Benefit of Gene Expression Profiling in Gastrointestinal Neuroendocrine Tumors of Unknown Primary Origin

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Abstract. Background/Aim: Cancer of unknown primary (CUP), representing 3-5% of all newly diagnosed cancers in the United States, is a presumptive, non-definitive diagnosis rendered when a primary tumor site cannot be identified after exhaustive diagnostic evaluation, including cases of neuroendocrine neoplasms (NENs). CUPs are characterized by findings that are challenging to reconcile, including inconclusive immunohistochemical (IHC) stains, an undifferentiated morphologic phenotype, history of multiple cancers, a clinical presentation that is discordant from histologic findings, an atypical distribution of metastases, or lack of expected response to treatment. For a significant subset of NENs (10%), traditional diagnostic evaluation is unable to determine a primary tumor site using histomorphology and IHC stains. Gene expression profiling (GEP) of either mRNA or microRNA is the technique utilized in the three commercially available platforms that provide a molecular prediction of tumor type in cases of diagnostic uncertainty and CUPs, including CUPs with neuroendocrine differentiation. Case Series Report: Here we present four cases of NENs, where the diagnosis based upon histomorphological and IHC features presented a unique challenge that ultimately benefited from the integration of molecular tumor classification using the validated assay. CancerTYPE ID by Biotheranostics is based on a quantitative RT-PCR assay that uses a computational algorithm to measure the collective expression of 92 genes (87 cancer-related genes and 5 control genes). This case series reports five appropriate clinical scenarios that highlight the utility of a GEP-based assay to effectively provide a molecular tumor classification to identify NEN subtypes and tumor primary site of origin. Conclusion: These cases demonstrated that the CancerTYPE ID test was able to resolve challenging diagnoses for primary and metastatic NENs. These cases emphasize the clinical need of utilizing a GEP-based assay for determining the anatomic site of origin and NEN subtyping, both essential for the appropriate clinical management of NENs.

Cancer of unknown primary (CUP; 3–5% of all newly diagnosed cancers in the United States) is a presumptive, non-definitive diagnosis rendered when a primary tumor site cannot be identified after exhaustive diagnostic evaluation (1-3). CUPs are characterized by findings that are challenging to reconcile, including inconclusive immunohistochemistry (IHC) stains, an undifferentiated morphologic phenotype, history of multiple cancers, a clinical presentation that is discordant from histologic findings, an atypical distribution of metastases, or lack of expected response to treatment (1). Gene expression profiling (GEP) of either mRNA or microRNA is the technique utilized in the three major commercially available platforms that provide a molecular prediction of tumor type in cases of diagnostic uncertainty and CUPs, including CUPs with neuroendocrine differentiation (1, 4-6).

Neuroendocrine tumors (NETs) are graded depending on their mitotic rate. The high-grade (poorly differentiated) tumors are referred to as neuroendocrine carcinomas (NECs), which occur at a significantly lower frequency than NETs. NECs usually present with metastatic disease and portend a worse prognosis. There are few reliable prognostic factors that demonstrate clinical utility with this type of tumors. For a significant subset of NENs (10%), traditional diagnostic evaluation is unable to determine a primary tumor...
site using histomorphological tests and immunohistochemistry (IHC) (7). Regardless of anatomic site of origin, observation of exclusively large cell features, increased mitotic count and a high Ki-67 proliferation index are associated with unfavorable outcomes, whereas microsatellite instability (MSI) is associated with favorable outcomes (8-11). Determination of the anatomic site of origin as well as the grade and level of differentiation of NENs are important regarding guidance of clinical management. There are cases where, despite exhaustive work up, there is still ambiguity and GEP may help in determining the site of origin and/or grade.

