113 Cancer of Unknown Primary Site

F. Anthony Greco and John D. Hainsworth

INTRODUCTION

Cancer of unknown primary (CUP) site is a clinical syndrome that includes many types of advanced cancers. Patients are considered to have CUP if no anatomical primary site is identified after clinical evaluation. As diagnostic techniques improve, the spectrum of patients with CUP continues to evolve.

Patients with CUP are common. The exact incidence is unknown because many of these patients are assigned other diagnoses and therefore are not accurately represented in tumor registries. Nonetheless, in the United States, CUP accounted for approximately 2% of all cancer diagnoses reported by Surveillance, Epidemiology, and End Results (SEER) registries.[1] International registries from seven other countries have reported incidences ranging from 2.3% to 7.8%.[2] The authors believe a more realistic estimate of the incidence of these patients is 5% of all invasive cancers, or approximately 80,000 to 90,000 patients per year in the United States.

Patients in this heterogeneous group have a wide variety of clinical presentations and histologic tumor types. Most patients have metastatic carcinoma; however, many neoplasms are difficult to categorize using histologic features alone. At autopsy, primary sites (usually <2 cm in size) can be located in the majority of patients; the molecular basis of this unusual biologic behavior is undefined. The preponderance of poorly treated tumor types in autopsy series (lung, pancreas, stomach, colon, liver) has led to negativity surrounding the diagnosis of CUP.[3]

Until recently, the major advance in the management of CUP was the recognition of several important patient subsets, identified by clinical and/or pathologic features and shown to benefit from specific first-line therapy. For the remainder of CUP patients (about 80%), empiric chemotherapy regimens were developed and resulted in modest benefit. At the time these regimens were developed, most types of solid tumors were poorly treated, and considerable overlap existed in the chemotherapy regimens used to treat sensitive tumor types. In this context, the possibility of developing a broad-spectrum chemotherapy regimen to adequately treat most of the treatable tumor types within the CUP population seemed feasible. However, during the last 20 years, treatments have not only improved for many tumor types, but have also become more site specific. Therefore, the idea of providing optimal treatment to patients with a diverse group of solid tumors using a single chemotherapy regimen is no longer feasible.

An accurate prediction of the tissue of origin is now possible for the majority of patients with CUP, using either improved panels of immunohistochemical (IHC) stains or molecular gene expression tumor profiling (MTP). Although the anatomical primary sites cannot be found in most patients even after the tissue of origin is predicted, increasing clinical experience confirms that these predictions can effectively guide site-specific therapy for patients with CUP.

This chapter is divided into three major sections. The first section reviews the pathologic evaluation of patients with CUP. New information regarding the emerging and important role of MTP assays is included. In the second section, the clinical evaluation of CUP patients is summarized. Situations in which results from the pathologic evaluation direct the clinical evaluation are addressed. Finally, the treatment of patients with CUP is discussed, with an emphasis on the favorable prognostic subsets and the new paradigm of site-specific therapy directed at the tissue of origin.

PATHOLOGIC EVALUATION

Histologic examination by hematoxylin and eosin (H&E) staining of a biopsy tumor specimen remains the gold standard for the initial evaluation and provides a practical classification system on which to base a subsequent evaluation. In the broad category of CUP, there are five major light microscopic histologic diagnoses: (1) poorly differentiated neoplasm, (2) poorly differentiated carcinoma (with or without features of adenocarcinoma), (3) well-differentiated and moderately well-differentiated adenocarcinoma, (4) squamous cell carcinoma, and (5) neuroendocrine carcinoma. Sarcoma and melanoma are also occasionally diagnosed without an obvious primary tumor site, and management of these patients follows established guidelines. These histologic diagnoses define patient groups that vary to some extent with respect to clinical characteristics, a recommended diagnostic evaluation, treatment, and prognosis (Fig. 113.1). Because a histologic examination rarely allows for the identification of the site of tumor origin, an additional pathologic evaluation is important in almost every patient with CUP. A fine-needle aspiration biopsy usually does not contain enough of a biopsy specimen for the necessary pathologic evaluation, and should not be performed as the initial diagnostic procedure. Close communication between the oncologist and pathologist is critical to ensure that the most important diagnostic studies are obtained with the available biopsy material.

Poorly Differentiated Neoplasms of Unknown Primary Site

If the pathologist cannot differentiate a general category of neoplasm (e.g., carcinoma, lymphoma, melanoma, sarcoma), the tumor is designated a poorly differentiated neoplasm. A more precise diagnosis is essential because many patients in this category have responsive tumors. Approximately 5% of all patients with CUP (4,000 patients annually) present with this diagnosis after a standard histologic evaluation. However, only a few remain without a defined lineage after specialized pathologic studies including IHC staining, electron microscopy, and, recently, MTP.[4–7] (These techniques are discussed separately.) In reported series, 35% to 65%

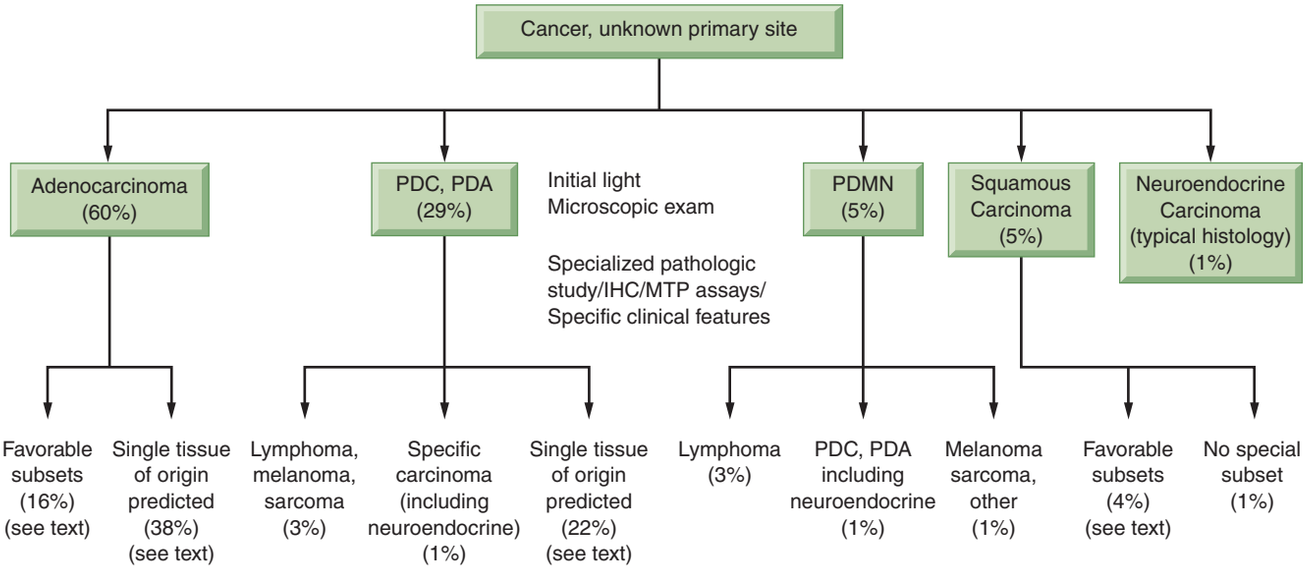


Figure 113.1 Relative size of various subgroups of patients as determined by clinical, specialized pathologic, and molecular evaluations. PDC, poorly differentiated carcinoma; PDA, poorly differentiated adenocarcinoma; IHC, immunohistochemistry; MTP, molecular tumor profiling; PDMN, poorly differentiated malignant neoplasm.

of poorly differentiated neoplasms were found to be lymphomas, which were highly responsive to specific therapy.[4,5] Most of the remaining tumors were carcinomas, including poorly differentiated neuroendocrine tumors. Melanoma and sarcoma together account for less than 15% of all patients.

Poorly Differentiated Carcinoma

Poorly differentiated carcinomas (PDC) account for approximately 30% of CUP (about 25,000 patients annually). In approximately one-third of these patients, some features of adenocarcinomatous differentiation can be identified (poorly differentiated adenocarcinoma). Some patients have extremely responsive neoplasms, and therefore, a careful pathologic evaluation is crucial.

Histopathologic features that can differentiate chemotherapy-responsive tumors from nonresponsive tumors have not been identified.[8] Even with a careful retrospective review of these tumors, responsive tumors of well-defined types (e.g., germ cell tumor, lymphoma) are only rarely identified.

All PDCs should undergo additional pathologic study for the purposes of (1) identifying other tumor types (e.g., lymphoma, sarcoma, melanoma) occasionally mistaken for carcinoma, (2) identifying neuroendocrine tumors, and (3) to determine the tissue of origin. Additional pathologic studies should include IHC stains, an MTP assay, and occasionally, electron microscopy and a karyotypic/cytogenetic analysis.

Adenocarcinoma

Well-differentiated and moderately differentiated adenocarcinomas are the most common tumors identified by light microscopy and account for 60% of CUP diagnoses (about 50,000 patients annually). These are the patients that many physicians associate with the entity of CUP. Typically, patients are elderly and have metastatic tumors at multiple sites. The sites of metastasis frequently determine the clinical presentation; common metastatic sites include the lymph nodes, the liver, the lung, and the bone.

The diagnosis of adenocarcinoma is based on light microscopic features, particularly the formation of glandular structures by neoplastic cells. All adenocarcinomas share histologic features, and the primary tumor site usually cannot be determined by a histologic examination. Certain histologic features are typically associated with a particular tumor type (e.g., papillary serous features with ovarian cancer and signet ring cells with gastric cancer). However, these features are not specific enough to be used as definitive evidence of their origin.

The identification of relatively cell-specific proteins by IHC staining has improved the ability to predict the tissue of origin in CUP patients.[7,9,10] Panels of IHC stains are most useful and are often directed by clinical features (e.g., sites of metastases, gender). Molecular tumor profiling assays are relatively accurate and often provide additional diagnostic information. Both of these diagnostic modalities should be utilized in the pathologic evaluation of adenocarcinoma of unknown primary site.

Squamous Carcinoma

Squamous carcinoma represents approximately 5% of patients with CUP (about 4,000 patients annually). Approximately 90% of these patients have specific clinical syndromes for which effective treatment is available; therefore, an appropriate clinical evaluation is important.

A definitive diagnosis of squamous carcinoma is usually made by a histologic examination. On occasion, an MTP assay may diagnose the tissue of origin. An additional pathologic study with IHC and MTP should be considered in patients with poorly differentiated squamous carcinoma, particularly if the clinical presentation is atypical.

Neuroendocrine Carcinoma

Neuroendocrine carcinoma with widely varying clinical and histologic features account for approximately 3% of all CUP (about 3,500 patients annually). Improved pathologic methods for diagnosing neuroendocrine tumors have resulted in the recognition of an increased incidence and wider spectrum of these neoplasms.[11]

Two general subgroups can be routinely recognized by histologic features. Low-grade tumors share the same histologic features as carcinoids and islet cell tumors and may secrete bioactive substances. An MTP assay may point to the tissue of origin[12,13]; in some instances (e.g., pancreatic origin), defining the site of origin carries treatment implications. A second histologic group

(variously described as small-cell carcinoma, atypical carcinoid, or poorly differentiated neuroendocrine carcinoma) has typical neuroendocrine features and a high-grade histology.

A third group cannot be recognized on a histologic examination, because neuroendocrine features are absent. These tumors appear histologically as poorly differentiated neoplasms or PDCs; an accurate identification requires IHC staining, an MTP assay, or occasionally, electron microscopy.

Immunohistochemical Tumor Staining

Immunohistochemical staining is the most widely available specialized technique for the classification of neoplasms.[7,9–22] Staining usually can be done on formalin-fixed, paraffin-embedded tissue, which broadens its applicability. Most IHC antibodies are directed at normal cellular proteins that are retained during neoplastic transformation. The ongoing development of antibodies to increasingly tissue-specific proteins makes this area of diagnostic pathology a dynamic and evolving field.

Several important questions can usually be answered by IHC staining. The correct lineage of poorly differentiated neoplasms can usually be identified (Table 113.1).[7,9,10,14–16] In particular, lymphomas (common leukocyte antigen staining) and poorly differentiated neuroendocrine carcinomas (chromogranin, synaptophysin, and CD56 staining) can be recognized,[10,15] and staining for germ cell tumors (octamer-binding transcription factor 4 [OCT4], placental alkaline phosphatase [PLAP]) may be diagnostic in an appropriate clinical situation.

