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Case Report

Cancer of Unknown Primary: When Imaging, Pathology, and Molecular Biology Do Not Match

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Cancer of unknown primary · Occult primary · Molecular testing · Next-generation sequencing · Multidisciplinary team

Abstract

Introduction: Cancers of unknown primary are aggressive and rare malignancies with a complex diagnosis and management. Here we present a case in which imaging, pathology, and molecular biology did not match for a specific tumor site and the importance of a multidisciplinary team for these complicated cases. ***Case Presentation:*** A man in his 70s with strong smoking history under workup for suspicion of metastatic lung cancer underwent lung mass biopsy. Immunohistochemical stains corresponded to hepatocellular/cholangiocarcinoma or germ cell tumor; however, dedicated liver and testicular studies including imaging and iscochrome 12p FISH were negative. Additionally, somatic variant profiling was not specific for any malignancy nor targetable variants. Given the pattern of disease, risk factors, and patient history, the patient received treatment for lung adenocarcinoma (carboplatin, pemetrexed, and pembrolizumab). The patient had a drastic improvement in dyspnea, weight gain, and was able to return to work. ***Conclusion:*** This report describes a case in which immunohistochemistry and molecular profiling did not identify the tissue of origin and highlights the importance of a multidisciplinary team to reach a diagnosis and guide treatment without delaying patient care in patients with these diagnoses.

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Introduction

Cancers of unknown primary (CUPs), also known as occult primary, are histologically proven metastatic tumors for which a primary site cannot be identified. They occur equally in men and women, with a mean age at diagnosis of 60–75 years, and account for <5% of cancers [1]. The American Cancer Society estimated 30,620 cases of CUP in 2022, with 47,770 deaths, roughly 2% of all cancer diagnosis [2]. Their most common histology is adenocarcinoma (50%), and associated risk factors include smoking with a 1.8–4.1-fold increase in risk, high BMI, alcohol, and family history [3, 4].

Prognosis is poor, with a median survival of 8–12 months, and is worse in adenocarcinoma, undifferentiated carcinoma, poor performance status, male gender, >65 years old, and those with multiple organs involved [5–7]. Favorable prognostic factors include resectable tumors, squamous histology, and low metastatic/lymph node involvement [7]. Molecular testing has been used and studied to aid in establishing a site of primary origin, with accuracy being reported even higher than immunohistochemistry (IHC) [3]. It has been reported that CUPs have at least 1 genomic alteration, with a mean of 4.2 alterations per tumor, between 13 and 32% having an actionable variant with common reported amplifications being ERBB2, EGFR, and BRAF and alterations involving TP53, MAPK, and PI3K [8, 9]. Additionally, high TMB and PD-L1 overexpression are rare in CUP, with an 8% and 21–63% prevalence being reported, respectively [10].

Due to their rarity, complex diagnosis, and management of CUP, both the National Comprehensive Cancer Network (NCCN) and European Society of Medical Oncology (ESMO) offer guidelines regarding workup and treatment [1, 11]. Here, we present a case of a patient with CUP where pattern of metastatic spread, histology, IHC, and molecular testing did not indicate a unifying diagnosis. The CARE Checklist has been completed by the authors for this case report, attached as online supplementary material (for all online suppl. material, see https://doi.org/10.1159/000539650).

Case Report

A male patient in his 70s presented to his primary care provider with fatigue and dyspnea on exertion over a period of 2 months. He reported a persistent dull posterior headache, decreased appetite, and ten-pound weight loss. He did not report fevers, cough, hemoptysis, chest pain, leg swelling, hematemesis, or melena. At baseline, he was an active and independent person with mild limitation in physical activity. His past medical history includes hypertension, atrial fibrillation, chronic kidney disease, and muscle non-invasive bladder cancer treated more than 10 years prior to the current presentation. He was an active 50 pack-year smoking and chronic alcohol user. His father and sister had smoking-associated lung cancer.

He underwent a chest radiograph, which showed a suspicious left upper lobe nodule, which was further evaluated with a chest CT scan demonstrating two left upper lobe irregular masses measuring 2.9 cm and 1.6 cm in their largest diameters. Given the presence of lung masses and significant smoking history, our primary suspicion was lung cancer.

