

Molecular Profiling in Unknown Primary Cancer: Accuracy of Tissue of Origin Prediction

F. ANTHONY GRECO,^a DAVID R. SPIGEL,^{a,b} DENISE A. YARDLEY,^{a,b} MARK G. ERLANDER,^c XIAO-JUN MA,^c JOHN D. HAINSWORTH^{a,b}

^aTennessee Oncology, PLLC, Nashville, Tennessee, USA; ^bSarah Cannon Research Institute, Nashville, Tennessee, USA; ^cbioTheranostics Inc., San Diego, California, USA

Key Words. Carcinoma of unknown primary site • Molecular profiling • Reverse transcriptase-polymerase chain reaction • Site-directed therapy

Disclosures: F. Anthony Greco: *Consultant/advisory role:* bioTheranostics; *Honoraria:* bioTheranostics; **David R. Spigel:** None; **Denise A. Yardley:** None; **Mark G. Erlander:** *Employment/leadership position:* bioTheranostics; *Ownership interest:* bioTheranostics; **Xiao-Jun Ma:** *Employment/leadership position:* bioTheranostics; *Ownership interest:* bioTheranostics; **John D. Hainsworth:** *Research funding/contracted research:* bioTheranostics.

The content of this article has been reviewed by independent peer reviewers to ensure that it is balanced, objective, and free from commercial bias. No financial relationships relevant to the content of this article have been disclosed by the independent peer reviewers.

ABSTRACT

Introduction. This retrospective, multi-institutional study evaluated the accuracy of tissue-of-origin prediction by molecular profiling in patients with carcinoma of unknown primary site (CUP).

Methods. Thirty-eight of 501 patients (7.6%) with CUP, seen in 2000–2008, had their latent primary site tumor subsequently identified during life. Twenty-eight of these patients (73.7%) had adequate initial tissue biopsies available for molecular profiling with a reverse transcriptase-polymerase chain reaction (RT-PCR) assay (Cancer Type ID; bioTheranostics, Inc., San Diego, CA). The assay was performed on formalin-fixed paraffin-embedded biopsy specimens in a blinded fashion, and the assay results were compared with clinicopathologic data and the actual latent primary sites.

Results. Twenty of the 28 (71.4%) RT-PCR assays were successfully completed (eight biopsies had either insufficient tumor or poorly preserved RNA). Fifteen of the 20 assay predictions (75%) were correct (95% con-

fidence interval, 60%–85%), corresponding to the actual latent primary sites identified after the initial diagnosis of CUP. Primary sites correctly identified included breast (four patients), ovary/primary peritoneal (four patients), non-small cell lung (three patients), colorectal (two patients), gastric (one patient), and melanoma (one patient). Three predictions were incorrect (intestinal, testicular, sarcoma) in patients with gastroesophageal, pancreatic, and non-small cell lung cancer, respectively, and two were unclassifiable in patients with non-small cell lung cancer. Clinicopathologic findings were helpful in suggesting the correct primary site in some patients and appear to complement the molecular assay findings.

Conclusions. These data validate the reliability of this assay in predicting the primary site in CUP patients and may form the basis for more successful site-directed therapy, when used in concert with clinicopathologic data. *The Oncologist* 2010;15:500–506

Correspondence: F. Anthony Greco, M.D., 250 25th Avenue North, Suite 110, Nashville, Tennessee 37203, USA. Telephone: 615-329-7272; Fax: 615-340-1535; e-mail: fgreco@tncnc.com, aso@scresearch.net Received December 21, 2009; accepted for publication March 26, 2010; first published online in *The Oncologist Express* on April 28, 2010. ©AlphaMed Press 1083-7159/2010/\$30.00/0 doi: 10.1634/theoncologist.2009-0328

INTRODUCTION

Cancer of unknown primary site (CUP) is a diverse syndrome representing about 5% of the advanced cancers in the U.S. per year. Therapy for the majority of patients in the absence of a specific tumor diagnosis has been empiric and relatively ineffective [1]. In the last three decades, therapies for patients with a number of advanced carcinomas have been developed, and continue to improve, including those originating from the breast, ovary, esophagus, stomach, lung, pancreas, bladder, prostate, colon, rectum, head/neck, kidney, and liver. Accurate diagnosis of the primary site in CUP patients would allow site-specific therapy and perhaps improve the outcome for many [2, 3].

