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ORIGINAL RESEARCH ARTICLE

Poorly Differentiated Neoplasms of Unknown Primary Site: Diagnostic Usefulness of a Molecular Cancer Classifier Assay

F. Anthony Greco[1] · Wayne J. Lennington[2] · David R. Spigel[1] · John D. Hainsworth[1]

Abstract

Purpose Definition of the lineage of poorly differentiated neoplasms (PDNs) presenting as cancer of unknown primary site (CUP) is important since many of these tumors are treatment-sensitive. Gene expression profiling and a molecular cancer classifier assay (MCCA) may provide a new method of diagnosis when standard pathologic evaluation and immunohistochemical (IHC) staining is unsuccessful.

Patients and Methods Thirty of 751 CUP patients (4 %) seen from 2000–2012 had PDNs without a definitive lineage diagnosed by histology or IHC (median 18 stains, range 9–46). Biopsies from these 30 patients had MCCA (92-gene reverse transcriptase-polymerase chain reaction mRNA) performed. Additional IHC, gene sequencing, fluorescent in situ hybridization for specific genetic alterations, and repeat biopsies were performed to support MCCA diagnoses, and clinical features correlated. Seven patients had MCCA performed initially and received site-specific therapy.

Results Lineage diagnoses were made by MCCA in 25 of 30 (83 %) patients, including ten carcinomas (three germ cell, two neuroendocrine, five others), eight sarcomas [three peritoneal mesotheliomas, one primitive neuroectodermal tumor (PNET), four others], five melanomas, and two lymphomas. Additional IHC and genetic testing [BRAF, i(12)p] supported the MCCA diagnoses in 11 of 16 tumors. All seven patients (two germ cell, two neuroendocrine, two mesothelioma, one lymphoma) responded to site-specific therapy based on the MCCA diagnosis, and remain alive (five progression-free) from 25+ to 72+ months.

Conclusion The MCCA provided a specific lineage diagnosis and tissue of origin in most patients with PDNs unclassifiable by standard pathologic evaluation. Earlier use of MCCA will expedite diagnosis and direct appropriate first-line therapy, which is potentially curative for several of these tumor types.

Key Points

Molecular cancer classifier assays (MCCA) made lineage diagnoses in 25 of 30 (83 %) patients with poorly differentiated neoplasms who were not diagnosed by histology or immunohistochemistry.

Seven patients had site-specific therapy based on MCCA diagnosis, and remain alive from 25+ to 72+ months.

Earlier use of MCCA can expedite diagnosis and direct appropriate first-line therapy.

1 Introduction

Cancer of unknown primary site (CUP) is a relatively common clinical syndrome, and accounts for approximately 3 % of all advanced cancers annually in the US [1]. The diagnosis is made by a biopsy of a metastatic site, and the general cancer type or lineage (i.e. carcinoma,

✉ F. Anthony Greco
fgreco@tnonc.com

[1] Sarah Cannon Research Institute and Cancer Center, Tennessee Oncology, PLLC, Suite 100, 250 25th Avenue North, Nashville, TN 37203, USA

[2] Associated Pathologists, Nashville, TN, USA

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melanoma, sarcoma, lymphoma) is defined in the vast majority of tumors. Recently, the ability to predict the site of tumor origin in patients with CUP, particularly adenocarcinoma or poorly differentiated carcinoma, has greatly improved with the introduction of more specific immunohistochemical (IHC) stains [2] and the development of molecular cancer classifier assays (MCCA) [3]. Recent studies have established the accuracy of these predictions, and site-specific therapy based on MCCA diagnoses, rather than empiric chemotherapy, is becoming the new standard of therapy [3, 4].

Rarely in CUP, diagnosis of the lineage of the neoplasm is not possible despite histopathologic examination and extensive IHC study [1]. Although this group of patients is small, correct diagnosis is important since the group contains many treatable (and some potentially curable) tumors [1]; however, the value of MCCA in determining the lineage of these tumors has not been evaluated

In this study, we identified CUP patients with undifferentiated neoplasms seen at our referral center, and performed gene expression profiling on archived biopsy tissue in an attempt to accurately identify the tumor lineage. Most of the patients identified were seen and treated empirically prior to the availability of MCCA for diagnosis; however, several of the more recent patients had MCCA performed at the time of the initial biopsy and received treatment based on the MCCA results.

