Cancer of unknown primary: progress in the search for improved and rapid diagnosis leading toward superior patient outcomes

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This paper explores the enigma of cancer of unknown primary (CUP) in relation to rapidly improving molecular diagnostic approaches. It is based on the first global collaboration meeting on improving research and clinical outcomes in CUP organized by the CUP Foundation. We review the difficulties of classifying this widely heterogeneous disease and the available diagnostic and pathological evaluative techniques, focusing on molecular profiling. Retrospective studies in CUP patients are shown to provide indirect validation of the accuracy of several platforms of gene expression profiling assays that may identify CUP subsets that respond favorably to active chemotherapy regimens. This review concludes that the recent major improvements in pathologic and molecular diagnostics, coupled with new improved therapies for several specific advanced solid tumors, need to be harmonized with more evidence from clinical–translational trials. All patients with CUP could thus be appropriately managed without the constant uncertainty that has previously severely hampered patient care and optimal outcomes. The longer-term objective is to understand the biology of highly metastatic disease, leading to the development of future global therapeutic programs. Current clinical studies, such as CUP-ONE, will address some of these issues.

Key words: cancer of unknown primary, diagnosis, immunohistochemistry, metastases, molecular profiling, therapeutic profiling

introduction

Cancer of unknown primary (CUP) has been traditionally considered as metastatic cancer in the absence of a clinically detectable anatomically defined primary tumor site after an ‘adequate’ diagnostic evaluation [1]. There is poor consensus on the extent of the diagnostic evaluation necessary. It is essential for patients to have a confirmed pathologic diagnosis. Recent major improvements in pathologic and molecular diagnostics coupled with new improved therapies for several specific advanced solid tumors need to be harmonized so that all patients with CUP can be appropriately managed without the constant uncertainty that has severely hampered patient care. This article is a summary of the first global conference on CUP, organized by the CUP Foundation, which brought together people of different disciplines to share understanding of the biology and treatment of patients with CUP. The emphasis was on newer diagnostic techniques and developments with the aim of standardizing and improving patient care and stimulating further CUP research.

definition, epidemiology, biology, and prognosis

About one-third of all advanced cancers present with metastatic disease and the diagnosis of the primary site of origin is uncertain initially in many of these patients. In the majority, the primary tumor site becomes evident after clinical and pathologic evaluation. The remainder of these patients have CUP without an anatomically defined primary tumor and can be divided into two major groups. In the first group, there is a strong suspicion of the primary tumor site of origin based on clinical and pathologic features. In the second group, even after substantial evaluation, the primary tumor site remains uncertain. In both of these groups, unless a primary site is initially categorically found, the patients are classified as CUP. There is no definitive published evidence concerning treatment decisions and outcomes comparing these two groups. In those with a highly likely primary site of origin, it is logical to assume that treatment directed at the suspected primary site may
produce outcomes similar to those in patients with that type of advanced cancer. These data are not available, except in small numbers of patients. With improving diagnostic technologies, including more specific immunohistochemical (IHC) stains [2] and molecular profiling assays [3], the number of patients with highly likely primaries will increase. Generating further data on outcomes for these patients is a priority.

The wide heterogeneity of both clinical and pathologic presentations has understandably confounded attempts at classifying CUP. Diagnosis and treatment is hampered by a lack of agreement on the definition of this entity. The interpretation of published data is thus challenging and the lack of a clear definition explains in part why the epidemiology, diagnostic algorithms, clinical outcomes, and biology seem to have wide variances. Cancer registries around the world report the incidence of CUP in the range of 2%-10% of all cancer diagnoses [1] and CUP therefore ranks among the top 10 commonest malignancies. According to the USA Surveillance, Epidemiology and End Results data, CUP accounts for 2.3% of all cancers in both sexes or ~30,000 patients each year [1]. A recent analysis carried out by Tong et al. [4] concluded that the number may actually be much higher than previously thought, revealing that in the United States there may be up to 53,000 new CUP Medicare patients each year. Some CUP patients are treated for pragmatic reasons as ‘known primaries’, but usually these diagnoses are uncertain and the primary site is suspected, rather than conclusively identified. These patients are coded in various tumor registries as known primaries, i.e. lung, pancreas, etc., and thus are not registered as CUP. In this context, there may be as many as 100,000 CUP patients per year in Europe and a similar number in the United States.

