Comprehensive Genomic Profiling of Carcinoma of Unknown Primary Site
New Routes to Targeted Therapies

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IMPORTANCE For carcinoma of unknown primary site (CUP), determining the primary tumor site may be uninformative and often does not improve outcome.

OBJECTIVE To discover opportunities for targeted therapies in patients with CUP not currently searched for in routine practice.

DESIGN, SETTING, AND PARTICIPANTS Comprehensive genomic profiling on 200 CUP formalin-fixed paraffin-embedded specimens (mean, 756× coverage) using the hybrid-capture–based FoundationOne assay at academic and community oncology clinics.

MAIN OUTCOMES AND MEASURES Presence of targetable genomic alterations (GAs) in CUP and responses to targeted therapies.

RESULTS There were 125 adenocarcinomas of unknown primary site (ACUPS) and 75 carcinomas of unknown primary site without features of adenocarcinoma (non-ACUPS). At least 1 GA was found in 192 (96%) of CUP specimens, with a mean (SD) of 4.2 (2.8) GAs per tumor. The most frequent GAs were in TP53 (110 [55%]), KRAS (40 [20%]), CDKN2A (37 [19%]), MYC (23 [12%]), ARID1A (21 [11%]), MCL1 (19 [10%]), PIK3CA (17 [9%]), ERBB2 (16 [8%]), PTEN (14 [7%]), EGFR (12 [6%]), SMAD4 (13 [7%]), STK11 (13 [7%]), SMARCA4 (12 [6%]), RBL1 (12 [6%]), RICTOR (12 [6%]), MLL2 (12 [6%]), BRAF (11 [6%]), and BRCA2 (11 [6%]). One or more potentially targetable GAs were identified in 169 of 200 (85%) CUP specimens. Mutations or amplifications of ERBB2 were more frequent in ACUPS (13 [10%]) than in non-ACUPS (3 [4%]). Alterations of EGFR (10 [8%] vs 2 [3%]) and BRAF (8 [6%] vs 3 [4%]) were more common in ACUPS than in non-ACUPS. Strikingly, clinically relevant alterations in the receptor tyrosine kinase (RTK)/Ras signaling pathway including alterations in ALK, ARAF, BRAF, EGFR, FGFR1, FGFR2, KIT, KRAS, MAP2K1, MET, NFI, NF2, NRAS, RAF1, RET, and ROS1 were found in 90 (72%) ACUPS but in only 29 (39%) non-ACUPS (P < .001).

CONCLUSIONS AND RELEVANCE Almost all CUP samples harbored at least 1 clinically relevant GA with potential to influence and personalize therapy. The ACUP tumors were more frequently driven by GAs in the highly druggable RTK/Ras/mitogen-activated protein kinase (MAPK) signaling pathway than the non-ACUP tumors. Comprehensive genomic profiling can identify novel treatment paradigms to address the limited options and poor prognoses of patients with CUP.
between 2% and 9% of all cancer diagnoses present as a metastatic carcinoma of unknown primary site (CUP),1-4 and in the United States alone, more than 30,000 patients receive a diagnosis of CUP each year.1 Approximately two-thirds of CUP tumors are adenocarcinomas of unknown primary site (ACUPS) with mucin production, tubule formation, and immunohistochemistry (IHC) findings that can define histologic subtype of adenocarcinoma but cannot identify the exact primary site of origin for the tumor. The remaining one-third of CUP tumors are a mix of nonadenocarcinomas (non-ACUPS) including squamous cell carcinomas, neuroendocrine carcinomas, and small- and large-cell undifferentiated carcinomas.3,4 Diagnostic workups often fail to locate the primary tumor site despite the use of multiple imaging modalities, invasive procedures (eg, endoscopy, colonoscopy), serum biomarker tests, and IHC staining and mRNA expression profiling of biopsy tissues. Additional postmortem analyses typically fail to confirm a primary site of origin in approximately one-quarter of patients who are autopsied.7 Carcinoma of unknown primary site occurs with equal frequency in men and women, and median age at presentation is approximately 60 years.8 Although there are no drugs specifically approved for treatment of CUP, there are multiple guidelines published for treating the disease using multiagent cytotoxic chemotherapy,2,5,6 but the response to nontargeted chemotherapy in this disease is generally poor, with 5-year survival currently at 11% and short progression-free and median overall survival ranging from only 11 weeks to 11 months.2,5,6

