Prospective Gene Signature Study Using microRNA to Identify the Tissue of Origin in Patients with Carcinoma of Unknown Primary (CUP)

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Running Title: microRNA-based diagnosis of Carcinoma of Unknown Primary

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Translational Relevance

Carcinoma of unknown primary (CUP) is described as metastatic cancer without a detectable primary – there is increasing evidence that these cancers are unrelated groups of site-specific tumors which happen to share the property of having a diminutive primary that escapes detection. Our research evaluates the role of microRNA profiling in CUP to determine the primary cancer profile and compare the assay results to the clinicopathologic presentations. There is an unmet need to study molecular profiling assays in prospective trials with true CUP patients (currently most data is with known cancers). This research has direct application to the future of CUP treatments which has undergone a paradigm shift from empiric to individualized therapy – it may allow leverage of promising treatments available for known cancers to CUP. As novel therapies are developed for site-specific cancers, they may be evaluated in appropriate CUP subtypes based on pathologic and profiling results in selected patients.
ABSTRACT

Purpose: Accurate identification of Tissue of Origin (ToO) for patients with Carcinoma of Unknown Primary (CUP) may help customize therapy to the putative primary and thereby improve the clinical outcome. We prospectively studied the performance of a microRNA-based assay to identify the ToO in CUP patients.

Experimental design: Formalin-fixed paraffin-embedded (FFPE) metastatic tissue from 104 patients was reviewed and 87 of these contained sufficient tumor for testing. The assay quantitates 48 microRNAs and assigns one of 25 tumor diagnoses using a biologically-motivated binary decision tree and a K-Nearest Neighbors (KNN). The assay predictions were compared to clinico-pathologic features and where suitable, to therapeutic response.

Results: 74 of the 87 cases were processed successfully. The assay result was consistent or compatible with the clinico-pathologic features in 84% of cases processed successfully (71% of all samples attempted). In 65 patients, pathology and immunohistochemistry (IHC) suggested a diagnosis or (more often) a differential diagnosis. Out of those, the assay was consistent or compatible with the clinico-pathological presentation in 55 (85%) cases. Of the 9 patients with non-contributory IHC, the assay provided a ToO prediction that was compatible with the clinical presentation in 7 cases.

Conclusions: In this prospective study, the microRNA diagnosis was compatible with the clinicopathologic picture in the majority of cases. Comparative effectiveness research trials evaluating the added benefit of molecular profiling in appropriate CUP
subsets are warranted. MicroRNA profiling may be particularly helpful in patients where the IHC profile of the metastasis is non-diagnostic or leaves a large differential diagnosis.

INTRODUCTION:
Carcinoma of unknown primary (CUP) patients poses a therapeutic challenge. When the putative site of origin cannot be assessed based on clinico-pathologic features, empiric treatment with ‘broad spectrum’ doublet chemotherapies is usually the standard of care.(1, 2) With the growing number of cytotoxic and targeted therapies shown to be effective against specific cancers,(3-7) innovative methods to identify the tissue of origin (ToO) of CUP cancers may permit the use of more targeted therapies for CUP patients.(8, 9) There is increasing evidence that CUP cancers, rather than being a distinct entity biologically and molecularly different from other cancers, are a group of unrelated site-specific tumors which happen to share the property of having a diminutive primary that escapes detection.(10) Thus, accurate identification of the putative tumor of origin may be helpful in optimizing patient management.