Molecular profiling of tumors is a promising technique to improve the site of origin diagnosis in CUP patients [4]. Recently, three reported studies suggested the usefulness of this approach [5–7]. However, validation of the origin as predicted by the assays was indirect, based on clinicopathologic features and response to treatment. A more direct study to validate the reliability of molecular profiling in predicting the primary site in CUP patients would be the correlation with an eventual primary tumor identified later at autopsy or during life.

The detection of a primary is rare during life in CUP patients, but clinically occult primaries have subsequently been detected at autopsy in about 75% of patients [8] (lung, pancreas, and colorectal cancer account for about half). Herein, we report molecular profiling performed on the initial diagnostic biopsy specimen in CUP patients who eventually had their primary site identified while still alive (latent primary). The aim of this study was to validate the accuracy of a molecular diagnostic assay in predicting the primary site of origin.

PATIENTS AND METHODS

Patient Selection

In total, 501 patients treated in Minnie Pearl Cancer Research Network clinical trials in 2000–2008 [1, 2], as well as those not on a clinical trial and referred to Tennessee Oncology, PLLC, were retrospectively reviewed. All patients fulfilled our definition of CUP and had no detectable primary site after a diagnostic evaluation consisting of: a complete history, physical examination, CBC, chemistry profile, and computed tomography (CT) scans of the chest, abdomen, and pelvis; mammography in women; and evaluation of serum prostate-specific antigen (PSA) in men. Further evaluation targeting any signs or symptoms was also often performed. Patients who had a latent primary cancer site recognized at least 2 months after their initial diagnosis of CUP were identified.

Sample Collection and Processing

Initial diagnostic biopsy specimens were preserved as formalin-fixed paraffin-embedded (FFPE) blocks. FFPE blocks were sectioned to obtain three unstained 10- μ m sections and one adjacent hematoxylin and eosin–stained section. All sections were sent to bioTheranostics, Inc. (San Diego, CA); for each tumor sample, one adjacent hematoxylin and eosin–stained slide was examined by a board-certified anatomical pathologist to confirm the presence of tumor and target areas enriched for tumor content. Specifically, tumor areas were circled (via Sharpie pen on the coverslip) that contained $\geq 40\%$ tumor after circling. The cutoff of $\geq 40\%$ was previously determined as a percentage of tumor cells in which the predictive accuracy was not compromised [9]. Circled areas of tumor were then deparaffinized and subsequently scraped from the adjacent unstained sections into an Eppendorf tube and incubated with proteinase K overnight. Subsequently, RNA was extracted, amplified, and analyzed for the expression of 87 cancer-associated genes and five reference genes using TaqMan real-time reverse transcriptase-polymerase chain reaction (RT-PCR) (commercially available as Cancer Type ID, bioTheranostics, Inc., San Diego, CA) [9]. Prediction was made from an algorithm using the 87-gene expression profile of the test sample to measure its similarity to each of the 39 tumor types in a reference database.

Specimens were coded, and those performing the assays did not have any specific clinicopathologic information, other than the sex, biopsy location, and one hematoxylin and eosin–stained slide of the initial biopsy specimen.

Statistical Considerations

The purpose of this retrospective observational study was to estimate the accuracy of the molecular profile assay in predicting the primary site. We arbitrarily decided that an accuracy rate $\geq 50\%$ determined solely by the assay with a $\leq 30\%$ inaccuracy rate was the target endpoint of this study. If met, this would warrant further clinical validation of the assay. Confidence intervals (CIs) comparing the accuracy rates were calculated. Sample size with latent primary cancers was not initially known, but we anticipated finding at least 16 patients. All successful assays constituted the intent-to-evaluate population.