To date, the underlying biology of CUP has been studied predominantly by IHC analysis and gene expression (mRNA) profiling.7,8 The use of molecular profiling and gene sequencing on a relatively small scale to search for potential therapeutic targets has just begun to emerge.9 In addition, currently available clinical trials for patients with CUP have rarely featured targeted therapeutic agents or required a specific biomarker for trial entry. Recent evidence for diseases such as primary non–small-cell lung cancer suggests that the use of targeted therapy whose selection was directed by information acquired from gene sequencing can significantly improve patient outcomes.10 Some oncologists have thus hypothesized that a test that can guide targeted therapy selection for patients with CUP “up front” would have utility in clinical management and could help avert the expensive and potentially futile search for the primary lesion that is often pursued. In the present study, we used a comprehensive genomic profiling assay based on next-generation sequencing (NGS) of tumoral DNA to evaluate a series of 200 consecutive CUP specimens to determine whether the genome-derived drug targets identified could be used to guide personalized, targeted treatment for this challenging disease.

Methods

Due to the retrospective nature of the study, the original written local site permissions to use clinical samples and approval by the Albany Medical College institutional review board to analyze patient data were obtained for this study. In this study, CUP was defined as a tumor from a patient presenting with metastatic carcinoma that did not unequivocally originate from a confirmed primary site on the basis of available diagnostic imaging, prior surgery, or pathology workup, including IHC analysis, fluorescence in situ hybridization (FISH), serum biomarker analysis, and/or mRNA transcriptional profiling. The 200 CUP cases in this series were evaluated in 2 ways, both as a single group and divided into 2 subsets: ACUP, in which features of adenocarcinoma were readily evident (gland formation, mucin production), and non-ACUP, in which adenocarcinoma features were absent. Metastatic carcinomas initially submitted as CUP but subsequently found to have local site positive staining for characteristic site-of-origin IHC markers (thyroid transcription factor 1, prostate-specific antigen, mammaglobin, calretinin, melanoma markers) or positive FISH-based detection of fusions characteristic of a specific tumor type (EML4-ALK, TMPRSS-ERG) were excluded from this study. All cases included in this study underwent an onsite pathology review to confirm histologic classification and tumor tissue adequacy, which required a minimum of 20% relative proportion of tumor cell nuclei and sufficient nucleated cells to create a minimum of 50 ng of extracted DNA.

Genomic profiling was performed in a Clinical Laboratory Improvement Amendments–certified, College of American Pathologists–accredited laboratory (Foundation Medicine) using the Illumina HiSeq 2500 instrument. At least 50 ng of DNA per specimen was isolated and sequenced to high, uniform coverage (mean, 756×), as previously described.11 The DNA extracted from CUP formalin–fixed paraffin–embedded (FFPE) tumor specimens was analyzed after hybridization capture of 3769 exons from 236 cancer-related genes and 47 introns of 19 genes commonly rearranged in cancer. The original 207 CUP cases were reduced to 200 cases when 7 (3%) of the tumors failed to yield acceptable sequencing results. Genomic alterations (GAs) simultaneously detected by this assay included base substitutions, short insertions and deletions, focal gene amplifications and homozygous deletions (copy number alterations), and select gene fusions and rearrangements.

Sequence analysis methods and validation of the comprehensive genomic profiling platform used in this study have been described previously, including extensive comparisons with orthogonal methodologies.11 Base substitution detection is performed using a Bayesian methodology, which allows detection of novel somatic mutations at low mutant allele frequency (MAF) and increased sensitivity for mutations...
at hot-spot sites through the incorporation of tissue-specific prior expectations.14 Reads with mapping quality less than 25 are discarded, as are base calls with quality 2 or less. Final calls are made at MAF of at least 5% (MAF ≥1% at hot spots) after filtering for strand bias (Fisher test, *P* < .05), read location bias (Kolmogorov-Smirnov test, *P* < .001), and presence in 2 or more normal controls. To detect short insertions or deletions (indels), de novo local assembly in each targeted exon is performed using the de Bruijn approach.12,13 After read pairs are collected and decomposed, the statistical support for competing haplotypes is evaluated and candidate indels are aligned against the reference genome. Filtering of indel candidates is carried out as described for base substitutions. Gene amplifications and homozygous deletions are detected by comparing complete chromosomal copy number maps to reference process-matched normal control samples. Finally, gene fusions and rearrangements are detected by analysis of chimeric read pairs.14 Statistical analysis of GAs identified in the ACUP and non-ACUP patient cohorts was performed with the Fisher exact test with the level of significance set at *P* < .05.