The biology and pathogenesis of CUP is poorly understood. In the majority of patients, the primary tumor site is very small and clinically undetectable yet has metastasized to yield clinically detectable metastases. This is apparent from autopsy studies, particularly in carcinomas, where small clinically undetectable primaries are found in ~75% of these patients [5]. In 25% of patients, the primary is not evident even at autopsy: in these, the primary was either missed or spontaneously resolved or there remains another explanation for the metastatic cancer. These other explanations include various theories, including stem cell and embryologic migration hypotheses [1]. The clinical biology of CUP appears to be different in some aspects in comparison with known advanced cancers. Metastatic sites are at times atypical and different from those expected from tumors arising from a known primary site. There have been no specific genetic differences found thus far in CUP compared with known primary cancers. It is likely that some genetic changes, particularly mutations early in the development of the primary tumor, may explain the propensity for early metastasis and lack of substantial growth of the primary tumor. However, the molecular signature of CUP appears similar to the corresponding known primary cancer and the evolving and improving IHC and molecular profiling assays have identified several CUP profiles that are often similar to the profiles of metastatic disease of known primary sites [2, 3, 6].

Although CUP as a whole has a poor prognosis, distinct subsets of patients (~20% of the whole group) are now recognized to have an improved survival after appropriate therapy (Table 1). These so-called ‘favorable’ subsets are identified on the basis of clinical features, metastatic patterns, and pathologic features [1, 7]. Some specific neoplasms are occasionally confused with CUP, particularly when they are poorly differentiated, such as lymphoma or germ-cell tumors, and these can usually be identified and patients can receive appropriate treatment. Several of the favorable subsets of CUP patients seem to mimic the clinical and pathologic features of particular known metastatic cancers. These include auxiliary adenocarcinoma in women (breast primary); squamous cancer in neck nodes (head and neck primary); squamous cancer in inguinal node (ano-genital primaries); peritoneal serous adenocarcinoma (ovarian primary); and poorly differentiated carcinoma in the mediastinum, lung, and/or retroperitoneum in young men (extragonadal germ-cell tumor primary). The majority of patients with CUP, however, have a poor prognosis and present with adenocarcinomas and poorly differentiated carcinomas. This group accounts for ~80% of CUP, often with multiple metastases, and there has been no consensus of defined clinical and pathologic investigations or treatment guidelines. Several empiric chemotherapeutic regimens have generally been used [1, 8]. Prognosis for these patients who are not in the favorable subsets is poor with a median survival of 8-12 months from diagnosis and 1-year survival probabilities ranging from 15% to 35%. There are no definitive phase III randomized prospective clinical trials reported and therefore no uniform treatment standard. There have been few studies addressing the evolving and improving IHC stains or molecular profiling assays in classification of this heterogeneous group of cancers.

Table 1. Favorable prognostic subsets of patients with unknown primary cancer recognized by clinical and pathologic features in the last three decades