Therefore, if at least eight of the 16 assay predictions were correct (95% CI, 35%–70%) and no more than four were incorrect (95% CI, 15%–35%), the assay would warrant further evaluation. An institutional review board (IRB) found official IRB review unnecessary for coded information regarding human subjects in this setting.

Table 1. Patient characteristics, response to initial therapy, and survival

Patient no.	Age (yrs)/sex	Sites of metastasis	Light microscopic histology of initial biopsy	Initial therapy	Response/survival (wks)
1	59/F	Right axilla	PDC	Paclitaxel, doxorubicin, cyclophosphamide	Not measurable/296+
2	65/F	Left axilla, left adrenal	PDA	Paclitaxel, doxorubicin, etoposide	Partial response/327
3	51/F	Bone, liver	PDC	Paclitaxel, bevacizumab	Progression/26
4	64/F	Left supraclavicular, mediastinum	PDA	Paclitaxel, carboplatin, bevacizumab	Partial response/44+
5	85/F	Chest wall, axilla, mediastinum, pleura, retroperitoneum	PDA	Paclitaxel, carboplatin	Partial response/26
6	69/F	Bilateral inguinal, retroperitoneum	Adenocarcinoma	Docetaxel, carboplatin	Partial response/152
7	87/F	Lung, abdominal mass	PDA	Paclitaxel, carboplatin	Partial response/102
8	63/F	Paratracheal, hilar, omental, retroperitoneum, pelvis	PDC	Paclitaxel, carboplatin, etoposide, gefitinib	Complete response/268+
9	49/M	Liver	PDA	Gemcitabine, irinotecan	Stable/37
10	47/F	Mesenteric nodes, liver, lung	PDA	Capecitabine, irinotecan, bevacizumab	Partial response/124+
11	42/F	Brain	PDA	Surgery, radiotherapy to brain	Not measurable/119+
12	67/M	s.c. scapular mass	Squamous carcinoma	Surgery, paclitaxel, carboplatin, etoposide, radiotherapy	Not measurable/143
13	59/M	Brain	PDA	Surgery, radiotherapy to brain	Not measurable/119+
14	74/M	Bone, lung	Adenocarcinoma	Radiotherapy to bone	Partial response/120
15	76/M	Axilla, lung, retroperitoneum	PDC	None	Progression/60
16	60/M	Small intestine	PDC	None	Progression/16
17	38/M	Mediastinum, bone	PDA	Radiotherapy, etoposide, cisplatin	Partial response/101
18	61/M	Supraclavicular, retroperitoneum	PDC	Gemcitabine, irinotecan	Partial response/36
19	62/M	Retroperitoneum, mediastinum	PDA	Paclitaxel, carboplatin, 5-fluorouracil	Progression/20
20	75/F	Chest wall, pleura	PDC	Docetaxel, gemcitabine	Progression/33

Abbreviations: F, female; M, male; PDA, poorly differentiated adenocarcinoma; PDC, poorly differentiated carcinoma.

Analysis of Clinical Information

Patients with successfully performed assays were reviewed in detail; clinical information was collected, including demographics, sites of metastases, laboratory and imaging data, pathologic evaluation, type of therapy, response to therapy, survival, and the time and details regarding the latent primary site. Although the specific immunohistochemical (IHC) stains, therapy, response to therapy, and survival times were not part of this direct validation study, all these data were compared with the molecular assay results. This retrospective multi-institutional study over 9 years precluded a uniform diagnostic approach in each patient, but all initially fit the definition of CUP (see Patient Selection).