Molecular profiling (MP) methods using various platforms including measuring mRNA, by DNA microarrays or qRT-PCR, and more recently microRNAs have been used to evaluate the ToO in metastatic samples. The data on known metastases has been validated using independent blinded sets of tumor samples, where the reference diagnosis is known, with an accuracy of about 80-90%.(11-15) The study
described in this paper extends this to the more challenging group of true CUP patients. Unlike known cancers, a unique challenge to CUP is the inability to directly validate the accuracy of a profiling test given that there is typically no primary identified and the low rate of detection of latent primary cancers. One could argue that the clinical utility of profiling assays would ideally be evaluated in randomized trials comparing survival outcomes in patients receiving therapy based on ToO prediction from MP versus those receiving standard empiric therapies. However, at this time, designing such a randomized trial is not feasible as an adequately powered trial would require more than 500 patients and still run the risk of ambiguous results due to the very heterogeneous presentations of CUP cancers. However, it is still important to evaluate the accuracy of MP in CUP in prospective trials because there is inadequate data demonstrating that results from MP assays performed on metastatic known cancer samples can be extrapolated to CUP. Hence in the trial described herein, we used clinico-pathologic presentations as a surrogate to evaluate MP predictions of ToO.

Multiple approaches have been used for molecular profiling. In this study, we describe a microRNA-based approach to determine the ToO in CUP patients. MicroRNAs, non-coding genes of between 21-23 nucleotides in length, have been shown to control gene expression by regulating translation of mRNA into protein. Interestingly, microRNAs have been found to be important for tissue differentiation(16, 17) and tumorigenesis(18, 19) and appear to demonstrate highly tissue-specific expression.(20-22) These features as well as their excellent
preservation in formalin-fixed and paraffin-embedded tissues (FFPE)(23, 24) suggest that microRNA expression profiles can serve as attractive markers for the molecular identification and characterization of tissues and tumors. The feasibility of using microRNA expression profiles from metastatic tumors to accurately identify ToO in patients with known primaries has been previously reported.(13) Subsequently, a qRT-PCR assay which is based on the expression levels of 48 microRNAs was developed and was shown to be able to identify correctly the ToO in 85% of the cases in an independent validation set.(14) This validation set was composed of tumor cases representing the 25 possible diagnoses corresponding to 17 distinct tissues and organs of origin in the assay’s tumor panel. By definition, the validation set was constructed to evaluate the performance of the assay comparing the assay diagnosis with a “gold standard” reference diagnosis in patients with a known primary.

The aim of the present study was to prospectively evaluate the clinical utility of ToO predictions generated by this microRNA-based assay for metastases in CUP patients in the context of currently available immunohistochemistry and clinico-pathologic “working diagnoses”.

PATIENTS and METHODS:

Patient and specimen inclusion criteria:

Patients were prospectively enrolled in this study between July 2008 and June 2010 at the University of Texas, M. D. Anderson Cancer Center (MDACC). All patients
were diagnosed with CUP at presentation, in that a primary cancer was not detected after a complete history and physical examination, detailed laboratory studies, imaging, and when indicated, invasive studies including endoscopy and colonoscopy as directed by symptoms, signs and pathology. Patients with epithelial malignancies were eligible including patients with poorly differentiated carcinomas. Pathology data including morphology and immunohistochemical stains (IHC) from MDACC or other referring institutions were available, as well as FFPE tissue sections of untreated or previously treated tumor biopsies or resection specimens. Patients with cytology-only specimens were not eligible. A unique study identifier was used to maintain patient anonymity. The study was approved by the Institutional Review Board of MDACC. The assays were performed at Rosetta Genomics' laboratory in Philadelphia, PA as described below.

Sample preparation and microRNA assay:

The assay was performed on FFPE tissue. H&E slides were reviewed by a surgical pathologist (TBE) for suitability regarding tumor cell content, surrounding tissue, amount of necrosis, inflammation, hemorrhage and fibrosis. Between 2 and 10 corresponding unstained sections were available for RNA extraction for each case. The method had been validated for a tumor cell content of at least 50%. When feasible, microdissection was performed to increase the tumor cell content to beyond 50% based on tumor size and histologic features.
Suitable samples were processed as previously described (14) to generate a putative ToO. Briefly, total RNA was extracted using acid phenol-chloroform extraction, and RNA was reverse transcribed. The expression levels of 48 microRNAs that had been identified as informative during the development of the assay (14) were determined in duplicates by qRT-PCR. Samples were processed in batches starting with the extraction. The following external negative controls were used with each batch: a "no-sample" control with each extraction batch that did not contain any FFPE tissue, to control for contamination of the extraction process, and a "no RNA" sample to control for contamination of the reverse transcription and/or the qPCR process. A well-characterized RNA sample was processed as an external positive control with each batch. In addition, internal quality parameters were monitored for the microRNA amplification of each patient specimen. qRT-PCR results for samples which passed the quality assessment criteria were analyzed using two different classifiers: a K-Nearest-Neighbor (KNN) classifier and a binary decision tree (Tree) with 1 to 3 microRNAs at each node. The assay quantitates 48 miRNAs, and the two classifiers assign the ToO based on these expression levels. The assay was trained to identify 25 different tumors from 17 ToO that include the most common origins for CUP. A single diagnosis was reported when the KNN and Tree classifiers agreed, and both diagnoses were reported when the two classifiers disagreed. When the two classifiers agreed regarding the ToO but not the histologic subtype, the ToO was reported without a histological subtype. Other tissues of origin have a lower probability but are not completely ruled out, as only the single best answer for each
classifier (KNN and binary tree) is reported. Since the assay always reports ToO from the training database, origins of samples that are not represented in the tumor panel cannot be identified by the assay.

As a first step towards a standardized ToO reporting system, a “level of agreement” tool was created (Table 1). Using this tool, level 1 indicates that the MP results are either consistent with a specific IHC (e.g. test result of colon cancer in CDX-2 positive and CK20 positive metastasis) or an IHC differential together with a specific clinical presentation or a latent primary. Level 2 agreement either indicates an MP result compatible with an IHC differential or with the clinical presentation (e.g. test result of biliary tract cancer in CK7 positive and CK20 negative tumor or a test result of renal cell carcinoma in a patient with lytic osseous metastases) or cases with non-contributory IHC (usually a large differential, e.g. undifferentiated carcinomas) in which the clinicopathologic presentation cannot rule out the MP diagnosis. Level 3 indicates that the MP results either disagree with the clinicopathologic features or it is uncertain whether the test result is likely in the context of an atypical presentation.

RESULTS:

104 patients (66 females) were enrolled in the study. Seventeen samples (16%) were excluded from further analysis since the tumor cell content in the block did not meet the criteria for the analysis. These samples typically consisted of very small biopsies with inadequate tissue left after extensive IHC work-up. The remaining 87
of 104 samples (84%) were considered suitable for analysis based on the tumor cell content and underwent processing (of these, 39% were from small biopsies). (Fig. 1) Microdissection was performed in 43 samples. 74 of the 87 samples (85%) passed all QA criteria and yielded a 'putative primary' result. Table 2 depicts the demographics and tumor characteristics of these patients.

Level 1 or 2 agreement of the molecular profile with the clinico-pathological diagnosis was obtained in 62 of 74 (84%) successfully processed samples which amounts to 62 of 87 samples (71%) where profiling was attempted. In 33 (45%) patient samples the MP assay showed level 1 agreement. Two of these samples, lymph node metastases from the inguinal and pelvic areas, were correctly identified as squamous cell carcinoma by the assay, but the clinically most likely primary sites in the ano-genital area were not assigned because the algorithm had not been trained to recognize primary squamous cell carcinoma locations in the ano-genital area. In one patient, the metastases manifested as abdominal carcinomatosis demonstrated concordant 'colon' IHC and molecular profile. A primary tumor was later found in the terminal ileum suggesting a molecular signature overlap between small bowel and colorectal cancer (in practice, they also have similar therapies). In 29 patient samples (39%) the agreement level was 2. In 12 patients (16%) level 3 disagreement /uncertainty was reported.