The ability of IHC staining to identify the origin of various neoplasms has improved, but in most cases, the staining results must be interpreted in the context of clinical and histologic features. An exception is the prostate-specific antigen (PSA) stain, which is very specific for prostate carcinoma.[7] Stains suggestive of other primary sites are summarized in Table 113.1. The use of panels improves specificity[7,9,10,19–22]; several classic staining patterns have been described that are usually diagnostic of the tissue of origin (e.g., CK7+/CK20-/TTF-1+ in lung adenocarcinoma or CK7-/CK20+/CDX2+ in colorectal carcinoma). Four stains (CK7, CK20, thyroid transcription factor-1 [TTF1], CDX2) form the

TABLE 113.1

Immunohistochemical Tumor Staining Patterns in the Differential Diagnosis of Cancer of Unknown Primary[a]

Tumor Type	Immunohistochemical Staining
Carcinomas	Pan-cytokeratin AE1/3 (+), EMA (+), S100 (–), CLA (–), vimentin (–), CK7, 20 (variable)
Lymphomas	CLA (+), pan-cytokeratin AE1/3 (–), EMA (–), S100 (–)
Melanoma	S100 (+), HMB45 (+), melan-A (+) (all variable), pan-cytokeratin (–), CLA (–)
Sarcoma	Vimentin (+), desmin (+), CD117 (+), myogen (+), factor VIII antigen (+) (all variable), pan-cytokeratin AE1/3 (usually –), S100 (usually –), CLA (–), HMB45 (–), melan-A (–)
Neuroendocrine	Epithelial stains (+), chromogranin (+), synaptophysin (+), CD56 (+) (all variable)
Specific Sarcomas	
Gastrointestinal stromal tumor	CD117 (+), CD34 (+), DOG1 (+)
Mesothelioma	Calretinin (+), CD5/6 (+), WT1 (+), mesothelin (+)
Specific Carcinomas	
Colorectal	CK20 (+), CK7 (–), CDX2 (+)
Lung: adenocarcinoma	CK7(+), CK20 (–), TTF1 (+), napsin A (+)
Lung: squamous	CK7 (+), CK20 (–), P63 (+), CK5/6 (+)
Lung: neuroendocrine (small cell/large cell)	TTF1 (+), chromogranin (+), synaptophysin (+), CD56 (+)
Breast	CK7 (+), ER (+), PR (+), GCDFP-15 (+), Her2/neu (+), mammaglobin (+), GATA3 (+) (all variable)
Ovary	CK7 (+), ER(+), WT1 (+), PAX8 (+), mesothelin (+) (all variable)
Bladder (transitional cell)	CK20 (+), CK5/6 (+), p63(+), GATA3 (+), urothelin (+) (all variable)
Prostate	PSA (+), CK7 (–), CK20 (–)
Pancreas	CK7 (+), Ca19-9 (+), mesothelin (+)
Renal	RCC (+), PAX8 (+), CD10 (+), pan-cytokeratin AE 1/3 (+) (all variable)
Liver	Hepar1 (+), CD10 (+)
Adrenocortical	Alpha-inhibin (+), melan-A (+), CK7(–), CK20(–)
Germ cell	PLAP (+), OCT4 (+)
Thyroid/follicular/papillary	Thyroglobulin (+), TTF1 (+), PAX8 (+)

[a]Derived from references 7, 9, 10, and 14–23.
EMA, epithelial membrane antigen; S100, calcium-binding protein expressed in melanocytes; CD56, neural cell adhesion molecule; CLA, common leukocyte antigen; CK, cytokeratin; HMB-45, anti-human melanosome antibody; melan-A, melanoma antigen; CD34, cluster of differentiation molecule; CD117, tyrosine kinase receptor (c-kit); CDX2, intestinal specific transcription factor; DOG1, calcium dependent chloride channel; Napsin A, novel aspartic proteinase of the pepsin family; TTF1, thyroid transcription factor 1; ER, estrogen receptor; PR, progesterone receptor; GCDFP-15, gross cystic fluid protein 15; GATA3, zinc finger transcription factor family; WT1, Wilms' tumor transcription factor; PAX8, paired box gene 8; p63, tumor suppression gene protein; PSA, prostate specific antigen; RCC, brush border of proximal kidney tubule antibody; CD10, common acute lymphocytic leukemia antigen; hepar1, hepatocyte paraffin 1 marker; PLAP, placental alkaline phosphatase; OCT4, octamen-binding transcription factor 4.

basis of several diagnostic patterns or suggest other possibilities and are appropriate initial stains on most CUP biopsies.

Several problems are associated with the IHC stains. Technical expertise is required to perform these tests accurately; interpretation is subjective and requires an experienced pathologist. False-positive and false-negative results can occur with any of these stains. For example, some carcinomas stain with vimentin, some sarcomas stain with cytokeratins, and a wide variety of carcinomas do not always stain in the expected patterns.[7,22] Some classic staining patterns (see Table 113.1) can overlap with staining patterns of other carcinomas, forcing the pathologist to consider two or three possible primary sites. It is not feasible to routinely perform multiple unselected stains on biopsy specimens, and management of the tissue is becoming extremely important. Consideration of the clinical setting helps to direct the selection of stains and may narrow the spectrum of possibilities if patterns are not completely specific. For example, in a patient with mucin-positive adenocarcinoma and metastases to the liver, a CK20+/CK7–/CDX-2+ staining pattern provides strong evidence for colorectal carcinoma. The IHC findings may lead to additional diagnostic procedures; in the previous example, a colonoscopy should be performed and may identify the anatomical primary site.

In many cases, a single tissue of origin site cannot be identified with certainty even after an histologic examination, IHC staining, and correlation with clinical features. An electron microscopy or karyotypic analysis is occasionally helpful in this setting. However, the use of MTP assays allows for the identification of the tissue of origin in many cases where IHC is nondiagnostic.

Electron Microscopy

A diagnosis can be made by electron microscopy in some poorly differentiated neoplasms, but should be reserved for the study of neoplasms whose lineage is unclear after a routine light microscopy, IHC staining, and an MTP assay. Electron microscopy may be useful in undifferentiated sarcoma. Ultrastructural features such as neurosecretory granules (neuroendocrine tumors) or premelanosomes (melanoma) can suggest a particular tumor. Undifferentiated tumors can lose these specific ultrastructural features; therefore, the absence of a particular ultrastructural finding cannot be used to rule out a specific diagnosis. Electron microscopy is not able to distinguish among various adenocarcinomas and cannot usually identify a tissue of origin.

Karyotypic or Cytogenetic Analysis

The existence of specific chromosomal abnormalities is well characterized in several neoplasms. Most B-cell non-Hodgkin's lymphomas are associated with tumor-specific immunoglobulin gene rearrangements, and typical chromosomal changes have been identified in some B-cell and T-cell lymphomas and in Hodgkin's lymphoma.[23,24] In rare instances, these studies or an MTP assay is necessary when a diagnosis of lymphoma cannot otherwise be established.

The recognition of specific chromosome 12 abnormalities in germ cell tumors (e.g., i[12p], del[12p], multiple copies of 12p) occasionally allowed for the identification of extragonadal germ cell tumors in young men with poorly differentiated carcinoma of unknown origin. Motzer et al.[25] performed a karyotypic analysis on tumors in 40 young men with the extragonadal germ cell syndrome or midline carcinomas of uncertain histogenesis. In 12 of the 40 patients, abnormalities of chromosome 12 (e.g., i[12p]; del [12p]; multiple copies of 12 p) were diagnostic of a germ cell tumor. Other specific abnormalities were diagnostic of melanoma (two patients) and one patient each with lymphoma, peripheral neuroepithelioma, and desmoplastic small cell tumor. Of the germ cell tumors diagnosed on the basis of genetic analysis, five patients achieved a complete response to cisplatin-based chemotherapy. These data confirm the authors' previously formulated hypothesis that some of these patients have histologically atypical germ cell tumors.

•AQ1•

A few other nonrandom chromosomal rearrangements have been identified and occasionally can be useful in the diagnosis of CUP. Some examples include: t(11:22) in peripheral neuroepitheliomas, desmoplastic small round cell tumors, and Ewing's tumor[26–28]; t(15:19) in children and young adults with carcinoma of midline structures of uncertain histogenesis[29]; chromosome 12 abnormalities in germ cell tumors[30–32]; t(2:13) in alveolar rhabdomyosarcoma; 3p deletion in small-cell lung cancer; 1p deletion in neuroblastoma; t(X:18) in synovial sarcoma; and 11p deletion in Wilms' tumor. Epstein-Barr viral genomes in the tumor cells of CUP patients with cervical node metastases highly suggest nasopharyngeal primaries.[33,34]

However, most of these neoplasms can now be identified using methods that are more widely available (IHC, MTP assays), and karyotypic analyses should be reserved for patients with the histologic diagnoses of poorly differentiated neoplasm or PDC, in whom other studies fail to narrow the diagnostic spectrum.

Molecular Tumor Profiling Assays and Cancer Classification

Gene expression or molecular profiling of human neoplasms arose from a DNA microarray analysis described about 20 years ago,[35,36] and subsequent studies have expanded genomic understanding of neoplasms.[37–45] A pivotal study in cancer classification was reported by Golub et al.,[37] and demonstrated for the first time that patterns of gene expression alone could discriminate acute myeloid leukemia from acute lymphoblastic leukemia. Other investigators demonstrated that numerous cancer types could be classified accurately by measuring the differential expression of specific gene sets.[41–61] The basis of molecular profiling in recognizing specific cancer types is the identification of the genes responsible for the synthesis of proteins required for specific normal cellular functions or relatively specific cytoplasmic microRNAs in the many different normal cell types in humans. Cancer cells often retain some of the functional characteristics specific to their tissues of origin, and can be recognized by their gene expression profiles.[59] Therefore, molecular profiling assays designed to determine the type of cancer measure gene expression dynamics in relation to cell lineage, rather than cancer-specific molecular abnormalities.

Patients with CUP represent a large heterogeneous group with clinically undefined anatomical primary tumor site and are ideal candidates for classification by molecular profiling.[38] Several MTP assays (molecular cancer classifiers) have been validated in the identification of known cancers and studied in CUP.

Two such assays are commercially available.[59,61] One of these is a 92-gene reverse transcription polymerase chain reaction (RT-PCR) mRNA assay[59] (Cancer TYPE ID; bioTheranostics, Inc.), whereas the other uses microarray methodology[61] to measure tissue-specific microRNAs (Cancer of Origin Test, Rosetta Genomics). A third microarray mRNA assay was previously also offered (Tissue of Origin Test), but is no longer available.[49]

Two studies of more than 100 tumors each have compared the accuracy of IHC and MTP assays in determining the tissue of origin of known primary cancers.[62,63] These blinded studies usually had generous amounts of tissue to test and allowed the participating pathologists to do numerous IHC stains. The MTP assay diagnostic accuracy was superior to IHC, particularly when tumors were poorly differentiated, or when the IHC diagnosis was unclear following the first round of IHC stains. In addition, the molecular diagnosis required much less tumor tissue (two to three unstained slides). These data support a further evaluation of MTP assays in CUP, particularly when IHC staining is inconclusive or when limited biopsy tissue is available.

STUDIES OF MOLECULAR GENE EXPRESSION TUMOR PROFILING ASSAYS IN CANCER OF UNKNOWN PRIMARY DIAGNOSIS

Accuracy in Tissue of Origin Diagnosis

The determination of the tissue of origin in CUP patients has fundamental importance and has always been the goal of their evaluation. Improved IHC and MTP assays have the potential, when used in conjunction with other clinicopathologic data, to determine the tissue of origin in most CUP patients. However, in order to feel confident in the use of an MTP assay, three questions needed to be addressed: (1) Are they accurate in diagnosing known primary cancers? (2) Are they accurate in diagnosing the tissue of origin in CUP? (3) Are the outcomes of CUP patients improved by site-specific therapies directed by MTP diagnoses? Substantial evidence supports the accuracy of MTP in diagnosing known cancers and CUP, and some of these data are reviewed here. The third question has also been addressed and is discussed in a later section (see Site-Specific Treatment Directed by Results of Molecular Gene Expression Tumor Profiling Assays).

When applied to biopsy specimens from metastatic sites or primary tumors of known cancers, various MTP assays have correctly predicted the tissues of origins and/or primary sites in about 85% of patients.[46–51,59–61] All these studies were blinded and used the known cancer type as the reference. The accuracy of the various MTP assays has not been directly compared.

Accurate identification of the tissue of origin in CUP by MTP is difficult to validate, because the anatomical primary tumor site is unknown and only rarely becomes apparent during the subsequent clinical course of these patients. It would seem reasonable to assume that these assays have a similar accuracy rate in CUP because they have previously demonstrated in metastatic cancers of known types. This assumption is supported by the results of several studies in CUP patients (Table 113.2).[46,47,53–58,64–68] Most studies of the accuracy of MTP assay diagnoses have been indirect, based on the correlation of the assay diagnosis with clinical features and other pathology studies (particularly IHC). In these studies, the MTP predictions correlated closely with the diagnoses suspected on the basis of standard clinical/pathologic findings. In a total of 698 CUP patients from a dozen studies using various MTP assays/platforms, an average of 80% of the molecular diagnoses were consistent with one of the suspected tissues of origin.[46,47,53–58,64–68]

The authors and associates have studied the initial version of the 92-gene RT-PCR MTP assay in CUP patients to determine the accuracy and ability to complement standard pathology.[69] Three methods were used to assess the accuracy of the MTP assay, including a direct validation (gold standard reference) in patients who had their anatomical primary sites (latent primaries) found months to years after their initial CUP diagnoses. Two other indirect methods were used: (1) a comparison of the MTP and IHC diagnoses when IHC was able to predict a single tissue of origin, and (2) additional supportive directed IHC and clinical/histologic findings obtained after the MTP assay diagnoses.

A total of 171 CUP patients were evaluated: 151 prospectively seen from 2008 through 2010, and 20 others with latent primaries identified retrospectively from 501 patients seen from 2001 through 2008. The molecular diagnoses of these patients are listed in Table 113.2. Although the assay requires only 300 to 500 tumor cells, there was insufficient biopsy remaining (RNA) to perform the assay in 22 patients (12.9%). In 5 others (3%), the assay results were unclassifiable or not diagnostic of a single site. Of the 149 patients with sufficient tumor specimens, 144 (96%) were diagnosed with a single tissue of origin (23 tumor types).

Twenty-four patients (20 from retrospective group,[58] and 4 from prospective group) had their latent primary sites detected at a median time of 12 months (range: 2.2 to 78.5 months) after their initial diagnosis/evaluation of CUP. In 75% (18 of 24 patients), the MTP assay diagnosis of their initial biopsies matched the latent primary tumor sites. In contrast, the IHC evaluation was successful in identifying the site of tumor origin in only 6 of 24 patients (25%).

TABLE 113.2

Molecular Gene Expression Tumor Profiling Assay Diagnoses

Site	Number	%
Insufficient tumor	22	12.9
Unclassifiable	5	3
Lung/adeno, large cell	18	10.5
Colorectal	26	15.2
Lung/small cell	6	3.5
Lung/squamous cell	1	0.6
Hepatocellular	10	5.8
Breast	15	8.8
Pancreas	9	5.2
Ovary	9	5.2
Bladder	7	4
Kidney	7	4
Gallbladder	6	3.5
Melanoma	5	3
Skin/squamous	5	3
Endometrium	3	1.7
Sarcoma	4	2.3
Testicle	3	1.7
Stomach	2	1.2
Thyroid	2	1.2
Mesothelioma	2	1.2
Others	4	2.4

Note: N = 171.
Adapted from Greco FA, Lennington WJ, Spigel DR, et al. Molecular profiling diagnosis in unknown primary cancer; accuracy and ability to complement standard pathology. *J Natl Cancer Inst* 2013;105:782–790.