RESULTS

Patients and Molecular Assay Results

Thirty-eight of 501 patients (7.6%) had a latent primary site clinically identified as previously defined following their

initial diagnosis of CUP. Ten had only a fine needle aspiration specimen and were excluded. Twenty-eight of the 38 patients (73.7%) had excisional/incisional biopsies or core needle biopsies, and their initial diagnostic tissue specimens were the subject of molecular profiling.

Assay results were obtained for 20 of the 28 biopsy specimens (eight biopsies yielded RNA of insufficient quality or quantity to meet the quality control threshold for the assay). The patient characteristics, including sites of metastasis, histopathology, initial therapy, response, and survival time, are detailed in Table 1. The biopsy sites, initial IHC stains performed, primary sites suspected, molecular assay diagnosis, and latent primary site are illustrated in Table 2.

Clinical features and IHC stains suggested a single primary site in six of the 20 patients (patients 1, 3, 9, 10, 11, and 13) and were correct in five patients (patients 1, 9, 10, 11, and 13) and incorrect in one patient (patient 3). In eight others (patients 2, 6, 7, 12, 16, 17, 18, and 20), one of the

Table 2. Comparison of immunohistochemical staining, suspected primary site, molecular assay diagnosis, and latent primary tumor site found

Patient no.	Biopsy site	Immunohistochemical stains on initial diagnostic biopsy	Primary site suspected	Molecular assay diagnosis on initial diagnostic biopsy	Latent primary tumor site found ^a
1	Axilla	CK 7 ⁺ , CK20 ⁻ , ER ⁻ , PR ⁻ , TTF-1 ⁻ , HER-2/neu ⁻	Breast	Breast	Breast
2	Axilla	EMA ⁺ , S100 ⁻ , HER-2/neu ⁺ , CK7 ⁺ , ER ⁻ , PR ⁻	Breast, lung	Breast	Breast
3	Bone	Cytokeratin AE1, 3 ⁺ , ER ⁻ , mammaglobin ⁻	Lung	Breast	Breast
4	Supraclavicular	CK7 ⁺ , CK20 ⁻ , ER ⁻ , PR ⁻ , TTF-1 ⁻	Lung, pancreas, gastric	Breast	Breast
5	Chest wall	CK7 ⁺ , ER ⁻ , PR ⁻ , TTF-1 ⁻ , CA125 ⁺	Lung, breast	Ovary ^b	Primary peritoneal
6	Inguinal node	CK7 ⁺ , CK20 ⁻ , CDX-2 ⁻ , CA125 ⁺	Lung, breast, ovary	Ovary	Primary peritoneal
7	Abdominal mass	CK7 ⁺ , ER ⁺	Lung, ovary, breast	Ovary	Primary peritoneal
8	Paratracheal node	CK7 ⁺ , TTF-1 ⁻	Lung, pancreas	Ovary	Ovary
9	Liver	CK7 ⁻ , CK20 ⁺ , CDX-2 ⁺	Colorectal	Intestinal ^c	Colon
10	Mesenteric node	CK7 ⁻ , CK20 ⁺ , CDX-2 ⁺	Colorectal	Intestinal	Colon
11	Brain	CK7 ⁺ , CK20 ⁻ , TTF-1 ⁺	NSCLC	NSCLC	NSCLC
12	s.c. mass	Not done (squamous cell)	Lung, head/neck	NSCLC	NSCLC
13	Brain	CK7 ⁺ , CK20 ⁻ , TTF-1 ⁻	NSCLC	NSCLC	NSCLC
14	Bone	CK7 ⁺ , vimentin ⁺ , TTF-1 ⁻	Lung, renal, pancreas	Gastric	Gastric
15	Axilla	Cytokeratin AE1,3 ⁻ , HMB45 ⁻ , S100 ⁻ , CK7 ⁻	Unknown	Melanoma	Melanoma
16	Small intestine	CK7 ⁺ , CK20 ⁻ , TTF-1 ⁻ , HMB45 ⁻ , S100 ⁻	Lung, pancreas	Indeterminate	NSCLC
17	Mediastinum	CK7 ⁺ , TTF-1 ⁻ , PLAP ⁻ , CK20 ⁻ , chromogranin ⁻	Lung, pancreas	Indeterminate	NSCLC
18	Supraclavicular	CK7 ⁺ , CK20 ⁻ , TTF-1 ⁻	Lung, pancreas	Testes	Pancreas
19	Retroperitoneum	CK7 ⁺ , CK20 ⁺	Colorectal, pancreas	Intestinal	Gastric
20	Chest wall mass	CK7 ⁺ , CK20 ⁻ , chromogranin ⁻	Lung, pancreas, gastric	Sarcoma	NSCLC