Interestingly, pathology and IHC alone suggested a diagnosis or (more often) a number of differential diagnoses in 65 of the 74 cases. (Fig 1) Of these 65, 55 (85%) resulted in a microRNA profile that was consistent or compatible with the pathology
diagnosis and/or the clinical data. 46 of these 55 cases matched the IHC data without taking into account clinical information. The remaining 9 out of 74 cases were not classifiable with conventional pathology and exhaustive IHC. In these diagnostically most challenging cases, the assay results were compatible with the clinical presentation in 7 cases (78%).

As the assay can return either one or two answers, depending on the agreement between the two classifiers, we turned to studying the two classifiers separately. Overall, the KNN classifier provided a level 1 or 2 agreement with clinico-pathological findings in 58 cases (78%) and the Tree provided a level 1 or 2 agreement in 46 cases (62%). For 34 samples (46%), the histologic diagnosis rendered by the two classifiers (KNN and Tree) agreed. Out of these, in 28 cases a single overall diagnosis was generated by the test. In the remaining 6 cases the KNN and Tree diagnoses were squamous cell carcinoma of head and neck and squamous cell carcinoma of the lung. The assignments were treated as squamous cell carcinoma of head and neck/lung. We looked to see if the concordance with the clinico-pathologic diagnosis was greater in patients where KNN and Tree gave the same ToO assignment versus those where they provided different results. 27 (79%) of these 34 single histology results as rendered by the assay were consistent or compatible with the clinico-pathologic findings. 35 (88%) of the 40 tumors where the ToO prediction differed between the 2 classifiers demonstrated a match of the clinico-pathological findings with at least one of the two assay predictions from KNN and Tree. When analyzing KNN and Tree separately in these 40 cases where the
two classifiers differed, the KNN provided a better match with the clinico-pathologic diagnosis than the tree in 17/40 cases (43%) whereas the tree alone provided a better match than the KNN in 10/40 cases (25%). In 8 cases the answers from both classifiers, even though different, were both equally consistent and compatible with the clinico-pathological presentation. The remaining 5 cases (12% of the 40 cases where the two classifiers provided different results) were discordant with the clinico-pathological presentation for both answers rendered by the assay.

Pertinent clinical data, pathology/IHC, molecular results for all patients whose specimens were processed successfully are detailed in the supplementary table (Suppl A, online version). Table 3 highlights and summarizes IHC features and treatment responses for patients in selected diagnostic categories whose molecular profiles were in agreement with the clinico-pathological cancer diagnosis.

DISCUSSION:

Until recently, despite the large numbers of patients diagnosed with CUP, innovative research and individualized approaches to managing these patients have lagged behind many other solid tumors. The availability of ToO molecular profiling assays hold promise for the increasing individualization of therapy for CUP patients.(12, 14, 25, 26) Our study confirms that microRNA profiling can be successfully performed on CUP patients with clinical FFPE tissue samples including decalcified bone specimens. Small sample size and extensive IHC analysis sometimes precludes successful MP analysis; ~ 15% of the blocks may be exhausted and unacceptable
for further testing. Additionally, an assay failure rate of 15%, mostly due to insufficient RNA is not uncommon in clinical practice. Even under the more favorable conditions of a clinical test validation, where primary tumors and metastases from known origins are tested, similar failure rates have been reported (27). Enrichment of the specimen for tumor cells using microdissection allows inclusion of biopsies and resection specimens that contain a high percentage of non-tumor cells. However, adequate sample acquisition remains a challenge in CUP patients.

MicroRNA profiling, using the assay described, results in an agreement with the clinical and/or pathologic presentation in 62 of 74 samples (84%) that were processed successfully. In the cohort presented here, performance of the KNN was better than of the binary decision tree. However, both classifiers are important as they jointly provide a better match with the clinicopathologic diagnosis. In clinical practice the assay is reported as two ToO predictions whenever the two classifiers disagree. The KNN result is reported as the "most likely tumor of origin" and the Tree result is reported as the "second most likely tumor of origin", reflecting the observation that the KNN is a more likely match but that the Tree result should also be considered in the differential diagnosis.