Imaging, Pathology, and Gene Expression Profiling

A PET/CT scan highlighted the two left upper lobe masses measuring 3.5 × 2.7 cm (SUV max 18.4) and 1.8 × 1.4 cm (SUV max 15.9) along with an infiltrative mass at the right clivus (SUV max 8.5). A chest CT at the same time showed two lung lesions measuring 3.3 × 2.5 cm and 1.8 × 2.3 cm (shown in Fig. 1a). Additionally, to complete staging, a brain MRI

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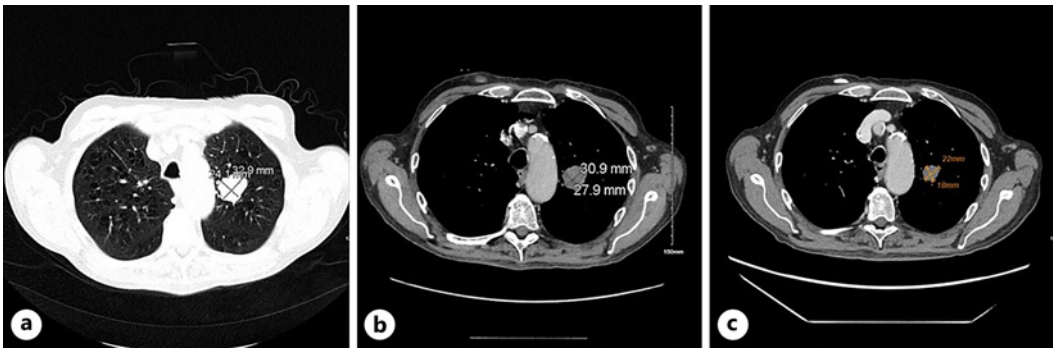

Fig. 1. a CT scan of main lung lesion at time of diagnosis measuring 3.3 × 2.5 cm. **b** CT scan of main lung lesion after 3 cycles of treatment demonstrating stable disease. **c** CT scan of main lung lesion at the end of chemo-immunotherapy treatment demonstrating decrease in tumor size (2.2 × 1.8 cm).

demonstrated a 3 mm enhancing lesion in the left cerebellar hemisphere and a T1/T2 hypointense infiltrating mass involving the right clivus. To establish the diagnosis, an endobronchial ultrasound-guided biopsy of the larger left upper lobe mass was performed, which proved a metastatic poorly differentiated adenocarcinoma IHC positive for cytokeratin markers (AE1/3 and CAM5.2), glypican-3, SALL-4, and HepPar-1 and focal positivity for glutamine synthetase and arginase-1 (Fig. 2). The tumor was negative for OCT3/4, napsin, TTF-1, cytokeratin-7 and 20, p40, GATA3, CDX2, RCC, PSA, and NKX3.1. Based on the IHC profile, metastatic hepatocellular/cholangiocarcinoma or germ cell tumor were suspected. Additional studies with serum tumor markers demonstrating elevated alpha fetoprotein (AFP) of 112.9 ng/mL, normal levels of lactate dehydrogenase (LDH) 143 IU/L, carcinoembryonic antigen (CEA) 2.8 ng/mL, beta hCG <5 mUI/mL and prostatic-specific antigen (PSA) 1 ng/mL, dedicated liver and testicular imaging were performed. These dedicated imaging studies did not show evidence of a primary tumor.

Somatic gene expression profiling was done via next-generation sequencing (NGS) using FoundationOne®CDx (F1CDX) of tumor sample to further characterize the tumor and find potentially actionable alterations. F1CDx is a qualitative NGS based on in vitro diagnostic test that uses targeted high throughput hybridization-based capture technology for detection of substitutions, insertion and deletion alterations, and copy number alterations in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin-fixed, paraffin-embedded tumor tissue specimens. NGS reported *KEAP1 R320Q*, *TSC2* loss of exons 3–26, *CDKN2A/B* p16INK4a and p14ARF loss of exons 1–2, *STK11* splice site 290 + 1_290+2delGT, and *TP53* P72 fs*51. The TMB was 6 muts/mb, PD-L1 (IHC 22C3) TPS of 0%, and MSI stable. None of these variants were tissue specific nor target specific. On consultation with pathology, FISH testing for isochrome 12p (associated with germ cell tumors) was performed and negative.