<table>
<thead>
<tr>
<th>No.</th>
<th>Subset Description</th>
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<tbody>
<tr>
<td>1.</td>
<td>Extragonadal germ cell tumor</td>
</tr>
<tr>
<td>2.</td>
<td>Poorly differentiated malignant neoplasms (60% lymphoma)</td>
</tr>
<tr>
<td>3.</td>
<td>Retroperitoneal, mediastinal and/or peripheral lymph node involvement</td>
</tr>
<tr>
<td>4.</td>
<td>Squamous cell carcinoma (head/neck or inguinal area)</td>
</tr>
<tr>
<td>5.</td>
<td>Isolated axillary carcinoma (women, rare in men)</td>
</tr>
<tr>
<td>6.</td>
<td>Peritoneal carcinoma (women, rare in men)</td>
</tr>
<tr>
<td>7.</td>
<td>Blastic bone METS or increased PSA in serum or tumor (men)</td>
</tr>
<tr>
<td>8.</td>
<td>Neuroendocrine carcinoma (low-grade or well differentiated-carinoid /islet cell type)</td>
</tr>
<tr>
<td>9.</td>
<td>Neuroendocrine carcinoma (high-grade or poorly differentiated-small cell, large cell, others)</td>
</tr>
<tr>
<td>10.</td>
<td>Single site of involvement (one lesion)</td>
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</table>
There is concern that many patients are currently being treated on a probability of primary tumor site diagnosis, based on pathology and clinical features combined, but the false-negative rate of this approach and the impact on treatment choice and patient outcomes are unclear. New approaches for diagnosing the primary tumor site in CUP are emerging, as IHC and molecular diagnostic technologies improve. The ultimate aim would be that these technologies could be utilized and thus predict the best treatment options for particular IHC/molecular–clinical subsets of CUP patients. Studies to evaluate patient outcomes of this approach are ongoing.

**diagnostic approaches to CUP**

CUP presents as one or multiple metastatic tumors. Even after exhaustive clinical and pathologic investigation, the primary is suggested in only a minority of cases ante-mortem. At autopsy, an occult primary can anatomically be found in ~75% of cases [5]. Of these, the most common primary tumor sites found are in the pancreas and lung, followed by liver/bile ducts, kidney/adrenal, and large bowel. Therefore, the majority of CUP patients have a clinically undetectable primary tumor site.

Prediction of the likely primary tumor site by testing the biopsy specimen of the metastatic tumor is improving through the use of a panel of multiple IHC stains, and the diagnostic usefulness of molecular profiling assays is emerging. It is difficult to validate the accuracy of IHC and molecular assays in CUP since by definition the primary tumor site is usually not found (except rarely later in the clinical course or commonly at autopsy). The confidence in these new diagnostic methods comes from testing in metastatic cancers where the primary site is known and also in CUP by comparing the predicted primary site with clinicopathologic features and therapeutic response and outcome to tailored regimens. However, more data about the utility of IHC and molecular profiling assays in CUP are needed, particularly comparisons of the clinical features, IHC staining patterns, and molecular assay results. Accurate diagnosis of the primary tumor site is the necessary first step to testing the clinical outcome to site-specific therapeutic regimens now and in the future.

**pathologic evaluation**

IHC stains are an important complement to light microscopy in the investigation of tumors of uncertain or unknown origin [2]. The pathologic investigation goes through a systematic approach: first, establish whether cancer is present in the tissue sample; and second, describe the broad cancer type, whether carcinoma, melanoma, lymphoma, or sarcoma: the majority of CUP patients have carcinomas. The third step is to identify the subtype of carcinoma: squamous, adenocarcinoma, solid carcinoma (thyroid, liver, renal, or adrenal), neuroendocrine (high-grade small-/large-cell or low-grade carcinoid), germ-cell tumors, or mesothelioma that may resemble carcinoma. Most CUP patients have adenocarcinomas. The fourth step is to try to predict the primary site from the histopathology and IHC staining pattern of antigens (proteins). Several rather specific stains or panels of stains are now recognized as important in the diagnosis of specific types of carcinomas. Carcinomas can have an IHC profile highly suggestive of a single primary site based on CK-7, CK-20, CDX-2, TTF-1, and breast/ovarian markers (estrogen receptor, progesterone receptor, mammoglobin, and GCDFP-15). Histopathologic investigation continues to evolve and improve. Several examples of stains of diagnostic importance include GCDFP-15 and mammoglobin in breast, TTF-1 in lung (particularly with CK-7+, CK-20−), HEPAR-1 in hepatocellular, renal cell carcinoma in renal cell, thyroglobulin/TTF-1 in thyroid, placental alkaline phosphatase/OCT-4 in germ-cell tumors, CDX-2 in colorectal (particularly with CK-7−, CK-20+), and WT-1/PAX-8 in ovary. Synaptophysin and chromogranin stains are important in diagnosing neuroendocrine tumors, particularly in those that have poorly differentiated histology. The clinicopathologic features of each patient should help decide which IHC stains should be done. In turn, the findings from IHC may suggest additional clinical evaluation. In using IHC, in particular subsets of patients with CUP, it appears possible to identify some who may respond better to site-specific therapy. Carcinomas that express CDX-2 and CK-20 and are negative for CK-7 have a ‘colon cancer profile’ and there is preliminary evidence that these patients respond better to colon cancer chemotherapy regimens than empiric ‘CUP regimens’ [9]. Prospective studies are underway to further document this subset of patients identified by IHC and/or molecular profiling.

