestinal (3) Ovary (4) Sarcoma (2) Melanoma (2) Skin/squamous (1) Gastric (1) Testes (1) Indeterminate (2)	75

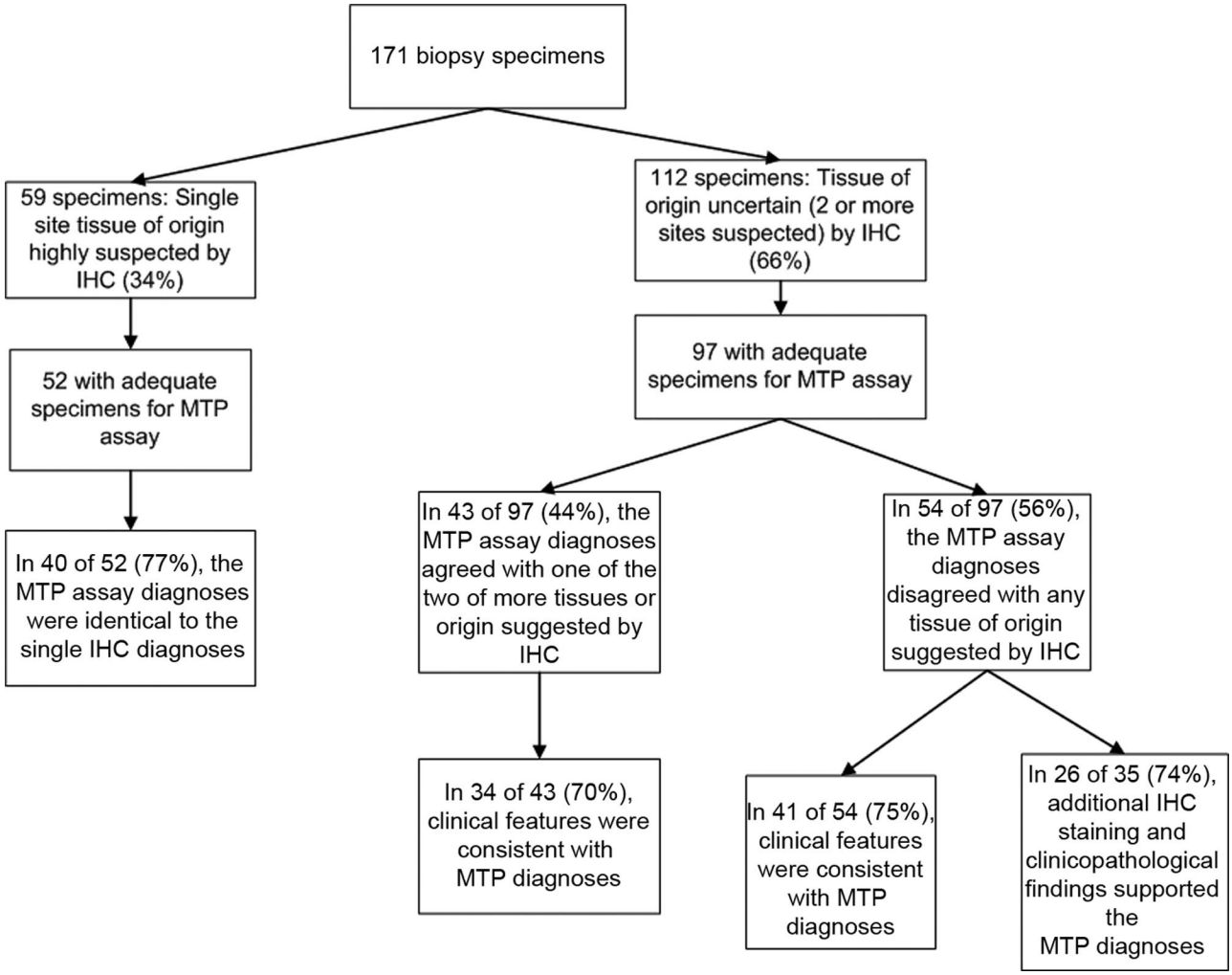

Figure 2. Comparison of molecular profile assay diagnoses with immunohistochemistry (IHC) diagnoses and clinicopathological features in all patients. MTP = molecular tumor profiling.

The agreement of MTP and IHC single diagnoses also provided firm support for the diagnostic accuracy of MTP. In 52 patients with a single diagnosis by IHC (Table 4), the MTP assay diagnosis matched the single IHC diagnosis in 40 (77%). The concordance was particularly noteworthy in colorectal (n = 15 of 16; 93%) and breast cancers (n = 5 of 5; 100%). Similar results in smaller numbers of patients have been reported by others using various MTP assays (4,7,19). The acceptance of IHC single diagnoses in CUP and the agreement of the MTP diagnosis in the majority of patients (77%) help validate the accuracy of the MTP.