The second method to assess the accuracy of MTP involved a comparison with IHC predictions in the 52 cases (30%) in which IHC suggested a single tissue of origin (Table 113.3). In 40 of these 52 patients (77%), the MTP and IHC diagnoses were identical. Others have reported similar results from five studies in a total of 65 patients (78% with identical IHC and MTP assay diagnoses) using various assays.[54,55,57,67,70]

The third method to access MTP assay accuracy involved performing additional directed diagnostic studies not included in the initial evaluation (IHC studies, clinical testing, histology review) to support the MTP assay diagnoses. Fifty-four patients (32%) had MTP assay diagnoses that did not match any of the suggested IHC diagnoses; 35 of these patients had remaining biopsy specimens available for additional studies. In 74% of these patients (26 of 35), additional findings supported the accuracy of the MTP assay diagnoses (Table 113.4).

All three methods used in this study support the accuracy (75% to 80%) of the 92-gene RT-PCR assay in determining the tissue of origin in CUP. The accuracy is similar to that seen with MTP assays when tested in known cancer types. The aggregate data from many other studies in patients with CUP[46,47,53–58,64–68] provide additional support of the value of molecular diagnoses. Molecular tumor profiling complements IHC, and usually provides a single

TABLE 113.3

Comparison of MTP Assay Diagnosis with IHC in Tumors with a Single Site Predicted by IHC

Diagnosis	Single Diagnosis by IHC Staining	IHC Staining Pattern	Agreement of MTP Assay Diagnoses with IHC Diagnoses	% Agreement
Colorectal	16	CK20+, CK7–, CDX-2+	15	93
Lung/adeno/large cell	19	CK20–, CK7+, TTF-1+	14	74
Lung/neuroendocrine	3	CK7+, TTF1+, chromogranin+ or synaptophysin+ or CD56+	2	66
Breast	5	CK20–, CK7+, mammaglobin+ or ER+ or GCDFP-15+	5	100
Melanoma	3	S100+, Melan-A+ or HMB45+	2	66
Germ cell	2	PLAP+ or OCT4+	1	50
Ovary	1	CK20–, CK7+, CA125+, WT1+, ER+	0	0
Hepatocellular	1	CD10+, Hepar-1+	1	100
Sarcoma	1	Vimentin+, CK7–, S100–, desmin+, CK20–	0	0
Prostate	1	CK20–, CK7–, PSA+	0	0
Total	52		40	77

Note: N = 52.
Adapted from Greco FA, Lennington WJ, Spigel DR, et al. Molecular profiling diagnosis in unknown primary cancer; accuracy and ability to complement standard pathology. *J Natl Cancer Inst* 2013;105:782–790.
•AQ2• CK, cytokeratin; CDX, ???; S100, calcium-binding protein expressed in melanocytes; ER, estrogen receptor; GCDFP-15, ???; CA125, ???; WT1, Wilms' tumor transcription factor; hepar-1, hepatocyte paraffin 1 marker. •AQ2•

diagnosis when IHC is inconclusive. When IHC predicted a single tissue of origin (as in 33% of patients in our series) the MTP assay prediction has high concordance and is probably not necessary.

As MTP is incorporated into the diagnostic evaluation of CUP patients, several potential pitfalls should be considered.[69] First, tumor biopsy specimens are often small, and the medical oncologist and pathologist should use available tissue judiciously. Performing multiple IHC stains in a tumor that is difficult to classify can deplete the biopsy and preclude MTP. In some cases, a repeat biopsy should be considered. Second, MTP assay diagnoses are not 100% accurate, even in the identification of known cancer types. MTP predictions should always be considered in context with clinical features and results of other pathologic studies. If the MTP diagnosis is inconsistent with other findings, additional directed IHC stains or a clinical evaluation may help to support the diagnosis. Third, several neoplasms have overlapping gene expressions, and a misdiagnosis may occur in these circumstances (e.g., breast, salivary gland, and skin adnexal tumors have similar gene expression profiles). Fourth, any tumor types that are not included in the MTP assay panel cannot be diagnosed, and may be considered unclassifiable or misdiagnosed as a cancer with an overlapping gene expression profile.

Diagnosis in Poorly Differentiated Neoplasms

The use of modern IHC staining is usually effective at defining the tumor lineage in CUP patients with poorly differentiated neoplasms. In the uncommon tumor that defies classification, MTP usually clarifies the lineage and, in some cases, can identify the tissue of origin.[71]

From 2000 through 2012, 751 CUP patients were seen at the authors' referral center,[71] and 30 (4%) had no definitive lineage determined after extensive IHC (median of 18 stains; range: 9 to 51). The archival biopsies were tested with the 92-gene RT-PCR assay and when feasible, the additional evaluation was performed to support the molecular diagnoses (e.g., directed IHC not previously done, fluorescence in situ hybridization (FISH) for specific molecular abnormalities, gene sequencing, repeat biopsies, and correlation with clinical features). Four tumors were unclassifiable by MTP, and one additional tumor had insufficient biopsy material available (17%). A lineage diagnosis was made in 25 of 30 patients (83%), including carcinoma in 10 (germ cell: 3, neuroendocrine: 2, others: 5), sarcoma in 8 (mesothelioma: 3, primitive neuroectodermal tumor [PNET]: 1, others: 5), melanoma in 5, and hematopoietic neoplasm in 2 (lymphoma: 2). Additional directed IHC, genetic testing (BRAF, i12p), or repeat biopsies done after MTP confirmed the MTP diagnoses in 11 of 15 patients (73%). Earlier use of MTP in the diagnosis of these difficult cases would have resulted in the expedited identification of tumor lineage in almost all patients, and often in a prediction of the specific tissue of origin. Because this group of tumors contains germ cell tumors, lymphoma, neuroendocrine tumors, and melanomas, an accurate diagnosis is critical. Molecular tumor profiling appears superior to IHC in the diagnosis of these undifferentiated cancers.

Diagnosis of the Cancer of Unknown Primary Renal Cell Carcinoma Subset

The renal carcinoma subset of CUP has not been previously systematically addressed. A few CUP cases have been reported[72,73] after recognition by pathologic features or MTP. The diagnosis of renal carcinoma is of practical importance, because these tumors are usually insensitive to cytotoxic chemotherapy, yet may often be treated with good control by a number of approved targeted/biologic drugs. When clear cell histology is seen in a CUP biopsy, the possibility of renal carcinoma is considered, and IHC may be diagnostic. However, histologies of adenocarcinoma or poorly differentiated carcinoma may not suggest renal carcinoma, and IHC stains to support the diagnosis of renal carcinoma may not be done.

In order to further characterize the renal cancer subset, 488 CUP patients seen from 2008 to 2012 had MTP of their biopsies using the 92-gene RT-PCR assay.[74] In many of these patients, MTP results were available for patient management. A renal carcinoma diagnosis was made in 22 patients (4.5%), including the subtypes papillary in 8, clear cell in 7 and unknown in 7. None of these patients had a primary site detected by abdominal computed tomography, and the metastatic sites most often included retroperitoneal nodes (63%), mediastinal nodes (31%), lung (22%), and bone

TABLE 113.4

Results of Additional IHC Stains and/or Clinicopathologic Studies Performed to Support MTP Assay Diagnoses in Tumors with Uncertain Initial IHC Diagnoses

MTP Assay Diagnoses (all with uncertain IHC diagnoses)	Additional Subsequent IHC and/or Clinicopathologic Findings
1. Kidney	CD10+, CA-9+, vimentin+
2. Kidney	RCC+
3. Kidney	Histologic review: scattered papillary and chromophobe features, vimentin+
4. Kidney	CD10+, CA-9+
5. Hepatocellular	Serum α-fetoprotein 5259, reticulin stain+
6. Hepatocellular	Serum α-fetoprotein 1326
7. Hepatocellular	Serum α-fetoprotein 649, Hepar1+
8. Hepatocellular	Serum α-fetoprotein 810
9. Hepatocellular	Serum α-fetoprotein 501
10. Ovary/serous	ER+, PR+, WT1+
11. Ovary/clear cell	New ascites, WT1+
12. Mesothelioma	Abdominal and pelvic masses, calretinin+
13. Mesothelioma	Abdominal mass, calretinin+
14. Sarcoma	CK7–, CK20–, S100–, LCA–, vimentin+, isolated bone/soft tissue lesion
15. Sarcoma	Desmin+, vimentin+, rapid growth chest wall and lung masses
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+
19. Lung/neuroendocrine	Chromogranin+, synaptophysin+
20. Endometrium	Pelvic mass, PR+, ER+
21. Intestine/carcinoid	CK20+, synaptophysin+, CDX2+
22. Bladder	CK7–, CK20–, p63+, histologic review: areas of transitional cell carcinoma
23. Breast	ER+
24. Intestinal	CDX2+
25. Seminoma	CK7–, CK20–, PLAP+
26. Prostate	Developed sclerotic bone lesions, serum PSA 32 (initially normal)
27–35. Various diagnoses	No additional supportive data found

Note: N = 35.

Adapted from Greco FA, Lennington WJ, Spigel DR, et al. Molecular profiling diagnosis in unknown primary cancer; accuracy and ability to complement standard pathology. *J Natl Cancer Inst* 2013;105:782–790.

•AQ2•
•AQ2•
CD10, ???; CA-9, ???; RCC, brush border of proximal kidney tubule antibody; hepar1, hepatocyte paraffin 1 marker; ER, estrogen receptor; PR, progesterone receptor; WT1, Wilms' tumor transcription factor; CK, cytokeratin; S100, calcium-binding protein expressed in melanocytes; LCA, ???; CDX, ???.

(18%). Only 1 biopsy had clear cell histology; 15 were PDC and 6 were adenocarcinomas (4 with papillary features). Because the histology did not suggest renal carcinoma in most patients, only •AQ2• three tumors (14%) had renal directed IHC (RCC, PAX8 stains) performed. However, additional IHCs performed to support the MTP assay diagnoses were typical of a renal origin in seven of nine tumors where a remaining biopsy was available. Sixteen patients received first-line treatment for advanced renal carcinoma; the objective response was 18% and the median survival was 13.4 months.

Renal cell carcinoma is a subset of CUP, which can be diagnosed by MTP assay and/or IHC. When the histologic examination does not identify clear cell features, the diagnosis of renal carcinoma may not be considered, and IHC staining for renal cancer may not be done. Papillary renal carcinoma was a relatively common subtype in the group studied. These patients are important to identify because they may benefit from renal cell–directed targeted drugs but not from empiric chemotherapy.

Diagnosis of the Cancer of Unknown Primary Colorectal Subset

These patients have been characterized recently and are a favorable subset. These data are discussed later (see section Treatment: Favorable Subsets: Colorectal Profile).

Summary

Molecular tumor profiling can predict the tissue of origin in a majority (about 95%) of patients with CUP when there is sufficient biopsy material available. These predictions are accurate in 75% to 80% of patients, as determined by several methods, including an evaluation of CUP patients who developed latent primaries. The correlation between IHC and MTP diagnoses is good (about 80%) when IHC predicts a specific tissue of origin; in patients with

diagnostic IHC, MTP is not necessary. However, in the majority of patients, IHC is inconclusive and MTP provides valuable additional diagnostic information. The optimal identification and evaluation of several subgroups of potential therapeutic importance, including colorectal, renal cell, and poorly differentiated neoplasms now requires the use of MTP in conjunction with a standard pathologic evaluation. A specific diagnosis of some tissues of origin by MTP should trigger additional molecular analysis, because effective targeted therapy for patient subsets is becoming common. For example, a prediction of non–small-cell lung cancer, should prompt an analysis for treatable molecular alterations, including epidermal growth factor receptor (EGFR)-activating mutations and anaplastic lymphoma kinase (ALK) or ROS1 rearrangements.

CLINICAL FEATURES AND EVALUATION

Most patients with CUP develop signs or symptoms at the site of a metastatic lesion and are diagnosed with advanced cancer. The subsequent clinical course is usually dominated by symptoms related to metastases; a latent primary site becomes obvious in about 5% of patients during their lifetime. At autopsy, a primary site is identified in about 75% of patients.[3,6] Primary sites in the pancreas, lung, colon/rectum, and liver account for approximately 60% of those identified. Primary sites in the breast, ovary, and prostate are uncommon in autopsy series, but data from MTP assay series suggest that the biliary tract, the urothelial tract, the breast, and the ovarian tissues of origin are more common than previously recognized.[6,52]

Although some clinical differences exist, there is substantial overlap between the clinical features of patients with adenocarcinoma and PDC. Patients with PDC have a somewhat younger median age and usually exhibit rapid tumor growth. These patients may also have a more frequent location of dominant metastatic sites in the mediastinum, retroperitoneum, and peripheral lymph nodes. The clinical evaluation of patients with these histologies should follow the same guidelines. Patients with neuroendocrine carcinoma and squamous carcinoma are discussed separately.