Clinically relevant alterations were defined as those GAs that could be targeted using anticancer drugs currently on the market for any tumor type with known primary site or GA required for entry in mechanism-driven registered clinical trials.

## Results

Of the 200 CUP cases included in this study, 125 (63%) were ACUP and 75 (38%) were non-ACUP, which included 47 carcinomas (not otherwise specified), 18 large-cell neuroendocrine carcinomas, 8 squamous cell carcinomas, and 2 small-cell undifferentiated carcinomas (Table 1). Tumor samples were isolated from 108 (54%) women and 92 (46%) men with CUP with a median (range) age of 58.5 (27-88) years. The most frequent sources of tissue for comprehensive profiling were obtained from the following metastatic sites: liver (25%), lymph node (19%), peritoneum (7%), soft tissue (6%), bone (5%), brain (5%), skin (4%), and pleura (3%). One hundred ninety-nine (95%) of the 200 CUP cases were analyzed by IHC analysis and 6 (3%) underwent mRNA profiling, neither of which confirmed a specific site of cancer origin.

Within these 200 samples, a total of 841 alterations were identified in 121 genes (401 base substitutions, 217 gene amplifications, 140 short indels, 66 gene homozygous deletions, and 17 gene rearrangements) for a mean (SD) of 4.2 (2.8) GAs per CUP (Table 2). One hundred ninety-two (96%) of the total CUP cases harbored at least 1 alteration. At least 1 clinically relevant GA that could potentially guide decisions for targeted treatment was identified in 169 (85%) of the 200 total CUP cases, with 113 of 125 (90%) in the ACUP group and 56 of 75 (75%) in the non-ACUP group. The frequency of clinically relevant GAs per tumor in patients with ACUP (1.9) and non-ACUP (2.1) tumors was virtually identical (Figures 1, 2, and 3, Table 2, eFigures 1 and 2 in the Supplement). Of the 58 unique genes found to be altered in 1 or more CUP cases, 30 (52%) were categorized as clinically relevant. The most common clinically relevant alterations potentially affecting treatment decisions included KRAS (40 [20%]), CDKN2A (37 [19%]), MCL1 (19 [10%]), PTEN (14 [7%]), PIK3CA (17 [9%]), ERBB2 (16 [8%]), RICTOR (12 [6%]), BRAF (11 [6%]), and NF1 (8 [4%]) (eTable in the Supplement).

Disparities in the distribution of genes altered in ACUP vs non-ACUP tumors were repeatedly observed. Alterations in ERBB2, including both sequence alterations and gene amplification, were identified in 13 (10%) of ACUPs but only in 3 (4%) of non-ACUPs. Alterations in EGF R were seen in 10 (8%) of ACUPs and 2 (3%) of non-ACUPs, and BRAF alterations were seen in 8 (6%) of ACUPs and 3 (4%) of non-ACUPs. In contrast, alterations in MLL2 were observed in 8 (10%) of non-ACUP tumors but were present in only 4 (3%) of ACUPs. Strikingly, actionable alterations in the receptor tyrosine kinase (RTK)/Ras signaling pathway were identified in 90 (72%) of ACUP cases, compared with 29 (39%) of the non-ACUP cases (Fisher exact test, *P* < .001) and affected the following genes: ALK, ARAF, BRAF, EGFR, FGFR1, FGFR2, KRAS, MAP2K1, MET, NF1, NF2, NRAS, RAF1, RET, and ROS1. In almost all cases, alterations within the RTK/Ras pathway were mutually exclusive. The most common biologically relevant alterations that cannot currently be linked to a targeted treatment option were found in TP53 (110 [55%]), MYC (23 [12%]), ARID1A (21 [11%]), SMAD4 (12 [6%]), SMARCA4 (12 [6%]), RBB1 (12 [6%]), and PBRM1 (7 [4%]).