Panels of IHC markers appear superior to single biomarkers in classifying the possible primary tumor sites in adenocarcinomas. Dennis et al. [10] demonstrated that an IHC panel using 10 different marker stains, including those mentioned above, can correctly classify the site of origin in ~88% of adenocarcinomas. These data are from known primary cancers but may have important diagnostic implications for CUP. There are, however, many primary tumors for which site-specific markers with high sensitivity and specificity are lacking, including most upper gastrointestinal cancers such as pancreaticobiliary and gastroesophageal cancers, which are also anatomically close. This difficulty provides one of the biggest challenges to conventional diagnostic methods.

There are few published results in CUP addressing the ability of IHC to predict a single primary tumor site, and validation of their accuracy is difficult since the primary site usually remains unknown. Horlings et al. [11] evaluated 38 patients with CUP with some accompanying IHC staining, and in 16 (42%), a single primary site was suspected. In 15 of these 16 patients, a molecular assay diagnosis was in agreement (94%) with the IHC diagnosis. The results of this study revealed not only that a single primary tumor site was predicted in a minority of patients by IHC (albeit possibly incomplete) in CUP but also that the agreement of the molecular profiling assay to IHC is notable. Greco and Hainsworth [12] also have evaluated a molecular profile assay in 171 patients with CUP, most having fairly comprehensive IHC. In 59 (35%) of 171 patients, a single primary tumor site diagnosis was predicted by IHC staining and the molecular profile assay diagnosis agreed in 40 of 52 evaluable tumors (77%). State-of-the-art IHC staining can accurately predict a single primary site in ~35%–40% of CUP patients and molecular profile assays are in agreement in these...
tumors most of the time. Further investigation of molecular profile assays in the majority of CUP patients not given a single primary tumor site diagnosis by IHC staining is certainly a priority. Regardless of the type of diagnostic technology utilized, the diagnosis of the possible primary tumor site must be interpreted with knowledge of the clinical context to enable optimal tumor characterization and thus potentially impact patient management. Communication between clinician and pathologist is therefore essential.

**clinical evaluation**

Morphologic examination of a biopsy tumor specimen is a critical first step and provides a practical classification system on which to base subsequent investigations. All patients should have a complete history and physical examination and a full-body computed tomography scan at the onset in addition to complete blood counts, renal and liver function tests, and a urinalysis. Women should have mammography and men should have a serum prostate-specific antigen determination. These examinations may lead to finding the primary tumor site. Most of the positron emission tomography (PET) scan literature in CUP patients is retrospective with a small sample size and it is unclear now if it impacts therapy and survival in the majority of patients. PET scanning can be helpful in selected patients, including those with squamous cancer presenting in cervical lymph nodes where a primary is suspected from PET in ~30% of these patients. In patients with a single site of metastatic disease, PET scanning can be useful, particularly before embarking on local therapy. Patients should certainly have appropriate endoscopic evaluations to investigate symptoms, positive review of system findings, or laboratory abnormalities. The pattern of metastasis in CUP is an important part of the puzzle and should always be considered in concert with specialized pathologic studies before a treatment plan is formulated.

