Case Report: Partial Response Following Nivolumab Plus Docetaxel in a Patient With EGFR Exon 20 Deletion/Insertion (p.N771delinsGF) Mutant Lung Adenocarcinoma Transdifferentiated From Squamous Cell Carcinoma

Lingling Zhu†, Yanyang Liu†, Honglin Gao2, Jiewei Liu1, Qinghua Zhou1* and Feng Luo1*

1Lung Cancer Center, West China School of Medicine, West China Hospital of Sichuan University, Chengdu, China, 2Key Laboratory of Drug Targeting and Drug Delivery System of the Education Ministry, Sichuan Engineering Laboratory for Plant-Sourced Drug, Sichuan Research Center for Drug Precision Industrial Technology, College of Polymer Science and Engineering, West China School of Pharmacy, Sichuan University, Chengdu, China

The histological transformation from lung squamous cell carcinoma (LUSC) to lung adenocarcinoma (LUAD) and p. N771delinsGF mutations in EGFR exon 20 (ex20) are exceedingly rare in non–small cell lung carcinoma (NSCLC). EGFR ex20 mutations are insensitive to EGFR tyrosine kinase inhibitors in NSCLC. Here, we present a 76-year-old male smoker harboring LUAD with a novel p. N771delinsGF deletion/insertion mutation in EGFR ex20 transdifferentiating from advanced LUSC after chemoradiotherapy. The patient presented reduced hydrothorax and relieved tightness with the treatment of nivolumab plus docetaxel and carboplatin after the failure of second-line chemotherapy. The case highlights the importance of rebiopsy and molecular retesting after the progression of lung cancer and supports the idea that the combination of immune checkpoint blockade and chemotherapy may be an attractive option for patients with EGFR ex20 mutations associated with LUSC–LUAD transformation.

Keywords: non-small cell lung cancer, immune checkpoint blockade, EGFR exon 20 mutation, histological transformation, immune therapy

INTRODUCTION

Lung cancer has the highest lethality rate among all cancers. Non–small cell lung carcinoma (NSCLC) is a major subtype of lung cancer, accounting for 85% of lung cancer cases, including lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and large cell carcinoma histologic subtypes. LUAD and LUSC harbor different features (Krause et al., 2020; Wang et al., 2020). Accumulating evidence from animal experiments and clinical observations indicates phenotypic plasticity in lung cancer cells, such as transdifferentiation of LUAD to LUSC or to small cell lung cancer. This phenotypic transition is a novel cellular mechanism for drug resistance in chemotherapy and in EGFR-targeted therapy (Hou et al., 2017). However, information regarding the transformation from LUSC to LUAD remains limited.
Studies have shown that approximately 12% of patients with EGFR-mutant NSCLC harbor insertion mutations in exon 20 (ex20ins), which are insensitive to first- and second-generation EGFR tyrosine kinase inhibitors (TKIs) (Riess et al., 2018). LUAD and LUSC of adenosquamous carcinomas (ASCs) also share a monoclonal origin, with EGFR mutations being the most common oncogenic driver in the Asian population (Lin et al., 2020). However, no information is available regarding the relationship between EGFR ex20ins mutations and the molecular mechanisms of driving LUSC transdifferentiating to LUAD.

Here, we describe a case of a patient with LUAD harboring a novel p. N771delinsGF deletion/insertion (delins) mutation in EGFR ex20 who experienced transdifferentiation from LUSC after failure of chemotherapy and subsequently responded favorably to immune checkpoint blockade (ICB) plus chemotherapy.

**CASE PRESENTATION**

In June 2019, a 76-year-old male smoker weighing 50 kg with no notable medical, family, or psychosocial history was admitted to the hospital with a complaint of intermittent cough with sputum for >1 month. Chest computed tomography (CT) revealed a left upper lobe lung mass with multiple metastases involving the left pleura, left pleural effusion, and left mediastinal and hilar lymph node enlargement. Lung fine-needle aspiration biopsy confirmed the diagnosis of low-differentiated LUSC at stage IVA (cT4N2M1a) (Figures 1A, 2A). The immunohistochemistry of pulmonary biopsy samples was positive for PCK, P40, CK7, CK5/6, and PD-L1 (40%) and was negative for TTF1. Genetic abnormalities such as amplification of CCND1, CCND2, FGF19, FGF3, FGF4, FGFR1, KRAS, and PIK3CA; ex2 missense mutation of CDKN2A; and ex5 code-shift mutation of TP53 were observed in the primary lung cancer lesion by using next-generation sequencing (NGS; 520-gene panel, Oncoscreen plus, Burning Rock Biotech, Guangzhou, China). Plasma NGS demonstrated amplification of CCND1, FGF19, FGF3, and FGF4; ex2 missense mutation of CDKN2A; ex5 shift mutation of TP53; and 11.1 mutations/Mb (Table 1).