2 Patients and Methods

Patients with CUP seen at the Sarah Cannon Cancer Center and the clinics of Tennessee Oncology in Nashville and Middle Tennessee between 2000 and 2012 were retrospectively reviewed. A total of 751 CUP patients were seen, and in 30 (4 %) of these patients a definitive lineage could not be determined by standard histopathologic evaluation and extensive IHC testing of the biopsy specimens. Patients were not included if IHC staining defined a specific lineage. All pathologic specimens were re-reviewed (WJL) to confirm that the tumor lineage could not be determined and that the diagnosis of poorly differentiated neoplasm was the most specific diagnosis possible.

Twenty-eight of 30 patients presented with advanced cancers (two patients had single-site lesions) and had no anatomical primary site detected after a standard work-up for CUP. Archival biopsy specimens were obtained and, when possible, additional biopsies were performed. Biopsies were tested by a 92-gene MCCA (reverse transcriptase-polymerase chain reaction (RT-PCR) mRNA, CancerTYPE ID, bioTheranostics, Inc., San Diego, CA, USA), as previously described [5–10]. When feasible (if remaining biopsy specimens were available or repeat biopsies were performed), additional evaluation of the tumors was carried out after obtaining the MCCA results, in an attempt to confirm or substantiate the MCCA diagnoses. These additional studies were prompted by the MCCA diagnoses and included directed IHC staining not initially performed, fluorescent in situ hybridization (FISH) for specific genetic alterations, genetic sequencing, and re-review of specific clinical features. The MCCA diagnoses were also correlated with the clinical features.

In the majority of patients, the MCCA was obtained months to years after the initial pathologic evaluation and diagnosis; therefore, the use of this diagnostic information to help determine therapy was not possible. These patients were usually treated with empiric chemotherapy regimens for CUP, with the exception of two patients with BRAF V600E mutations (one with an MCCA diagnosis of melanoma, and one with an indeterminate MCCA diagnosis). However, in 11 patients seen between 2008 and 2012, the MCCA assay was obtained contemporaneously on the initial biopsy specimens, and 7 of these 11 patients had specific MCCA diagnoses (four had indeterminate/unclassifiable diagnoses); these seven patients were treated according to the MCCA diagnosis.

3 Results

The histology in all 30 initial biopsies was poorly differentiated neoplasm without a diagnosis of a definitive lineage. The lineage of these tumors remained undefined, even after multiple IHC studies (median 18 stains; range 9–46 stains), although in several instances the lineage was debated by two or more pathologists involved in the initial evaluation of the biopsies. Upon re-review of the histopathology and IHC evaluation (WJL), there was no consensus concerning the precise lineage in any of these tumors.

An MCCA diagnosis was made in 25 of the 30 biopsy specimens (83 %). The diagnoses by MCCA was indeterminate (unclassifiable) in four tumors (13 %) and in one (4 %) there was insufficient tissue. Some details of these 30 patients are illustrated in Table 1. The MCCA diagnoses included carcinoma in ten (40 %) patients, including three germ cell tumors, two neuroendocrine tumors, and five others. Sarcoma was diagnosed in eight (32 %) patients, including mesothelioma in three, primitive neuroectodermal tumor (PNET) in one, and four others. Melanoma was diagnosed in five (20 %) patients, and hematopoietic neoplasms in two (8 %) patients, both lymphomas.

The MCCA diagnoses prompted additional evaluation of the biopsy specimens when sufficient tissue was available in paraffin blocks, or available patients (three) consented to repeat biopsies; these additional studies were

Table 1 Poorly differentiated neoplasms of unknown primary site ($N = 30$)

