

Evolving understanding and current management of patients with cancer of unknown primary site

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Patients with cancer of unknown primary site (CUP) represent a clinical challenge since their diagnosis, classification, and management have been frustrating and difficult. Over the past several years, many more relatively specific immunohistochemical marker stains have been developed, and the field of gene expression profiling has emerged. With these improvements, the diagnosis of the likely primary tumor site is possible in many patients, providing an opportunity for site-specific or tailored treatment, rather than empiric therapy for all patients. Although more validation of the usefulness of molecular profiling assays is required in CUP, the approach to these patients has changed, and now there is a real prospect for improving patient outcomes.

Cancer of unknown primary site (CUP) is not rare. About 80,000 new patients are diagnosed yearly in the United States. CUP is not a single entity but rather a clinical syndrome representing many types of cancers.¹ Autopsy series have documented primary sites in about 75% of CUP patients, but these primary sites are clinically undetectable, and most are adenocarcinomas.²

A fundamental definition of CUP is the presence of cancer and no clinically detectable primary tumor site of origin. The recommended evaluation of patients with suspected CUP to search for a primary tumor site has varied over the years and continues to evolve with newer and improved diagnostic technologies.³⁻⁵ There is no consensus on the diagnostic tests required for all patients at the time of clinical presentation. The large majority are carcinomas and the other lineages are occasionally confused with carcinoma (lymphoma, sarcoma, melanoma) or present without a known primary site (sarcoma, melanoma). These patients are excluded from further discussion here, since once recognized, they are treated ac-

ording to established guidelines for these neoplasms.

Diagnostic approach

The overall diagnostic approach to a possible CUP is illustrated in Figure 1. Note that particular clinical findings and pathologic findings may suggest additional diagnostic testing (the arrows go in both directions). The findings from various medical imaging tests may suggest additional specialized pathology be performed and vice versa. Most patients should have a biopsy (incisional, excisional, or core needle preferred) before embarking on more extensive evaluation.

The specific initial diagnostic evaluation recommended is listed in Table 1. In men with an elevated serum prostate-specific antigen (PSA) level, treatment should be for advanced prostate carcinoma. Needle biopsy of the prostate may confirm the diagnosis, but even if the biopsy is negative, the appropriate approach would not change, since an elevated PSA represents metastatic prostate in almost 100% of men.

Some would include PET scanning in the initial evaluation of CUP, but firm supporting data regarding

primary site detection are rather scant. Squamous cell carcinoma presenting in cervical lymph nodes is an exception, where a primary site is identified by PET in the head and neck region in about 50%–60% of these patients.¹ The most variable portion of the initial evaluation is the last bullet point listed in Table 1. Depending on the clinical findings and pathology, many additional tests can be considered in an attempt to find the primary site.

There is no uniform agreed-upon algorithm, but Table 2 lists suggested additional supplemental directed evaluation based on several clinicopathologic features. Any leads should be investigated further, such as colonoscopy and esophagogastroduodenoscopy, in any patient with occult blood in the stool or an MRI of the breasts in women presenting with isolated axillary carcinoma. There are many other examples, and the results

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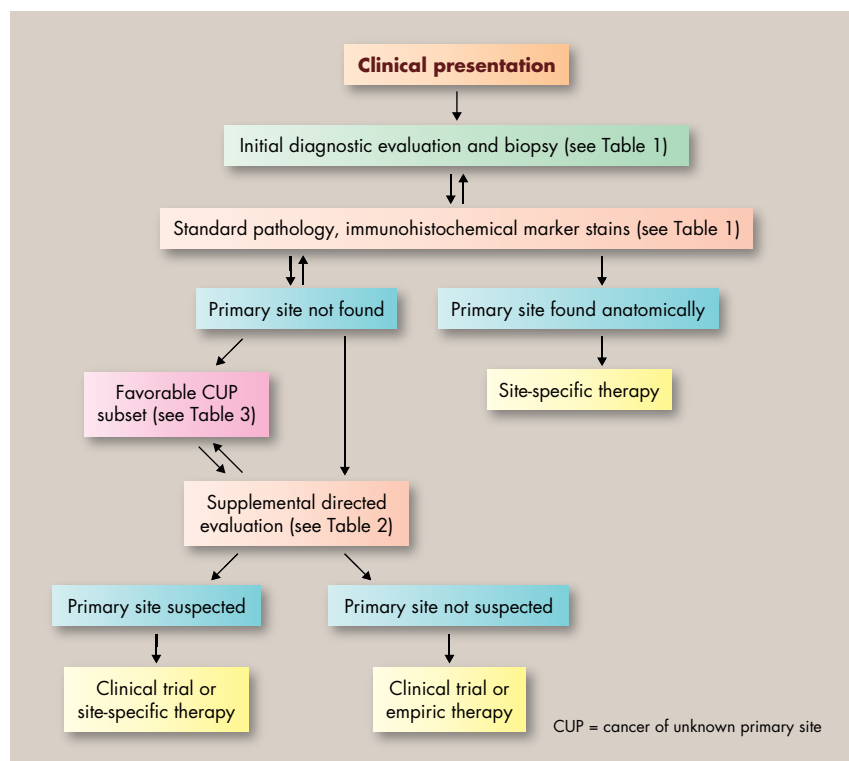


