Journal of Cancer Research and Practice

journal homepage: https://journals.lww.com/jcrp

Case Report

Next-generation Sequencing Helps to Make the Definite Diagnosis of Adenocarcinoma of Unknown Primary Site: A Case Report

Ling-Jen Hung[1,2], Wei-Pang Ho[1], Chiao-En Wu[1,3]*

[1]Division of Hematology-Oncology, Department of Internal Medicine, Linkou Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan

[2]Division of Hematology-Oncology, Department of Internal Medicine, Taoyuan General Hospital, Ministry of Health and Welfare, Taoyuan, Taiwan

[3]Division of Hematology-Oncology, Department of Internal Medicine, New Taipei Municipal TuCheng Hospital, New Taipei City, Taiwan

Abstract

We report the case of a 52-year-old Taiwanese male who presented with a left lower neck mass for 2 months. Computed tomography and positron emission tomography scans revealed malignant lymphadenopathies in the left neck, supraclavicular fossa, mediastinum, and pulmonary hilum, without definite lung lesions. Thoracic lymph node dissection pathologically proved metastatic nonsmall cell carcinoma. Immunohistochemistry markers were positive for CK7, CK20 (focal), TTF-1, and TdT, and negative for CD5, PAX8, and GATA3, suggesting a pulmonary origin. Crucially, an EGFR exon 19 deletion (E746_T751) was identified using next-generation sequencing (NGS)-based assay (TruSight Oncology 500). This pivotal finding confirmed primary lung cancer with lymph node metastasis (T0N3M1, stage IV), and indicated EGFR-targeted therapy. This case underscores the crucial role of NGS in diagnosing adenocarcinoma of unknown primary site and guiding optimal treatment.

Keywords: Adenocarcinoma of unknown primary site, cancer of unknown primary, next-generation sequencing

Introduction

Cancers of unknown primary (CUPs) refer to metastatic malignancies that have been histologically confirmed, but in which the primary tumor site remains unidentified despite comprehensive evaluations. Conventionally, the diagnosis of CUPs has relied on history taking, physical examinations, laboratory tests (e.g., tumor markers), imaging studies (e.g., Computed tomography [CT]), and pathological evaluations (e.g., immunohistochemistry [IHC] markers). Unfortunately, these traditional diagnostic approaches have limited sensitivity and specificity. Globally, CUPs account for approximately 2%–5% of all diagnosed cancers and are characterized by early and aggressive metastatic spread.[1]

Submitted: 20-Jul-2024 **Revised:** 16-Nov-2024
Accepted: 23-Dec-2024 **Published:** 27-Mar-2025

Access this article online

Quick Response Code:

Website: https://journals.lww.com/jcrp

DOI: 10.4103/ejcrp.eJCRP-D-24-00020

Address for correspondence: Prof. Chiao-En Wu, Division of Hematology-Oncology, Department of Internal Medicine, Linkou Chang Gung Memorial Hospital, Chang Gung University College of Medicine, No 5, Fu-Hsing Street, Kwei-Shan, Taoyuan 333, Taiwan. E-mail: 8805017@cgmh.org.tw

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Hung LJ, Ho WP, Wu CE. Next-generation sequencing helps to make the definite diagnosis of adenocarcinoma of unknown primary site: A case report. J Cancer Res Pract 2025;12:16-9.

Adenocarcinoma is the most common type of CUP, constituting about 70% of all cases. The inability to determine the origin of the tumor prevents access to specific evidence-based therapies or clinical trials, significantly impacting life expectancy. The median overall survival (mOS) for patients with adenocarcinoma of an unknown primary site is only 3 months.[2] Even guided by histologic features and metastatic patterns, standard combination chemotherapy achieves an objective response rate of only 25%–45% and a mOS of 7–10 months.[3]

While IHC is a crucial tool in diagnosing CUPs, it has several limitations.[4] For example, the accuracy of IHC is limited by variability in marker expressions and the overlap of immunoprofiling among different tumors. In addition, tissue sample quality and handling can significantly impact the results. IHC may require multiple sequential steps, making the process time-consuming and costly. These limitations underscore the need for complementary diagnostic tools such as next-generation sequencing (NGS), which can optimize CUP diagnosis and aid in identifying the primary site, driver mutations, and clinically actionable genomic alterations. However, only a few reports have demonstrated the benefits of NGS in the management of adenocarcinoma of unknown primary site.

In this report, we present a case of adenocarcinoma of an unknown primary site, initially manifesting as a neck mass. The identification of an EGFR exon 19 deletion through NGS not only confirmed the diagnosis of primary lung cancer but also guided optimal treatment with EGFR-targeted therapy.