Recent molecular investigations are bringing more clarity to the genomic profiles in NETs and NECs (12-17). A study using a combination of whole genome and targeted exome sequencing demonstrated similarity between the genomic profiles of large and small cell pancreatic NECs, which were distinct from well differentiated pancreatic neuroendocrine tumors (PNETs) (14). This same study of small cell and large cell PNECs reported alterations in the p53 and the Rb/p16 pathways, as well as BCL2 overexpression, which were not dysregulated in PNETs. PNETs had genomic alterations (GAs) causing dysregulation in genes involved with chromatin remodeling, such as MEN1, DAXX and ATRX, genes involved with DNA damage repair and telomere maintenance, and genes in the PI3K/Akt/mTOR pathway (14). Although loss of PTEN, ATRX and DAXX expression are characteristic of lower grade PNETs, their loss may carry a worse prognosis (14). Moreover, GEP of PNETs identified a subgroup associated with HIF signaling (18). PNETs have also been reported to upregulate epithelial mesenchymal transition (EMT) by signaling involving SLUG, through increased expression of the cancer stem cell marker DCLK1 as well as Cathepsin Z (19-21). PNECs have similarly been shown to upregulate EMT, however the mechanism of EMT upregulation observed in PNECs has been reported to involve a relative decrease in expression of the adhesion molecules, a relative decrease in E-cadherin/β-catenin complex integrity and a relatively higher expression of transcriptional repressors (Snail1, Snail2, Twist and Foxc2) when compared with PNETs (22-24). NETs and NECs have been shown to differ in terms of biologic behavior and responsiveness to different therapies, based on GEP and types of GAs that are oncogenic drivers, when compared to other NETs and NECs from different anatomic sites of origin (8-14, 17, 18, 25-82).

Evaluation by histomorphology and IHC may be of significant value in confirming neuroendocrine differentiation for the majority of cases. However, being solely reliant on histomorphology of a limited number of lineage markers such as CDX-2, PDX-1, NESP-55, TTF-1 and PAX8 presents challenges in definitively determining the NEN subtype (43, 62).

A straight-forward diagnosis may become further complicated by the possibility of considering mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs), which is an entity found throughout the gastrointestinal tract (83-88). Identification of a non-neuroendocrine component is important in distinguishing NEC from MiNEN, while intratumoral heterogeneity may present a challenge in definitively determining whether a tumor is composed of at least 30% neuroendocrine and non-neuroendocrine component.

Efforts to utilize IHC to evaluate NENs prognostically have reported results that do not warrant mainstream use to inform clinical decision-making. Preliminary studies initially supported the role of CD117 and Cytokeratin 19 (CK19) as potential biomarkers with utility as poor prognostic factors (45,89). However, further studies demonstrated inter-study variability regarding the association of CD117 and CK19 with prognosis; thus, further investigation should be performed in order to accurately assess the utility of CD117 and CK19 as potential metrics as prognostic biomarkers (17, 45, 49, 89). Interestingly, in a study of 109 patients with gastroenteropancreatic neuroendocrine carcinoma (GEP-NEC), multivariate analysis demonstrated that elevated lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) were factors that were significantly associated with unfavorable prognosis and were determined to be more useful than histomorphological markers (44). With the paucity of available clinical data for prospective IHC-based and serological prognostic biomarkers, reliable GEP-based assays would significantly facilitate the clinical management of NENs.

GEP is an effective adjunctive means of prognostic assessment in the assignment of probability of subtype categories that represent lower-grade NENs (carcinoids) as well as higher-grade NECs (small cell carcinomas and large cell carcinomas). Their grade and level of differentiation also carries treatment implications (12). GEP is an effective method in determining the anatomic site of origin, which is important in determining the grading and staging of NENs (63, 74, 90-95).

Here we present four cases of NENs, where the diagnosis based upon histomorphological and IHC features presented a unique challenge that ultimately benefited from the integration of molecular tumor classification using CancerTYPE ID®. CancerTYPE ID® (Biotheranostics, San Diego, CA, USA) is a validated assay based on quantitative RT-PCR assay that uses a computational algorithm to measure the collective expression of 92 genes (87 cancer-related genes and 5 control genes) and a reference database of more than 2,000 well characterized tumors to classify a tumor sample into 28 different main tumor types and 50 tumor sub-types. In cases where imaging, pathological workup including IHC (83, 96-98), and other diagnostic tests are unable to identify the NEN subtype, molecular tumor classification by CancerTYPE ID® has shown particular utility in correctly classifying the NENs with obvious impact on patient therapy. Furthermore, we propose a diagnostic algorithm that incorporates molecular tumor classification for NEN subtyping and anatomic primary site identification.
Case Series Report

Case selection and pathological evaluation. The objective of this retrospective study was to identify cases of metastatic neuroendocrine neoplasms (NENs) where molecular tumor classification by the 92-gene assay was used to reach the final diagnosis and likely impacted patient treatment. An initial review of clinical cases undergoing pathological evaluation for metastatic NEN identified at least four cases where integration of the 92-gene assay CancerTYPE ID® (Biotheranostics Inc.) significantly contributed to the final diagnosis. Pathological evaluation for NEN included a review of the clinical case history, histomorphological examination of biopsy material and immunohistochemical (IHC) analysis using protocols that are standardized within the CLIA-certified, CAP-accredited laboratory within the Department of Pathology at Moffitt Cancer Center. Information on the antibodies used for IHC can be found in Table I. Molecular tumor classification by CancerTYPE ID® was ordered as part of the diagnostic work up for these cases.