A total of 26 alterations identified in this study are associated with targeted therapies approved for use in patients with a known primary tumor type (Table 3). An additional 14 CUP cases harbored “off-target” GAs associated in multiple reports with targetable activity, such as MET amplification and ERBB2 activating substitutions.14-16 Finally, the remaining actionable GAs are not currently considered specific targets for approved therapies but were nonetheless linked to hundreds of registered clinical trials and could direct patient entry into

### Table 1. Clinical Characteristics of Patients and Tissue Samples in 200 Cases of Carcinoma of Unknown Primary Site

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (N = 200)</th>
<th>ACUP (n = 125)</th>
<th>Non-ACUP (n = 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (SD), y</td>
<td>58.5 (12.4)</td>
<td>57.2 (12.3)</td>
<td>59.0 (12.5)</td>
</tr>
<tr>
<td>Male sex, No.</td>
<td>92</td>
<td>62</td>
<td>30</td>
</tr>
<tr>
<td>Female sex, No.</td>
<td>108</td>
<td>63</td>
<td>45</td>
</tr>
</tbody>
</table>

Abbreviation: ACUP, adenocarcinoma of unknown primary site.

### Table 2. Genomic Alterations in 200 Cases of Carcinoma of Unknown Primary Origin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ACUP (n = 125)</th>
<th>Non-ACUP (n = 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total GAs, No.</td>
<td>494</td>
<td>347</td>
</tr>
<tr>
<td>GAs per sample, mean</td>
<td>4.0 (2.1)</td>
<td>4.6 (3.7)</td>
</tr>
<tr>
<td>Base substitutions, No. (%)</td>
<td>250 (51)</td>
<td>151 (44)</td>
</tr>
<tr>
<td>Indels, No. (%)</td>
<td>74 (15)</td>
<td>66 (19)</td>
</tr>
<tr>
<td>Amplifications, No. (%)</td>
<td>119 (24)</td>
<td>98 (28)</td>
</tr>
<tr>
<td>Homozygous deletions, No. (%)</td>
<td>39 (8)</td>
<td>27 (8)</td>
</tr>
<tr>
<td>Rearrangements/fusions, No. (%)</td>
<td>12 (2)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>Samples with no GA detected, No.</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

Abbreviations: ACUP, adenocarcinoma of unknown primary site; GA, genomic alteration; indel, short insertion or deletion.
studies testing targeted agents in both early and later stages of clinical development.

In this recent retrospective study, clinical outcome information was available only for a relatively small subset of cases to date. Examples of positive CUP tumor responses to targeted therapy include MET amplification (16 copies) in a white woman in her 50s with CUP who presented with new-onset seizures (Figure 4). Treatment with crizotinib led to a sustained complete clinical response currently at greater than 3 years.17 A second example of comprehensive genomic profiling being used to direct targeted therapy is shown in Figure 5. This patient with CUP also achieved a major response to targeted therapy when comprehensive profiling was performed upfront and revealed an ALK fusion that responded dramatically to crizotinib therapy.18

Discussion

In this study, almost all patients with CUP harbored at least 1 clinically relevant GA with potential to influence and personalize therapy selection. Clinicopathologic studies indicate that patients with CUP can be separated into 2 groups: (1) those with metastatic disease for which the primary tumor site cannot be identified and (2) those for whom the primary tumor site is uncertain because of limitations in tissue histologic analysis including immunohistochemistry.17 The latter category includes both primary tumors of known origin with undifferentiated appearance and cases of liver cholangiocarcinoma that present as a metastatic CUP.19 Regardless of disease classification, an important discussion has recently emerged surrounding the need to locate the primary tumor site when a patient presents with metastatic disease. The cost of a complete diagnostic workup for CUP—including multimodality diagnostic imaging procedures,20-23 tissue IHC panels,24-26 serum tumor marker panels,27,28 and mRNA profiling28-32—commonly exceeds $10 000. For 25% or more of these patients, after a thorough workup, the primary site will remain unknown, and no therapies will be considered “on label.”

A variety of commercial and proprietary mRNA and microRNA profiling platforms have been used to predict the most likely site of origin for a patient whose tumor presents as a CUP.20 Although it is generally well accepted that molecular methods to locate the likely site of origin in a case of CUP will play a substantial role in therapy selection for some patients, the impact on overall survival is estimated to be at most several months, as reported in a large clinical outcome study.29 The purpose of our study was not to identify the primary site...
of origin in the CUP case, which rarely specifies a unique therapy option. This study was restricted to the search for druggable GAs that could lead to the use of a targeted agent with either a drug on the market or one available through a clinical trial. These factors have led oncologists to ask whether a test that can guide therapy selection for all patients with CUP up front would have more value for clinical management than the potentially futile and expensive search for the primary lesion that is currently undertaken (Figure 5). Thus, there is substantial interest in evaluating the use of genomic profiling at the time of diagnosis to identify targeted treatment options and improve response rate, progression-free survival, and perhaps overall survival without ever searching for the primary tumor site.