Case Report

A 52-year-old Taiwanese male with no underlying conditions or family history of cancer presented with an enlarging mass on the lower left side of his neck for 2 months. He had a history of smoking 2 packs/day for 20 years but had stopped 10 years ago. Aspiration of the left lateral neck lymph node was initially performed, which cytologically confirmed the presence of metastatic carcinoma. IHC markers were positive for CK7, CK20 (focal), and TTF-1, and negative for p40 and thyroglobulin, suggesting a pulmonary origin. CT and positron emission tomography scans revealed lymphadenopathies in the left neck (levels III, IV, and V), supraclavicular fossa, mediastinum, and pulmonary hilum [Figures 1 and 2]. However, no pulmonary nodules suggestive of a primary lung tumor were detected. Laboratory tests showed elevated tumor markers, with carcinoembryonic antigen (CEA) and CA19-9 levels at 5.8 ng/mL (normal range: ≤5 ng/mL) and 319 U/mL (normal range: ≤27 U/mL), respectively. To further confirm the diagnosis, the patient underwent thoracic lymph node dissection, which pathologically proved metastatic nonsmall cell carcinoma. IHC markers were positive for CK7, CK20 (focal), TTF-1, and TdT, and negative for CD5, PAX8, and GATA3, further supporting a pulmonary origin. Nevertheless, the definitive diagnosis of primary lung cancer remained uncertain. We discussed the situation with the patient and decided to perform an NGS-based assay. The TruSight Oncology 500 NGS assay analyzes multiple variant types and key biomarkers in 523 genes across both DNA and RNA in a single test,[5] including various mutations in exons 18-21 of EGFR. In our patient, the assay identified an EGFR exon 19 deletion (E746_T751) with an allele frequency of 18%, and no other co-occurring mutations were found, confirming primary lung cancer. Brain magnetic resonance imaging revealed no evidence of brain metastasis. Consequently, he was diagnosed with lung cancer with lymph node metastasis, clinical T0N3M1, Stage IV. He underwent concurrent chemoradiotherapy (CCRT) with radiotherapy (5940 cGy/33 fractions) targeting the lymph nodes, along with intravenous cisplatin (20 mg/m^2) and vinorelbine (15 mg/m^2) weekly for six cycles for lung cancer with limited metastasis. Two weeks after completing CCRT, he initiated EGFR-targeted therapy with osimertinib at a daily dose of 80 mg. After 3 months of osimertinib treatment, a CT scan revealed a complete response according to RECIST version 1.1.

Discussion

This report describes a unique case of a patient who presented with a left lower neck mass. Subsequently, he was diagnosed with adenocarcinoma of an unknown primary site with lymph node metastasis through a series of imaging and pathological studies. The primary site and driver mutations of the tumor were uncertain before NGS. Crucially, NGS identified an EGFR exon 19 deletion (E746_T751), leading to the definitive diagnosis of primary lung cancer and optimal treatment with osimertinib.

Comprehensive testing such as NGS is recommended for CUPs[6] to identify multiple clinically actionable genomic alterations (e.g. NTRK fusions, BRAF V600E mutations, RET alterations, MSI-high, and TMB-high).[7] A retrospective study found potentially targetable genomic alterations in 85% of CUP patients.[8] Furthermore, the phase 2 CUPISCO study reported that NGS-guided therapy after induction chemotherapy significantly improved progression-free survival (PFS) in patients with newly diagnosed unfavorable CUP compared to standard platinum-based chemotherapy.[9] NGS potentially enables targeted therapy and minimizes the use of unnecessary treatments, leading to better outcomes and lower long-term healthcare costs. However, accessibility to NGS testing varies significantly, particularly in resource-limited settings, and the cost and need for specialized equipment and trained personnel can limit its widespread adoption.[10] In addition, matched targeted therapies can be expensive, posing a barrier for many patients. More research is needed to validate the clinical benefits of NGS in patients with adenocarcinoma of unknown primary site.