IHC is helpful in CUP(28, 29) but is not without limitations – most importantly IHC may not show a staining pattern that results in a specific diagnosis. This might be due to the nature of the tumors, e.g. tumor locations for which no specific markers are available, dedifferentiated tumors which have lost expression of characteristic markers, technical factors, or selection of markers that are unsuitable to make the
correct diagnosis. In this study, IHC in conjunction with histology, imaging and clinical presentation gave a strong working diagnosis in 27 patients (36%) and IHC was non-contributory in 9 cases (12%). In the remaining cases, the IHC provided a large differential diagnosis, e.g. in the case of CK7+ adenocarcinoma, which may include upper GI, pancreatico-biliary, breast, lung, gynecologic and other carcinomas. In addition, CUP tumors might have a different tumor biology that is reflected in IHC results that are different from their "non-CUP" counterparts, e.g. CUP cancers may have a higher rate of TTF-1 negative adenocarcinoma (table 3).

The results reported here suggest that in the near term, microRNA assay may be most helpful in guiding management when IHC studies are non-diagnostic or provides a large differential diagnosis. Therefore, where applicable, MP tests may complement instead of compete against IHC in selected patients.

This study identifies some limitations to the current microRNA-based profiling methods. A disagreement with the clinical and pathological findings of 16% may probably be acceptable in the context of CUP because of the lack of a true gold standard in most cases, and it certainly lies within the performance of other molecular profiling assays for CUP. Second, since the assay may report two diagnoses, the clinician may be confronted with two different ToO predictions including some where the therapeutic management may differ significantly. Even though the KNN results, which are reported as "more likely", have been found to have a higher level of concordance, clinical judgment and integration of other clinical and pathologic data is necessary for determination of the most appropriate patient
management. Third, since the assay can only report ToOs that it has been trained to recognize, samples with origins that are not in the tumor panel by definition cannot be classified correctly with the assay. This was noted with two samples from the pelvic area, where squamous cell cancer metastases were correctly identified as squamous cell cancers, but the clinically most likely primary location in the genital or anorectal area was not suggested by the algorithms because they had not be trained to recognize these primary tumor locations. Finally, 6 patients presented with a renal carcinoma profile which poses a challenge and an opportunity for molecular profiling because IHC can be non-contributory in renal cancers (RCC). Given that RCC has specific therapeutic needs, this CUP subset warrants additional study. These observations highlight the fact that it is important to put assay results in context of the patient’s clinico-pathologic presentation.

CUP physicians are excited about the emerging role MP is assuming in the identification of ToO. The microRNA assay reported herein does not replace any of the traditional diagnostic procedures performed in CUP patients, but does offer additional information in cases where the clinical and pathologic impressions remain ambiguous. Moreover, MP may provide further clarity when all other clinicopathologic efforts have failed. Given the field’s continued growth and evolution, it is important that investigators gain better understanding of two key questions: (i) how consistent is a diagnosis derived from an MP assay with other data that suggest a specific ToO (e.g. are profiling results concordant or discordant with IHC or the diagnosis of a primary that emerges during the observation of the
patient (a latent primary) or further detailed work-up; and (ii) will the profile results allow us to abandon empiric first-line cytotoxic chemotherapy and replace it with more specific and effective therapy? Currently, first line therapies overlap in several cancers, though with emerging newer agents, there will be a growing number of opportunities to use therapies known to be effective against specific cancers. Depending on their perceived robustness, profiling assays could help direct additional lines of therapies. A profiling assay result may also direct further search for the ToO and the use of specific molecular tests that may inform the therapy of specific CUP subsets (e.g. Her-2 IHC/FISH, EGFR or ALK mutational analysis). Finally, CUP has no gold standard, thus in selected patients, independent confirmation of IHC and other diagnostic results with completely independent diagnostic modalities can assist the treating physician in discussing prognosis and the best management with a patient. As an example, Fig. 2. Illustrates the essence of a CUP cancer – this patient’s radiology depicts a typical primary lung cancer; IHC suggested a GI tract cancer with no primary identified on endoscopy and colonoscopies. The microRNA assay complemented the IHC data and increases the confidence to pursue colorectal therapy options for this patient.