Table 5. Comparison of molecular tumor profiling (MTP) assay diagnosis with immunohistochemistry (IHC) in patients with a single site predicted by IHC (n = 52)*

Diagnosis	No. predicted by IHC staining	IHC profile	Agreement of MTP assay diagnoses with IHC diagnoses	% agreement
Lung/adeno/large cell	19	CK7$^+$, CK20$^-$, TTF-1$^+$	14	74
Lung/neuroendocrine	3	CK7$^+$, TTF-1$^+$, synaptophysin$^+$ or chromogranin$^+$ or CD56$^+$	2	66
Colorectal	16	CK7$^-$, CK20$^+$, CDX-2$^+$	15	93
Breast	5	CK7+, CK20$^-$, mammaglobin$^+$ or GCDFP-15$^+$ or ER$^+$	5	100
Melanoma	3	S100$^+$, Melan-A$^+$ or HMB45$^+$, CK7$^-$, CK20$^-$	2	66
Germ cell	2	AFP$^+$, HCG$^+$, PLAP$^+$ or OCT4$^+$	1	50
Hepatocellular	1	Hepar-1$^+$, CD10$^+$	1	100
Ovary	1	CK7$^+$, CK20$^-$, WT1$^+$, CA125$^+$, ER$^+$	0	0
Prostate	1	CK7$^-$, CK20$^-$, PSA$^+$	0	0
Sarcoma	1	vimentin$^+$, S100$^-$, CK7$^-$, CK20$^-$, desmin$^+$	0	0
Total	52		40	77

* AFP = alpha-fetoprotein; Ca125 = cancer antigen; CD10 = commom acute lymphocytic leukemia antigen; CD56 = neural cell adhesion molecule; CDX-2 = caudal type homeobox gene; CK7 = cytokeratin7; CK20 = cytokeratin20; ER = estrogen receptor; GCDFP-15 = gross cystic disease fluid protein; HCG = human chorionic gonadotropin; Hepar-1 = hepatocyte paraffin; HMB45 = anti-human melanosome antibody; Melan-A = melanoma antigen; OCT4 = octamer binding transcription factor; PLAP = placental alkaline phosphatase; PSA = prostate specific antigen; S100 = calcium binding protein; TTF-1 = thyroid transcription factor; WT1 = Wilms tumor.

Table 6. Comparison of molecular tumor profiling (MTP) assay diagnoses with additional clinicopathological findings noted and/or immunohistochemistry (IHC) performed after MTP assay diagnoses in patients with uncertain initial IHC diagnoses (n = 35)*

MTP assay diagnoses (all not suspected initially)	Additional subsequent IHC and/or clinicopathological findings
1. Hepatocellular	Serum α fetoprotein 1326
2. Hepatocellular	Reticulin stain$^+$, serum α fetoprotein 5259
3. Hepatocellular	Hepar1$^+$, serum α fetoprotein 649
4. Hepatocellular	Serum α fetoprotein 501
5. Hepatocellular	Serum α fetoprotein 810
6. Kidney	RCC$^+$
7. Kidney	CA-9$^+$, CD10$^+$, vimentin$^+$
8. Kidney	CA-9$^+$, CD10$^+$
9. Kidney	Vimentin$^+$, histological review—scattered papillary and chromophobe features
10. Mesothelioma	Calretinin$^+$, abdominal mass
11. Mesothelioma	Calretinin$^+$, abdominal and pelvic masses
12. Ovary/clear cell	WT-1$^+$, new ascites
13. Ovary/serous	WTI$^+$, ER$^+$, PR$^+$
14. Sarcoma	Vimentin$^+$, desmin$^+$, rapid growth chest wall and lung masses
15. Sarcoma	Vimentin$^+$, CK7$^-$, CK20$^-$, S100$^-$, LCA$^-$, isolated bone/soft tissue lesion
16. Skin/squamous (also breast signature) suggests skin adnexal carcinoma	Isolated epidermal lesion (primary adnexal skin adenocarcinoma); initially felt to be metastatic
17. Skin/squamous (also breast signature) suggests skin adnexal carcinoma	Isolated epidermal lesion (primary adnexal skin adenocarcinoma); initially felt to be metastatic
18. Lung/neuroendocrine	Synaptophysin$^+$, chromogranin$^+$
19. Lung/neuroendocrine	Synaptophysin$^+$
20. Intestine/carcinoid	CDX2$^+$, CK20$^+$, synaptophysin$^+$
21. Endometrium	ER$^+$, PR$^+$, pelvic mass
22. Bladder	p63$^+$, CK7$^-$, CK20$^-$, histological review—areas of transitional cell carcinoma
23. Intestinal	CDX2$^+$
24. Breast	ER$^+$
25. Prostate	Serum PSA 32 (initially WNL), developed sclerotic bone lesions
26. Seminoma	PLAP$^+$, CK7$^-$, CK20$^-$
27– 35. Various diagnoses	No additional supportive data found