**molecular profiling and classification of human cancers**

Microarray technology has progressed rapidly in the last 15 years and has enabled the quantification of whole genome or sets of gene expression in human tumors [13, 14]. This technology forms a foundation for the characterization of many cancer-activated pathways. These genomic technologies have led to the discovery of diagnostic, prognostic, and drug response signatures for several cancer types. The distinction between acute myeloid leukemia and acute lymphoblastic leukemia was the first example of how a gene expression pattern could be used to classify and therefore discriminate between cancer types [15]. Subsequently, there have been numerous reports demonstrating the ability to use gene expression profiles to classify tumors [16, 17]. The basis of this approach is the existence of cell type-specific differential gene expression profiles: malignant tissues still retain some of these signature gene expression profiles that define their normal counterpart. There is an urgent clinical need to validate these methods to predict the primary tumor site in CUP. Validation in known primary cancers is established [18–23], but the genetic signatures in CUP may not always be the same as known primary cancers. Molecular approaches have the potential to become a more readily standardized objective process, given its quantitative nature, and could ultimately determine targeted treatment pathways as well. A first-generation gene expression-based classifier for six specific cancer types plus ‘other undesignated types’ demonstrated that identifying a small subset of patients with CUP having a colorectal-like gene expression profile predicted responses to treatment similar to those of known colon cancer patients [24]. As more targeted and effective therapy for specific cancer types has become available (renal, lung, breast, colorectal, stomach, and others), a greater number of patients with an initially uncertain or unknown primary tumor site have the potential to benefit if their cancer type is accurately determined. Appropriate personalized treatment of each CUP patient in the future is a realistic expectation. Indeed, the diagnostic and treatment-predictive pathways could ultimately be integrated in one process.

Currently, there are several commercial tests available with gene expression-based assays that classify tumors of unknown or uncertain origin (Table 2): Pathworks [microarray for messenger RNA (mRNA)], Rosetta Genomics and Prometheus (RT-PCR for microRNA), and bioTheranostics (RT-PCR for mRNA). All tests have claimed prediction accuracies in known

<table>
<thead>
<tr>
<th>Test</th>
<th>Platform</th>
<th>Material</th>
<th>No. of genes profiled</th>
<th>No. of tumor classes</th>
<th>Strategy</th>
<th>Claimed % sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>bioTheranostics</td>
<td>CancerTYPE ID® Pathwork® Tissue Of Origin Test</td>
<td>RT-PCR</td>
<td>FFPE</td>
<td>92</td>
<td>54</td>
<td>Nearest Neighbor ‘Similarity Score’ to rule in or rule out certain tumor types</td>
</tr>
<tr>
<td>Pathworks</td>
<td></td>
<td></td>
<td>Fresh/frozen</td>
<td>&gt;1500</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Rosetta Genomics–Prometheus</td>
<td>miRview™ mets (ProOnc Tumour SourceDxT)</td>
<td>RT-PCR for microRNA</td>
<td>FFPE</td>
<td>48</td>
<td>25; 42 *</td>
<td>Binary decision tree combined with K nearest neighbors</td>
</tr>
</tbody>
</table>

*Forty-two tumor classes in second-generation test of Rosetta Genomics.

CUP, cancer of unknown primary; FFPE, formalin-fixed paraffin-embedded.
primary cancers between 80% and 90%. However, each test has different specimen requirements and a wide range in their ability to identify cancer types/subtypes (15–54 types/subtypes). In general, a clinically viable test needs to be compatible with formalin-fixed paraffin-embedded (FFPE) tissues with low numbers of tumor cells and to have the ability to discriminate large number of tumor types since metastatic cancers can arise from many cancer types and primary tumor sites.
new technologies in practice