Differential Diagnosis

Based on the patient's history of bladder cancer and smoking, bladder cancer had to be excluded; however, given the location of the lesions, male sex, and smoking history, metastatic lung cancer was also in our differential. Based on PET/CT, SUV max values, location of the lesions, male sex, and smoking history, metastatic lung cancer was the suspected diagnosis. However, on IHC, lung adenocarcinomas have positive CK7, napsin-A, TTF-1, mucicarmine, and PAS-D, which were all negative for this patient. This also ruled out bladder cancer, which generally are IHC CK7, GATA, and p63 positive. Prostate cancer was also excluded based on

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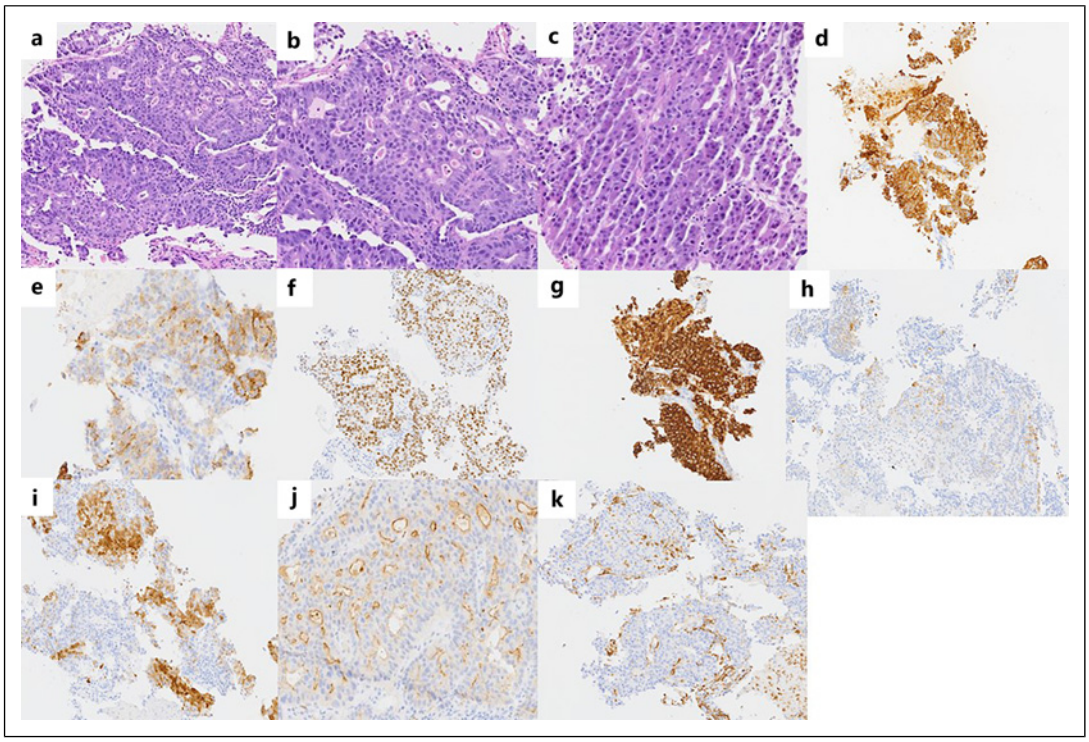

Fig. 2. Poorly differentiated adenocarcinoma. On lower power (**a**), this area of the biopsy demonstrates solid and glandular architecture. On higher power (**b**), the tumor is composed of large eosinophilic cells high nuclear to cytoplasm ratio. The cytoplasm is solid and eosinophilic, while the nuclei are large, hyperchromatic, and some contain prominent nucleoli. Moderate nuclear pleomorphism and scattered apoptotic and mitotic activity can be noted. A representative high-power field of a different biopsy area (**c**) demonstrates a more solid morphology. By IHC, lesional cells are positive for a cytokeratin cocktail AE1/3 and CAM5.2 (**d**), glypican-3 (**e**), SALL-4 (**f**), and HepPar-1 (**g**) and focally positive for glutamine synthetase (**h**) and arginase-1 (**i**). Unabsorbed CEA (**j**) and CD10 (**k**) show equivocal canalicular staining patterns.