^a14 of 20 latent primary sites were biopsied and six were documented by medical imaging (mean, 49 weeks; range, 9–314 weeks).

^bOvary or primary peritoneal (indistinguishable by assay).

^cColorectal or small intestine (indistinguishable by assay).

Abbreviations: CK, cytokeratin; EMA, epithelial membrane antigen; ER, estrogen receptor; HER, human epidermal growth factor receptor; HMB, human melanoma black; NSCLC, non-small cell lung cancer; PLAP, placental alkaline phosphatase; PR, progesterone receptor; TTF, thyroid transcription factor.

suspected primary sites proved to be correct, but in five patients (patients 4, 5, 8, 14, and 19) the suspected primary sites were incorrect.

The latent primary sites were identified 9–314 weeks (mean, 49 weeks) after the initial diagnosis of CUP. They were biopsy proven in 14 of the 20 patients and similar or identical to the initial biopsies. In the remaining six patients, various medical imaging testing documented the primary sites.

The initial therapy is listed in Table 1, with response and survival results. In those with measurable tumors, 10 of 14 patients had an objective response to therapy, and the mean

survival duration of all patients was 108 weeks (range, 16–327 weeks).

Fifteen of 20 predictions of the site of origin (75%) made by the molecular assay were correct (95% CI, 60%–85%), corresponding to the latent primary sites later identified (Table 2). Primary sites were correctly identified in patients 1–15 and included breast (four cases), ovary/primary peritoneal (four cases), non-small cell lung (three cases), colorectal (two cases), gastric (one case), and melanoma (one case) primaries. Three predictions (patients 18–20) were incorrect (15%), predicting colorectal cancer, testicular cancer, and sarcoma in patients with gastric, pan-

creatic, and non-small cell lung primaries (95% CI, 5%–25%), and in two assays (patients 16 and 17) prediction could not be made (unclassifiable) (10%) in patients with non-small cell lung cancer (95% CI, 2%–18%).

Specific Patient Examples

Correct Prediction: Ovarian Carcinoma

A 63-year-old woman (patient 8; Tables 1 and 2) developed a cough, and a chest radiograph revealed paratracheal masses. A mediastinoscopic biopsy showed poorly differentiated carcinoma (PDC) (IHC stains: cytokeratin [CK]7⁺, thyroid transcription factor [TTF]-1⁻). CT scans of the chest, abdomen, and pelvis showed right hilar, bilateral iliac, retroperitoneal adenopathy and a few questionable omental nodules. Primary sites suspected were the lung and pancreas. Treatment in a clinical trial was started with paclitaxel, carboplatin, and etoposide followed by gefitinib, and she had a complete response. During follow-up, 20 months later, a positron emission tomography (PET)/CT scan showed a new 2.5-cm left pelvic mass felt to involve the left ovary. She underwent a laparotomy and a left ovarian mass was removed. Ovarian carcinoma was identified and the histology was identical to the paratracheal mass.

Comment. This patient had advanced ovarian carcinoma presenting 20 months before as CUP. The molecular profile assay predicted ovarian carcinoma from the initial diagnostic biopsy. Her complete response to the type of empiric chemotherapy administered was also consistent with ovarian carcinoma.