FIGURE 1 Approach to the patient with possible cancer of unknown primary site.

TABLE 1

Initial diagnostic evaluation of a patient with cancer of unknown primary site

- Complete history, including detailed review of symptoms
- Complete physical examination, including stool for occult blood
- Complete blood count, comprehensive metabolic panel, lactate dehydrogenase level, and urinalysis
- CT scans of chest, abdomen, and pelvis; PET scan in selected patients
- Mammography in women
- Serum PSA value in men
- Pathology, including screening immunohistochemical marker stains (CK7, CK20, TTF-1, and CDX-2)
- Further clinical and pathologic evaluation based on clues from history, physical examination, laboratory testing, medical imaging, and specialized pathology (see also Table 2)

CT = computed tomography; PET = positron emission tomography; PSA = prostate-specific antigen; CK = cytokeratin; TTF = thyroid transcription factor; CDX = intestine-specific transcription factor

of immunohistochemistry (IHC) marker staining and molecular profiling, which is now commercially available, frequently suggest additional specific or directed clinical evaluation

(Table 2).

If a primary site is not anatomically defined after the initial diagnostic evaluation or in selected patients by supplemental directed evaluation, the diagnosis of CUP is established. Regardless of the findings of IHC and/or molecular profiling assays, which may seem to “resolve” or “establish” the primary site, patients still should be considered as having CUP. It is now appropriate to identify CUP patients who have a tumor with IHC and/or molecular profile characteristics highly suggesting a particular primary site as CUP-colorectal, CUP-non-small cell lung, CUP-breast, etc. Perhaps with further study, including comparative therapeutic outcome data, this aspect of the definition may change.

About 20% of CUP patients can be categorized as “favorable subsets,” (Table 3) since considerable data in the past 3 decades have established their improved prognosis, usually with specific therapies, compared

with the majority (80%) of the other patients with CUP.

Therapy for the patients not defined in a favorable subset has been difficult; although, in the past decade, empiric regimens using combinations of broad-spectrum antineoplastic agents appear to have improved their overall long-term survival (40% at 1 year, 20% at 2 years, 10% at 3 years and beyond).^{6,7}

Current evaluation and management

My clinical approach to patients with CUP has evolved and continues to change with the rather rapid development of more precise IHC marker stains and molecular profiling assays,³⁻⁵ as well as the documented improvement in site-specific therapy for most known advanced solid tumor patients over the past decade (Table 4).⁷ Although the favorable subsets (Table 3) were as easy to recognize a decade ago as they are today, for the majority of the other CUP patients, the stakes are much higher than a decade ago, since many of these patients may have better outcomes if treated with site-specific therapeutic regimens.

For many patients with advanced carcinomas (including non-small cell lung, breasts, ovaries, esophagus, stomach, pancreas, bladder, prostate, colon, rectum, uterine cervix, anal canal, and head and neck), systemic therapy has improved considerably. One of the most dramatic improvements has occurred in advanced colon and rectal carcinomas, where the median survival for patients with advanced disease has increased from 8 months to near 2 years. Furthermore, in renal and hepatocellular carcinomas, targeted drugs improve overall survival, and cytotoxic therapy is not useful. The use of targeted drugs in combination with chemotherapy has also improved the overall survival, in patients with colorectal, non-small cell lung, pancreatic and breast carcinomas. Therefore, the use of appro-

TABLE 2

Supplemental directed evaluation for patients with suspected cancer of unknown primary site