* CA-9 = carbonic anhydrase; CD10 = common acute lymphocytic leukemia antigen; CDX-2 = caudal homeobox gene; CK7 = cytokeratin7; CK20 = cytokeratin20; ER = estrogen receptor; Hepar1 = hepatocyte paraffin; LCA = leucocyte common antigen; p63 = tumor suppressor protein 63; PLAP = placental alkaline phosphatase; PR = progesterone receptor; PSA = prostate specific antigen; RCC = renal cell carcinoma antigen; S100 = calcium binding protein; WNL = within normal limits; WT-1 = Wilms tumor.

In the majority of biopsies in this series (67%), IHC could not confidently diagnose a single tissue of origin. When two or more diagnostic possibilities were suggested by IHC, the MTP diagnoses did not match any of the suggested diagnoses in 54 patients (57%). Although the "correct" primary site diagnosis can only be inferred, additional information obtained in many of the patients strongly supports the accuracy of the MTP diagnoses. Thirty-five of these 54 patients with adequate biopsy specimens remaining had additional targeted IHC and/or clinicopathologic studies prompted by the MTP assay results. In 26 patients (74%), the MTP diagnoses were supported by the additional clinical and pathologic information. The additional IHC stains obtained as well as specific clinical and histologic findings are summarized in Table 6. The stains included renal cell carcinoma (RCC) antigen (kidney), WTI (ovary), calretinin (mesothelioma), hepar1 (liver), and PLAP (seminoma), among others.

The clinical features observed frequently were consistent with the molecular diagnoses (Figure 2). In most patients, metastatic sites in the subgroups defined by MTP diagnoses were very similar to those expected for known advanced primary cancers. Although clinical features may be similar or overlap for several advanced cancers, the consistency with the MTP diagnoses is reassuring and also supports the accuracy of these diagnoses. We and others have previously noted positive clinical and pathologic correlations in smaller retrospective studies (4,5,7,19). Recently, a large prospective study (9) using this same MTP assay in CUP patients revealed improved survival with MTP assay–directed site-specific therapy, and these data also lend additional support to the accuracy of this MTP assay in making diagnoses of the tissue of origin.

The MTP assay evaluated in this study appears to accurately identify the tissue of origin in 75% to 80% of patients with CUP. This level of accuracy is similar to that already documented with all three commercially available MTP assays when tested on biopsies from patients with known primary cancer (10–15).

In general, there are limitations for all MTP diagnostic assays. In a small fraction, no information is provided because of either technical failure (usually from inadequate biopsy) or an indeterminate result. The assay diagnoses are not 100% accurate even when performed on known cancers. The mean accuracy is about 85% (10–15). Another shortcoming relates to overlapping gene expression of several neoplasms, which may cause incorrect diagnosis of the tissue of origin. One example of this phenomenon is the cross-reactivity seen with some breast, salivary gland, and adnexal skin cancers (20). Finally, MTP assays depend on panels of known cancers for comparison with the gene expression profiling of the unknown sample. There are several cancers (particularly less-common types) not represented in the panels, and these particular "off-panel" neoplasms may be incorrectly diagnosed. All of these limitations were at play to some extent in the study reported here. Because the MTP assays may give an incorrect diagnosis, the clinical features/setting and pathologic findings need to be considered in concert with the MTP diagnosis before making a decision regarding patient management. If possible, additional directed IHC stains and/or clinical/histologic evaluation should be performed to support or refute the MTP diagnosis. A MTP diagnosis is of particular importance when standard pathologic evaluation, including appropriate IHC staining, is unable to make a single tissue of origin diagnosis. In these patients, an MTP assay appears to complement standard pathology and improves the ability to diagnose a single tissue of origin.

Confidence in the relative accuracy of MTP assay diagnoses in CUP is necessary before this test is accepted as a standard part of the evaluation. These data from several evaluative methods reported here support the accuracy of this MTP assay in CUP diagnosis. Site-specific therapy based upon accurate prediction of the tissue of origin appears to improve the outcome for some patients (9), but for other tumor types there is currently no effective therapy available. Accurate diagnosis of the tissue of origin will provide important information to better manage all these patients and to guide appropriate therapy in the future as therapy for these tumor types improves.