Our ultimate goal is to provide a helpful framework where profiling and pathology are integrated in a cost and clinically effective algorithm with a positive impact on patient survival and quality of life. This study provides encouraging indications of the value of molecular profiling in CUP and suggests that this modality should be evaluated further.
FIGURE LEGENDS:

**Fig. 1** Algorithm illustrating Patient Flow through the Study.

* KNN or Tree

**Fig. 2** Pre- and post-treatment CT scan of a 73-year-old woman, non-smoker, who presented with a solitary lung mass (white arrow) with no other evidence of disease. Biopsy-proven moderately differentiated adenocarcinoma with an IHC profile suggestive of metastatic colon cancer - cytokeratin (CK) 20 and CDX-2 were positive, and CK7 and TTF-1 were negative. Two colonoscopies have failed to show a tumor. The microRNA assay diagnosis was colon adenocarcinoma. Patient was treated with 5FU and oxaliplatin based therapy followed surgery. The final pathology performed on the metastatectomy specimen again confirmed the metastatic colon cancer profile.
Table 1: “Level of agreement” designation to ToO Molecular profiling results

<table>
<thead>
<tr>
<th>Level of Agreement</th>
<th>Criteria</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>microRNA profile consistent with</td>
</tr>
<tr>
<td>&quot;Consistent&quot;</td>
<td>- Histology and specific IHC* profile OR</td>
</tr>
<tr>
<td></td>
<td>- IHC differential and specific clinical presentation OR</td>
</tr>
<tr>
<td></td>
<td>- Latent Primary</td>
</tr>
<tr>
<td>2</td>
<td>microRNA profile compatible with</td>
</tr>
<tr>
<td>&quot;Compatible&quot;</td>
<td>- IHC differential OR</td>
</tr>
<tr>
<td></td>
<td>- Clinical presentation with IHC non-contributory</td>
</tr>
<tr>
<td>3</td>
<td>Disagree: microRNA profile not compatible with</td>
</tr>
<tr>
<td>&quot;Disagree&quot;</td>
<td>- latent primary cancer OR</td>
</tr>
<tr>
<td>Or</td>
<td>- histology and IHC</td>
</tr>
<tr>
<td>&quot;Uncertain&quot;</td>
<td>Uncertain: difficulty in validating microRNA profile</td>
</tr>
<tr>
<td></td>
<td>- non-contributory IHC AND</td>
</tr>
<tr>
<td></td>
<td>- unusual clinical presentation</td>
</tr>
</tbody>
</table>

*IHC - Immunohistochemistry*
Table 2: Patient and Tumor characteristics (for patients with assay results)

<table>
<thead>
<tr>
<th>Patient or Tumor Characteristic</th>
<th>No. of patients (N=74)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>29</td>
<td>39%</td>
</tr>
<tr>
<td>Female</td>
<td>45</td>
<td>61%</td>
</tr>
<tr>
<td>Median Age (years)</td>
<td>58 yrs</td>
<td>(range 20-83)</td>
</tr>
<tr>
<td>Metastatic sites at presentation *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>44</td>
<td>59%</td>
</tr>
<tr>
<td>Liver</td>
<td>31</td>
<td>42%</td>
</tr>
<tr>
<td>Lung</td>
<td>24</td>
<td>32%</td>
</tr>
<tr>
<td>Bone</td>
<td>17</td>
<td>23%</td>
</tr>
<tr>
<td>Pelvic mass/adnexae</td>
<td>16</td>
<td>22%</td>
</tr>
<tr>
<td>Skin/subcutaneous</td>
<td>9</td>
<td>12%</td>
</tr>
<tr>
<td>Omentum/Peritoneum</td>
<td>25</td>
<td>34%</td>
</tr>
<tr>
<td>Adrenal</td>
<td>5</td>
<td>7%</td>
</tr>
<tr>
<td>Other</td>
<td>18</td>
<td>24%</td>
</tr>
<tr>
<td><strong>Tumor Differentiation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well differentiated</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>30</td>
<td>41%</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>41</td>
<td>55%</td>
</tr>
<tr>
<td>Unavailable</td>
<td>3</td>
<td>4%</td>
</tr>
</tbody>
</table>