Small-scale gene-sequencing methods restricted to hot spots of well-characterized genes associated with cancer have been applied to CUP samples but with inconsistent results and limited success. Recent investigations of KRAS and TP53 status during the workup of CUP failed to identify the primary site of the disease and could not guide therapy selection.34-37 Tyrosine kinase inhibitor therapy targeting EGFR in unselected patients with CUP has been used with limited benefit.36,37 Although these studies described high frequencies of EGFR expression as measured by IHC analysis, the activating EGFR mutations typically associated with benefit from EGFR-specific tyrosine kinase inhibitors, such as erlotinib hydrochloride, were rarely identified.38,39 In contrast, the present study identified 6 (3%) CUP samples featuring an activating EGFR alteration not detectable by IHC analysis, including both the activating missense mutation L858R and the exon 19 deletion alterations that predict clinical benefit from EGFR-targeting agents such as erlotinib and afatinib dimaleate with high fidelity. Finally, the Cancer Genome Atlas and other cancer-sequencing groups have not, to date, studied CUP and there is no available published database of GAs that has been created for this group of malignant neoplasms.40

Slide-based assays such as IHC and FISH, which estimate protein overexpression and gene amplification, respectively, are the predominant methods used to detect ERBB2 (HER2) status in CUP.14,41 Promising results have been reported for the subset of patients with tumors that tested positively and who were treated with anti–human epidermal growth factor receptor 2 (HER2) therapies.14,41 In this study, 6 (3%) of the CUP tumors featured ERBB2 amplifications that might have been detected by FISH or IHC if these assays had been performed, but a larger fraction of the cases (9 of 200 [5%]) harbored activating mutations in ERBB2 that would not be detectable by either IHC or FISH. These nonamplification alterations included missense mutations in either the extracellular or kinase domains. Recent studies have shown that carcinomas driven by activating ERBB2 mutation, vs amplification, can respond to anti-HER2 targeted therapies including trastuzumab, lapatinib ditosylate, and afatinib.14,42-43

Similar promising scenarios apply to several other genes assayed in this study. Although BRAF mutation profiling has also been performed on CUP samples, reports on the efficacy of BRAF inhibitors such as vemurafenib in BRAF-mutated CUP are not available.44 Notably, BRAF V600E–specific inhibitors such as dabrafenib and vemurafenib have shown clinical efficacy in diverse, but not all, tumor types such as non–small-cell lung carcinoma, malignant peripheral nerve sheath tumors, and papillary thyroid cancers, offering hope that they would be efficacious in CUP. In addition, patients with RET alterations, including mutations and rearrangements, have been shown to benefit from anti-RET targeted therapies.45-47 Finally, detection of MET overexpression or MET amplification in CUP, detected predominantly by IHC analysis and FISH, has been performed, but detailed reports of responses to MET inhibitors such as crizotinib have not been published.48-50 However, the sustained response of a patient with a MET-amplified CUP illustrated in Figure 4 is an example of a major, biomarker-predicted response to the targeted crizotinib
therapy. In the present study, multiple classes of alterations in targetable genes such as *EGFR*, *ERBB2*, *BRAF*, *ALK*, and *RET* were simultaneously detected by a single genomic profiling test, rather than as a series of individual hot-spot assays each requiring a separate tissue sample for evaluation. Furthermore, 40 (20%) of the CUP cases harbored either GAs considered targetable by therapies currently on the market for specific known primary tumors or mutations that suggested eligibility for entry into late-stage, mechanism-based clinical trials (Table 3). When these therapeutic opportunities are combined with earlier-stage, biomarker-driven clinical trials available for many of the additional actionable GAs, the “long tail” observed in eFigures 1 and 2 in the Supplement raises the possibility that, over time, more than three-fourths of patients with CUP could be treated with targeted therapies alone or targeted therapies combined with cytotoxic agents rather than with cytotoxic agents alone.

