EDITORIAL

A Primary Approach to Cancers of Unknown Primary

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Most solid tumors are diagnosed in their organ of origin; however, approximately 20% of patients will present with a tumor identified in one or more metastatic sites. In the overwhelming majority of cases, a clinical history, physical examination, laboratory tests, functional and radiographic imaging (positron emission tomography/computed tomography), and histologic assessment will disclose the primary site, enabling site-directed chemotherapy. Yet, again, in approximately 20% of cases, the primary site eludes determination, even after examination of broad panels of immunohistochemical assays. These cancers of unknown primary organ (CUP), defined as metastatic cancers whose anatomic origin is clinically not detectable even after a thorough diagnostic evaluation, represent a heterogeneous group of malignancies and account for approximately 4% of cancer diagnoses (1,2). Interestingly, in our experience, even after postmortem examination, 20% of CUPs, or about 1% of all cancers, are never anatomically defined. Although these cancers present as metastases and represent a spectrum of biological behavior, oncologists have stratified them into favorable (approximately 20%) and poor (approximately 80%) prognostic groups based on such factors as clinical presentation, host factors, tumor histology, number and location of metastatic sites, and their sensitivity to chemoradiation treatment (1–3). In general, patients with CUP have an overall survival of 6 to 9 months, although the favorable prognostic group may have a median survival of nearly 36 months.

The pathologic diagnoses of CUPs in metastatic sites tend to be carcinomas, of which the majority are adenocarcinomas. The initial diagnostic approach seeks to exclude atypical but benign reactive process and then classify the malignancy as a carcinoma or other malignancies such as sarcomas, lymphomas, and melanomas. An immunohistochemical (IHC) panel can separate the majority of these tumor types. Histopathologic features combined with histochemical mucin stains usually permit distinction between adenocarcinomas and other cancers, namely, squamous cell carcinomas, poorly differentiated carcinomas, germ cell cancers, neuroendocrine carcinomas, and the occasional mesotheliomas that mimic a sarcoma or adenocarcinoma. The increasing versatility of IHC panels based on the pairwise findings of CK7 and CK20, and the more refined organ-specific panels, have increased the pathologist's ability to narrow the field of primary organ sites of cancer or in some cases to readily define it (1,3–5). Tumors with unique immunohistochemical signatures can provide high probability of primary sites such as the panel of $CK7^-$, $CK20^+$, $CDX2^+$ for colorectal adenocarcinomas, and $CK7^+$, $CK20^-$, $TTF-1^+$, $Napsin-A^+$ for pulmonary adenocarcinomas. As more organ-specific and

therapy-directed IHC markers (such as human epidermal growth factor receptor-2 and estrogen receptor) are introduced and validated, they should increasingly enable CUPs to be better defined and managed. Despite these advances, as a result of tumor heterogeneity, IHC only supports a differential diagnosis, and additional and alternative modalities for primary site designation are clearly needed. In this regard, several attempts at molecular profiling have been initiated that have focused on multiple gene expression profiles or microRNA signatures using sequencing, reverse-transcription polymerase chain reaction (RT-PCR), or microarray platform technologies. Some reports have claimed an 80% overall accuracy at organ-specific identification based on a focused group of tumor types (6–11).Commercial assays and reference laboratories advertise to help the oncologist in this endeavor and support the hypothesis that identification of the primary site will focus therapy and improve clinical outcome.

In this issue of the Journal, Greco and colleagues at the Sarah Cannon Research Institute in Nashville, Tennessee, have contributed to the clinical laboratory science of organ site prediction of CUPs based on a molecular profiling approach that complements the results of diagnostic pathology (12). Much of the work and the thrust of molecular tumor profiling (MTP) technology have already been published with the demonstration of the improved organ site identification over IHC findings (6,7). The authors generated a retrospective review of 171 CUPs and performed MTP using a proprietary technology (CancerTYPE ID, BioTheranostics, San Diego, CA) utilizing a 92-gene quantitative RT-PCR for 54 tumor classes on archival material. They compared the MTP results with clinical follow-up primary site identification and generic and focused IHC panels. The authors considered two patient groups: one consisting of 151 patients followed prospectively from 2008 to 2010, and a second prospective cohort of 24 patients whose primary sites were identified during clinical follow-up. Among these groups, the MTP assay was able to identify a single tissue of origin in 80%–90% of cases, compared with IHC, which could identify a single site in only 30%. Focusing on the cohort of 24 cases where the subsequent evaluation identified the anatomic primary site, MTP provided a single site in 22 cases and was correct in 18. IHC identified a single site in seven cases and was correct in six; in 16 cases, IHC offered a differential diagnosis and included the identified site in nine. Importantly, MTP is a multiparameter technique with a complicated algorithmic analysis, whereas IHC represents a set of binary decisions based on the staining characteristics. In a separate evaluation, 52 of 59 cases from the overall patient group in which IHC initially identified a single

putative primary site and adequate tissue was available for MTP assay; the concordance was 77% (40 of 52 cases). In cases where IHC provided a differential diagnosis among several primary sites, there was agreement with the specific MTP results in 44% (43 of 97 cases), and, in the majority of these cases, clinical features supported the MTP diagnosis.

The results of Greco and colleagues (12) are encouraging and demonstrate that molecular profiling is capable of arriving at a correct single designation of the primary site in a large majority (80%) of cases. Interestingly, when IHC is definitive for a single site, it is also correct in a similarly high percentage of cases; however, it arrives at a single site in only the minority (30%) of cases. It is more likely that IHC provides a differential set of choices than a single identifiable site. The authors acknowledge that the current test characteristics of sensitivity and specificity need to be improved based on additional tumors for their test and validation assays. In addition, cost-effectiveness of the assays, standardization of the protocol algorithm for determination of the primary site, and the turn-around time need to be addressed. Currently, the National Comprehensive Cancer Network guidelines do not recommend the routine testing of gene expression profiles for the identification of tumor primary site (2).

The authors also appreciate that even with an improved assay protocol, tumor sufficiency will be a limiting condition preventing the utilization of fine-needle aspirations and needle core biopsies, material well suited for IHC. Even tissue from formalin-fixed, paraffin-embedded blocks will require pathologic determination for specimen adequacy and minimization of tumor necrosis. In their study, 13% of cases had insufficient tumor for assay diagnosis. Greco et al. have previously attempted to address the fundamental hypothesis of this endeavor—namely, that knowledge of the primary site will provide the correct treatment for improved clinical outcome—yet that study used historical controls and had some selection bias (13).

The primary site of malignant tumors represents a surrogate marker for oncological management. Further development of molecular modalities to determine chemotherapeutic sensitivity and resistance and targeted therapy can be expected to enhance management of CUPs. These cancers may be biologically different from their cognate primary tumors with predictable tumor progression and, consequently, identification of primary site of CUPs may be only one component of optimal cancer management.

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