CancerTYPE ID® testing. Formalin fixed paraffin embedded (FFPE) tumor blocks were shipped to Biotheranostics, a CLIA-certified, CAP-accredited laboratory to perform molecular tumor classification using CancerTYPE ID®. CancerTYPE ID® consists of a standardized laboratory workflow where the sample undergoes histological evaluation based on H&E staining by a board-certified pathologist to designate tumor regions for enrichment by laser microdissection. Total RNA collected from tumor-enriched cells served as the input for the 92-gene RT-PCR assay, a validated gene expression-based classifier that uses a pre-specified computational algorithm that applies linear discriminant analysis to generate probabilities for candidate tumor types based on the degree of similarity of the queried sample to the reference tumor database (99). The assay classifies 50 different tumor types and tumor subtypes from 105 different morphologies. The CancerTYPE ID® test report is structured as a two-level labeling scheme: A Main Tumor Type (i.e., Neuroendocrine) and a second tier Tumor Subtype (i.e., Small/large cell lung carcinoma). In some cases, additional main tumor type(s) with ≥5% probability are included in the test report as rule-in tumor type(s) with lower probabilities. Tumor types with a combined probability of <5% that can be ruled out with 95% confidence are also included (i.e., rule-out tumor types).

Patient 1

Clinical presentation, imaging, and surgical intervention. An 84-year-old woman presented to the hospital with severe anemia of uncertain etiology. Initial chest and abdominal CT scan showed a lung node as well as several hepatic lesions. A liver core biopsy obtained suggested an initial interpretation of spindle cell neoplasm, favoring Gastrointestinal Stromal Tumor (GIST) based on spindle features and CD117 positivity.

Pathology evaluation. Evaluation of H&E sections at Moffitt Cancer Center described a neoplasm composed of spindle cells with a high nuclear-cytoplasmic (N/C) ratio,
abundant mitoses and tumor necrosis (Figure 1A). Considerations in the differential diagnosis included spindle cell tumors (leiomyosarcoma, GIST), melanoma, spindle cell carcinoma of the lung and NET. To further narrow the wide differential diagnosis, additional IHC was performed. Tumor cells stained positive for MOC31, CD117, synaptophysin, chromogranin and AE1/AE3, but negative for S100 and HMB45. Of note, CD117 has been shown to be positive in a subset of colorectal NETs (Figure 2B and Table I) (42). Ki67 staining demonstrated a 15-20% proliferation index, which is at the upper end for an intermediate Grade 2 tumor. Whereas the IHC profile is consistent with a NET, the lack of protein markers for NET subtypes combined with the clinical presentation of metastatic disease limited further NET subtyping by immunomorphology alone.

Molecular tumor classification by CancerTYPE ID®. FFPE tissue from the liver biopsy was submitted to Biotheranostics for testing with the CancerTYPE ID® platform. Within 5 days of receiving the tissue, Biotheranostics returned the CancerTYPE ID® report that indicated a Main Tumor Type of Neuroendocrine (96% probability) and a Main Tumor Subtype of lung carcinoid (Table II). The other NET subtypes reported by CancerTYPE ID® (Merkel cell carcinoma, Small/Large cell carcinoma, GI carcinoid and Islet cell carcinoma) have a combined probability of <5% but cannot be statistically excluded.

Impact of molecular tumor classification and treatment. In the context of the patient’s history of a lung mass with multiple liver lesions, the results of the tumor’s GEP helped to inform the diagnosis, which was determined to be a well-differentiated (grade 2) NET originating from the lung. This result guided therapy and impacted this patient’s outcome.