From July to December 2019, the patient was treated with six cycles of paclitaxel plus cisplatin chemotherapy and subsequent radiotherapy (60 Gy/30 fractions at 2 Gy per day). He experienced an initial partial response with a decrease in tumor size (from 8.5 × 9.6 × 10.4 cm to unmeasurable). However, he was readmitted to our hospital with breathlessness and tightness of the chest. Chest CT in October 2020 showed that the amount of pleural effusion in his left chest had notably increased. LUAD cells (EC-positive, TTF-1–positive, napsin A–positive, P40-negative, P63-negative, D2-40–negative) were found by cytological examination of pleural fluid (Figure 1B). Unfortunately, the patient showed poor compliance resulting from the side effects of kidney function and gastrointestinal symptoms of cisplatin during first-line chemotherapy. We have demonstrated the safety and efficacy of the combination of paclitaxel plus oxaliplatin in pretreated advanced NSCLC (Xie et al., 2004); oxaliplatin was administered in the second-line treatment instead of cisplatin.

However, after one cycle of second-line treatment (albumin-bound paclitaxel plus oxaliplatin) and four rounds of recombinant endostatin (an antiangiogenesis agent), the disease continued to progress. Chest CT and B-mode ultrasound revealed an increase in the amount of pleural effusion (from 4.2 cm in November 2020 to 6.4 cm in February 2021; Figures 2A, B). A novel p. N771delinsGF indel mutation in EGFR ex20, a p. E17k mutation in AKT1 ex3 and 6.72 mutations/Mb were identified in the pleural fluid
ctDNA by a hybrid-capture NGS method using a 1,021-gene panel (Table 1).

Five cycles of nivolumab plus docetaxel and carboplatin therapy as third-line therapy were initiated on March 19, 2021. The amount of pleural effusion then decreased (from 6.4 cm in March to 3.7 cm in May by B-mode ultrasound, Figure 2B) with well-controlled symptoms of breathlessness and chest tightness, and the lung lesion appeared stable. Moreover, an improved Eastern Cooperative Oncology Group performance status score (from 2 to 0) was observed in the patient after treatment with nivolumab plus chemotherapy. Therapeutic response was stable until the last follow-up on August 31, 2021, according to the well-controlled and unmeasurable lung lesion, decreased pleural effusion tested by chest CT, and well-controlled symptoms (Figure 2A). During the whole process of treatment with immunochemotherapy, the patient experienced no severe side effects.

**DISCUSSION**

We report a patient who was initially diagnosed with LUSC and was then found to have LUAD cells in the pleural effusion after chemotherapy resistance. One explanation for this is that the LUAD cells may have arisen from an original LUAD component of ASCs in our case. However, the patient had no shared mutations between the two genomic profiles of LUAD and LUSC detected by NGS. This is not in line with previous studies suggesting that LUAD and LUSC of ASCs share a monoclonal origin (Lin et al., 2020). Such a case may present more like two different pathological types. Recently, emerging data have provided convincing evidence supporting a phenotypic transition in lung cancer, such as adenocarcinoma–to–squamous cell transdifferentiation (AST) (Hou et al., 2017). Animal studies suggest that the mechanisms of AST are associated with Kras activation and Lkb1 deletion. Moreover, AST is observed during...
the development of chemotherapy resistance, suggesting that the transdifferentiation of lung cancer represents a novel mechanism of drug resistance (Han et al., 2014). Thus, we suggest that there may have been a transdifferentiation from LUSC to LUAD which led to chemotherapy resistance in our case. Although this hypothesis needs to be further confirmed, the high plasticity of lung cancer indicates this possibility.

Notably, LUSC and LUAD in our report were diagnosed using bronchoscopy biopsy specimens and cytological examination of exfoliated cells in pleural effusion, respectively. This highlights the importance of repetitive and comprehensive sampling and molecular testing, including that of tissues (biopsy specimens or surgery), plasma, and body fluid, both before and after treatments. The overall genomic profile of both LUAD and LUSC in our case were detected by NGS, which allows sequencing of a high number of nucleotides (Mosele et al., 2020) and facilitated identification of the novel EGFR ex20 p. N771delinsGF mutation in our case. However, it has been reported that each fluid type (blood, urine, or saliva) still retains its own characteristic signature based on its unique molecules (El-Mogy et al., 2018). For instance, cell-free DNA from body fluid supernatants has a higher detection rate and sensitivity for tumor-specific mutations than cell-free DNA from sedimented tumor cells and plasma from body fluid (Guo et al., 2019; Zhang et al., 2019). LUAD was detected by pleural effusion in our case, indicating that more information may be revealed from a pleural effusion sample compared with LUSC diagnosed by biopsy specimens and plasma before treatment.