The NGS technology–based approach such as the hybrid-capture–based assay used in the present study enables the detailed and comprehensive characterization of clinical specimens and is emerging as a major approach for allowing the selection of targeted therapies for a variety of cancers.45-47 Recently, Tothill and colleagues5 identified therapeutic targets in 12 of 16 (75%) cases from a small-scale NGS-based study of Australian patients with CUP, concluding that genomic profiling combined with clinical history, routine pathologic analysis, serum tumor marker analysis, and IHC data, but not mRNA profiling results, could substantially inform the identification of a primary tumor site.9 The 169 of 200 (85%) frequency of potentially clinically relevant alterations identified in our much larger CUP study is similar to that reported by Tothill et al,9 supporting the conclusion that data describing DNA copy number variations and mutational signatures provide significant clinical value for a majority of patients with CUP by identifying treatment options.

In addition to analysis of the cohort as a whole, the samples were evaluated as 2 subgroups: CUP with histologic subtype of adenocarcinoma (ACUP) and CUP with histologic

### Table 3. Selected Actionable Genomic Alterations in Adenocarcinoma of Unknown Primary Site (ACUP) and Non-ACUP Carcinomas of Unknown Primary Site (CUPs) Associated With Targeted Therapies on the Market or in Clinical Trials

<table>
<thead>
<tr>
<th>Genomic Alteration</th>
<th>ACUP</th>
<th>Non-ACUP</th>
<th>Total CUP</th>
<th>Associated Targeted Therapies</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR substitution</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>Erlotinib, afatinib, gefitinib, lapatinib, cetuximab, panitumumab</td>
</tr>
<tr>
<td>ERBB2 amplification</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>Trastuzumab, lapatinib, pertuzumab, trastuzumab-DM1, afatinib</td>
</tr>
<tr>
<td>BRAF substitution</td>
<td>8</td>
<td>3</td>
<td>11</td>
<td>Vemurafenib, dabrafenib, regorafenib, sorafenib, trametinib</td>
</tr>
<tr>
<td>ALK substitution</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>Crizotinib, ceritinib</td>
</tr>
<tr>
<td>RET fusion/substitution</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>Cabozantinib, ponatinib, sorafenib, sunitinib, vandetanib, regorafenib</td>
</tr>
</tbody>
</table>

**Altersations Associated With Active Clinical Trials for Novel Targeted Therapies**

<table>
<thead>
<tr>
<th>Genomic Alteration</th>
<th>ACUP</th>
<th>Non-ACUP</th>
<th>Total CUP</th>
<th>Associated Targeted Therapies</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3CA substitution, amplification</td>
<td>10</td>
<td>7</td>
<td>17</td>
<td>Temozolomide, everolimus, novel mTOR inhibitors (clinical trials), novel PI3K inhibitors (clinical trials), novel dual PI3K/mTOR inhibitors (clinical trials)</td>
</tr>
<tr>
<td>PTEN loss, substitution, truncation</td>
<td>8</td>
<td>6</td>
<td>14</td>
<td>Temozolomide, everolimus, novel mTOR inhibitors (clinical trials), novel PI3K inhibitors (clinical trials), novel dual PI3K/mTOR inhibitors (clinical trials)</td>
</tr>
<tr>
<td>STK11 truncation</td>
<td>7</td>
<td>6</td>
<td>13</td>
<td>Everolimus, temsirolimus</td>
</tr>
<tr>
<td>ATM substitution, truncation</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>DNA-PKcs inhibitors, PARP inhibitors</td>
</tr>
<tr>
<td>NF1 loss, truncation</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>Temsirolimus, everolimus, novel mTOR inhibitors, trametinib</td>
</tr>
<tr>
<td>BRC2 substitution, truncation</td>
<td>6</td>
<td>5</td>
<td>11</td>
<td>PARP inhibitors</td>
</tr>
<tr>
<td>NOTCH1 substitution, truncation</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>γSecretaseinhibitors</td>
</tr>
<tr>
<td>CCND2 amplification</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>CDK inhibitors</td>
</tr>
<tr>
<td>FGFR1 substitution, amplification, fusion</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>Pazopanib, ponatinib</td>
</tr>
<tr>
<td>FGFR2 substitution, fusion</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>Pazopanib, ponatinib</td>
</tr>
<tr>
<td>RICTOR amplification</td>
<td>5</td>
<td>7</td>
<td>12</td>
<td>Temozolomide, everolimus, novel mTOR inhibitors</td>
</tr>
<tr>
<td>ROS1 fusion</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>Crizotinib, ceritinib</td>
</tr>
</tbody>
</table>