TABLE 113.5

Initial Diagnostic Evaluation

- Complete history: including detailed review of systems
- Complete physical examination: including pelvic examination, stool for occult blood
- Complete blood cell count, comprehensive metabolic panel, lactate dehydrogenase, urinalysis
- Computed tomography scans of chest, abdomen, and pelvis
- Mammography in women
- Serum prostate-specific antigen in men
- Positron emission tomography scan in selected patients
- Pathology: including screening immunohistochemistry marker stains (CK7, CK20, TTF-1, CDX2)
- MTP assay if small biopsy specimen

Clinical Evaluation

The recommended clinical evaluation for all patients is summarized in Table 113.5. In actuality, many of these procedures are usually done in the process of diagnosing CUP. The goal is to find the anatomical primary site or, if not possible, the tissue of origin. Although positron-emission tomography (PET) scanning may be useful in some patients,[57] it should not be considered routine in the initial CUP evaluation; definitive data in large numbers of patients have not been published[75] and PET was not superior to computed tomography (CT) in finding a primary site in one comparative study.[76]

A further evaluation of patients should be directed by results of the initial clinical and pathologic evaluations. A focused evaluation may: (1) identify an anatomical primary site, (2) narrow the spectrum of possible tissues of origin, (3) identify specific favorable subsets of patients, or (4) identify the tissue of origin even if the anatomical primary site is undetectable.

Table 113.6 summarizes the additional evaluation indicated for several common clinical presentations. An additional focused

TABLE 113.6

Focused Diagnostic Evaluation of Patient Subsets Defined by Initial Clinicopathologic Evaluation

Initial Evaluation	Additional Evaluation
Women with features of possible breast cancer (bone, lung, liver metastases, CK7+)	Breast magnetic resonance imaging ER, GCDFP-15, GATA3 stains, FISH for HER2 (MTP assay if necessary)
Women with features of possible ovarian cancer (pelvic/peritoneal metastases; CK7+)	Pelvic/intravaginal ultrasound WT1, PAX8 stains (MTP assay if necessary)
Mediastinal/retroperitoneal mass	Testicular ultrasound Serum HCG, AFP PLAP, OCT4 stains; FISH for i(12p) (MTP assay if necessary)
Features of lung cancer (hilar/mediastinal adenopathy; CK7+, TTF1+, Napsin A+)	Bronchoscopy (MTP assay if necessary), Genetic studies for EGFR mutations, ALK/ROS1 rearrangements
Features of colon cancer (liver/peritoneal metastases; CK20+/CK7–, CDX2+)	Colonoscopy (MTP assay if necessary), Genetic study for KRAS mutation
Poorly differentiated carcinoma, with or without clear cell features	Stains for chromogranin, synaptophysin, RCC, hepar-1, HMB-45, Melan-A (If melanoma stains +, genetic study for BRAF mutation; if hepar-1+, obtain serum AFP; if neuroendocrine stains +, obtain octreotide scan (MTP assay if necessary)

•AQ2• CK, cytokeratin; ER, estrogen receptor; GCDFP-15, ???; GATA3, zinc finger transcription factor family; WT1, Wilms' tumor transcription factor; PAX8, paired box gene 8; HCG, human chorionic gonadotropin; AFP, α-fetoprotein; OCT4, octamen-binding transcription factor 4;
•AQ2• HMB-45, ???;

evaluation should be triggered by either clinical findings or IHC results during the initial evaluation. An MTP assay is indicated when IHC or other testing is not conclusive. Panels of IHC stains and an MTP assay are complementary and when considered in conjunction with all other data provide a tissue of origin diagnosis in the majority of CUP patients.

Neuroendocrine Carcinoma

Although the initial clinical evaluation is the same (see Table 113.5), patients with neuroendocrine carcinoma require special consideration in determining appropriate treatment. Of major importance is the separation of this group into tumors with low-grade histology (classic carcinoid) and indolent clinical course versus those with high-grade histology (small or large cell with neuroendocrine features) and an aggressive clinical course. Some of the high-grade carcinomas may not be recognized as neuroendocrine by H&E staining, but are usually diagnosed by IHC or MTP assay.

Low-grade neuroendocrine carcinomas, when presenting with an unknown primary site, most frequently involve the liver. Other metastatic sites include the lymph nodes (usually abdominal or mediastinal) and bone. Some are associated with various syndromes caused by the secretion of bioactive peptides (carcinoid syndrome, glucagonoma syndrome, VIPomas, Zollinger-Ellison syndrome). An additional clinical evaluation in these patients should include serum or urine screening for these substances. In addition to the evaluation listed in Table 113.5, an octreotide scan as well as an upper and lower gastrointestinal endoscopy should be performed, because some of these patients have detectable primary sites in the gastrointestinal tract. An MTP assay may diagnose the tissue of origin in some patients[12,13]; the identification of a pancreatic site of origin is potentially important because targeted drugs (sunitinib, everolimus) are useful in this setting.

High-grade neuroendocrine carcinomas of unknown primary site are usually found in multiple metastatic sites and rarely secrete bioactive peptides. Patients with small- or large-cell histology and a history of cigarette smoking should be suspected of having an occult lung primary and a fiber optic bronchoscopy should be considered. Patients with a positive tumor cell IHC stain for TTF1 should also be considered for a bronchoscopy. Extrapulmonary small-cell carcinomas arising from a variety of other primary sites (salivary glands, paranasal sinuses, esophagus, pancreas, colon/rectum, bladder, prostate, uterus, cervix) have been described and are occasionally identified during a clinical evaluation. A colonoscopy should be considered in patients with tumor IHC staining positive for CDX2.

The origin of these high-grade neuroendocrine carcinomas remains unclear. The tissue of origin may be determined in some patients by an MTP assay, but this knowledge now usually does not change therapy for most patients. Some patients with small-cell histology may have occult small-cell lung cancer. However, more than half of these patients have no smoking history, which makes this diagnosis unlikely. It has been speculated that these undifferentiated tumors share the same origin as the low-grade neuroendocrine tumors, and are at opposite ends of a *spectrum* of tumor biology. However, it now seems more likely that these high-grade neuroendocrine tumors have a different oncogenesis; many share the chromosomal abnormalities commonly seen in small-cell lung cancer (deletions of chromosomes 3p, 5q, 10q, and 17p), whereas no shared molecular abnormalities have been found with indolent carcinoid-type tumors.[77,78]

Anaplastic or atypical carcinoid tumors arising in the gastrointestinal tract are responsive to platinum-based chemotherapy, whereas carcinoid tumors with typical histology are usually resistant.[79] A few reports of patients with extrapulmonary small-cell carcinomas of unknown primary site have also documented chemotherapy responsiveness and occasional long-term survival after systemic therapy.[80,81] However, the term *extrapulmonary small-cell carcinoma* implies the existence of a known primary site; some CUP neuroendocrine tumors may have arisen from an occult extrapulmonary site, but are aptly described as neuroendocrine carcinoma of unknown primary site.

Squamous Carcinoma

Squamous carcinoma, as opposed to other histologies, often presents with isolated metastases in the cervical or inguinal lymph nodes. The cervical nodes are the most common metastatic site. Patients are usually middle aged or elderly, and frequently, they have abused tobacco or alcohol, although recently it has also been associated with human papilloma virus infection. When the upper or middle cervical nodes are involved, a primary tumor in the head and neck region should be suspected. The clinical evaluation should include an examination of the oropharynx, hypopharynx, nasopharynx, larynx, and upper esophagus by direct endoscopy, with a biopsy of any suspicious areas. CT of the neck better defines the disease in the neck and occasionally identifies a primary site. PET scanning in this subset is indicated, because it can frequently identify primary tumor sites.[82] Detection of Epstein-Barr virus genome in the tumor tissue is highly suggestive of a nasopharyngeal primary site,[33,34,83] particularly in poorly differentiated carcinomas. When the lower cervical or supraclavicular nodes are involved, a primary lung cancer should be suspected. A fiber optic bronchoscopy may identify a lung primary if other evaluations are unrevealing.[84]

An ipsilateral or bilateral tonsillectomy has been advocated as a diagnostic modality in patients with single nodal or bilateral nodal involvement, respectively.[85] In one series of 87 patients who had tonsillectomy as part of their workup for cervical node presentations, 26% had a tonsillar primary identified.[86] The identification of the primary site has several advantages in this group of patients, including the ability to develop a specific treatment plan, a reduction of the radiation therapy fields, more accurate assessment of prognosis, and easier follow-up.

Most patients with involvement of inguinal nodes have a detectable primary site in the anogenital area. Careful examination of the anal canal, vulva, vagina, uterine cervix, penis, and scrotum is important, with biopsy of any suspicious areas. The identification of a primary site in these patients is inconsequential because curative therapy is available for carcinomas of the vulva, vagina, cervix, and anus, even after it has spread to regional nodes.

Metastatic squamous carcinoma in areas other than the cervical or inguinal nodes usually represents metastasis from an occult lung cancer, but metastases from several other sites (the esophagus, skin, uterine cervix, and anal canal) are also possible. An MTP assay may be useful in the diagnosis of the tissue of origin and provides the basis of site-specific therapy.

TREATMENT

The heterogeneous group of patients with CUP contains some patients who experience long-term survival after appropriate treatment and others for whom treatment makes little or no impact. Patients who have an anatomical primary site defined during their clinical evaluation do not have CUP and should be treated appropriately for their defined tumor type. A second group of patients can be identified as having favorable subsets and, in most, their tissues of origin may be presumed, even when the anatomical primary site is not identified (Table 113.7). The management of these subsets is detailed in this section. Most CUP patients (approximately 80%) do not fit into a favorable subset, even after an appropriate clinical and pathologic evaluation. Empiric chemotherapy has been the treatment standard for many years, and will be briefly reviewed. However, there is now ample evidence to support the use of site-specific therapy for most patients, guided by IHC and MTP predictions of the tissue of origin. These new data will also be reviewed.

TABLE 113.7

Carcinoma of Unknown Primary Site: Summary of Evaluation and Therapy of Favorable Subsets

Carcinoma	Clinical Evaluation[a]	Special Studies	Subsets	Therapy	Prognosis
Adenocarcinoma (well-differentiated or moderately differentiated)[b]	Chest, abdominal CT scan; PET scan Men: Serum PSA Women: Mammogram Additional studies to evaluate symptoms, signs	Men: PSA stain Women: ER, PR, Other IHC (see text) MTP assay (see text)	1. Women, axillary node involvement[b] 2. Women, peritoneal carcinomatosis[b] 3. Men, blastic bone metastases, high serum PSA, or PSA tumor staining 4. Single metastatic site[b] 5. Colon cancer profile	1. Treat as primary breast cancer 2. Surgical cytoreduction plus chemotherapy 3. Hormonal therapy for prostate cancer 4. Lymph node dissection, radiotherapy 5. Treat as metastatic colon cancer	Survival improved with specific therapy
Squamous carcinoma	Cervical node presentation[b] Panendoscopy PET scan Supraclavicular presentation[b] Bronchoscopy PET scan Inguinal presentation[b] Pelvic, rectal examinations, anoscopy PET scan	Genetic analysis including MTP assay (see text)	1. Cervical adenopathy; nasopharyngeal cancer identified by PCR for Epstein-Barr viral genes 2. Supraclavicular 3. Inguinal adenopathy	1. Radiation therapy, neck dissection, chemotherapy 2. Radiation therapy, chemotherapy 3. Inguinal node dissection, radiation therapy, chemotherapy	Survival improved 1. 25%–50% 5-y survival 2. 5%–15% 5-y survival 3. 15%–20% 5-y survival
Poorly differentiated carcinoma, poorly differentiated adeno-carcinoma	Chest, abdominal CT scans, serum HCG, AFP; PET scan; additional studies to evaluate symptoms, signs	IHC; electron microscopy; genetic analysis; MTP assay (see text)	1. Atypical germ cell tumors (identified by chromosome 12 abnormalities) 2. Extragonadal germ cell syndrome (two features) 3. Lymph node–predominant tumors (mediastinum, retroperitoneum, peripheral nodes) 4 Gastrointestinal stromal tumors (identified by CD117 stain) 5. Other groups (see text)	1. Treatment for germ cell tumor 2. Cisplatin/etoposide 3. Treat with site-specific therapy (see text) 4. Imatinib 5. Treat with site-specific therapy (see text)	1. 40%–50% cure rate 2. Survival improved (10%–20% cured) 3. Survival improved 4. Survival improved 5. Survival improved
Neuroendocrine carcinoma	Chest, abdominal CT	IHC Electron microscopy Genetic analysis including MTP assay (see text)	1. Low-grade 2. Small-cell carcinoma (or Ewing's family of tumors) 3. Poorly differentiated	1. Treat as advanced carcinoid 2, 3. Carboplatin/etoposide or platinum/etoposide (or other)	1. Indolent biology/long survival 2, 3. High response rate survival improved; rarely cured

[a]In addition to history, physical examination, routine laboratory tests, and chest x-ray films.
[b]May also present with poorly differentiated carcinoma, and management and outcome are similar.
PSA, prostate-specific antigen; ER, estrogen receptor; PR, progesterone receptor; HCG, human chorionic gonadotropin; AFP, α-fetoprotein.

Favorable Subsets

Women with Peritoneal Carcinomatosis

Adenocarcinoma, particularly serous adenocarcinoma, causing diffuse peritoneal involvement, is typical of ovarian carcinoma, although carcinomas from the gastrointestinal tract, lung, or breast can occasionally produce this clinical syndrome. On occasion, women with diffuse peritoneal carcinomatosis have no primary site found in the ovaries or elsewhere in the abdomen at the time of laparotomy. These patients frequently have histologic features typical of ovarian carcinoma, such as papillary serous configuration or psammoma bodies, and also share clinical features, such as elevated serum cancer antigen 125 (CA-125) levels. These tumors are more common in women with a family history of ovarian cancer, and prophylactic oophorectomy does not always protect them from this tumor.[87] Like ovarian carcinoma, the incidence of primary peritoneal carcinoma is increased in women with *BRCA1* mutations.[88]

It is now clear that many of these carcinomas arise from the peritoneal surface (primary peritoneal carcinoma) or from the fimbriated end of the fallopian tubes.[89,90] Many of these tumors have characteristic IHC findings (ovary pattern) or diagnostic MTP assays. Carcinomas arising from the peritoneal (mesothelial) surface or the uterine tubes share a common lineage (müllerian derivation) and biology with ovarian carcinoma. Support for this hypothesis has been strengthened by the demonstration of gene expression profiles nearly identical to ovarian carcinoma.[58] Treatment of these women with standard ovarian carcinoma regimens (surgical cytoreduction followed by taxane/platinum chemotherapy) produces results similar to those seen in ovarian cancer.[91–93] This entity has very rarely been seen in men.[94]

Women with Axillary Lymph Node Metastases

Breast cancer should be suspected in women who have metastatic carcinoma in an axillary lymph node.[95] Men with occult breast cancer can present in this fashion, but these are very rare. The initial lymph node biopsy should be stained for IHC breast markers. When positive, these findings provide strong support of the diagnosis.[96] An MTP assay may also be diagnostic in this setting.