negative IHC for PSA and prostatic acid phosphatase and normal serum PSA. Regarding colorectal cancer, negative stain for CK-20, CDX-2, and normal serum CEA ruled out this diagnosis. Due to the smoking history, pancreatic and renal cell carcinoma had to be excluded. Pancreatic cancer was excluded as IHC staining was negative for CA19-9, CK-7, CDX-2, CK17 and having normal levels of serum CA19-9 and renal cell carcinoma due to netative IHC staining for CD-10, vimentin, CK7, Pax-8. Based on glypican-3 and SALL-4 positivity, germ cell tumor was suspected. However, testicular ultrasound was negative with no masses or signs of tumor exhaustion and negative isochrome 12p via FISH, characteristic of germ cell tumors. Positivity of HepPar-1 and arginase-1 led us to suspect a hepatobiliary tumor. Nevertheless, liver imaging was normal by multiple modalities.

The AACR Project GENIE database, a publicly accessible cancer registry of clinical-genomic data from 19 international cancer centers helping in treatment decision guidance via millions of cancer sequencing, was used with the goal of recognizing a common primary site based on somatic variants and/or a target variant to drive therapy. *KEAP1 R320Q* loss is present in 0.02% of all tumors, *TSC2* loss in 0.10%, *CDKN2A* loss in 8.05%, and *STK11* variant in 3.04% with the most common tumor in all these alterations being non-small cell lung (NSCLC) adenocarcinoma. The patient also had a TMB <10 mut/mb, PD-L1 negative, and

stable MSI. These findings are expected for CUP with only 11.8% having TMB high, 1.8% MSI high, and 22% PD-L1 >5% [12]. Ultimately, based on metastatic pattern and clinicopathologic features of the patient we decided to treat the patient as a metastatic NSCLC adenocarcinoma.

Management

Given his persistent headache, prior to starting systemic therapy, the patient was treated with stereotactic radiosurgery for the brain and clivus. Thereafter, the patient started combination chemo-immunotherapy based on carboplatin AUC 5, pemetrexed 500 mg/m^2, and pembrolizumab 200 mg IV every 3 weeks as frontline therapy for metastatic NSCLC adenocarcinoma.

After completion of three cycles, interval imaging showed the left upper lobe lesion to be stable with no new concerning areas of disease (shown in Fig. 1b). Brain MRI showed decrease size of the cerebellar and clivus lesions. Treatment course was complicated by chemotherapy-induced nausea and decreased appetite. With additional supportive care, we continued to treat the patient for an additional three cycles of chemo-immunotherapy. Repeat imaging 5 months after starting treatment showed decrease in size of the left upper lobe lesion (2.2 × 1.8 cm) and stable size in the other mass, indicating a partial response (shown in Fig. 1c). After six cycles, treatment was changed to maintenance pemetrexed-pembrolizumab which the patient continues to be on 7 months since diagnosis. The patient reported improvement in dyspnea and gained seven pounds in weight.

Discussion

The diagnosis and management of patients with CUP is complex. The NCCN and ESMO publish guidelines for the workup of these patients [1, 11]. In addition to a complete history and physical examination, one must perform a complete blood count, serum chemistries, serum PSA (for males), and CT scan, as performed with our patient. PET/CT is a useful modality to identify the primary site with a reported detection rate between 25 and 75% [1, 11]. These tests have the goal of identifying a primary site; nevertheless, a biopsy is required. The most common histopathologic type of CUP is adenocarcinoma, as reported in our patient. As the main objective is to identify a primary site, in addition to imaging modalities, IHC and molecular assays are useful tools. In our case, there was a dilemma as imaging and patient history suggested a lung primary tumor; however, IHC pattern suggested either a germ cell or hepatobiliary tumor and molecular assay was not tissue specific.

As our patient had positive IHC markers for germ cell tumors, elevated AFP, and imaging was negative for a primary testicular mass or signs of tumor exhaustion, it was important to exclude an unusual presentation; therefore, isochrome 12p via FISH was performed. This study is a useful adjuvant tool to confirm the origin of these tumors, with a prevalence between 87 and 89% in germ cell tumors, sensitivity of 77.2%, and specificity of 97.3% [13]. Our patient had negative results excluding this primary site. Regarding hepatocellular carcinoma, imaging did not find any liver masses. Even though AFP elevation is common, the absence of liver lesions ruled out HCC as a primary site.