**Abbreviations:**

CDK, cyclin-dependent kinase; mTOR, mammalian target of rapamycin; PARP, polyadenosine diphosphate ribose polymerase; PI3K, phosphatidylinositol 3-kinase; PKcs, protein kinase catalytic subunit.
subtype of squamous, undifferentiated, or neuroendocrine carcinoma (non-ACUP). The ACUP tumors commonly demonstrated alteration of \( \text{ERBB2} \) (10%) (both mutations and amplifications), \( \text{EGFR} \) (8%), and \( \text{BRAF} \) (6%), although alterations in these 3 genes were not observed in non-ACUP (0%) cases. In contrast, a variety of gene sequence alterations in \( \text{MLL2} \) were identified in 10% of non-ACUP tumors, but \( \text{MLL2} \) alterations were not found in any of the ACUP samples. This significant difference in the profiles of actionable GAs within the RTK/Ras signaling pathway in the gland-forming, mucin-producing ACUP cases compared with the non-ACUP tumors suggests that major biologic differences exist that might be exploited in the development of targeted therapeutic strategies.

Historically, the prognosis has been poor for patients who present with metastatic carcinoma for which no confirmed primary site can be identified after either routine or extensive diagnostic workup and who are treated with traditional untargeted cytotoxic drugs such as the regimens featuring platinum and taxane.\(^2,5,54-56\) A meta-analysis of currently used treatments for CUP showed no significant benefit for any one treatment group over the others.\(^{57}\) Thus, given the fact that no chemotheraphy has been solidly proven to prolong survival in patients with CUP, and the resulting poor prognosis for patients with CUP when treated with cytotoxic chemotherapy protocols not driven by biomarker status, oncologists are anxious to assess whether molecular testing to identify therapeutic targets could alter the outcomes for this disease. In the present study, the use of a validated and sensitive comprehensive genomic profiling assay on routine clinical FFPE specimens yielded an unexpectedly high frequency of actionable GAs in CUP that was greater than seen for tumors profiled by the same assay for which the primary site of origin was known.\(^{11}\) Currently, when patients present with CUP, traditional oncologic practice has focused on exhaustive and expensive analyses searching for the primary tumor site. Although these analyses are costly in terms of both time and expense, the rate of eventual primary tumor site detection is high enough that lead-

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**Figure 4. MET Amplification in a White Woman in Her 50s With Carcinoma of Unknown Primary Site Who Presented With New-Onset Seizures**

A. 16-copy \( \text{MET} \) amplification. The x-axis shows the number of targets (exons); the left y-axis, the log ratio; and the right y-axis, gene copy number (CN). Vertical lines indicate divisions between chromosomes (numbered at lower left corner of each section). B. Imaging with 18F-fluorodeoxyglucose positron-emission tomography/computed tomography (PET/CT) showed a 2 × 4 cm left mid-abdominal mass. The brain lesion was resected, and pathologic examination of the specimen revealed a poorly differentiated metastatic carcinoma with weak, inconclusive staining for thyroid transcription factor 1 and negative staining for other potential tumor-specific antigens. Diagnostic imaging was negative for disease in the thoracic region. Reverse transcription-polymerase chain reaction assays interrogating specific codons ("hot-spot testing") revealed a \( \text{KRAS} \) G12V alteration and wild-type \( \text{EGFR} \). Break-apart fluorescence in situ hybridization for \( \text{ALK} \) rearrangement had negative results. The patient was treated with multiple cycles of carboplatin and docetaxel but subsequently experienced disease progression. The original brain metastasis was analyzed by means of next-generation sequencing for potential therapy targets\(^{11}\) and was found to contain a 16-copy amplification of \( \text{MET} \), \( \text{CCND1} \) (9 copies), and \( \text{MYC} \) (9 copies), as well as \( \text{KRAS} \) G12V, \( \text{TP53} \) R273L, and \( \text{CARD11} \) N184S. The patient began treatment with crizotinib (250 mg orally twice a day). C and D. Subsequent scans at 3-month intervals demonstrated an ongoing durable response exceeding 15 months with the fused PET-CT revealing a complete normalization of tumor metabolic activity for the abdominal mass. Images republished with permission from Karger, Inc.\(^{15}\)
In the field have suggested that empiric broad-spectrum chemotherapies such as paclitaxel-carboplatin-gemcitabine for CUP are now indicated in only a small minority of patients.\(^{58}\) However, given that with a single assay NGS can discover unanticipated therapeutic targets for patients presenting with CUP, clinical trials are needed to compare the front-line use of comprehensive genomic profiling to identify targeted regimens with the conventional use of generic cytotoxic chemotherapy.\(^{59}\) The value of this approach is bolstered by recent evidence suggesting that determining the tissue of origin of an unknown primary cancer may be less relevant than delineating the driver GAs that are fueling disease progression.\(^{60}\) Only by routinely investigating the genomic landscape of a tumor can we realize the full clinical impact of refocusing diagnostic testing away from costly laboratory and imaging studies toward the comprehensive identification of driver alterations and their associated therapies made possible by efficient, high-throughput sequencing technologies. In the past several years, substantial interest has emerged in the potential clinical utility of massively parallel, so-called