* Most patients presented with > 2 sites of disease
Table 3: MicroRNA results and correlation with IHC and therapy (patients with Level 1 and 2 agreement)

<table>
<thead>
<tr>
<th>Molecular Classification (Profile)</th>
<th>Immunohistochemistry (N, %) *</th>
<th>1st Line Treatment</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon (n=13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CK7 + (6/13, 46%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CK20 + (11/13, 85%)</td>
<td>5-FU + Oxaliplatin</td>
<td>PR (4), SD</td>
</tr>
<tr>
<td></td>
<td>CDX2 + (11/13, 85%)</td>
<td>(10/13, 77%)</td>
<td>(2)**, NA (2)</td>
</tr>
<tr>
<td>Ovarian (n=6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CK7 + (6/6, 100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CK20 - (6/6, 100%)</td>
<td>Paclitaxel +</td>
<td>PR (5) NA (1)</td>
</tr>
<tr>
<td></td>
<td>WT1+ (3/6, 50%)</td>
<td>Carboplatin (6/6,)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ER + (3/6, 50%)</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Pancreaticobiliary (n=10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CK7+ (9/10, 90%)</td>
<td>Gemcitabine based</td>
<td>PR (4), SD (3)</td>
</tr>
<tr>
<td></td>
<td>CK20 - (7/10, 70%)</td>
<td>(7/8), FOLFOX (1/8) ^</td>
<td>PD (3)</td>
</tr>
<tr>
<td></td>
<td>TTF1 - (7/10, 70%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CDX2 + (4/10, 40%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung Adeno (n=6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CK7 + (5/6, 83%)</td>
<td>Paclitaxel or</td>
<td>PR (2), PD (3)</td>
</tr>
<tr>
<td></td>
<td>CK20 - (3/6, 50%)</td>
<td>Gemcitabine +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TTF1 - (5/6, 83%)</td>
<td>Carboplatin (5/6, 83%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CK7 + (5/6, 83%)</td>
<td>Paclitaxel + surgery, radiation in PR (2), PD (2)</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------</td>
<td>-----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>vs. Lung</td>
<td>CK20 - (5/6, 83%)</td>
<td>Carboplatin (4/6, 67%)</td>
<td></td>
</tr>
<tr>
<td>Squamous (n=6)</td>
<td>TTF1 - (5/6, 83%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* IHC data including CK7, CK20, TTF-1, CDX-2, WT-1 / ER (where applicable) available on most patients

** 2 additional patients received FOLFOX in the Stage IV adjuvant setting after bilateral oophorectomy for ovarian metastases, currently with no evidence of disease

NA – not available

^ - patient died with rapidly progressive disease with no therapy

Supplemental table (Suppl A) (online version): Clinicopathologic, immunohistochemistry and molecular profiling data for all patient samples processed successfully.
REFERENCES:


104 patients enrolled

87 processed

74 processed successfully

17 rejected

13 failed processing

74 processed successfully

65 with contributory IHC

55: MP* matched clinico-pathologic presentation

10: MP* did not match clinico-pathologic presentation

9 with non-contributory IHC

7: MP* matched clinico-pathologic presentation

2: MP* did not match clinico-pathologic presentation

Total of 62/74 (84%): MP* matched clinico-pathologic presentation
Fig. 2

Pre treatment

Post treatment
Clinical Cancer Research

Prospective Gene Signature Study Using microRNA to Identify the Tissue of Origin in Patients with Carcinoma of Unknown Primary (CUP)


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