The identification of an EGFR exon 19 deletion strongly indicates lung cancer in patients with adenocarcinoma of unknown primary site due to its high prevalence in nonsmall cell

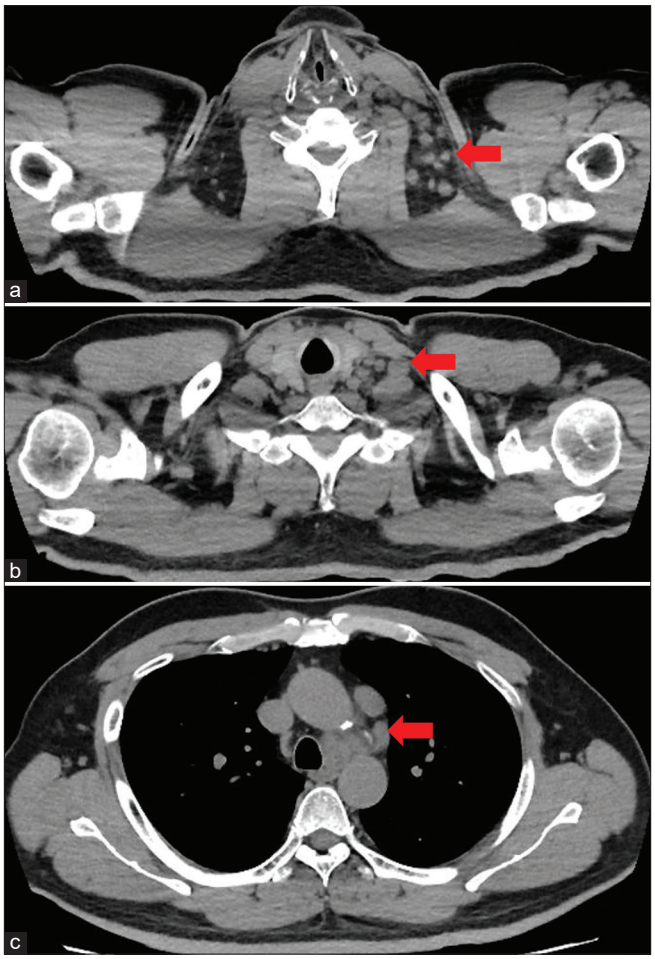

Figure 1: Computed tomography scan at diagnosis. Transverse view showed lymphadenopathies in the left neck at Levels IV and V (a), left supraclavicular fossa (b), and mediastinum (c)

lung cancer (NSCLC), its role in driving cancerous behavior, and its utility as a predictive biomarker for targeted therapies. The EGFR exon 19 deletion is found in 1.57% of all cancers globally, predominantly in lung adenocarcinoma (10.71%), followed by small cell lung carcinoma (2.39%), and squamous cell lung carcinoma (0.68%).[11] In contrast, a high frequency of EGFR mutations (51.4%) has been reported in lung adenocarcinoma in Asian populations.[12] The deletion in exon 19 of the EGFR gene leads to a constitutively active EGFR protein, which promotes uncontrolled cell proliferation and survival through the activation of downstream signaling pathways such as the RAS/RAF/MEK/ERK and PI3K/AKT pathways.[13] Furthermore, the EGFR exon 19 deletion is clinically significant as it is associated with a favorable response to EGFR tyrosine kinase inhibitors (TKIs), which have shown significant efficacy in treating NSCLC.[14]

The standard treatment for Stage IV EGFR-mutated NSCLC typically involves palliative EGFR-TKIs. However, aggressive local treatments such as surgery or radiation were suggested in a meta-analysis to enhance outcomes.[15] Definitive CCRT has been shown to potentially be a curative therapy for locally advanced NSCLC, highlighting the importance of

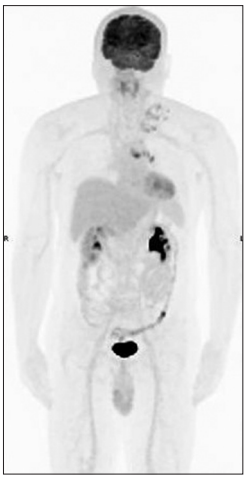

Figure 2: Positron emission tomography scan at diagnosis. Coronal view showed high uptake of fluorodeoxyglucose in enlarged lymph nodes located in the left neck at Level II, III, IV, V, left supraclavicular fossa (maximum standardized uptake value [SUVmax]: 5.97), subaortic space (SUVmax: 11.91), para-aortic space of mediastinum, and left pulmonary hilum (SUVmax: 9.79)

local treatment for advanced cases. Although our patient was diagnosed with Stage IV NSCLC, the disease involved only neck lymph nodes, allowing all tumors to be covered within a single radiation field. Therefore, CCRT was discussed as a potentially curative option. Following CCRT, durvalumab may be considered based on the PACIFIC trial.[16] However, subgroup analysis has shown no benefit of durvalumab for EGFR-mutated NSCLC.[17] Given the presence of an EGFR exon 19 deletion in our patient, osimertinib was suggested following CCRT, partially supported by the LAURA trial.[18] Although he was diagnosed with Stage IV NSCLC we performed potentially curative CCRT, and so subsequent treatment with osimertinib was suggested.