Figure 5. Never-Smoker White Woman in Her Fifties Who Presented With a 3-Week History of Worsening Fatigue and Severe Exertional Dyspnea

Physical examination identified a 3-cm nontender, hard, subcutaneous, proximal right upper extremity mass with erythematous discoloration of the overlying skin. A 1-cm nontender, subcutaneous nodule was also identified in the left parietal area of the scalp. Positron-emission tomography and computed tomography imaging revealed multiple metabolically active masses in the right lung, with the largest mass in the upper lobe measuring 5.8 × 5.0 cm (standardized uptake value [SUV], 23.4); a left lung mass measuring 2.3 × 2.2 cm (SUV, 12.8) was also identified. Right hilar and mediastinal lymphadenopathy and subcarinal lymphadenopathy were also noted, with the subcarinal nodal mass measuring 4.4 × 4.3 cm (SUV, 21.7). Extrathoracic, metabolically active lesions were also noted, including a mass located in the celiac nodal basin and a mass in the anterior right upper arm that correlated with the mass identified during physical examination. Magnetic resonance imaging of the brain showed a 3-cm transcranial lesion in the left frontal bone that was 1.8 cm thick.

A, Low-power (original magnification, ×40) and high-power (original magnification, ×200 [inset]) photomicrographs of hematoxylin-eosin-stained biopsy specimen from right upper extremity. B, EML4-ALK fusion. EML4 (in blue) exons 1 through 6, which includes the coiled-coil domain (CC), is fused to ALK (in red) exons 20 through 29, which includes the kinase domain. C, Pretreatment CT scan of the chest shows a right hilar mass measuring 6.5 × 7 cm. D, Posttreatment CT scan was taken 1 month after initiation of crizotinib treatment.
NGS of tumoral DNA to discover therapy targets not currently searched for in routine clinical practice. The present study uncovers an unexpectedly high frequency of clinically relevant GAs in CUP, a disease that, until now, has rarely undergone extensive genomic analysis. Despite this potential to affect the clinical outcome for CUP, the present study has definite limitations: (1) it is a retrospective analysis of the genomic landscape of CUP cases; (2) the exact number of patients whose therapy was changed on the basis of the genomic profiling results is not known; (3) the number of cases in which it is known that the genomic analysis led to a major clinical response to targeted therapy as shown in Figures 4 and 5 is limited; (4) prospective randomized clinical trials are needed to confirm the observations described in the present study; and (5) regulatory oversight is needed to optimize and advance the evolution of genomic-derived therapeutic targets.

Conclusions

Carcinoma of unknown primary site accounts for 3% of adult malignant neoplasms in the United States, and evaluation for a primary anatomic site of origin is often unrevealing and may involve invasive studies. In this study of 200 CUP cases that were uniformly negative for site-specific IHC, FISH, or mRNA biomarkers, clinically relevant GAs with the potential to affect therapy selection were identified in 169 (85%) of the sequenced tumors. Given the poor prognosis of CUP treated by nontargeted conventional therapies, comprehensive genomic profiling shows promise to identify targeted therapeutic approaches to improve outcomes for this disease while potentially reducing the often costly and time-consuming search for the tumor’s anatomic site of origin.

REFERENCES