Patient	Sex/age (years)	Site of biopsy	Initial lineage diagnoses based on standard pathologic evaluation	Number of IHC stains before MCCA	MCCA diagnosis
1	M/62	Pelvic mass	Unknown	12	Mesothelioma
2	M/56	Abdominal mass	Unknown	15	Sarcoma
3	F/55	Inguinal mass	Carcinoma versus sarcoma	15	Mesothelioma
4	F/57	Chest wall/abdominal nodes	Carcinoma versus sarcoma	16	Uterine cervix adenocarcinoma
5	F/53	Breast	Sarcoma versus carcinoma	18	Indeterminate
6	M/54	Scalp mass, lung nodules	Sarcoma versus carcinoma	24	Indeterminate
7	F/84	Skin, subcutaneous mass	Carcinoma versus sarcoma	9	Skin/neuroendocrine–Merkel cell
8	F/58	Axillary mass	Carcinoma versus mesothelioma	26	Breast adenocarcinoma
9	M/60	Scalp mass	Sarcoma versus carcinoma	15	Lung adenocarcinoma
10	M/86	Liver	Carcinoma versus melanoma	12	Gall bladder adenocarcinoma
11	M/63	Liver	Unknown	15	Indeterminate
12	M/36	Neck mass, lung nodule	Carcinoma versus sarcoma	26	Sarcoma/PNET
13	F/39	Mediastinal mass	Carcinoma versus sarcoma	39	Sarcoma/osteosarcoma
14	M/65	Brain, lung nodule	Unknown	16	Germ cell tumor/non-seminoma
15	M/61	Chest wall mass	Unknown	16	Indeterminate
16	F/68	Omental masses	Carcinoma versus mesothelioma	15	Urothelial carcinoma/bladder
17	F/28	Soft tissue/subcutaneous nodule	Sarcoma versus melanoma	21	Melanoma
18	M/33	Paratracheal mass	Unknown	46	Lymphoma
19	F/71	Mediastinal mass	Unknown	9	Lung/neuroendocrine
20	M/38	Lung nodules	Unknown	19	Melanoma
21	F/46	Axillary mass	Unknown	18	Sarcoma
22	M/74	Bone, soft tissue mass	Unknown	18	Lymphoma[a]
23	M/24	Abdominal and pelvic masses	Unknown	20	Mesothelioma
24	F/59	Bone, soft tissue mass	Carcinoma versus sarcoma	16	Sarcoma
25	F/79	Inguinal mass	Sarcoma versus melanoma	9	Melanoma
26	M/38	Soft tissue, subcutaneous masses	Unknown	11	Melanoma
27	F/46	Brain	Carcinoma versus melanoma	14	Melanoma
28	M/44	Neck mass	Lymphoma versus carcinoma	14	Germ cell/seminoma
29	M/50	Retroperitoneal masses	Sarcoma versus carcinoma	14	Germ cell/seminoma
30	M/64	Lung, abdominal mass	Unknown	13	Assay unsuccessful

IHC immunohistochemical, *MCCA* molecular cancer classifier assay, *M* male, *F* female, *PNET* primitive neuroectodermal tumor, *CML* chronic myelogenous leukemia

[a] Proved to be granulocytic sarcoma/CML (chloroma) with further diagnostic testing

performed in an attempt to provide confirmation of the MCCA diagnoses (Table 2). In 12 of 16 tumors (75 %) further studied, the MCCA diagnoses were confirmed or supported. These diagnoses included germ cell tumor/seminoma (two), melanoma (three), mesothelioma (two), sarcoma (two), neuroendocrine tumor (two), and lymphoma (one).

The clinical features in 21 of the 25 patients with specific MCCA diagnoses were consistent with these diagnoses and, in a minority, were supportive of the MCCA diagnoses.

The seven patients who received site-specific therapy based on the MCCA diagnosis are detailed in Table 3. The specific IHC staining performed before the MCCA was obtained is illustrated, along with the MCCA diagnosis, confirmatory/supportive testing carried out after the MCCA, treatments, and outcomes.

Five of these seven patients who received site-specific therapies based on the MCCA diagnoses had favorable, very treatable diagnoses; in six of the seven, additional testing after the MCCA diagnoses supported the molecular diagnoses. All seven patients remain alive (five

progression-free; two germ cell tumors, two neuroendocrine tumors, one lymphoma) from 25+ to 72+ months.