Retrospective studies in CUP patients have provided indirect validation of the accuracy of several platforms of gene expression profiling assays [11, 18, 19, 24–26]. These studies have judged the accuracy of the prediction of the molecular assay based on correlation with the clinical and pathologic (including IHC) findings. A retrospective study was recently reported using a molecular profiling assay (RT-PCR) by bioTheranostics on the initial diagnostic biopsy specimen of 20 patients with CUP, retrospectively collected from a group of >500 CUP patients, most treated in prospective clinical trials over an 8-year period [27]. These patients eventually had their primary site detected later during the course of their disease (latent primary). The accuracy of the assay was based on the latent known primary sites found between 2 and 54 months following the initial diagnosis of CUP. The molecular profile assay prediction was accurate in 15 of the 20 patients (75%). Detailed clinical information was gathered on these patients, including their demographics, sites of metastasis, and laboratory and imaging data. Pathologic evaluation including IHC staining, type of therapy, response to therapy, and survival were all documented. The molecular assay compared favorably with IHC marker stains, more often providing a single correct prediction of the primary tumor site (75% versus 25%). Of the 15 patients that were accurately predicted by the molecular assay, the treatment would have been different than the actual treatment administered in eight (52%) patients. A recent prospective study [28] compared the molecular profiling of 89 CUP patients using the Rosetta Genomics–Prometheus assay with clinicopathologic features and with therapeutic response. This study showed feasibility in retrieving microRNA and determining tissue of origin from FFPE tissue of CUP patients and the assay results correlated with clinicopathologic features in 84% of cases.

The value of molecular profiling will be enhanced when used in concert with appropriate IHC stains and the clinical features. Clinical judgment currently remains important when making decisions regarding CUP patient management based on the emerging diagnostic technologies. Additional clinical research is critical to help validate the accuracy of molecular assay diagnoses and to show that site-specific therapy, based on these assays, can indeed now improve the outcome or survival of some patients with CUP. In the future, patients with CUP as well as other metastatic tumors are likely to be treated with therapy proven to be useful for their specific tumor type and/or with therapeutic agents targeting important regulatory sites discovered in their tumor cells by gene expression profiling signatures, regardless of the primary tumor site.

Ultimately, the aim in CUP is for better patient outcomes because at present most patients have relatively poor outcomes. It is critical to determine whether CUP patients respond and do as well as their counterparts with known primary cancers when treated with site-specific therapy, based upon robust IHC staining and molecular profiling assays, preferably within randomized studies. An appropriate diagnosis, particularly if the tumor shares the biology of the known primary cancers, may be critical in the future since it is expected that all types of cancers will eventually have improved therapy. Major progress may require global cooperation and coordination to carry out the appropriate prospective therapeutic studies to validate the usefulness of molecular profiling in reference to CUP patient management and outcomes. A reasonable current framework for the evaluation of CUP patients is illustrated in Figure 1.

**Conclusion**

A single definition of CUP has been difficult since this is not a single neoplasm but a clinicopathologic syndrome with a range of neoplasms and clinical presentations. The immediate future of CUP patients appears to rest on a better understanding of their primary site of origin as well as, and perhaps more important, the molecular intricacies driving the neoplastic process in each individual patient. With further knowledge of these details, it is hoped that the ultimate model of personalized treatment of patients with metastatic cancers will be based on an intimate knowledge of their individual tumor regardless of the primary site of origin (Figure 2).

Technology to predict the primary tumor site of origin is improving rapidly and will eventually improve the management and treatment of patients. Molecular profiling assays and/or IHC may be able to identify CUP subsets that respond favorably to active chemotherapy regimens as used against the corresponding anatomically defined cancers. The performance of molecular assays appears broadly similar to optimal IHC: direct comparisons are few as yet, but molecular assays may provide additional and clinically important information for patients with tumors not adequately classified by IHC. It remains unclear whether
molecular assays should be used routinely and if so in which cases, perhaps where optimal standard/current IHC assessment yields no clear single tumor type or primary site or where the tumor classification is doubtful and important treatment options exist? The comparison of optimal pathology and molecular assays in CUP patients remains an important question. It is likely that these two important diagnostic methods will be complementary. Clinical and cost-effectiveness studies evaluating the impact of these new diagnostic tools on therapy decisions and survival are warranted. It appears we are now entering a new era of better understanding of the biological enigma of CUP, and further research will lead toward superior patient outcomes.

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FAG is on the speaker’s bureau for bioTheranostics. KO has acted as advisor to bioTheranostics. ME is a full-time employee of bioTheranostics, which offers a commercial assay (miRview). All remaining authors have declared no conflicts of interest.

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