Patient 2

Clinical presentation, imaging, and surgical intervention. A 67-year-old male smoker with a history of hypertension, hyperlipidemia and peripheral vascular disease presented to the hospital Emergency Department for right-sided numbness and tremors, with occasional loss of motor control in recent weeks. A head CT demonstrated a low-density lesion in the inferior left thalamus, extending into the left cerebral peduncle with surrounding vasogenic edema. An MRI of the brain illustrated left-sided dural enhancement, with a 1 cm uniformly enhancing nodule in the anterior right temporal lobe, and a second 1.7×1.2 cm lesion with central necrosis at the juncture of the left thalamus and left cerebral peduncle. A CT scan of the chest, abdomen and pelvis showed no evidence of metastatic disease or suspicious adenopathy, with a non-contributory medical history. Resection of the right temporal lobe lesion for tissue diagnosis was performed 3 days later, as well as metastatic work up to obtain an accurate diagnosis and guidance for adjuvant treatment.

Pathology evaluation. Intraoperative evaluation demonstrated highly cellular sheets of a patternless neoplasm with abrupt borders, with mildly reactive brain and subtle infiltration. The neoplastic cells had scant eosinophilic cytoplasm with indiscernible borders and irregular hyperchromatic nuclei lacking nucleoli, with frequent mitoses (12 in a single High Power Field) (Figure 2). Immunohistochemical analysis demonstrated that the tumor cells stained mildly positive for CAM5.2, had strong uniform nuclear staining for TTF1, and weak inconsistent vimentin and pan-keratin positivity (Table I). Tumor cells were negative for CK7 and CK20. Synaptophysin highlighted normal brain, and at the tumor-brain interface there was delicate granular positivity extending between clusters of tumor cells. GFAP was
expressed in scattered neoplastic cells, as well as in reactive astrocytes. Nuclear p53 overexpression was noted in approximately 50% of tumor cell nuclei, and Ki-67 demonstrated a proliferative index of 60%. Mutant IDH1R132H and BRAFV600E were both negative; and retained expression was observed for both ATRX and INI1

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*+ represents tumor cells with positive staining. - represents tumor cells with no expression. **Main type and main subtype are specified with the probability of diagnosis (%).
The observed tumor histomorphology and inconclusive IHC stains in this case presented the possibility of either a glioma or a NEC that could not be further classified.

Molecular tumor classification by CancerTYPE ID\textsuperscript{\textregistered}. FFPE tissue from the brain biopsy was submitted to Biotheranostics for testing with the CancerTYPE ID\textsuperscript{\textregistered} platform. Within 4 days of receiving the tissue, Biotheranostics returned the CancerTYPE ID\textsuperscript{\textregistered} report that indicated a main tumor type of neuroendocrine (96% probability) and a main tumor subtype of small/large cell lung carcinoma (Table I). The other NET subtypes reported by CancerTYPE ID\textsuperscript{\textregistered} (Merkel cell carcinoma, Small/Large cell carcinoma, GI carcinoid and Islet cell carcinoma) have a combined probability of <5% but cannot be statistically excluded. Importantly, CancerTYPE ID\textsuperscript{\textregistered} ruled out brain as a possible main tumor type with 95% confidence.

Impact of molecular tumor classification and treatment. Based on the patient’s clinical history histological analysis including positive TTF-1 staining, the molecular tumor
Figure 3. Hematoxylin and eosin (H&E) and immunohistochemical (IHC) stains for Patient 2. The tumor is highly cellular with an abrupt margin with focal geographic micronecrosis. The tumor cells have scant eosinophilic cytoplasm with irregular hyperchromatic nuclei lacking nucleoli. ATRX expression is retained, and a manual semiquantitative assessment of Ki-67 labelling shows a proliferative index of 60%. Nuclear p53 overexpression is noted in about 50% of tumor cell nuclei. CAM5.2 shows delicate positive expression, synaptophysin highlights normal brain, and at the tumor-brain interface there is delicate granular positivity extends between clusters of tumor cells, and TTF1 shows strong uniform nuclear expression.
subtype classification helped to resolve a diagnosis of small/large cell neuroendocrine carcinoma of the lung for this metastatic patient.

**Patient 3**

**Clinical presentation, imaging, and surgical intervention.** A 59-year-old female suffered a pathologic fracture of her right humerus, and upon initial assessment was subsequently determined to have an associated soft tissue mass. She underwent resection of the mass with reconstruction of her right humerus using an intercalary prosthetic graft. Her staging CT in the thorax/abdomen revealed right axillary, left supraclavicular, and mediastinal lymphadenopathy with an epicardial mass, which were concerning for metastatic disease.