3.1 Illustrative Cases

Patient 18: A 33-year-old man presented with right chest pain and hemoptysis. Computed tomography (CT) scans of the chest/abdomen/pelvis showed a 4 cm right hilar mass, right paratracheal mass, and several right lung nodules. Serum human chorionic gonadotropin (HCG) and α-fetoprotein (AFP) levels were normal. Bronchoscopy was normal, and biopsy of the paratracheal mass revealed a poorly differentiated malignant neoplasm (PMN). Multiple IHC stains were not diagnostic of the lineage (see Table 3). The MCCA revealed a 96 % probability of lymphoma. From studies performed, it was not possible to determine if the lymphoma was of B- or T-cell lineage. There was scattered necrosis in the tumor which may have accounted for the negative immunostains, particularly CD45. Additional confirmatory testing, including T-cell receptor/immunoglobulin gene rearrangement study, was considered but the biopsy specimen was exhausted and re-biopsy was not feasible due to the need to proceed with therapy. Chemotherapy with six cycles of CHOP (cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone) produced a complete response and the patient has been relapse-free for 47+ months.

Patient 28: A 43-year-old man presented with back pain and renal failure (serum creatinine 13 mg/dL). He was found to have a neck mass and, by CT scanning, multiple lung nodules, a superior mediastinal mass, retroperitoneal masses, and bilateral hydronephrosis. Serum HCG and AFP were normal. He was started on hemodialysis and bilateral ureteral stents were inserted; biopsy of the neck mass showed poorly differentiated neoplasms (PDNs), although one consulting pathologist favored lymphoma. Multiple IHC stains were not diagnostic (see Table 3). An MCCA revealed germ cell tumor/seminoma (95 % probability). He was treated with four cycles of cisplatin, etoposide, and bleomycin, and had a marked reduction of tumor masses. The decision was made to follow the patient with no other treatment and he has remained relapse-free for 57+ months.

4 Discussion

Identification of tumor lineage is accomplished by standard histopathologic examination in approximately 95 % of CUPs [1]. In most of the remaining 5 %, IHC staining

Table 2 Additional confirmatory/supportive diagnostic testing ($N = 16$)

Patient	Sex/age (years)	MCCA diagnosis	Additional diagnostic testing	Able to support MCCA diagnosis
1	M/62	Mesothelioma	IHC: calretinin	Yes
2	M/56	Sarcoma	IHC: calretinin, WT-1	No (lineage correct)
3	F/55	Mesothelioma	IHC: calretinin, WT-1	No
7	F/84	Skin/neuroendocrine–Merkel cell	IHC: neurofilament, CK20, CD56, synaptophysin	Yes
12	M/36	Sarcoma/PNET	FISH; 11:22 translocation	No
13	F/39	Sarcoma/osteosarcoma	IHC: multiple	No
17	F/28	Melanoma	IHC: multiple; electron microscopy	No
19	F/71	Lung/neuroendocrine	IHC: synaptophysin	Yes
21	F/46	Sarcoma	IHC: desmin; clinical features	Yes
22	M/74	Lymphoma[a]	IHC: CD45R0, CD43, CD33	Yes[b]
23	M/24	Mesothelioma	IHC: calretinin	Yes
25	F/79	Melanoma	Repeat biopsy later; IHC:S100, HMB45	Yes
26	M/38	Melanoma	Repeat biopsy later; IHC: melan A	Yes
27	F/46	Melanoma	Repeat biopsy later; BRAF mutation (V600E)	Yes
28	M/44	Germ cell/seminoma	IHC: PLAP, CD117	Yes
29	M/50	Germ cell/-seminoma	FISH; i(12)p	Yes

IHC immunohistochemical, *MCCA* molecular cancer classifier assay, *M* male, *F* female, *PNET* primitive neuroectodermal tumor, *FISH* fluorescent in situ hybridization, *CML* chronic myelogenous leukemia

[a] Proved to be granulocytic sarcoma/CML (chloroma) with further diagnostic testing

[b] Lymphoma closest gene expression match; leukemia not in panel of tumors recognized (other granulocyte markers diagnosed granulocytic sarcoma)

Table 3 Patients treated prospectively with site-specific therapies based on MCCA diagnosis ($N = 7$)