Molecular cancer classifier assays (MCCAs) and gene expression profiling have the goal of identifying the tissue of origin via analysis of thousands of genes. These techniques have an accuracy between 85 and 90% in identifying the tissue of origin, but as it is difficult to confirm the site of origin, the accuracy of these molecular tests is not clear [14]. Trials have compared patient outcomes between an empiric chemotherapeutic and an MCCA site-specific approach. The GEFCAPI04 trial compared site-specific versus empiric cisplatin + gemcitabine obtaining similar results in progression-free survival and overall survival (OS) [15]. However, a subset analysis of those unlikely to respond to empiric therapy (e.g., liver, kidney, breast, colorectal, sarcoma)

demonstrated a benefit in favor of site-specific therapy [15]. A Japanese trial demonstrated no significant difference at 1-year survival between site-specific treatment and empiric chemotherapy [16]. Due to this conflicting evidence, neither the NCCN nor ESMO guidelines recommend the use of MCCAs outside research as clinical benefit remains to be determined.

Another approach is the use of comprehensive molecular profiling via NGS, which has the goal of identifying actionable variants to guide therapy. This approach is useful and recommended in the setting of metastatic scenarios. NGS can be used to identify the tissue origin with a prediction rate of 71.7% and actionable variants in 20%–85% of CUP patients [8]. One study analyzed the presence of targetable genomic alterations in 200 CUP (125 adenocarcinoma) and reported at least 1 genomic alteration found (mean 4.2 per tumor), with 13% of tumors having actionable variants [8]. NGS can also identify MSI, TMB, and PD-L1, which have therapeutic implications. High TMB has been reported in 8% of CUP adenocarcinomas and PD-L1 overexpression in 21–63% of CUP [10]. Promising results are expected from the ongoing CUPISCO trial comparing NGS-directed therapy versus empiric platinum-based chemotherapy (NCT03498521). Even though the NCCN guidelines only recommend NGS under clinical trials, the ESMO guidelines do suggest the use of NGS routinely in CUP (IV, B recommendation). NGS was performed in our patient and unfortunately no targetable alterations nor a molecular pattern specific to a primary site were identified.

Even though a site-specific tumor nor targetable variants were identified, the use of ICI was still considered. The use of ICI in CUP is generally used in CUP patients who do not respond to first-line chemotherapy, especially in those with TMB ≥10 mut/Mb or ≥7.75 mut/Mb [17]. Our patient did not have an elevated TMB, nor positive PD-L1 or high MSI; however, ICI can still be considered especially in scenarios suggesting a primary cancer in which ICI treatment is established (e.g., NSCLC). Based on our patient imagining pattern, clinical presentation, risk factors, and no clear IHC nor molecular profile, pembrolizumab was added to the chemotherapeutic regimen as NSCLC adenocarcinoma was the suspected primary cancer and chemoimmunotherapy is considered first-line therapy. This regimen was chosen over the empiric therapy for CUP platinum-based doublet chemotherapy with gemcitabine or taxane with reported response rates between 25 and 45% and 7–10-month median OS due to the high suspicion of primary metastatic NSCLC adenocarcinoma, which would offer a greater benefit [1, 11].

Due to their poor prognosis, one of the most important clinical decisions is to identify prognostic factors in patients with CUP. Clinical risk factors include performance status (ECOG), male sex, higher number of organs involved, visceral metastases, and adenocarcinoma [18–20]. Laboratory prognostic parameters include elevated ALP and LDH, low albumin, lymphopenia, and elevated neutrophil to lymphocyte ratio [6, 18–20]. An easy way to assess unfavorable CUP should take in consideration ECOG and LDH (86). Additionally, normal albumin and absence of liver metastases are considered favorable prognostic factors while poor performance status (ECOG ≥2) and bone metastasis are identified as poor prognostic factors [18, 21]. Molecular markers that have poor prognosis include *KRAS*, *NRAS* activation, *CDKN2A* deletion, chromosomal copy number losses, deleterious *TP53*, deletion of Chr17p [4, 22]. Finally, CUP patients with high PD-L1 expression and TMB have better response and survival to ICI [17]. Therefore, determining MSI, PD-L1, and TMB status is important to risk stratify patients when ICI is considered.