Correct Prediction: Colorectal Carcinoma

A 47-year-old woman (patient 10; Tables 1 and 2) presented with abdominal pain and weight loss. A CT scan of the abdomen and pelvis showed liver lesions and mesenteric adenopathy, and a PET scan showed hypermetabolic activity in liver lesions, upper abdominal nodes, and a left hilar lung nodule. A mesenteric node biopsy revealed poorly differentiated adenocarcinoma (PDA) (IHC stains: CK7⁻, CK20⁺, CDX2⁺). Mammograms were negative and her serum carcino-embryonic antigen (CEA) was 4.9 ng/mL. A colonoscopy was normal. She was treated with capecitabine, irinotecan, and bevacizumab and had a partial response. Four months later occult blood was detected in her stool and a repeat colonoscopy revealed a small intraluminal lesion in the cecum. A biopsy was nondiagnostic and she had a laparotomy. A 0.7-cm nodule was found in the cecum. She underwent right hemicolectomy. Pathology revealed invasive adenocarcinoma (with two positive mesenteric lymph nodes), similar to the previously biopsied node.

Comment. This patient had a latent small colon adenocarcinoma. Initially she had liver and nodal metastases and an occult colorectal primary was suspected based on the pattern of metastasis and IHC staining. She responded favorably to colorectal cancer–directed therapy. The molecular profile assay performed on her initial node biopsy predicted intestinal carcinoma.

Correct Prediction: Breast Cancer

A 64-year-old woman (patient 4; Tables 1 and 2) noticed a lump in her left supraclavicular area. A biopsy revealed PDA (IHC stains: CK7⁺, CK20⁻, estrogen receptor [ER]⁻, progesterone receptor [PR]⁻, TTF-1⁻). PET/CT scanning of the chest, abdomen, and pelvis showed enlarged hypermetabolic nodes in the left supraclavicular area and anterior mediastinum. She had negative esophagogastroduodenoscopy and mammograms, and was treated with paclitaxel, carboplatin, and bevacizumab and had a partial response. A cystic lesion was palpated in her left breast 4 months later. An ultrasound showed a small smooth benign-appearing cyst. Magnetic resonance imaging (MRI) of the breasts revealed a 1.4-cm irregular lesion separate from the cyst deep in the subareolar portion of the left breast. A core needle biopsy showed invasive adenocarcinoma of the breast, similar to the initial biopsy.

Comment. She had a breast adenocarcinoma that was detectable only by MRI 4 months after her presentation with metastatic carcinoma. The clinical features and IHC stains suggested lung and upper gastrointestinal primaries. The molecular profile assay performed on her initial biopsy predicted breast carcinoma.

Incorrect Prediction: Testicular Cancer

A 61-year-old man (patient 18; Tables 1 and 2) developed abdominal pain. He had a palpable left supraclavicular mass, and a CT scan of his abdomen and pelvis showed retroperitoneal adenopathy. A CT scan of his chest was normal, but a PET scan showed activity in the left supraclavicular and retroperitoneal nodes. His serum cancer antigen (CA) 19-9, PSA, CEA, and α -fetoprotein levels were normal; his β -human chorionic gonadotrophin (hCG) level was slightly elevated at 15 mIU/mL. An esophagogastroduodenoscopy, colonoscopy, and testicular ultrasound were all normal. Biopsies of retroperitoneal and supraclavicular masses showed PDC (IHC stains: CK7⁺, CK20⁻, TTF-1⁻). He was treated in a clinical trial with gemcitabine and irinotecan and had a partial response. Four months later, he had a CT scan of his abdomen and a new lesion was seen in the pancreatic head. The serum CA 19-9 measurement was repeated and it was found to be elevated to 170 U/mL; his

β -hCG level was <5 mIU/mL. Four months later the pancreatic mass was larger. A supraclavicular biopsy was sent for fluorescence in situ hybridization testing for chromosome 12 abnormalities, and isochromosome of 12 [i (12)p], deletion of 12p, or multiple copies of 12p, often seen in germ cell tumors, were not identified.