LUAD with a novel p. N771delinsGF mutation occurring in EGFR ex20 was found in our case. The EGFR ex20ins mutations are diverse, accounting for approximately 12% of patients with EGFR mutations. The V769_D770insASV is the most common in EGFR ex20ins mutation (23.0%) (Tsiambas et al., 2016; Riess et al., 2018). Similar to other EGFR mutation subtypes, EGFR ex20ins functions as an oncogenic driver. However, compared with those with EGFR ex19 deletion or ex21 L858R mutation, NSCLC patients with EGFR ex20ins mutations have reduced affinity for EGFR-TKIs. They experienced a shorter median survival than patients with EGFR-TKI–sensitive mutations (16.5 vs. 33.0 months) during EGFR-TKI therapy (Naidoo et al., 2015; Yang et al., 2020). Nowadays, targeted therapeutics such as amivantamab, mobocertinib, and poziotinib have shown initial efficacy in NSCLC patients with EGFR ex20ins (Köhler and Jänne, 2021); however, no targeted therapies have been approved for EGFR ex20ins–driven NSCLC by the US Food and Drug Administration. Currently, chemotherapy remains the suitable treatment for patients harboring these mutations in China (Naidoo et al., 2015). One study showed that metastatic NSCLC patients with EGFR ex20ins treated with platinum chemotherapy presented a more favorable overall survival (OS; median 20 vs. 12 months) compared with NSCLC without targetable alterations (Choudhury et al., 2021).

| Mutant genes | Primary lung cancer before treatment (lung squamous cell carcinoma) | Plasma before treatment (lung squamous cell carcinoma) | Pleural effusion cDNA after chemoradiotherapy (lung adenocarcinoma) |
|--------------|---------------------------------------------------------------|------------------------------------------------------|---------------------------------------------------------------|
| Mutant type  | Mutation location    | Mutation abundance | Mutation type | Mutation location | Mutation abundance | Mutation type | Mutation location | Mutation abundance |
| CDKN1        | Amplification       | 11q13.3            | CN: 8.9      | Amplification   | 11q13.3           | CN: 3.1      | —                | —                |
| CDKN2A       | Amplification       | 12p11.22           | CN: 4.9      | —                | —                | —            | —                | —                |
| FGFR1        | Amplification       | 8p11.22            | CN: 4.9      | —                | —                | —            | —                | —                |
| KRAS         | Amplification       | 12p12.1            | CN: 4.0      | —                | —                | —            | —                | —                |
| PIK3CA       | Amplification       | 3p26.32            | CN: 3.9      | —                | —                | —            | —                | —                |
| TPS3         | Missense mutation   | exon 2 c.242c>T    | 65.05%       | Missense mutation| exon 2 c.242c>T    | 6.14%        | —                | —                |
| TMB          | —                  | —                  | —            | —                | —                | —            | —                | —                |
| EGFR         | Deletion insertion  | exon 20 p.N771delinsGF | — | — | — | — | — | — |
| AKT1         | —                  | —                  | —            | —                | —                | —            | —                | —                |

TABLE 1 Next-generation sequencing results of pulmonary biopsy and plasma before treatments and of pleural fluid–exfoliated cells after chemoradiotherapy.
in advanced NSCLC patients (Leonetti et al., 2019). Although compared with EGFR wide-type LUAD, cases of NSCLC with EGFR 19del or 21exon L858R mutations have a lower clinical response rate to ICB combined with chemotherapy. NSCLC patients with EGFR ex20ins mutation achieved both better PFS and OS than NSCLC patients with no targetable oncogenes after ICB and chemotherapy treatment (Remon et al., 2020). Therefore, chemotherapy in combination with nivolumab was administered to our patient, resulting in a significant reduction of pleural effusion and relieved dyspnea for more than 5 months. Likewise, in a retrospective single-center case series of patients with NSCLC and EGFR ex20ins mutation, two patients received platinum-doublet chemotherapy in combination with pembrolizumab, and both had stable disease (Shah et al., 2021). Thus, according to these reports and our case, ICB combined with chemotherapy can be considered an effective therapeutic strategy in NSCLC patients with EGFR ex20 p. N771delinsGF mutation. However, there is a lack of clinical trials to validate this hypothesis, and future clinical trials could be conducted to clarify the efficacy of ICB in combination with chemotherapy in NSCLC patients harboring EGFR ex20ins mutation.