Patient	IHC before MCCA	MCCA diagnosis	Confirmatory testing	Treatments	Outcome
1	CK7+, CK20-, CK8/18+, CKAE1/AE3+, melan A-, HMB45-, CDX2-, TTF-1-, CD117-, synaptophysin-, desmin-, actin (focal+)	Mesothelioma	IHC: calretinin+	1. Surgery 2. Pemetrexed, carboplatin chemotherapy 3. Surgery, intraperitoneal chemotherapy 4. Surgery	Partial response Resection of visible tumor Progression suspected Alive 40+ months
2	panCK+, NSE+, CEA (focal +), CD15+, Ber-EP4+, MOC31+, chromogranin-, synaptophysin-, CK5/6+, WT-1+, D2-40+, CK7-, CK20-, RCC-	Sarcoma	IHC: calretinin+, WT-1+ (all data support mesothelioma)	1. Pemetrexed, carboplatin chemotherapy 2. Gemcitabine chemotherapy 3. Clinical trial, mesothelin antibody	Partial response to initial chemotherapy Progressive tumor Alive 55+ months
7	CKAE1/AE3-, S100-, HMB45-, mart 1-, CK7-, CK20-, CD45-, MiTF-, TTF-1-	Skin/neuroendocrine carcinoma—Merkel cell	IHC: CK20+ dot pattern, CKAE1/AE3+, CD56+, synaptophysin+	Surgery, radiotherapy	No progression Alive 35+ months
18	CKAE1/AE3-, CAM 5.2-, OSCAR keratin-, CD45-, CD10-,CD 20-, CD 23-, CD 138-, CD 3- CD4-, CD8-, CKA.C-, CK7-, calretinin-, TTF-1-, synaptophysin-, BCL1-, kappa/lambda light chains-, CD5-, CBAC-, CD30-, CD35-, CD43-, CD45-, CD56-, CD68-, CD 79A-, CD 99-, CD163-, S100-, melan A-, TdT-, PLAP-, Oct-4-, CD117-, ALK-1-, chromogranin-, MUM1+, EBER-ISH-, desmin-, actin-, myogen-, lysozyme-	Lymphoma	None (biopsy exhausted)	CHOP chemotherapy	Complete response No progression Alive 46+ months
19	panCK-, CD45-, S100-, CD3-, CD20-, HCG-, AFP-, PLAP-, melan A-	Lung/neuroendocrine	IHC: synaptophysin+	Paclitaxel, carboplatin chemotherapy; radiotherapy	Complete response No progression Alive 72+ months
28	CD45-, CAM 5.2+, CK7 (focal+), CKAE1/AE3 (focal +), EMA-, CK20-, S100-, TTF-1-, melan A-, CD3-, CD43-, CD7-, CD20-, CD15-	Germ cell carcinoma—seminoma	IHC: PLAP+	Etoposide/cisplatin/bleomycin chemotherapy	Partial response No progression Alive 58+ months
29	CAM 5.2+, CKAE1/AE3+, CD45-, S100-, CD117+, CD30+, CD34+, CD10+, PLAP-, vimentin+, CD3-, CK7-, CK20-, TTF-1-	Germ cell carcinoma—seminoma	FISH; i(12)p present	1. Etoposide/cisplatin/bleomycin chemotherapy 2. Surgery—residual carcinoma resected 3. Vinblastine/ifosfamide/cisplatin chemotherapy	Partial response Complete response Alive 25+ months Lost to follow-up

IHC immunohistochemistry, *MCCA* molecular cancer classifier assay, *FISH* fluorescent in situ hybridization, *CHOP* cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone

clarifies the tumor lineage; therefore, a small number of cancers remain with undefined lineage after complete pathologic examination, and are characterized as poorly differentiated or undifferentiated neoplasms. Although these tumors are uncommon, successful identification of lineage is critical since this group contains many treatable tumors, including hematopoietic neoplasms, germ cell tumors, neuroendocrine tumors, and melanoma [1, 11–13].