The information shown in Table 1 summarizes the most important risk factors and prognostic models in the literature regarding CUP. As seen in the table, our patient has both poor and good prognostic factors. Using the nomogram by Raghav et al. [6], our patient score of 25 points corresponds to a 1-year survival of 75% and 2-year survival of 55%. Using the Culine et al. [20] model assessing ECOG and LDH, our patient corresponds to the "good risk group," with a median survival of 12 months. Similarly, using the Seve et al. [18] and Kodaira et al. [21] models, our patient is classified in the "good risk group" with a median survival of 371 days and 1-year OS of 36.8%, respectively, for each model. On the other hand, the Huang et al. [19] model classified our patient on the intermediate risk group with a median survival

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Table 1. Literature-reported risk factors and risk models correlated with our patient characteristics

	Our patient	Reported outcome
Literature-reported risk factors		
Risk factors by Kambhu et al. [22]		
Male	Male	Poor response to chemotherapy
Female		Better response to chemotherapy
With visceral metastases below diaphragm		Median survival: 5.9 months
Without visceral metastases below diaphragm	No visceral metastases below diaphragm	Median survival: 11.8 months
With liver metastases		Median survival: 5.9 months
Without liver metastases	No liver metastases	Median survival: 9.1 months
1–2 organs affected	1–2 organs affected	Median survival: 9.1 months
≥ 3 organs affected		Median survival: 7.2 months
Risk factors by Hainsworth et al. [3]		
Primary site in retroperitoneum or peripheral nodes, tumor limited to 1–2 sites, normal serum CEA, normal serum LDH, no tobacco history, age <35 years old	Tumor limited to 1/2 sites, normal CEA, normal LDH	Better response to chemotherapy
Risk factors by Abbruzzese et al. [23]		
Peritoneal involvement, lymph node involvement, and neuroendocrine tumor		Longer survival
Male sex, adenocarcinoma, hepatic involvement, and increasing number of organ sites	Male, adenocarcinoma	Poor survival
Risk factors by Bochtler et al. [21]		
Male, ECOG ≥2, unfavorable CUP, higher number of involved organs, RAS activation, CDKNA deletions	Male, unfavorable CUP, CDKN2A deletion	Decreased event-free survival and OS
Literature-reported prognostic models		
Raghav et al. [6] nomogram considers the following factors to have the worse outcome		
Male, ECOG >2, adenocarcinoma, ≥3 sites of metastasis, and high N:L ratio (≥5)	Male, adenocarcinoma, 1–2 sites of metastasis	Poor survival
Culine et al. [19] model		
Good risk group: ECOG 0–1 + normal LDH	Good risk group	Median survival: 12 months, 53% 1-year survival rate
Poor risk group: ECOG ≥2 + elevated LDH		Median survival: 7 months, 23% 1-year survival rate

(Continued on following page)

Table 1 (continued)

	Our patient	Reported outcome
Seve et al. [17] prognostic model		
Good risk group: no liver metastasis + normal albumin	Good risk group	Median survival: 371 days
Poor risk group: liver metastasis + low albumin		Median survival: 103 days
Huang et al. [18] prognostic model (ECOG, visceral organ involvement, N:L ratio)		
Good risk group = 0 points		Median survival: 1,086 days
Intermediate risk group = 1–2 points	Intermediate risk group	Median survival: 305 days
Poor risk group = 3–4 points		Median survival: 64 days
Kodaira et al. [20] prognostic model (ECOG and bone metastasis)		
Good risk group (ECOG 0–1, no bone metastasis)	Good risk group	1-year OS: 36.8%
Poor risk group (ECOG ≥2, with bone metastasis)		1-year OS: 67.1%

LDH, lactate dehydrogenase; CEA, carcinoembryonic antigen; CUP, cancer of unknown primary; ECOG, Eastern Cooperative Group; OS, overall survival.

of 305 days. Regarding response to ICI, we expect our patient not to have a response superior to cases with high PD-L1, TMB, and MSI; however, we expect a better response than chemotherapy alone due to the high suspicion of lung adenocarcinoma as the primary tumor.