Clinical feature	Additional immunohistochemical testing suggested ^a	Additional diagnostic evaluation suggested
Hepatic lesions	If CK7-, add Hep par 1	Serum aFP, EGD, ERCP
Women	If CK7+, add ER, GCDFP-15, WT1	If ER+ and/or GCDFP-15+, breast MRI; if WT1+, intravaginal ultrasonography
Mediastinal/retroperitoneal or diffuse adenopathy		
Men	PLAP, OCT4	Serum aFP, HCG; If PLAP and/or OCT4+, add FISH for i12p and testicular ultrasonography
Women	If CK7+, add ER, GCDFP-15, WT1	If ER+ and/or GCDFP-15+, breast MRI; if WT1+, add intravaginal ultrasonography
Peritoneal/omental	If CK7+	EGD, ERCP
Women	If CK7+, add ER, GCDFP-15, WT1	If ER+ and/or GCDFP-15+, breast MRI; if WT1+, add intravaginal ultrasonography
Bone Lesions	If CK7+	EGD, ERCP
Women	If CK7+, add ER, GCDFP-15, WT1	If ER+ and/or GCDFP-15+, breast MRI; if WT1+, add intravaginal ultrasonography
Brain lesions		
Women	If CK7+, add ER, GCDFP-15	If ER+ and/or GCDFP-15+, breast MRI
Poorly differentiated carcinoma, with or without clear cell	Chromogranin and synaptophysin; if CK7-, add RCC, Hep par 1, HMB45	If chromogranin and/or synaptophysin +, serum chromogranin, octreotide scan; if Hep par 1+, serum aFP
Women	If CK7+, add ER, GCDFP-15, WT1	If ER+ and/or GCDFP-15+, breast MRI; if WT1+, add intravaginal ultrasonography

CK = cytokeratin; ER = estrogen receptor; GCDFP = gross cystic disease fluid protein; WT = Wilms' tumor; aFP = alpha-fetoprotein; EGD = esophagogastroduodenoscopy; ERCP = endoscopic retrograde cholangiopancreatography; PLAP = placental-like alkaline phosphatase; OCT = octamer transcription factor; HCG = human chorionic gonadotropin; FISH = fluorescence in situ hybridization; HMB = human melanosome antibody

^a A molecular profile assay of the biopsy specimen should be considered for most patients without strong immunohistochemical evidence of a primary site (see Table 5).

appropriate therapy for the specific type of carcinoma is much more important now for many CUP patients than a decade ago. Single empiric based regimens administered to all patients would likely provide inferior treatment compared to a more individualized site-specific therapy for each patient.

Our ability to make a highly probable diagnosis of the occult primary cancer has improved by the use of improved IHC marker stains and molecular profiling assays. Further validation of the clinical usefulness of site-specific therapy for CUP patients is ongoing. Preliminary findings in CUP patients with IHC and molecular assay predictions of colorectal carcinoma do suggest this approach may be successful.⁸

The new and evolving diagnos-

TABLE 3

Treatment of favorable subsets of cancer of unknown primary site patients

Subset	Appropriate treatment
Extragenital germ cell syndrome (men; rare in women)	Treat as germ cell tumor
Predominant lymph node involvement	Treat with empiric chemotherapy or site-specific therapy (based on predicted primary site)
Squamous cell carcinoma (head/neck or inguinal area)	Treat as head/neck or anogenital carcinoma
Isolated axillary adenopathy/carcinoma (women; rare in men)	Treat as breast carcinoma
Peritoneal serous carcinoma (women; rare in men)	Treat as ovarian carcinoma
Neuroendocrine carcinoma (high grade/low grade)	Treat with etoposide/platinum or as carcinoid
Single small site of involvement (one lesion)	Treat with definitive local therapy and empiric chemotherapy or site-specific therapy (based on predicted primary site)

tic technology is critical in helping facilitate a personalized approach to therapy for each patient. However,

the clinical context (including gender, specific historical details, sites of metastases, standard pathology and

TABLE 4

The decade of change in diagnosis and treatment of cancer of unknown primary site

Characteristic	1999	2009
Immunohistochemistry	Few "specific" markers; not helpful in most patients	Several "specific" markers; helpful in some patients
Molecular profiling	Not developed/available	Helpful in some patients; complements immunohistochemistry
Primary site suspected based on all data	Occasionally, mainly in favorable subsets	Commonly
Empiric systemic treatment	Most patients	Minority of patients
Systemic treatment for common solid tumors	Useful for a few types	Useful for many types
Clinical trials	Few available	Few available