**Pathology evaluation.** Histological evaluation of the fracture site of her right humerus revealed a high-grade malignancy composed of blue round cells, with frequent mitotic activity and extensive necrosis (Figure 4A and B). Immunohistochemical studies demonstrated that the tumor cells were strongly and diffusely positive for synaptophysin (Figure 4C), focally positive for chromogranin, and negative for keratin AE1/AE3/CAM 5.2, CEA (polyclonal), KER903, CK7, CK20, CD99, TTF-1, Desmin, actin, CD45, S-100 and bcl-2. Ki-67 was positive in 80% of the tumor cell nuclei (3+) by manual quantitative analysis (Figure 4D). Cytogenetics demonstrated a normal female karyotype and the possibility of a hematopoietic malignancy was ruled out. Molecular testing by RT-DNA amplification did not detect a fusion transcript for EWSR1/FLI1, and there was no detection of any rearrangements involving the EWSR1 (22q12) locus.

**Molecular tumor classification by CancerTYPE ID®.** A CancerTYPE ID® test was ordered, which determined that there was a 96% probability that the main cancer type was neuroendocrine in nature. Of the potential subtypes that were determined, the CancerTYPE ID® test had assigned a 63% probability that the tumor was a small/large cell lung carcinoma, and 33% probability that the tumor was a Merkel cell carcinoma.

**Impact of molecular tumor classification and treatment.** These subtypes correspond to the high-grade description of the tumor’s morphology. After treatment, restaging
radiographs of her right humerus demonstrated improvement consistent with a response to her therapy.

**Patient 4**

*Clinical presentation, imaging, and surgical intervention.* A 57-year-old woman with history of vertigo and migraines presented to the Emergency Department of an outside hospital with diplopia, an intractable headache and nausea. An MRI of the brain demonstrated metastatic disease to the left skull with extension into the left cavernous sinus and encasement of left cavernous carotid artery. The tumor extended to the left posterior orbit and superior orbital fissure with involvement of the distribution of maxillary division of left trigeminal nerve. There was impingement observed upon compression of left optic nerve by tumor at the level of the optic foramen. A CT of the abdomen and pelvis identified hepatomegaly with numerous solid masses throughout the liver.

A CT of the thorax identified multiple lung masses associated with extensive mediastinal adenopathy. An MRI of the abdomen identified small bilateral pleural effusions, multiple hepatic lesions with unusual enhancement, at least 6 lesions in left hepatic lobe and multi-segmental multifocal

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**Figure 5. Patient 4. A CT-guided biopsy of a liver mass was morphologically described on hematoxylin and eosin (H&E) as a poorly differentiated malignant neoplasm. (A) The malignant cells were cohesive, growing in sheets with enlarged vesicular nuclei and variable amounts of cytoplasm. (B) Abundant mitotic activity and apoptosis were observed. The malignant cells were immunohistochemically negative for (C) Synaptophysin and (D) Chromogranin; while demonstrating (E) faint positivity for OSCAR; as well as demonstrating (F) a Ki-67 proliferation rate of greater than 80%.**
lesions in the right lobe with a large confluençe of lesions in central right liver. A PET scan demonstrated near complete collapse of the left lung with large space-occupying pleural effusion and hypermetabolic left perihilar soft tissue mass, multiple hypermetabolic liver metastases, with increased uptake in the distal esophagus and gastric cardia, a hypermetabolic focus in the region of the left cavernous sinus, hypermetabolic thoracic, right supraclavicular, infraclavicular and peripancreatic lymph nodes. A liver biopsy was obtained for diagnosis and treatment planning.

Pathology evaluation. A CT-guided biopsy of a liver mass was morphologically described on H&E as a poorly differentiated malignant neoplasm (Figure 5A and B). The malignant cells were initially characterized as negative for expression of AE1/AE3, CK7, CK20, PAX8, TTF1, GATA3, CD45, Hepatocyte Specific Antigen (Hep Par-1), S100, CD56, Synaptophysin and Chromogranin. Per a review at John's Hopkins Hospital, further immunostains characterized the malignant cells as focally positive for CAM 5.2 and AE1/AE3; as well as negative for expression of CK903, INSM1, CD99, CD34, ERG, Arginase, Glypican-3 and p40 with retained expression of INI-1.