If no other metastases are identified, these patients may have stage II breast cancer with an occult primary, which is potentially curable with appropriate therapy. Magnetic resonance imaging and PET have occasionally identified primary breast cancer even with normal mammography.[97–99] A modified radical mastectomy has been recommended, even when physical examination and mammography are normal. An invasive breast primary has been identified after mastectomy in 44% to 80% of patients. Primary tumors are usually less than 2 cm in diameter and may measure only a few millimeters; occasionally in patients, only a noninvasive tumor is identified in the breast. Prognosis after primary therapy is similar to that of other patients with stage II breast cancer.[95] Radiation therapy to the breast after axillary lymph node sampling and chemotherapy represents a reasonable alternative primary therapy.[100] Either neoadjuvant or adjuvant systemic therapy is indicated in this setting, following guidelines established for the treatment of stage II breast cancer.

Women with metastatic sites in addition to the axillary lymph nodes, particularly when supported by IHC and/or a MTP assay diagnosis, should be managed as metastatic breast cancer. Hormone receptor and *HER2* status are of particular importance in these patients because they may derive major benefit from hormonal therapy, chemotherapy, or HER2-targeted agents.

Men with Elevated Serum Prostate-Specific Antigen or Prostate-Specific Antigen Tumor Staining

Serum prostate-specific antigen (PSA) concentrations should be measured in men with adenocarcinoma of unknown primary site. These tumors can also be stained for PSA. Even when clinical features (i.e., metastatic pattern) do not suggest prostate cancer, a positive PSA (serum or tumor stain) is reason for a trial of androgen deprivation.[101,102] In some of these patients, a needle biopsy of the prostate might confirm the primary site but may not be necessary for optimal clinical management. Osteoblastic bone metastases in the absence of a defined origin and other metastatic sites are also an indication for an empiric hormone trial, regardless of the PSA findings. Although IHC is usually diagnostic, an MTP assay may also provide a definitive diagnosis.

Extragonadal Germ Cell Cancer Syndrome

The extragonadal germ cell cancer syndrome was first described in 1979.[103–105] The full syndrome, which is seen in only a minority of patients, has the following features: (1) occurrence in men less than 50 years of age; (2) predominant tumor location in the midline (mediastinum, retroperitoneum) or multiple pulmonary nodules; (3) short duration of symptoms (less than 3 months) and a history of rapid tumor growth; (4) elevated serum levels of human chorionic gonadotropin (HCG), α-fetoprotein (AFP), or both; and (5) good response to previously administered radiation therapy or chemotherapy. These tumors may be definitively diagnosed by IHC and/or an MTP assay or if testing for specific chromosome 12 abnormalities. If the diagnosis remains uncertain, patients with this syndrome may still have atypical germ cell tumors, and treatment with cisplatin-based chemotherapy is recommended.

Single Site of Neoplasm

When only one site of neoplasm is identified (e.g., one node group, one mass), the possibility of an unusual primary tumor mimicking metastatic disease should be considered. Several unusual tumors could present in this fashion, including Merkel-cell neuroendocrine tumors, skin adnexal tumors (e.g., apocrine, eccrine, and sebaceous carcinomas), sarcomas, melanomas, or lymphomas that are mistakenly interpreted as metastatic carcinoma. Patients with a clinically detectable single metastasis (brain, liver, adrenal, subcutaneous tissue, bone, intestine, lymph node, skin, or other sites) usually have other undetectable sites. Some of these patients may have a primary tumor at the single site that developed from embryonic rest cells or adult stem cells. A PET scan may be helpful to exclude other unsuspected metastatic sites.[106]

These patients without other documented metastasis should be treated with aggressive local therapy because a minority enjoy long-term, disease-free survival. If their tissue of origin is determined by IHC or MTP, site-specific systemic chemotherapy should be considered in either the neoadjuvant or adjuvant setting.

Patients with a single small site of metastasis frequently survive 1 year or longer, regardless of their tissue of origin, and thus represent a favorable prognostic subset. In a reported group of patients presenting with single brain metastasis of unknown primary site, 15% remained progression free 5 years after definitive therapy.[107] The authors have treated and followed 36 patients with single site metastases (unpublished observations). All patients had local therapy (resection with or without radiotherapy) and most also received empiric chemotherapy regimens. The median survival in this group is 17 months; 1-, 2-, and 3-year survivals are 65%, 40%, and 28%, respectively. These overall results may be improved with site-specific systemic therapy.

Squamous Carcinoma Involving Cervical or Supraclavicular Lymph Nodes

Squamous carcinoma most frequently presents with unilateral involvement of the cervical lymph nodes. The recommended clinical evaluation (previously described) results in the identification of a head and neck primary site in almost 85% of patients. In those without a defined anatomical primary site, an occult primary site in the head and neck may be presumed.

When no primary site is identified, local treatment should be given to the involved neck. Results have been reviewed in more than 1,400 patients, derived primarily from retrospective single-institution experiences and treated with a variety of local treatment modalities.[108] In many of these series, a large minority of patients had PDC or adenocarcinoma. Long-term, disease-free survival was achieved in 30% to 40% of patients following treatment with local modalities. The results obtained using radical neck dissection, high-dose radiation therapy, or a combination of these modalities have been similar. The volume of tumor in the involved neck influences outcome, with N1 or N2 disease having a significantly higher cure rate than N3 or massive neck involvement.[109] Poorly differentiated carcinoma also represents a poor prognostic factor in these patients. When resection is used alone, a primary tumor in the head and neck subsequently becomes apparent in 20% to 40% of patients. Primary tumors surface less commonly when radiation therapy is used, presumably because of the eradication of occult head and neck primary sites within the radiation field. Radiation therapy

dosages and techniques should be similar to those used in patients with primary head and neck cancer, and the nasopharynx, oropharynx, and hypopharynx may be included in the irradiated field.

The role of chemotherapy for carcinoma in cervical lymph nodes is now generally accepted. No randomized studies have been performed, but a nonrandomized comparison favored chemotherapy plus radiotherapy versus local therapy[110] (median survival: 37 months versus 24 months). Concurrent treatment with chemotherapy and radiotherapy is now standard in locally advanced head and neck carcinoma, and should be the treatment of choice for squamous cell carcinoma in cervical lymph nodes.

Patients with low cervical and supraclavicular nodes do not do as well because lung cancer is a frequent site of occult primary tumors, although the skin, uterine cervix, esophagus, and anus are also possible primary sites. Molecular assays may be helpful in predicting the primary site. Patients with no detectable disease below the clavicle should be treated with aggressive local therapy, and 10% to 15% have long-term, disease-free survival. Concurrent chemotherapy should also be considered for these patients.

Squamous Carcinoma Involving Inguinal Lymph Nodes

Most patients with squamous carcinoma involving inguinal lymph nodes have a detectable primary site in the anogenital areas. For the unusual patient in whom no primary site is identified, inguinal lymph node dissection with or without radiation therapy to the inguinal area sometimes results in long-term survival.[111] An MTP assay may diagnose the tissue of origin and suggests appropriate therapy. These patients should also be considered for neoadjuvant or adjuvant chemotherapy, because occult primaries from the uterine cervix and anal canal are likely to be responsive to chemotherapy.

Low-Grade Neuroendocrine Carcinoma

These tumors usually exhibit an indolent biology, and slow progression over the years is likely. Management should follow guidelines established for metastatic carcinoid or islet cell tumors from known primary sites. Treatment with octreotide long-acting release (LAR) results in an increase in time to tumor progression with low toxicity.[112] Depending on the clinical situation, appropriate management may also include local therapy (resection of isolated metastasis, hepatic artery ligation or embolization, cryotherapy, radiofrequency ablation). Several cytotoxic agents have some activity (streptozotocin, doxorubicin, 5-fluorouracil, capecitabine, temozolomide), and results with targeted agents (sunitinib, everolimus) from pancreatic primaries are promising. These neoplasms are usually refractory to intensive systemic chemotherapy, and cisplatin-based chemotherapy produces low response rates.[104]

High-Grade Neuroendocrine Carcinomas

Patients with aggressive neuroendocrine carcinomas are those with either small-cell carcinoma or PDC (often large cell) with neuroendocrine staining by IHC or a diagnosis by MTP assay. Tumors with these histologies are initially responsive to combination chemotherapy, and patients should be considered for a trial of treatment.

The initial report of 29 patients with poorly differentiated neuroendocrine tumors[113] was updated to include 99 patients, with 94 treated with combination chemotherapy.[108] These patients had clinical evidence of rapid tumor growth and multiple metastases. Of 87 assessable patients, 59 (68%) responded to a platinum-based combination regimen. Nineteen patients (22%) had complete responses, and 13 remained continuously disease free more than 2 years after the completion of therapy.

The results of a prospective trial using the combination of paclitaxel, carboplatin, and oral etoposide in 48 patients (of the 99 previously listed) have been reported.[114] The majority of these cancers were initially called PDC (about 20% were small-cell carcinoma) and were later defined as neuroendocrine by IHC staining or electron microscopy. These patients usually had several sites of metastasis, often with a predominant tumor in the bones, liver, and nodes (particularly retroperitoneum and mediastinum). The overall response rate was 55%, with six complete responses (13%). The median survival was 14 months and 10 patients survived beyond 2 years (range: 2 to 6 years).

Data remain limited in this uncommon group of patients; however, current first-line chemotherapy should include the platinum-based regimens used for small-cell lung cancer. In the uncommon patient with a single site of involvement, radiation therapy with or without resection should be added to combination chemotherapy.

In some of these patients, MTP assays may diagnose the tissue of origin,[12,13] but it remains uncertain if this knowledge can be applied to improve therapies in the high-grade tumors, although the gastrointestinal primaries may respond to site-specific regimens such as folinic acid, fluorouracil, and oxaliplatin (FOLFOX).

Poorly Differentiated Carcinoma

Although patients with PDC form a large and heterogeneous group, the inclusion of patients with highly treatable neoplasms within this group has been recognized since the late 1970s.[103–105] At that time, several young men with mediastinal tumors were reported who had complete responses to combination chemotherapy. Elevated serum levels of HCG or AFP were common in these young men. Although the histology was not diagnostic, these patients were thought to have histologically atypical extragonadal germ cell tumors. Several other specific cancers have also subsequently been identified in some of these patients (i.e., thymic neoplasms, neuroendocrine tumors, midline carcinoma with t[15;19], sarcomas, melanomas, lymphomas), but others defy precise classification.

Further evidence for the responsiveness of tumors in many other patients has accumulated over the years. Based on the encouraging results in a few patients treated from 1976 to 1978, the authors prospectively studied the role of cisplatin-based therapy. In a series of reports, the authors documented a high overall response rate and long-term disease-free survival in a minority of these patients[115–118]; a small cohort (5% to 10%) were long-term disease-free survivors. Other investigators also demonstrated the responsiveness of selected patients with PDCs.[119–124]

Several years ago, many PDC patients included in these reports had neoplasms that are now identifiable and included either in the favorable subsets already discussed or other recognized responsive neoplasms. These patient subsets included (1) the extragonadal germ cell syndrome, (2) poorly differentiated neoplasms otherwise not specified, (3) anaplastic lymphomas misdiagnosed as carcinoma, (4) thymic carcinomas, (5) primary peritoneal carcinomas, (6) poorly differentiated neuroendocrine carcinomas, and (7) carcinomas with metastases predominantly involving the retroperitoneum, mediastinum, and peripheral lymph nodes. If a patient has PDC, the diagnostic evaluation should target these possibilities. After these subgroups are excluded, the remaining patients have a similar prognosis to the large majority of the adenocarcinoma group. These patients should be evaluated in the same fashion as those with adenocarcinomas, with particular attention given to determining the site of origin using IHC and/or MTP.

Colorectal Cancer Profile

With the introduction of more effective cytotoxic agents and targeted therapies, the median survival of patients with metastatic colorectal carcinoma has increased from 8 to about 24 months.[125,126] It is therefore important to identify the subset of CUP patients with colorectal cancer in order to administer appropriate therapies. The improved specificity of IHC staining for colorectal cancer, coupled with the recent availability of MTP assays, facilitates the identification of this patient subset. The

outcome data in these patients treated with colorectal chemotherapy are similar to known advanced colorectal cancer; therefore, this subset merits inclusion as a CUP favorable subset.

Patients with typical clinical features (liver, peritoneal metastases), histology compatible with a lower gastrointestinal primary, and typical IHC staining (CDX2+ and/or CK20+/CK7−) have been defined as having the *colon cancer profile*. Several such patients described by Varadhachary et al.[127,128] had excellent responses and survival when treated with colorectal cancer regimens.

Substantial data now indicate that CUP patients with a colorectal tissue of origin can be accurately identified using IHC stains and/or MTP.[53,127–131] A total of 172 CUP patients with colorectal profiles have been treated with site-specific colorectal regimens. The objective response rates were usually above 50%, and the combined median survival of all these patients was 26 months.

Although these data are largely retrospective, the outcome results are far superior than expected from empiric chemotherapy in CUP (ineffective in colorectal carcinoma) and similar to those achieved in patients with known metastatic colorectal cancer. Further prospective studies may confirm these results. In the meantime, these data are sufficient to recommend treatment with colorectal cancer regimens for CUP patients with either an IHC or MTP colorectal profile.