Conclusion

This case underscores the crucial role of NGS in diagnosing adenocarcinoma of unknown primary site and highlights the need for further research to validate its value in multidisciplinary diagnosis and individualized precision treatment.

Ethical Statement

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and its amendments, it was approved by the institutional review board of Chang Gung Memorial Hospital (202401527B0) on 2024/10/01. The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that his name and initials will not be published and due efforts will be made to conceal his identity, but anonymity cannot be guaranteed.

Data availability statement

The datasets generated during and/or analyzed during the

current study are available from the corresponding author on reasonable request.

Financial support and sponsorship

Nil.

Conflicts of interest

Prof. Chiao-En Wu, the Editor-in-Chief at Journal of Cancer Research and Practice, had no role in the peer review process of or decision to publish this article. The other authors declared no conflicts of interest in writing this paper.

References

1. Rassy E, Pavlidis N. The currently declining incidence of cancer of unknown primary. Cancer Epidemiol 2019;61:139-41.
2. 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;23:1854-63.
3. Greco FA, Pavlidis N. Treatment for patients with unknown primary carcinoma and unfavorable prognostic factors. Semin Oncol 2009;36:65-74.
4. Kandalaft PL, Gown AM. Practical applications in immunohistochemistry: Carcinomas of unknown primary site. Arch Pathol Lab Med 2016;140:508-23.
5. Zhao C, Jiang T, Hyun Ju J, Zhang S, Tao J, Fu Y, *et al.* TruSight oncology 500: Enabling comprehensive genomic profiling and biomarker reporting with targeted sequencing. BioRxiv 2020. https://doi.org/10.1101/2020.10.21.349100.
6. Chakravarty D, Johnson A, Sklar J, Lindeman NI, Moore K, Ganesan S, *et al.* Somatic genomic testing in patients with metastatic or advanced cancer: ASCO provisional clinical opinion. J Clin Oncol 2022;40:1231-58.
7. Mosele MF, Westphalen CB, Stenzinger A, Barlesi F, Bayle A, Bièche I, *et al.* Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: A report from the ESMO precision medicine working group. Ann Oncol 2024;35:588-606.
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;1:40-9.
9. Mileshkin L, Bochtler T, Pauli C, Durán-Pacheco G, Arslan C, Bigot F, *et al.* LBA16 primary analysis of efficacy and safety in the CUPISCO trial: A randomised, global study of targeted therapy or cancer immunotherapy guided by comprehensive genomic profiling (CGP) versus platinum-based chemotherapy (CTX) in newly diagnosed, unfavourable cancer of unknown primary (CUP). Ann Oncol 2023;34:S1254-5.
10. Ferreira-Gonzalez A, Ko G, Fusco N, Stewart F, Kistler K, Appukkuttan S, *et al.* Barriers and facilitators to next-generation sequencing use in United States oncology settings: A systematic review. Future Oncol 2024;20:2765-77.
11. Center V-IC. 2019 2024/07/08. EGFR Exon 19 Deletion. My Cancer Genome. Available from: https://www.mycancergenome.org/content/alteration/egfr-exon-19-deletion/. [Last accessed on 2024 Jul 08].
12. Shi Y, Au JS, Thongprasert S, Srinivasan S, Tsai CM, Khoa MT, *et al*. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). J Thorac Oncol 2014;9:154-62.
13. De Luca A, Maiello MR, D'Alessio A, Pergameno M, Normanno N. The RAS/RAF/MEK/ERK and the PI3K/AKT signalling pathways: Role in cancer pathogenesis and implications for therapeutic approaches. Expert Opin Ther Targets 2012;16 Suppl 2:S17-27.
14. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, *et al.* Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. N Engl J Med 2018;378:113-25.
15. Ashworth AB, Senan S, Palma DA, Riquet M, Ahn YC, Ricardi U, *et al.* An individual patient data metaanalysis of outcomes and prognostic factors after treatment of oligometastatic non-small-cell lung cancer. Clin Lung Cancer 2014;15:346-55.
16. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, *et al.* Durvalumab after chemoradiotherapy in stage III non-small-cell lung cancer. N Engl J Med 2017;377:1919-29.
17. Naidoo J, Antonia S, Wu YL, Cho BC, Thiyagarajah P, Mann H, *et al.* Brief report: Durvalumab after chemoradiotherapy in unresectable stage III EGFR-mutant NSCLC: A post hoc subgroup analysis from PACIFIC. J Thorac Oncol 2023;18:657-63.
18. Lu S, Kato T, Dong X, Ahn MJ, Quang LV, Soparattanapaisarn N, *et al.* Osimertinib after chemoradiotherapy in stage III EGFR-mutated NSCLC. N Engl J Med 2024;391:585-97.