Conclusion

The diagnosis and management of CUP is extremely complex and not standardized. Even though established guidelines exist, clinical knowledge and a personalized approach is required. Molecular sequencing to identify the primary location or guide treatment via targeted therapies is not yet guideline indicated; however, cases need to be individualized. Additionally, the inclusion of these patients into clinical trials is strongly encouraged.

Our case reports an unusual scenario of a patient in which imaging, histopathology, and molecular sequencing do not correlate, making it an unfavorable CUP with no indication of a clear primary nor guided therapy. Even though histopathology, imaging, and molecular sequencing did not indicate a primary tumor in our case, the strong clinical suspicion of a primary lung adenocarcinoma based on metastatic pattern, smoking history, and a molecularly diverse pattern (common in NSCLC) led to therapeutic selection. The patient has achieved a partial response to this treatment and is clinically improved.

Statement of Ethics

Ethical approval is not required for this study in accordance with local or national guidelines. Written informed consent for publication of this case report and any accompanying images has been obtained from the patient. All identifying information has been removed to preserve confidentiality.

Conflict of Interest Statement

J.J.J.-V.W., P.P., and Y.C.: none to declare. M.L.P.: none related to the submitted work and outside the submitted work, institutional research funding from Nucana and Helsinn Therapeutics.

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Author Contributions

J.J.J.-V.W. contributed to the conception, design, and medical record data acquisition and interpretation and had a major contribution in the writing of the manuscript. P.P. contributed to the design and medical record data acquisition and interpretation and had a major contribution in the writing of the manuscript. Y.C. contributed to the review and analysis of pathology and IHC slides and pictures. M.L.P. contributed to the conception and design, supervised the entirety of the manuscript and analysis of the data, as well as contributed to the writing of the manuscript. All authors read and approved the submitted version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material files. Further inquiries can be directed to the corresponding author.

References

1 Primary O. NCCN clinical practice guidelines in Oncology (NCCN guidelines ®). 2022. Available from: https://www.nccn.org/home/member-
2 American Cancer Society. Cancer facts & figures 2023: Am Cancer Soc; 2023.
3 Hainsworth JD, Greco FA. Cancer of unknown primary site: new treatment paradigms in the era of precision medicine. Am Soc Clin Oncol Educ Book. 2018;38(38):20–5. https://doi.org/10.1200/EDBK_100014
4 Bochtler T, Wohlfromm T, Hielscher T, Stichel D, Pouyiourou M, Kraft B, et al. Prognostic impact of copy number alterations and tumor mutational burden in carcinoma of unknown primary. Genes Chromosomes Cancer. 2022;61(9):551–60. https://doi.org/10.1002/gcc.23047
5 Binder C, Matthes KL, Korol D, Rohrmann S, Moch H. Cancer of unknown primary-epidemiological trends and relevance of comprehensive genomic profiling. Cancer Med. 2018;7(9):4814–24. https://doi.org/10.1002/cam4.1689
6 Raghav K, Hwang H, Jacome AA, Bhang E, Willett A, Huey RW, et al. Development and validation of a novel nomogram for individualized prediction of survival in cancer of unknown primary. Clin Cancer Res. 2021;27(12):3414–21. https://doi.org/10.1158/1078-0432.CCR-20-4117
7 Hemminki K, Bevier M, Hemminki A, Sundquist J. Survival in cancer of unknown primary site: population-based analysis by site and histology. Ann Oncol. 2012;23(7):1854–63. https://doi.org/10.1093/annonc/mdr536
8 Ross JS, Wang K, Gay L, Otto GA, White E, Iwanik K, et al. Comprehensive genomic profiling of carcinoma of unknown primary site: new routes to targeted therapies. JAMA Oncol. 2015;1(1):40–9. https://doi.org/10.1001/jamaoncol.2014.216
9 Kato S, Krishnamurthy N, Banks KC, De P, Williams K, Williams C, et al. Utility of genomic analysis in circulating tumor DNA from patients with carcinoma of unknown primary. Cancer Res. 2017;77(16):4238–46. https://doi.org/10.1158/0008-5472.CAN-17-0628