References

1. Greco FA, Hainsworth, JD. Cancer of unknown primary site. In: Devita VT, ed. *Cancer: Principles and Practice of Oncology*. 9th ed. Philadelphia: Lippincott, Williams and Wilkins; 2011:2033–2051.
2. Bender RA, Erlander MG. Molecular classification of unknown primary cancer. *Semin Oncol*. 2009;36(1):38–43.
3. Greco FA, Erlander MG. Molecular classification of cancers of unknown primary site. *Mol Diagn Ther*. 2009;13(6):367–373.
4. Bridgewater J, van Laar R, Floore A, et al. Gene expression profiling may improve diagnosis in patients with carcinoma of unknown primary. *Br J Cancer*. 2008;98(8):1425–1430.
5. Greco FA, Spigel DR, Yardley DA, et al. Molecular profiling in unknown primary cancer: accuracy of tissue of origin prediction. *Oncologist*. 2010;15(5):500–506.
6. Hainsworth JD, Pillai R, Henner WD, et al. Molecular tumor profiling in the diagnosis of patients with carcinoma of unknown primary site: retrospective evaluation of gene microarray assay. *J Mol Biomark Diagn*. 2011;2(2):106–109.
7. Horlings HM, van Laar RK, Kerst JM, et al. Gene expression profiling to identify the histogenetic origin of metastatic adenocarcinomas of unknown primary. *J Clin Oncol*. 2008;26(27):4435–4441.
8. Varadhachary GR, Talantov D, Raber MN, et al. Molecular profiling of carcinoma of unknown primary and correlation with clinical evaluation. *J Clin Oncol*. 2008;26(27):4442–4448.
9. Hainsworth JD, Rubin MS, Spigel DR, et al. Molecular gene expression profiling to predict the tissue of origin and direct site-specific therapy in patients with carcinoma of unknown primary site: a prospective trial of the Sarah Cannon Research Institute. *J Clin Oncol*. 2013;31(2):217–223.
10. Erlander MG, Ma XJ, Kesty NC, et al. Performance and clinical evaluation of the 92-gene real-time PCR assay for tumor classification. *J Mol Diagn*. 2011;13(5):493–503.
11. Kerr SE, Schnabel CA, Sullivan PS, et al. Multisite validation study to determine performance characteristics of a 92-gene molecular cancer classifier. *Clin Cancer Res*. 2012;18(14):3952–3960.
12. Ma XJ, Patel R, Wang X, et al. Molecular classification of human cancers using a 92-gene real-time quantitative polymerase chain reaction assay. *Arch Pathol Lab Med*. 2006;130(4):465–473.
13. Monzon FA, Lyons-Weiler M, Buturovic LJ, et al. Multicenter validation of a 1,550-gene expression profile for identification of tumor tissue of origin. *J Clin Oncol*. 2009;27(15):2503–2508.
14. Rosenfeld N, Aharonov R, Meiri E, et al. MicroRNAs accurately identify cancer tissue origin. *Nat Biotechnol*. 2008;26(4):462–469.
15. Talantov D, Baden J, Jatkoe T, et al. A quantitative reverse transcriptase-polymerase chain reaction assay to identify metastatic carcinoma tissue of origin. *J Mol Diagn*. 2006;8(3):320–329.
16. Oien KA. Pathologic evaluation of unknown primary cancer. *Semin Oncol*. 2009;36(1):8–37.
17. A'Hern RP. Sample size tables for exact single-stage phase II designs. *Stat Med*. 2001;20(6):859–866.

18. Pentheroudakis G, Golfinopoulos V, Pavlidis N. Switching benchmarks in cancer of unknown primary: from autopsy to microarray. *Eur J Cancer*. 2007;43(14):2026–2036.
19. Varadhachary GR, Spector Y, Abbruzzese JL, et al. Prospective gene signature study using microRNA to identify the tissue of origin in patients with carcinoma of unknown primary. *Clin Cancer Res*. 2011;17(12):4063–4070.
20. Greco FA. Cancer of unknown primary or unrecognized adnexal skin primary carcinoma? Limitations of gene expression profiling diagnosis [published online ahead of print February 26, 2013]. *J Clin Oncol*. 2013. doi:10.1200/JCO.2012.47.1615.

Funding

This work was supported in part by bioTheranostics, Inc. and the Minnie Pearl Cancer Foundation.

Notes

F. Anthony Greco is a member of the Speaker's Bureau for bioTheranostics, Inc.

Affiliations of authors: Sarah Cannon Research Institute and Tennessee Oncology, PLLC, Nashville, TN (FAG, DRS, JDH); Associated Pathologists, Nashville, TN (WJL).