TABLE 5

Immunohistochemical marker staining profiles supportive of primary site in CUP^a

Primary site	Staining profile
Colorectal	CK7-, CK20+, CDX-2+
Lungs (adenocarcinoma/large cell)	CK7+, CK20-, TTF-1+
Breasts	CK7+, ER+, GCDFP-2+
Ovaries	CK7+, ER+, WT-1+
Prostate	CK7-, CK20-, PSA+
Kidneys	CD10+, vimentin+, RCC+
Liver	CD10+, Hep par 1+
Melanoma	S100+, Melan-A+, HMB45+
Germ cell	PLAP+, OCT-4+
Thyroid	TTF-1+, thyroglobulin+

CUP = cancer of unknown primary site; CK = cytokeratin; ER = estrogen receptor; WT = Wilms' tumor; aFP = alpha-fetoprotein; PLAP = placental-like alkaline phosphatase; OCT = octamer transcription factor; HMB = human melanosome antibody; RCC = renal clear cell; CD = cytoplasmic domain; TTF = thyroid transcription factor

^a When the above IHC profiles are present, CUP should be designated as CUP-colorectal profile, CUP-lung (adenocarcinoma/large cell) profile, CUP-breast profile, etc. Tumors do not always express expected marker stains or may express unexpected marker stains. If the diagnosis is unclear after immunohistochemical staining, a molecular profile assay should be considered.

laboratory, and medical imaging findings) remains an important component in evaluating patients and needs to be used in concert with IHC marker stains and molecular profiling assays in primary site prediction. New and more specific IHC marker stains are available today compared with 10 years ago.³

In the initial diagnostic evaluation (Table 1); screening IHC stains

should include CK (cytokeratin) 7, CK20, TTF-1 and CDX-2 on all initial biopsies. For patients with tumors fitting a colorectal profile (CK7-, CK20+, CDX-2+), colonoscopy should be performed. In those with a non-small cell lung cancer profile (CK7+, CK20-, TTF-1+), bronchoscopy should be considered. Several IHC staining profiles suggestive of the primary tumor type are now well appreciated (Table 5),³ but even when these occult primaries are present, not all the stains listed in Table 5 will be positive or negative in the tumor cells of these patients. For example, TTF-1 is not positive in a substantial minority of known adenocarcinomas and large cell carcinomas of the lungs.

The clinical picture and initial diagnostic evaluation (including screening IHC stains) should guide further possible testing in each patient, including imaging tests, IHC stains, and molecular profile assays in select patients (Table 2). Oncologists need to be proactive in communicating the clinical picture with pathologists, and pathologists must discuss the implications of various IHC stains with oncologists. In many instances, the clinical context guides which additional IHC stains may be useful, and IHC staining patterns may suggest further additional clinical evaluation. A stepwise evaluation of each patient starts with the initial diagnostic eval-

uation of the patient within a particular clinical context.

Molecular profiling assays to predict the tissue of origin in CUP were developed from microarrays, a technology invented about 15 years ago.^{4,5} There have been several modifications, including reverse transcriptase-polymerase chain reaction (RT-PCR) assays, and several assays are now commercially available (bioTheragnostics, messenger RNA RT-PCR assay; Prometheus Therapeutics and Diagnostics, microRNA RT-PCR assay; Pathwork Diagnostics, messenger RNA microarray assay). Additional data are necessary to adequately validate the usefulness of predicting the primary site. It does appear that molecular profiling assays complement IHC stains and improve our ability to diagnose the occult primary site.^{5,8-11}

Gene expression assays detect distinct normal cellular functions retained in part by neoplastic cells from their tissue of origin. It is often difficult to decide when a new diagnostic technology should be utilized in clinical medicine and particularly when it should become a standard approach. At this time, I would consider molecular profiling of the biopsy specimen for most patients who do not have a classic IHC marker stain profile and clinical picture suggestive of the primary site.

Most of the molecular assays require only very small amounts of tissue and can be performed on formalin-fixed, paraffin-embedded biopsy specimens. Although cytologic specimens obtained from fine-needle aspiration may be adequate for some patients, core biopsies or more traditional incisional or excisional biopsies are necessary to ensure a successfully completed assay.

Assays currently commercially available,¹²⁻¹⁴ as well as several others in development, appear to be quite accurate (80%-89%) in identifying specific cancer types in patients with

known primary cancers.