10 Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, et al. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. Mol Cancer Ther. 2017;16(11):2598–608. https://doi.org/10.1158/1535-7163.MCT-17-0386
11 Krämer A, Bochtler T, Pauli C, Baciarello G, Delorme S, Hemminki K, et al. Cancer of unknown primary: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol. 2023;34(3):228–46, https://doi.org/10.1016/j.annonc.2022.11.013
12 Gatalica Z, Xiu J, Swensen J, Vranic S. Comprehensive analysis of cancers of unknown primary for the biomarkers of response to immune checkpoint blockade therapy. Eur J Cancer. 2018;94:179–86. https://doi.org/10.1016/j.ejca.2018.02.021
13 Freitag CE, Sukov WR, Bryce AH, Berg JV, Vanderbilt CM, Shen W, et al. Assessment of isochromosome 12p and 12p abnormalities in germ cell tumors using fluorescence in situ hybridization, single-nucleotide polymorphism arrays, and next-generation sequencing/mate-pair sequencing. Hum Pathol. 2021;112:20–34. https://doi.org/10.1016/j.humpath.2021.03.008
14 Greco FA, Lennington WJ, Spigel DR, Hainsworth JD. Poorly differentiated neoplasms of unknown primary site: diagnostic usefulness of a molecular cancer classifier assay. Mol Diagn Ther. 2015;19(2):91–7. https://doi.org/10.1007/s40291-015-0133-8
15 Fizazi K, Maillard A, Penel N, Baciarello G, Allouache D, Daugaard G, et al. A phase III trial of empiric chemotherapy with cisplatin and gemcitabine or systemic treatment tailored by molecular gene expression analysis in patients with carcinomas of an unknown primary (CUP) site (GEFCAPI 04). Ann Oncol. 2019;30:v851. https://doi.org/10.1093/annonc/mdz394
16 Hayashi H, Kurata T, Takiguchi Y, Arai M, Takeda K, Akiyoshi K, et al. Randomized phase II trial comparing site-specific treatment based on gene expression profiling with carboplatin and paclitaxel for patients with cancer of unknown primary site. J Clin Oncol. 2019;37(7):570–9. https://doi.org/10.1200/JCO.18.00771
17 Tanizaki J, Yonemori K, Akiyoshi K, Minami H, Ueda H, Takiguchi Y, et al. Open-label phase II study of the efficacy of nivolumab for cancer of unknown primary. Ann Oncol. 2022;33(2):216–26. https://doi.org/10.1016/j.annonc.2021.11.009
18 Seve P, Ray-Coquard I, Trillet-Lenoir V, Sawyer M, Hanson J, Broussolle C, et al. Low serum albumin levels and liver metastasis are powerful prognostic markers for survival in patients with carcinomas of unknown primary site. Cancer. 2006;107(11):2698–705. https://doi.org/10.1002/cncr.22300
19 Huang CY, Lu CH, Yang CK, Hsu HC, Kuo YC, Huang WK, et al. A simple risk model to predict survival in patients with carcinoma of unknown primary origin. Medicine. 2015;94(47):e2135. https://doi.org/10.1097/MD.0000000000002135
20 Culine S, Kramar A, Saghatchian M, Bugat R, Lesimple T, Lortholary A, et al. Development and validation of a prognostic model to predict the length of survival in patients with carcinomas of an unknown primary site. J Clin Oncol. 2002;20(24):4679–83. https://doi.org/10.1200/JCO.2002.04.019
21 Kodaira M, Takahashi S, Yamada S, Ueda K, Mishima Y, Takeuchi K, et al. Bone metastasis and poor performance status are prognostic factors for survival of carcinoma of unknown primary site in patients treated with systematic chemotherapy. Ann Oncol. 2010;21(6):1163–7. https://doi.org/10.1093/annonc/mdp583
22 Bochtler T, Reiling A, Endris V, Hielscher T, Volckmar AL, Neumann O, et al. Integrated clinicomolecular characterization identifies RAS activation and CDKN2A deletion as independent adverse prognostic factors in cancer of unknown primary. Int J Cancer. 2020;146(11):3053–64. https://doi.org/10.1002/ijc.32882
23 Abbruzzese JL, Abbruzzese MC, Hess KR, Raber MN, Lenzi R, Frost P. Unknown Primary Carcinoma: Natural History and Prognostic Factors in 657 Consecutive Patients. J Clin Oncol. 1994;12:1272–1280. https://doi.org/10.1200/JCO.1994.12.6.1272