Recently, gene expression profiling has emerged as a valuable new diagnostic method in the evaluation of CUP [3]. To date, gene expression profiling in CUP has focused on tumors already identified as being carcinomas after standard pathologic evaluation. In approximately 95 % of patients with carcinoma, MCCA is successful in predicting a tissue of origin [3, 10], and increasing evidence documents the improved efficacy of site-specific treatment for some patients based on these diagnoses [3, 4]. However, the value of MCCA as a diagnostic tool has not been previously evaluated in undifferentiated neoplasms of unknown primary site.

In this study, we identified 30 patients presenting with CUP who received a diagnosis of undifferentiated neoplasm after complete pathologic evaluation. These 30 patients represented 4 % of the 731 CUP patients seen at our referral centers over a 12-year period. All these tumors had undergone extensive evaluation with IHC staining as part of the initial pathologic evaluation, and the initial diagnoses were confirmed by a second pathologic review as part of this study. We successfully performed gene expression profiling using the 92-gene RT-PCR MCCA in 29 patients (in one patient, remaining biopsy material was insufficient).

The results of this study confirm the role of gene expression profiling and the MCCA in the evaluation of patients with undifferentiated neoplasm of unknown origin. The tumor lineage was established in 25 of the 29 patients (86 %) who were successfully profiled, and a site of origin was predicted in 10 of 10 patients with carcinoma. Sufficient biopsy material was available in 16 patients to perform additional studies (see Table 2) directed by MCCA results (e.g. specific IHC stains, FISH testing, gene sequencing); in 11 of 16 tumors, these further studies, as well as correlation of clinical features, confirmed or supported the diagnoses made by MCCA. In one tumor (patient 2), the correct general lineage was diagnosed as sarcoma rather than the specific type (mesothelioma), but IHC staining confirmed the specific diagnosis of mesothelioma.

Although this was a retrospective study, several of the patients were seen during the last several years of the study timeframe, and received site-specific therapy based on the MCCA diagnosis. These treatments were particularly important for the two patients diagnosed with germ cell tumors, two patients with locally advanced neuroendocrine carcinomas, and one patient with lymphoma; all five responded favorably to therapy and remain alive and progression-free 25+ to 72+ months following treatment.

Prior to the availability of specialized pathologic diagnostic techniques, the identity of PDNs of unknown primary site remained uncharacterized. With the introduction of lineage-specific IHC stains, a substantial number of lymphomas [11] and neuroendocrine tumors were identified in this group [1, 2]. Following recognition of the i(12p) chromosomal abnormality typical in germ cell neoplasms, these tumors were also recognized [12]. All of these patients proved to be highly responsive to appropriate therapy [11–13]. The patients addressed in this report had the nonspecific diagnosis of PMN, even after pathologic examination and multiple IHC stains. Although these patients represent a small percentage of CUP, the identity of their tumors has remained undetermined.

The use of IHC staining over the 12 years when these patients were seen has evolved with more recent specific stains and panels/patterns of stains. It is likely that at least some of the 30 cases reported in this study may have been resolved or diagnosed by the use of IHC marker stains that are available, assuming the appropriate stains are applied. There are limitations on how many IHC stains may be undertaken given the size of the biopsies available, and it is usually not possible to indiscriminately obtain dozens of stains. The MCCA offer a major advantage as the gene expression platforms tested represent the majority of specific cancers and require only a limited amount of biopsy material to perform. With the MCCA, we demonstrate that various treatable cancers remain in this group and are unidentifiable by standard pathologic evaluation. Some of these patients derive major benefit from appropriate first-line treatment, and would not be optimally treated by the empiric chemotherapy often administered to CUP patients.

5 Conclusions

The results of this study confirm the value of the MCCA in the diagnosis of PDN of unknown primary site, and broaden its application in the management of patients with CUP. In this uncommon group, gene expression profiling can diagnose the tumor lineage and the tissue of origin in the majority of patients. Accurate diagnosis and early institution of appropriate first-line therapy are critical in several of these aggressive tumors, some of which are curable with site-specific therapy. Based on the results of this study, MCCA should be included in the initial evaluation of patients with PDN of unknown primary site.

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Conflict of interest None to declare.

Author contributions The retrospective case review was designed and performed by F. Greco and J. Hainsworth. All authors contributed and reviewed the data. F. Greco had primary responsibility for writing the article, and all authors reviewed and approved the final content.

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