Since the specimen was morphologically consistent with a large cell neuroendocrine carcinoma, neuroendocrine markers were repeated (CD56, Synaptophysin and Chromogranin) and were negative (Figure 5C and D), which corroborated the initial Synaptophysin and Chromogranin negativity. Squamous markers (CK5/6, p63 and p40) were also negative. OSCAR was faintly positive and a Ki-67/CD45 (multiplex) immunohistochemical study demonstrated a proliferation rate of greater than 80% upon review at Moffitt Cancer Center (Figure 5E and F). The immunoprofile in this tumor was negative for repeated attempts at immunostains for neuroendocrine markers, which presented a diagnostic challenge, as large-cell neuroendocrine carcinoma (LCNEC) typically demonstrates positivity in at least one neuroendocrine marker.

Molecular tumor classification by CancerTYPE ID®. For further clarification, Biotheranostics CancerTYPE ID® was requested, which reported a 90% probability of small/large cell lung neuroendocrine carcinoma. Other cancer types with less than a 5% probability included cervical adenocarcinoma, lung adenocarcinoma, squamous cell carcinoma of the lung, head and neck, skin or cervix; as well as thyroid follicular/papillary or medullary carcinoma. Lack of PAX8 expression excluded cervical and thyroid primaries and lack of TTF1 expression excluded a lung primary as a consideration.

Impact of molecular tumor classification and treatment. The results of the Biotheranostics CancerTYPE ID® were invaluable in context of this uncommonly observed immunoprofile, as the tumor’s histomorphology, mitotic rate as well as the proliferative index were consistent with a LCNEC of pulmonary origin. These findings assisted in determining clinical management, as these results enabled treatment-based decisions to be made that could provide systemic treatment with greater precision when compared to making treatment-based decisions agnostic of the CancerTYPE ID® test results. The patient was subsequently treated with palliative chemotherapy (carboplatin and etoposide). Interim restaging scans revealed interval improvement in mediastinal lymphadenopathy, as well as a decrease in size of numerous liver metastases and there was no progression in other sites. Clinically, her ECOG improved dramatically with total independence in ADL, while she experienced a resolution of her nausea and she reported improvement in left eye vision. The patient has continued on palliative systemic chemotherapy with disease stability and increased quality of life.

Discussion

Here we report four appropriate clinical scenarios that highlight the utility of a GEP-molecular based assay to identify NEN subtypes and tumor sites of origin when dealing with metastatic NENs.

These cases demonstrated that the CancerTYPE ID® test facilitated in the resolution of particularly challenging diagnoses for primary and metastatic NENs. In a blinded study evaluating the performance of CancerTYPE ID® that included 44 metastatic and 31 primary NENs, the assay demonstrated a high level of accuracy for the classification of both well-differentiated (97%) and poorly differentiated NENs (87%). The CancerTYPE ID® test was determined to be significantly more accurate than the use of histology and IHCs to establish the neoplasms' primary sites (43, 62). Further analysis demonstrated that 15 of 87 cancer-related genes demonstrated sufficient discriminatory value for accurate subtyping of NENs. Given the significance of determining the anatomic site of origin for clinical management, the ability of CancerTYPE ID® to differentiate NEN subtypes supports the clinical utility of molecular tumor classification (93). Differentiating the NEN’s subtype and anatomic site of origin for an indeterminate NEN has significant implications for clinical management.

The importance of determining the tumor site of origin is further emphasized when considering that therapeutic indications granted by the Food and Drug Administration (FDA) are largely cancer type specific, with the recent approval for immunotherapy for microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) status in solid tumors being a notable unprecedented exception (100). For metastatic tumors the accurate diagnosis of their site of origin is important not only for treatment decisions but also in determining cancer risk for the patients’ relatives (101). Development of targeted therapies and precision-
based management has become increasingly relevant given the driver mutations that have been identified in NENs. Recent efforts have included investigation of inhibitors targeting signaling in the Wnt/β-catenin pathway, the PI3K/AKT/mTOR pathway (everolimus), the MET pathway and the vascular endothelial factor (VEGF) pathway (sunitinib) (11, 15, 17, 31-33, 37, 38, 59, 102-114). These investigative efforts were typically focused on specific organ systems as dictated by the requirement for the approval of targeted agents. Thus, the clinical need for determination of the tumor anatomic site of origin is a plausible reason to utilize a GEP-based assay as an effective tool for the clinical management of NENs (11, 17, 31-33, 35, 37-41, 63, 92, 103-109, 115, 116).

Conflicts of Interest

The Authors have no conflicts of interest to declare.

Authors’ Contributions

James Saller and Sameer Al Diffalha drafted the manuscript, Mintallah Haider revised the original draft, James Saller completed the references, Domenico Coppola planned, originated and supervised the study.

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