Empiric Therapy for CUP

Chemotherapy

Approximately 80% of CUP patients are not represented in any of the favorable prognostic clinical subsets (see Table 113.7). In the past, empiric chemotherapy was used for most of these patients because their tissue of origin could not be determined. The history and more recent results of empiric chemotherapy have been reviewed previously.[132–134] and will be briefly discussed here.

Several reports of survival in large groups of patients with CUP[135–142] help to establish a historical control and define the natural history of this syndrome. These historical series included a total of 31,419 patients. Because these reports were retrospective, treatments were not uniform, and some patients received no systemic therapy. The median survival was 5 months, with a 1-year survival of 22% and 5-year survival of 5%. Most patients who survived for 1 year or longer had clinical features now known to be associated with a favorable prognosis. Squamous cell carcinoma (usually in neck nodes) and well-differentiated neuroendocrine carcinoma (carcinoid, islet cell–type histology) reported from some of these series (N = 2,971) had median, 1-year, and 5-year survivals of 20 months, 66%, and 30%, respectively.

Between 1990 and 2000, the introduction of several new drugs with rather broad-spectrum antineoplastic activity improved treatments and prognoses for patients with several common epithelial cancers, and also resulted in a modest improvement in the empiric treatment of CUP.[143] A number of combinations containing these new drugs (taxanes, gemcitabine, vinorelbine, irinotecan, topotecan, oxaliplatin), often combined with a platinum agent, had modest activity in CUP patients and became standard regimens.

The Minnie Pearl Cancer Research Network/Sarah Cannon Oncology Research Consortium (MPCRN/SCORC) completed 10 sequential prospective trials[144–153] (nine phase 2 with 692 patients and one phase 3 with 198 patients), often incorporating platinums with paclitaxel,[144,145] docetaxel,[145,146] gemcitabine,[147,148] gemcitabine/irinotecan,[149,150] bevacizumab/erlotinib,[151] and oxaliplatin[152] into the first-line or second-line therapy for 890 patients with unfavorable prognostic features. The median survival of the 692 first-line patients was 9.2 months and the 1-, 2-, 3-, 4-, 5-, 8-, and 10-year survivals were 39%, 20%, 12%, 11%, 9%, 8%, and 8%, respectively.

Several trials of empiric chemotherapy reported by others over the past 15 years[119–124,132–134,154–164] revealed similar results. The primary end points of 12 of these trials were response rate or median survival.[133] The median survival of all these patients was 9.1 months. The 1-year survival (reported in 12 trials) ranged from 25% to 52% (mean: 34.4%), and at 2 years, survival (reported in 8 trials) ranged from 5% to 18% (mean: 12.3%). Only one study reported a 3-year survival rate (11%). These survival results are very similar to the 692 patients reported by the MPCRN/SCORC studies,[144–153] and superior to historical survival data and to the combined data from multiple prospective clinical trials reported from 1964 to 2002.[2,108] It is of note that in all studies of 100 or more patients, the median survival following empiric regimens is about 9 months.[134,147,150,153,157] The survival curve has been shifted to the right, and the survival at 2 years is comparable to the 1-year survival of historical control patients. Comparison of the existing trials does not allow for the definition of an optimum regimen; several two-drug combinations appear similar.

The era of empiric chemotherapy for most patients with CUP is nearing its end. Improved IHC stains and MTP assays accurately identify the tissue of origin in most patients and provide a more rational framework for decisions regarding therapy. The advantages of site-specific therapy are supported by an increasing amount of clinical data. In the small minority of CUP patients that remain without a defined tissue of origin, empiric chemotherapy remains the standard approach.

Targeted Therapy

A number of agents broadly targeting pathways critical to some cancers (i.e., vascular endothelial growth factor [VEGF] and EGFR inhibitors) have been incorporated into the standard therapy of various solid tumors. It is likely that some patients in the heterogeneous CUP group would also benefit from these targeted agents. Although there has been limited clinical experience with targeted agents, definite activity has been documented.

The combination of bevacizumab and erlotinib was evaluated in a group of 51 patients[151] with very poor prognostic features and the majority in the second-line setting. The median survival was 7.4 months with 33% of patients alive at 1 year and 18% at 2 years. Survival seemed superior to second-line chemotherapy previously reported and was similar to results of many first-line chemotherapy trials.

This trial was followed by a first-line phase 2 study evaluating standard empiric chemotherapy (paclitaxel and carboplatin) plus bevacizumab and erlotinib.[165] Sixty patients with good performance status were treated. The median survival was 12.6 months, and the 2-year survival was 27%. In neither trial were patients selected on the basis of molecular tumor abnormalities predictive of response to the study targeted therapy.

Future development of targeted therapy in CUP will depend on the documentation of molecular targets for which drugs are available. The genomic analysis of biopsy samples, particularly with next-generation sequencing technologies, has opened the door to this potential in many cancers. Because CUP represents many types of metastatic cancer, there is an opportunity to find a variety of actionable genetic alterations. A few CUP patients with EGFR-activating mutations or ALK rearrangements have responded to treatment with inhibitors of these targets.[166,167] Recently, a preliminary report[168] reviewed 1,350 biopsies of CUP patients who had molecular profiling by a number of techniques and several *actionable* biomarkers were identified, including targeted protein overexpression (steroid receptors, MET), activating mutations (BRAF, EGFR, PIK3CA), protein loss (PTEN), and gene copy number variations (HER2, TOP2A, MET amplification) in a large number of these specimens. •AQ2•

Although the therapeutic implications of these findings are largely unexplored, the identification of the tissue of origin should lead to a focused search for tumor-specific molecular abnormalities. Examples include BRAF in melanoma, EGFR and ALK/ROS1 in lung adenocarcinoma, HER2 in breast/gastric/gastroesophageal cancers, and KRAS in colorectal cancer. The identification of these abnormalities should guide patient management

and provide additional effective treatment options. The role of genomic testing in advanced cancer is rapidly evolving and is likely to play a larger role in the near future.

Prognostic Factors

The identification of prognostic factors in patients with CUP continues to evolve as the group is divided into an increasing number of subsets. By definition, patients who fit into the favorable subsets have better prognoses compared to the remaining patients. As new treatable subsets are identified, the clinicopathologic features of the remaining patients can be expected to change. The ability to determine the tissue of origin in most patients will make the specific type of cancer in each patient one of the most important prognostic factors. Therefore, results of previous analyses of prognostic factors, conducted primarily in CUP patients receiving empiric chemotherapy, may no longer be relevant to the current population.

Several investigators have analyzed prognostic factors and proposed models.[118,132,169–173] These patients had unfavorable prognostic features and most received empiric chemotherapy regimens. Liver metastasis, poor performance status, elevated serum lactate dehydrogenase and/or alkaline phosphatase levels, hypoalbuminemia, multiple visceral metastasis, lymphopenia, and male gender were negative factors.

Prognostic factors that have been repeatedly identified are related to tumor location, extent of tumor, performance status, and measures of general health status. None of these features is surprising, because most have been repeatedly identified as prognostic factors in patients with various known solid tumors. The tissues of origin in CUP can now usually be determined, and further study is necessary to see if prognoses are similar to their counterparts with known cancers after appropriate site-directed therapies.

Site-Specific Treatment Directed by Results of Molecular Gene Expression Tumor Profiling Assays

Because the tissue of origin can now be accurately predicted in most patients with CUP, the assumption that this information should result in better treatment seems reasonable. However, clinical data confirming this assumption have developed only recently, and some skepticism still remains. One cause for concern is the unique biology of CUP (evidenced by the fact that the primary site does not become apparent); it has been speculated that these cancers, regardless of their origins, will respond differently to treatment. If so, the ability to identify the tissue of origin may not lead to improved therapy.

At present, clinical data suggest that CUP represents a collection of cancer types, which, if identified, will respond to site-specific therapy in a predictable way. The largest experience comes from the treatment of patients in several of the favorable CUP subsets, where treatment follows guidelines for a specific cancer type based on a presumed (but unidentified) site of origin. Examples include women with serous adenocarcinoma involving the peritoneum who are treated for ovarian cancer, women with isolated axillary adenocarcinoma who are treated for breast cancer, and patients with squamous carcinoma involving cervical lymph nodes who are treated for head and neck cancer. In all of these subsets, treatment results are similar to results for the corresponding cancer types. Recently, CUP patients identified by IHC or MTP as having a colorectal site of origin (but with no identifiable colon anatomical primary site) have been demonstrated to respond well to treatment for advanced colorectal cancer.

A prospective evaluation of site-specific therapy selected on the basis of an MTP assay diagnoses has recently been published.[131] In this large prospective phase 2 multicenter study, CUP patients had their biopsies tested with the 92-gene RT-PCR assay and were treated with standard site-specific therapies based on the assay diagnoses of the tissues of origin. Of the 253 patients with successful assays performed, 242 (98%) had a single tissue of origin predicted. Twenty-six different tissues of origin were diagnosed. Assay-directed standard therapies were administered to these patients, and the median survival was 12.5 months, comparing favorably to the median 9-month survival expected with empiric chemotherapy.

Various patient subsets also had outcomes that supported the accuracy of the MTP predictions and the efficacy of assay-directed therapy. In 115 patients, the assay predicted tumor types relatively responsive to standard therapies (colorectal, breast, ovary, kidney, prostate, bladder, lung, germ cell, high-grade neuroendocrine, and lymphoma); this group of patients had a median survival of 13.4 months. When the assay predicted less responsive tumor types in 79 patients (biliary tract, pancreas, gastroesophageal, liver, sarcoma, uterine cervix, endometrium, mesothelioma, melanoma, skin, thyroid, head/neck, and adrenal) the median survival was only 7.6 months ($p = 0.04$) (Fig. 113.2). In addition, groups of

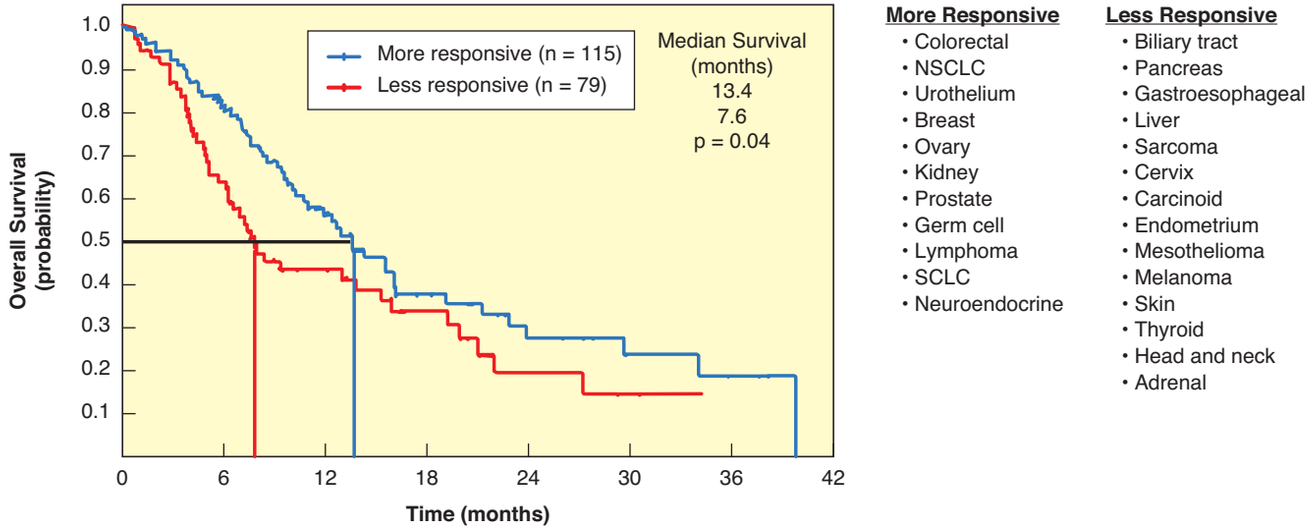


Figure 113.2 Survival of CUP patients after site-specific treatment directed by MTP assay: responsive tumor types versus less responsive tumor types. Median survival 13.4 versus 7.6 months ($p = 0.04$). NSCLC, non–small-cell lung cancer; SCLC, small-cell lung cancer. (Adapted from Hainsworth JD, Rubin 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* 2012; 31:217–223.)

patients with individual cancer types were assessed. Although the groups were small, the median survivals were generally within the range expected for these cancer types (median survival months: breast, 28; ovary, 30; non–small-cell lung, 15.9; colorectal, 12.5; pancreas, 8.2; biliary tract, 6.8).

These results are consistent with those from retrospective studies and provide evidence that site-specific therapy improves the efficacy of treatment for patients with CUP. As expected, patients with more responsive tumor types have greater benefit. Given the heterogeneity of CUP (at least 26 cancer types in the previous study and many more subtypes), it would be extremely challenging to perform a phase 3 randomized study comparing empiric chemotherapy to assay-directed site-specific therapy and difficult to interpret the results if it were accomplished. The varieties of cancers within CUP are diverging along different treatment pathways on the basis of their origin and knowledge of specific treatable molecular alterations, and there is no longer any reason to consider these diverse cancers for treatment with the same empiric chemotherapy regimen.

A sizable minority of patients with CUP will not currently benefit much, if at all, from site-specific therapies because therapy for their tumor types is relatively ineffective. Confidence in the tissue of origin diagnoses by IHC and/or MTP will allow these patients to receive more effective therapy once therapy for their tumor types improves.

SPECIAL ISSUES IN CARCINOMA OF UNKNOWN PRIMARY SITE

Biology of the Primary Tumor

The biology of the primary tumor in CUP remains an enigma. Most patients harbor a clinically undetectable anatomical primary tumor site, as demonstrated by autopsy series.[3,6] It is remarkable that many of these invasive primary tumors measure less than 1 cm. The mechanism explaining clinically occult invasive primary tumor sites remains unknown, but almost certainly will be clarified by a better understanding of the molecular mechanisms controlling primary tumor growth and metastasis. These mechanisms may be different than those found in their easily detected cognate primary cancers.