Comment. He presented with CUP, and a primary site later became obvious in the pancreas. The molecular profile prediction of testicular carcinoma was incorrect. The lack of characteristic chromosome 12 abnormalities and the emergence of a pancreatic head mass clarified his diagnosis. Molecular profiling predictions can be misleading. As with any diagnostic test, results may be incorrect and require interpretation within the clinical context.

DISCUSSION

Patients with CUP represent a very frustrating and difficult clinical problem. Most never have a latent primary site discovered, but at the time of diagnosis the majority do harbor a clinically occult primary cancer. Identification of their primary tumor may help to determine prognosis and more appropriate therapy. The molecular assay tested in this retrospective study was correct in 15 of 20 patients (75%), providing reasonable direct validation of the ability to accurately predict the primary site in CUP patients. Nonetheless, three of 20 patients (15%) were incorrectly predicted, and two (10%) were indeterminate. The accuracy of the assay was as good as or superior to the expectations of this study.

IHC staining of tumor cells [10] for several markers can suggest the type of cancer in some CUP patients, but often it is not highly specific for one tumor type. The comparisons of the suspected primary sites with the molecular assay results and the actual latent primaries found are of interest (Table 2). In six of the 20 patients, a single primary site was initially suspected based on clinical features and IHC markers, and this proved to be correct in five patients (25%). The molecular assay result was correct in all six of these patients. In 13 other patients, more than one possible primary site was suspected. One of the sites suspected proved to be correct in eight of these 13 patients. The molecular assay result was correct in eight of these 13 patients, and in one other patient without a suspected primary site. Therefore, the molecular assay results were correct in 15 of 20 patients (75%). Although the number of observations is small and not all of the best and more recently developed IHC stains were performed in this retrospective study, the molecular assay compares favorably with the IHC markers, more often providing a single correct prediction of the primary tumor site. The IHC markers and molecular assays validate

one another in several of these patients, and appear to be complimentary in predicting the correct primary site when used in concert with clinical features.

Previously, we and others have reported clinicopathologic correlative data that also suggested the usefulness of molecular assays in predicting correct primary sites [5–7]. Varadhachary et al. [5] reported on the use of a 10-gene (capable of recognizing six tumors) RT-PCR assay on biopsies of 120 patients with CUP. A tissue of origin was predicted for 63 patients (61%), and the clinicopathologic features and responses to treatment were compatible with the predicted site of origin in most patients.

Horlings et al. [6] used a gene-expression microarray assay on biopsies of 38 patients with CUP. Of the 38 patients, 22 remained unidentified by IHC. In 14 of these 22 patients (64%), the gene expression profile predicted the tissue of origin, and it was frequently consistent with the clinicopathologic features. In 15 of the 16 patients with primary sites “resolved” by IHC, the molecular assay was in agreement.

Bridgewater et al. [7] used a gene expression microarray assay on biopsies from 21 patients with CUP, and correlations were made with clinicopathologic features. A “clinically feasible” primary prediction was obtained in 18 of the 21 patients and would have potentially influenced the management in 12 patients. In all these reports [5–7], direct validation of the molecular assay in predicting the primary site was lacking, because none of the patients had an anatomically documented primary tumor site.

The accuracy of the RT-PCR assay reported on here in predicting the primary site is encouraging. The molecular assay results were generated in a blinded fashion without any knowledge of the clinical or pathologic data (including IHC stains), providing a direct independent estimate of the accuracy of the molecular assay prediction. The usefulness of primary tumor site prediction from this assay in clinical practice is likely to be further improved by considering all the clinical and pathological data for each patient.