My clinical experience with more than 100 patients in the past 3 years (unpublished observations) would also support the usefulness of the molecular assays in helping to clarify more difficult CUP cases and in many instances has suggested a different therapy. Site-directed or tailored therapy for CUP patients based on a more accurate diagnosis of the primary site determined by IHC staining and/or molecular profile assays will likely improve overall outcome in patients with CUP.

In 2008, a small group of CUP-colorectal profile subset of patients has been treated with site-specific therapy, and these patients had responses, and survival similar to those of known advanced colorectal cancer patients.⁸ In several other CUP patients with the same colorectal profile treated with empiric paclitaxel and carboplatin⁸ chemotherapy, the response rates and survival were poor. Prospective studies are ongoing to further document outcome data in CUP patients treated with site-specific therapy based on predicted primary sites from molecular profile assays and/or IHC marker stains. It is likely that our ability to accurately predict the primary site in CUP will reshape the field and help produce a new paradigm of patient management. However, data are still required to validate this concept.

Additionally, an important component of molecular profiling would be validation of the accuracy in predicting a particular occult primary. This is difficult, since primary sites are rarely found in patients with CUP. Patients who have postmortem examination or develop a latent primary site provide an opportunity to validate the accuracy of both molecular profiling and IHC marker staining.

Recently, my associates and I reported a retrospective study on the accuracy of an RT-PCR assay^{5,15}

TABLE 6

Changes in treatment based on accurate molecular profile predictions

Patient	Primary site(s) suspected based on clinicopathologic features	Treatment for	Molecular assay diagnosis	Latent primary site later found	Likely change in treatment and outcome
1	Breast	Breast	Breast	Breast	No
2	Breast, lung	Breast	Breast	Breast	No
3	Lung	NSCLC	Breast	Breast	Yes
4	Lung, pancreas, stomach	CUP	Breast	Breast	Yes
5	Lung, breast	NSCLC	Ovary ^a	Primary peritoneal	Yes
6	Lung, breast, ovary	CUP	Ovary	Primary peritoneal	Yes
7	Lung, ovary, breast	CUP	Ovary	Primary peritoneal	Yes
8	Lung, pancreas	NSCLC	Ovary	Ovary	Yes
9	Colon/rectum	Colorectal	Intestine ^b	Colorectal	No
10	Colon/rectum	Colorectal	Intestine	Colorectal	No
11	Lung	NSCLC	NSCLC	NSCLC	No
12	Lung, head/neck	NSCLC	NSCLC	NSCLC	No
13	Lung	NSCLC	NSCLC	NSCLC	No
14	Lung, kidney, pancreas	CUP	Gastric	Gastric	Yes
15	Unknown	None	Melanoma	Melanoma	Yes

CUP = cancer of unknown primary site; NSCLC = non-small cell lung cancer

^a Ovary or primary peritoneal (indistinguishable by assay)

^b Colorectal or small intestine (indistinguishable by assay)

(bioTheranostics) performed on the initial diagnostic biopsies in CUP patients that later had their latent primary sites discovered during the course of their disease (mean time, 49 weeks). The assay was correct in 15 of 20 patients (75%), providing some direct information as to the accuracy of this particular molecular profile assay. In 8 of the 15 tumors correctly diagnosed by the assay, the treatment that was recommended would have been different had the results been known at the time of diagnosis. This is illustrated in Table 6, where patients 3–8, 14, and 15 would have had a more appropriate therapy recommended if the assay results had been known at that time.

As site-specific therapies continue to improve for patients with many known solid tumors, these therapies could then be immediately applied and studied in CUP, further defined by molecular profile assays. By its nature, clinical oncology is an evolving

and fluid field, and the acceptance of new technology is often a gradual process and depends on confirmatory data. As in all new and evolving areas of medicine, clinical judgment should be exercised in the use of these as well as other new technologies.

Clinical trials are recommended for CUP patients, even though there are only a few available. Empiric therapy has modestly improved the survival of CUP patients in the past decade.^{6,7} In the off-study setting, empiric regimens such as paclitaxel/carboplatin or gemcitabine (Gemzar)/irinotecan occasionally are used for patients without a suspected occult primary site (Figure 1). In patients with suspected primary sites, a site-specific regimen may be recommended. Most patients with CUP will likely be treated in the near future with therapy proven to be useful for their specific tumor type or with other specific agents directed at targets documented in their tumors by

molecular methods, regardless of the primary site.

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