There are several other potential explanations for the apparent absence of a primary cancer in some of these patients. First, the primary cancers may inexplicably regress or involute entirely, despite the fact that metastasis already occurred. This theory is supported by the scarring seen occasionally in the testicle with metastatic germ cell neoplasms (i.e., *burned-out primary*). Second, the primary may have arisen from embryonic epithelial *rest cells* that are fully differentiated but did not complete their appropriate migration in utero to their designated tissue or organ. Extragonadal germ cell tumors with primaries in the mediastinum, retroperitoneum, or undescended testicle are known examples of this phenomenon. Third, some of these patients have unrecognized primary neoplasms, such as extragonadal germ cell tumors, thymic neoplasms, lymphomas, melanomas, or sarcomas, which arise from these cellular lineages virtually anywhere in the body. Fourth, the pathogenesis of some of these carcinomas may result from a specific germ line genetic lesion present in all cells, as is suggested by the unusual occurrence of CUP in monozygotic twin brothers with primary immunodeficiency disorder (X-linked hyperimmunoglobulin M syndrome).[174]

Finally, some of these neoplasms may arise from adult stem cells with an ability to differentiate to multiple lineages.[175–179] Hematopoietic stem cells appear to be able to give rise to or transform into liver cells as well as muscle, gastrointestinal, skin, and brain cells.[175] Reserve precursor stem cells exist within the connective tissue compartments throughout postnatal life[178] and can form any lineage in any tissue if they undergo neoplastic transformation. Therefore, some tumors might continue to reflect the differentiation or transformation of adult stem cells and may be *tumors of adult stem cells*. For example, seemingly metastatic adenocarcinoma in bone, liver, lymph node, or elsewhere may, in fact, arise in these sites from an adult stem cell with the capacity to become any type of cell and to develop as a *primary* neoplasm in any of these tissues.[176]

Although CUPs share a metastatic phenotype, it is currently unknown whether these tumors share specific unique molecular abnormalities. A genomic analysis of CUP demonstrates multiple abnormalities, but these are not unique and are shared with many advanced known solid tumors (e.g., various chromosomal 1p abnormalities).[180] Similarly, the expression of p53, bcl-2, cMYCc, RAS, NOTCH1-3, JAGGED1, phosphoMAPK, PTEN, pAKT, cMET, HER2, hypoxia-related protein, and MET mutations have been observed in some CUP tumors, but are not specific.[168,181–188] Although the search for a CUP-specific gene signature continues, none has yet been identified. At present, most evidence suggests that CUP retains typical site-specific markers and can often be identified by IHC and/or MTP assays; however, this does not preclude the coexistence of CUP group-specific molecular abnormalities. Techniques are now available to study the CUP genome (next-generation sequencing technologies, proteomics, and metabolomics), and the pathogenesis of this syndrome may be eventually explained by specific genetic/epigenetic alterations.

Carcinoma of Unknown Primary Site as a Distinct Clinical Syndrome

The authors have found it amazing over the past 3 decades how often patients and their referring physicians are frustrated by CUP. Physicians are often somewhat obsessed with finding the anatomical primary site or at least with giving the patient a site diagnosis. There are many reasons underlying these feelings. Some patients think their oncologist may not be a very good diagnostician and seek the advice of others. Some oncologists feel relatively inadequate and wonder what other test(s) they might order; some have been relatively tentative, not feeling confident in recommending therapy. With improved ability to accurately predict the tissue of origin, most of these issues should be alleviated. Patients are better served, and physicians eventually feel more comfortable, and therefore manage these patients more effectively once their patients accept and understand their diagnosis. Nonetheless, these patients will still lack anatomically defined primary sites and will therefore remain a distinct population.

A second practical issue in the United States is the reimbursement for chemotherapy by Medicare for cancer diagnoses. Other than U.S. Food and Drug Administration approval for a specific tumor type, reimbursement for chemotherapy is usually determined by Medicare (and some other third-party insurers) by consulting compendia—Medicare Drug Policies or the National Comprehensive Cancer Network Compendium. The list of *approved* drugs is based on published literature showing *effectiveness* or clinical benefit in a specific tumor type. For many years, CUP was not included in any of the listings. Four drugs are currently listed as indicated for these patients (paclitaxel, carboplatin, cisplatin, and etoposide). The magnitude of this problem should be substantially diminished because the tissue of origin can now usually be diagnosed and, with these data, specific coded cancer diagnoses can be recorded.

Isolated Pleural and/or Pericardial Effusion

An isolated malignant pleural and/or pericardial effusion is most frequently a manifestation of a peripheral lung adenocarcinoma.

The diagnosis of mesothelioma or a metastatic tumor from other sites (breast, ovary, primary peritoneal, others) should also be considered. In a series of 42 patients, a primary lung cancer was eventually found in 15 patients (36%).[189] The primary may not be apparent even after chest tube drainage. Cytology usually shows adenocarcinoma; positive TTF1 and CK7 stains support a diagnosis of lung carcinoma. Other IHC stains (i.e., calretinin in mesothelioma) or an MTP assay may also assist in defining a primary site. An MTP assay may be successfully performed on small numbers of cancer cells, and in these circumstances should be a preferred test.

In one small series of patients,[189] empiric chemotherapy produced symptomatic improvement in 29 of 37 patients, and 30 of 37 patients had their pleural effusion reduced by chemotherapy; median survival was 12 months (range: 3 to 60 months).

Germ Cell Tumors with Metastases of Other Histologies

On occasion, patients with germ cell tumors, particularly extragonadal primaries, may have a metastatic lesion that consists of only somatic tumor cells. This is particularly true for neuroendocrine or sarcomatous differentiation, but can include any histology. Therefore, patients may be diagnosed as having a neuroendocrine tumor or sarcoma. In these rare instances, a primary germ cell tumor (usually extragonadal) is present elsewhere and subsequently becomes clinically apparent. It is difficult to make the diagnosis initially. An elevated plasma AFP or HCG level and/or the presence of a mediastinal and/or retroperitoneal supports this possibility. Chromosomal analysis, IHC staining, or an MTP assay may confirm the diagnosis of germ cell tumor. The treatment of choice is cisplatin-based chemotherapy. Surgical resection should be pursued later if feasible. These patients have a worse prognosis than those with typical germ cell tumors, probably because the somatic cell tumors are less sensitive to chemotherapy.

•AQ2•

Melanoma and Amelanotic Melanoma

Approximately 10% to 15% of all melanomas that present with an unknown primary site are believed to be amelanotic. This diagnosis should be viewed with skepticism. At times, the only reason for the pathologic diagnosis is the similarity of the histologic pattern to melanoma, even though no pigment is demonstrated. Detailed pathologic and molecular study has occasionally revealed a group of other specific diagnoses, including lymphomas, neuroendocrine tumors, germ cell tumors, sarcomas, and PDC (not otherwise specified).

Melanosomes or premelanosomes seen on electron micrographs have been considered diagnostic of melanoma, but on rare occasions, these structures are seen in other tumors. Some believe amelanotic melanomas do not always form premelanosomes, raising the question as to whether they are really melanomas. Immunohistochemical panels and an MTP assay are also useful in supporting the diagnosis.

The history of a resected, abraded, or frozen pigmented skin lesion would favor melanoma. In addition, the rare primary visceral melanoma should be considered (e.g., eye, adrenal, bowel, anus, others) as the source of the disease in questionable cases. For patients with the diagnosis of amelanotic melanoma, particularly without diagnostic IHC stains, an MTP assay, or a BRAF mutation, an alternative diagnosis should be considered. Mutations of *BRAF* have been found in approximately 50% of melanomas, and if present, would also support a presumptive diagnosis of melanoma and consideration of therapy with a *BRAF* inhibitor.

UNKNOWN PRIMARY CANCER IN CHILDREN

There are limited data in children, and as expected, many of these neoplasms represent embryonal malignancies.[190] They are exceedingly rare. In patients with carcinoma, not otherwise specified, the authors favor following the same management plan as for adults.

Midline Carcinoma in Young Adults and Children with t(15;19) and *BRD4-NUT* Oncogene

A few young patients have been described with carcinomas arising from midline locations and an associated chromosomal translocation t(15;19) (q13,p13.1).[29,191] Patients with this syndrome are usually children or young adults; most have poorly differentiated carcinoma and widespread metastasis. The primary tumor site is difficult to identify in many patients. The *NUT* (nuclear protein in testes) oncogene is common to all these tumors and supports their possible origin from a specific cell type, perhaps an early epithelial progenitor cell that is more common in the first 2 or 3 decades of life. Perhaps these tumors are an example of *stem cell tumors* (see section Biology of the Primary Tumor in Special Issues in Carcinoma of Unknown Primary Site).

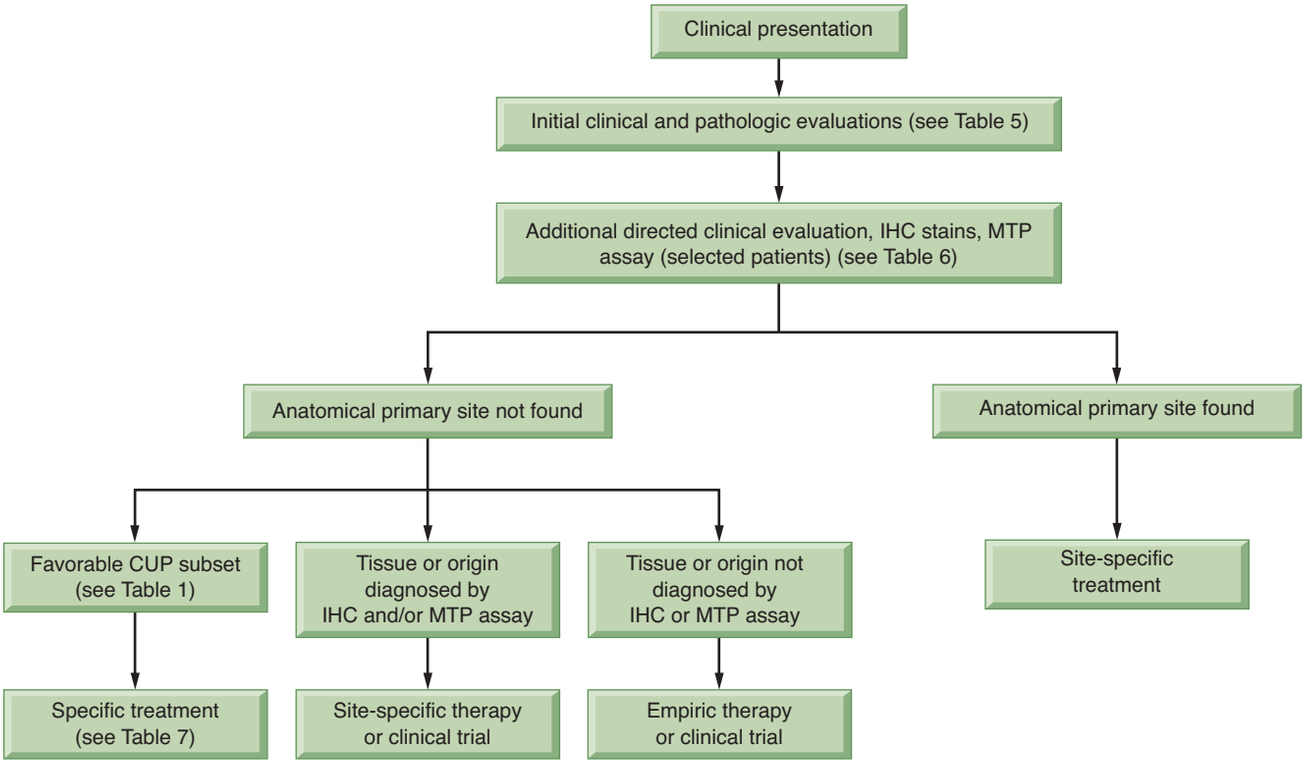
A recent review of 54 patients[191] revealed poor median survival (6.7 months) and 2-year survival of 19% despite surgery and radiation therapy when feasible and intensive chemotherapies. These patients are clinically similar to the extragonadal germ cell cancer syndrome and, without a positive t(15;19), some of these patients could be included in that clinical syndrome and vice versa. Further knowledge of these *NUT*-rearranged carcinomas and improved targeted therapies for these patients are likely to follow with their more broad recognition.

NEW TREATMENT PARADIGM

As described in this chapter, improved diagnostic methods have changed the clinical evaluation and therapy for most CUP patients. A change from empiric chemotherapy to site-specific therapy based on tissue of origin diagnosis is now indicated for the majority of patients.

In Figure 113.3, we summarize the management approach. After standard initial clinical and pathologic evaluations, selected patients should have an additional directed clinical evaluation, IHC staining, and/or an MTP assay of the tumor specimen. Patients with an identified anatomical primary site should be treated accordingly, and patients who fit into an identified favorable subset should receive appropriate subset-specific therapy (see section Favorable Subsets). Patients who have their tissue of origin diagnosed by IHC should receive site-specific therapy. Patients in neither of these categories should have MTP; site-specific therapy should then be based on the tissue of origin diagnosis. Diagnoses made by IHC and/or MTP should be interpreted in conjunction with clinical features and pathologic results. Empiric chemotherapy is indicated for the small minority without a defined tissue of origin, and clinical trials should always be considered.

Some may now think that CUP will be a rare entity once most patients are assigned a tissue of origin and become a subset or member of a recognized cancer type, albeit without a defined anatomical primary site. This may be a clinical reality once it is agreed upon that the survival of CUP patients with defined origins are similar to the patients with anatomically defined primary sites. However, the syndrome persists and the biologic phenomenon explaining CUP for now remains a mystery. Further knowledge of the genetic/epigenetic mechanisms may eventually explain CUP



•AQ4• **Figure 113.3** New management paradigm for the CUP patient.

and lead to new targeted therapies for patients with this enigmatic syndrome and perhaps for other patients with metastatic cancers.