There are several possible shortcomings of molecular diagnostic assays in CUP patients. The genetic profiles of tumors are not always homogeneous and may overlap with several other tumor types. This may account for incorrect predictions. The genetic profiles of occult cancers giving rise to CUP may also differ from latent primaries and known primary cancers [8]. The assays have only been validated in patients with known primary cancers [9, 11–13]. The response to site-specific therapy of a CUP diagnosed by the molecular assay may not be the same response expected in known primary cancers. The molecular assay prediction may be incorrect and result in ill-advised therapy. An awareness of these potential problems is essential in in-

terpreting the results, and emphasizes the importance of comprehensive evaluation and clinical judgment in these patients.

Empiric chemotherapy, for example, with paclitaxel and carboplatin (a popular regimen), administered to all CUP patients would not be expected to be useful in patients with several carcinomas (colorectal, renal, pancreatic, prostate, and hepatic) and would be less than optimal in others (breast, lung, esophagus, stomach, biliary tract, head and neck, and bladder). Patients reported here usually received empiric chemotherapy (Table 1), and the responses and survival times are consistent with the clinical features and the primary tumor sites ultimately found. This study is too small to make any other meaningful correlations or conclusions regarding the efficacy of therapy. A more site-directed therapy based on an accurate determination of the primary site may offer a more effective and individual ap-

proach to therapy. Prospective clinical trials are ongoing to determine whether treatment based on molecular profiling can improve CUP patient outcomes.

ACKNOWLEDGMENTS

Supported in part by the Minnie Pearl Cancer Foundation, Nashville, TN.

Presented in part at the American Society of Clinical Oncology Meeting 2009, Orlando, FL, May 30 to June 2.

AUTHOR CONTRIBUTIONS

Conception/Design: John D. Hainsworth, F. Anthony Greco

Provision of study material or patients: F. Anthony Greco, David R. Spigel, Denise A. Yardley

Collection and/or assembly of data: F. Anthony Greco, Mark G. Erlander, Xiao-Jun Ma

Data analysis and interpretation: F. Anthony Greco, Mark G. Erlander, Xiao-Jun Ma

Manuscript writing: John D. Hainsworth, F. Anthony Greco, Mark G. Erlander, Xiao-Jun Ma

Final approval of manuscript: John D. Hainsworth, F. Anthony Greco, David R. Spigel, Denise A. Yardley, Mark G. Erlander, Xiao-Jun Ma

REFERENCES

- 1 Greco FA, Hainsworth JD. Cancer of unknown primary site. In: DeVita VTJ, Hellman S, Rosenberg SA, eds. *Cancer: Principles and Practice Oncology*, Eighth Edition. Philadelphia: Lippincott, 2008:2363–2387.
- 2 Greco FA, Pavlidis N. Treatment for patients with unknown primary carcinoma and unfavorable prognostic factors. *Semin Oncol* 2009;36:65–74.
- 3 Greco FA. Therapy of adenocarcinoma of unknown primary: Are we making progress? *J Natl Compr Canc Netw* 2008;6:1061–1067.
- 4 Bender RA, Erlander MG. Molecular classification of unknown primary cancer. *Semin Oncol* 2009;36:38–43.
- 5 Varadhachary GR, Talantov D, Raber MN et al. Molecular profiling of carcinoma of unknown primary and correlation with clinical evaluation. *J Clin Oncol* 2008;26:4442–4448.
- 6 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;26:4435–4441.
- 7 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;98:1425–1430.
- 8 Pentheroudakis G, Greco FA, Pavlidis N. Molecular assignment of tissue of origin in cancer of unknown primary may not predict response to therapy or outcome: A systematic literature review. *Cancer Treat Rev* 2009;35:221–227.
- 9 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;130:465–473.
- 10 Oien KA. Pathologic evaluation of unknown primary cancer. *Semin Oncol* 2009;36:8–37.
- 11 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;8:320–329.
- 12 Rosenfeld N, Aharonov R, Meiri E et al. MicroRNAs accurately identify cancer tissue origin. *Nat Biotechnol* 2008;26:462–469.
- 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;27:2503–2508.