The evolution of improved, more personalized therapies for CUP patients is linked to the wave of precision therapies for many types of known cancer types based on genomic understanding; in addition, the dawn of clinically impressive and beneficial immunotherapy (such as CTLA-4, PD-1, PDL-1 inhibitors, and genetically engineered T cells) for many common solid tumor patients is here. There is no other reasonable option now but to further define in each patient the type and subtype of neoplasm he or she harbors within the CUP syndrome before planning definitive therapy.

The integration of molecular diagnostics into CUP patient management is already supported by clinical data, but continued investigation is necessary to further refine management recommendations. Even with the ability to identify the tissue of origin, further improvements in the treatment of many of these patients are dependent on the development of improved treatments for advanced solid tumors.

SELECTED REFERENCES

The full reference lists appears in the electronic version.

2. Pavlidis N, Briasoulis E, Hainsworth J, Greco FA. Diagnostic and therapeutic management of cancer of an unknown primary. *Eur J Cancer* 2003;39:1990–2005.
5. Horning SJ, Carrier EK, Rouse RV, et al. Lymphomas presenting as histologically unclassified neoplasms: characteristics and response to treatment. *J Clin Oncol* 1989;7:1281–1287.
7. Owen KA. Pathologic evaluation of unknown primary cancer. *Semin Oncol* 2009;36:8–37.
10. Oien KA, Dennis JL. Diagnostic work-up of carcinoma of unknown primary: from immunohistochemistry to molecular profiling. *Ann Oncol* 2012;23:271–277.
11. Stoyianni A, Pentheroudakis G, Pavlidis, N. Neuroendocrine carcinoma of unknown primary: a systematic review of the literature and a comparative study with other neuroendocrine tumors. *Cancer Treat Rev* 2011;37:358–365.
12. Kerr SE, Schnabel CA, Sullivan PS, et al. A 92-gene cancer classifier predicts the site of origin for neuroendocrine tumors. *Modern Pathology* 2014;27:44–54.
22. Anderson GG, Weiss LM. Determining tissue if origin for metastatic cancers: meta-analysis and literature review of immunohistochemistry performance. *Appl Immunohistochem Mol Morphol* 2010;18:3–8.
25. Motzer RJ, Rodriguez E, Reuter VE, et al. Molecular and cytogenic studies in the diagnosis of patients with midline carcinomas of unknown primary site. *J Clin Oncol* 1995;13:274–282.
29. French CA, Kutok JL, Faquin WC, et al. Midline carcinoma of children and young adults with NUT rearrangement. *J Clin Oncol* 2004;22:4135–4139.
32. Summersgill B, Goker H, Osin P, et al. Establishing germ cell origin of undifferentiated tumors by identifying gain of 12p material using comparative genomic hybridization analysis of paraffin-embedded samples. *Diagn Mol Pathol* 1998;7:260–266.
37. Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* 1999; 286:531–537.
38. Greco FA, Erlander MG. Molecular classification of unknown primary cancer site. *Mol Diagn Ther* 2009;13:367–373.
40. Sotiriou C, Piccart MJ. Taking gene-expression profiling to the clinic: when will molecular signatures become relevant to patient care? *Nature Rev Cancer* 2007;7:545–553.
41. Ramaswamy S, Tamayo P, Rifkin R, et al. Multiclass cancer diagnosis using tumor gene expression signatures. *Proc Natl Acad Sci U S A* 2001;98:15149–15154.
42. MacConaill LE. Existing and emerging technologies for genomic profiling. *J Clin Oncol* 2013;31:1815–1824.
43. Abaan OD, Polley EC, Davis SR, et al. The exomes of the NCI-60 panel: a genomic resource for cancer biology and systems pharmacology. *Cancer Res* 2013;73:4372–4382.
45. Bloom G, Yang IV, Boulware D, et al. Multi-platform, multi-site, microarray-based human tumor classification. *Am J Path* 2004;164:9–16.

48. Rosenfeld N, Aharonov R, Meiril E, et al. MicroRNAs accurately identify cancer tissue origin. *Nature Biotech* 2008;26:462–469.
49. 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.
50. Ma X-J, Pate R, Wang X, et al. Molecular classification of human cancers using a 92-gene real-time quantitative polymerase chain reaction array. *Arch Path Lab Med* 2006;130:465–473.
51. Pillai R, Deeter R, Rigl CT, et al. Validation of a microarray-based gene expression test for tumors with uncertain origins using formalin-fixed paraffin-embedded (FFPE) specimens. *J Mol Diagn* 2011;13:48–56.
53. Varadhachary G, Talantov D, Raber M, et al. Molecular profiling of carcinoma of unknown primary and correlation with clinical evaluation. *J Clin Oncol* 2008:26:4442–4448.
55. Horlings HM, van Laar R, Kerst J-M, et al. Gene expression profiling to identify the histogenetic origin of metastatic adenocarcinomas of unknown primary. *J Clin Oncol* 2008;26:4435–4441.
58. Greco FA, Spigel DR, Yardley DA, et al. Molecular profiling in unknown primary cancer: tissue of origin prediction. *Oncologist* 2010;15:500–506.
59. 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;13:493–503.
60. 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;18:3952–3960.
61. Meiri E, Mueller WC, Rosenwald S, et al. A second-generation microRNA-based assay for diagnosing tumor tissue origin. *The Oncologist* 2012;17:801–812.
62. Weiss LM, Cha PG, Schroeder BE, et al. Blinded comparator study of immunohistochemistry analysis versus 92-gene cancer classifier in the diagnosis of the primary site in metastatic tumors. J Molecular Diagn. 2013;15:263–269.
63. Handorf CR, Kulkarni A, Grenut JD, et al. A multisite study directly comparing the diagnostic accuracy of the gene expression profiling and immunohistochemistry for primary site identification in metastatic tumors. *Am J Surg Pathol*. 2013;37:1067–1075.
65. Pentheroudakis G, Pavlidis N, Fountzilas G, et al. Novel microRNA-based assay demonstrates 92% agreement with diagnosis based on clinicopathologic and management data in a cohort of patients with carcinoma of unknown primary. *Molecular Cancer* 2013; 12:57.
67. Varadhachary Gr, Spector Y, Abbruzzese JL, et al. Propective gene signature study using microRNA to identify the tissue of origin in patients with carcinoma of unknown primary. *Clin Cancer Res* 2011;17:4063–4070.
68. Ferracin M, Pedriali M, Veronese A, et al. Micro RNA profiling for the identification of cancers with unknown primary tissue of origin. *J Pathol* 2011;225:43–53.
69. Greco FA, Lennington WJ, Spigel DR, et al. Molecular profiling diagnosis in unknown primary cancer; accuracy and ability to complement standard pathology. *J Natl Cancer Inst* 2013;105:782–790.
71. Greco FA, Spigel DR, Hainsworth JD. Molecular tumor profiling of poorly differentiated neoplasms of unknown primary site. *J Clin Oncol* 2013;31:217–223.
72. Sorscher SM, Greco FA. Papillary renal carcinoma presenting as a cancer of unknown primary and diagnosed through gene expression profiling. *Case Rep Oncol* 2012;5;229–232.
74. Hainsworth JD, Spigel DR, Greco FA. Renal cell carcinoma presenting as cancer of unknown primary: diagnosis by molecular tumor profiling. *J Clin Oncol* 2013;31:abstract e15501.
76. Moller AK, Loft A, Berthelsen AK, et al. A prospective comparison of 18F-FDG PET/CT and CT as diagnostic tools to identify the primary tumor site in patients with extracervical cancer of unknown primary site. *Oncologist* 2012;17:1146–1154.
82. Rusthoven KE, Koshy M, Pauline AC. The role of fluorodeoxyglucose positron emission tomography in cervical lymph node metastases from an unknown primary tumor. *Cancer* 2004;101:2641–2649.
85. Varadhachary GR, Abbruzzese JL, Lenzi R. Diagnostic strategies for unknown primary cancer. *Cancer* 2004;100:1776–1785.
93. Pentheroudakis G, Pavidis N. Serous papillary peritoneal carcinoma: unknown primary tumor ovarian cancer counterpart or a distinct entity? A systematic review. *Crit Rev Oncol Hematol* 2010;75:27–42.
95. Pentheroudakis G, Lazaridis G, Pavlidis N. Axillary nodal metastases from carcinoma of unknown primary (CUPAx): a systematic review of published evidence. *Breast Cancer Res Treat* 2010;119:1–11.
108. Greco FA, Hainsworth JD. Cancer of unknown primary site. In: DeVita VT, Lawrence TS, Rosenberg SA, eds. *Cancer Principles and Practice of Oncology*, 8th ed. Philadelphia: Lippincott Williams & Wilkins; 2008: 2363.
110. De Braud F, Heilbrun LK, Ahmed K, et al. Metastatic squamous cell carcinoma of an unknown primary localized to the neck: advantages of an aggressive treatment. *Cancer* 1989;64:510–515.
112. Rinke A, Muller HH, Schade-Brittinger C, et al. Placebo-controlled double-blind, prospective, randomized study of the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors: a report from the PROMID Study Group. *J Clin Oncol* 2009;27:4656.
113. Hainsworth JD, Johnson DH, Greco FA. Poorly differentiated neuroendocrine carcinoma of unknown primary site: a newly recognized clinicopathologic entity. *Ann Intern Med* 1988;109:364–371.
114. Hainsworth JD, Spigel DR, Litchy S, Greco FA. Phase II trial of paclitaxel, carboplatin, and etoposide in advanced poorly differentiated neuroendocrine carcinoma: a Minnie Pearl Cancer Research Network Study. *J Clin Oncol* 2006;24:3548–3554.
115. Richardson RL, Schoumacher RA, Fer MF, et al. The unrecognized extragonadal germ cell cancer syndrome. *Ann Intern Med* 1981;94:181–186.
116. Greco FA, Vaughn WK, Hainsworth JD. Advanced poorly differentiated carcinoma of unknown primary site: recognition of a treatable syndrome. *Ann Intern Med* 1986;104:547–553.
117. Hainsworth JD, Greco FA. Treatment of patients with cancer of an unknown primary site. *N Engl J Med* 1995;329:257–263.
118. Hainsworth JD, Johnson DH, Greco FA. Cisplatin-based combination chemotherapy in the treatment of poorly differentiated carcinoma and poorly differentiated adenocarcinoma of unknown primary site: results of a 12 year experience at a single institution. *J Clin Oncol* 1992;10:912–922.
127. Varadhachary GR, Raber MN, Matamoros A, Abbruzzese JL. Carcinoma of unknown primary with a colon cancer-profile changing paradigm and emerging definitions. *Lancet Oncol* 2008;9:596–599.
128. Varadhachary GR, Karanth S, Qiao W, et al. Carcinoma of unknown primary with gastrointestinal profile: immunohistochemistry and survival data for this favorable subset. *In J Clin Oncol* 2014;19:479–484.
129. Greco FA, Lennington WJ, Spigel DR, et al. Carcinoma of unknown primary site: outcomes in patients with colorectal profile treated with site-specific chemotherapy. *J Cancer Ther* 2012;3:37–43.
130. Hainsworth JD, Schnabel CA, Erlander MG, et al. A retrospective study of treatment outcomes in patients with carcinoma of unknown primary site and a colorectal cancer molecular profile. *Clin Colorectal Cancer* 2012;11: 112–118.
131. 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* 2012; 31:217–223.
132. Greco FA, Hainsworth JD. Cancer of unknown primary site. In: DeVita VT, Lawrence TS, Rosenberg SA, eds, *Cancer Principles and Practice of Oncology*, 9th ed. Philadelphia: Lippincott Williams and Wilkins; 2011: 2033.
133. Greco FA, Pavlidis N. Treatment for patienmts with unknown primary carcinoma and unfavorable prognostic factors. *Semin Oncol* 2009;36:65–74.
143. Greco FA. Therapy of adenocarcinoma of unknown primary: are we making progress? *J Natl Compr Conc Netw* 2008;6:1061–1067.
144. Hainsworth JD, Erland JB, Kalman CA, et al. Carcinoma of unknown primary site: treatment with one-hour paclitaxel, carboplatin and extended schedule etoposide. *J Clin Oncol* 1997;15:2385–2393.
151. Hainsworth JD, Spigel DR, Farley C, et al. Bevacizumab and erlotinib in the treatment of patients with carcinoma of unknown primary site: a phase II trial of the Minnie Pearl Cancer Research Network. *J Clin Oncol* 2007;25: 1747–1752.
153. Hainsworth JD, Spigel DR, Clark BL, et al. Paclitaxel/carboplatin/etoposide versus gemcitabine/irinotecan in the first-line treatment of patients with carcinoma of unknown primary site: a randomized, phase III Sarah Cannon Oncology Research Consortium Trial. *Cancer J* 2010;16:70–75.
165. Hainsworth JD, Spigel DR, Thompson DS, et al. Paclitaxel/carboplatin plus bevacizumab/erlotinib in the first-line treatment of patients with carcinoma of unknown primary site. *Oncologist* 2009;14:1189–1197.
167. Penley WC, Spigel Dr, Greco FA, et al. Confirmation of non-small cell lung cancer diagnosis using ALK testing and genetic profiling in patients presenting with carcinoma of unknown primary site. *J Clin Oncol* 2013;31:abstract e115004.
176. McCulloch EA. Stem cells and diversity. *Leukemia* 2003;17:1042–1048.
191. Bauer DE, Mitchell CM, Strait KM, et al. Clinicopathologic features and long-term outcomes of NUT midline carcinoma. *Clin Cancer Res* 2012;18; 5773–5779.

QUERIES:

AQ1: Changed "occasional young men" to "occasionally allowed for." OK?
AQ2: Please spell out on first mention.
AQ3: "a mediastinal and/or retroperitoneal" what?
AQ4: Are the tables being referenced in this figure referring to the tables in this chapter? If so, please change figure numbers to include chapter (Table 5 to Table 113.5).

Global:
Please check/verify new arts in proofs.
Please verify all instances of genes are in italics per the Style Guide.