In conclusion, our case suggests that phenotypic transition between LUSC and LUAD may occur in lung cancer during treatment. When lung cancer progresses, biopsy is helpful in accurately understanding and treating the disease. Moreover, NSCLC with an EGFR ex20 p. N771delinsGF mutation can benefit from nivolumab plus docetaxel and carboplatin. ICB plus chemotherapy may be a favorable strategy for NSCLC with EGFR ex20 mutation.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

REFERENCES

Choudhury, N. J., Schoenfeld, A. J., Flynn, J., Falcon, C. J., Briti, H., Rudin, C. M., et al. (2021). Response to Standard Therapies and Comprehensive Genomic Analysis for Patients with Lung Adenocarcinoma with EGFR Exon 20 Insertions. *Clin. Cancer Res.* 27 (10), 2920–2927. doi:10.1158/1078-0432.CCR-20-4650

El-Mogy, M., Lam, B., Haj-Ahmad, T. A., McGowan, S., Yu, D., Nosal, L., et al. (2018). Diversity and Signature of Small RNA in Different Bodily Fluids Using Next Generation Sequencing. *BMC Genomics* 19 (1), 408. doi:10.1186/s12864-018-4785-8

Gao, Z., Xie, Z., Shi, H., Du, W., Peng, L., Han, W., et al. (2019). Malignant Pleural Effusion Supernatant Is an Alternative Liquid Biopsy Specimen for Comprehensive Mutational Profiling. *Thorac. Cancer* 10 (4), 823–831. doi:10.1111/1759-7714.13006

Han, X., Li, F., Fang, Z., Gao, Y., Li, F., Fang, R., et al. (2014). Transdifferentiation of Lung Adenocarcinoma in Mice with Lkb1 Deficiency to Squamous Cell Carcinoma. *Nat. Commun.* 5, 3261. doi:10.1038/ncomms4261

Hou, S., Zhou, S., Qin, Z., Yang, L., Han, X., Yao, S., et al. (2017). Evidence, Mechanism, and Clinical Relevance of the Transdifferentiation from Lung Adenocarcinoma to Squamous Cell Carcinoma. *Ann. J. Pathol.* 187 (5), 954–962. doi:10.1016/j.ajpath.2017.01.009

Köhler, J., and Jänne, P. A. (2021). Amivantamab: Treating EGFR Exon 20-Mutant Cancers with Bispecific Antibody-Mediated Receptor Degradation. *JCO* 39 (30), 3403–3406. doi:10.1200/JCO.21.01494

Krause, A., Roma, L., Lorber, T., Habicht, J., Lardinois, D., De Filippo, M. R., et al. (2020). Deciphering the Clonal Relationship between Glandular and Squamous Components in Adenosquamous Carcinoma of the Lung Using Whole Exome Sequencing. *Lung Cancer* 150, 132–138. doi:10.1016/j.lungcan.2020.10.013

Krause, A., Roma, L., Lorber, T., Habicht, J., Lardinois, D., De Filippo, M. R., et al. (2020). Deciphering the Clonal Relationship between Glandular and Squamous Components in Adenosquamous Carcinoma of the Lung Using Whole Exome Sequencing. *Lung Cancer* 150, 132–138. doi:10.1016/j.lungcan.2020.10.013

Leonetti, A., Wever, B., Mazzaschi, G., Assaraf, Y. G., Rolfo, C., Quaini, F., et al. (2019). Molecular Basis and Rationale for Combining Immune Checkpoint Inhibitors with Chemotherapy in Non-small Cell Lung Cancer. *Drug Resist. Updates* 46, 100644. doi:10.1016/j.drup.2019.100644

Lin, G., Li, C., Li, P. S., Fang, W. Z., Xu, H. P., Gong, Y. H., et al. (2020). Genomic Origin and EGFR-TKI Treatments of Pulmonary Adenosquamous Carcinoma. *Ann. Oncol.* 31 (4), 517–524. doi:10.1016/j.annonc.2020.01.014

Mosele, F., Remon, J., Mateo, J., Westphalen, C. B., Barlesi, F., Lolkema, M. P., et al. (2020). Recommendations for the Use of Next-Generation Sequencing (NGS) for Patients with Metastatic Cancers: a Report from the ESMO Precision Medicine Working Group. *Ann. Oncol.* 31 (11), 1491–1505. doi:10.1016/j.annonc.2020.07.014

Naidoo, J., Sima, C. S., Rodriguez, K., Busby, N., Nafa, K., Ladanay, M., et al. (2015). Epidermal Growth Factor Receptor Exon 20 Insertions in Advanced Lung Adenocarcinomas: Clinical Outcomes and Response to Erlotinib. *Cancer* 121 (18), 3212–3220. doi:10.1002/cncr.29493

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by West China Hospital of Sichuan University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LZ, YL, HG, and JL collected the clinical information, diagnostic information, therapeutic information, and images of the patients. LZ wrote the manuscript. LZ and YL identified the case and submitted the manuscript. FL, QZ, and HG revised the manuscript. FL and QZ proofread the manuscript. LZ and YL contributed equally to this work. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Wu Jieping Medical Foundation, China (312150082), Post-Doctor Research Project of West China Hospital and Sichuan University (LZ).

ACKNOWLEDGMENTS

We sincerely appreciate Yan Wang, Ting Cao, and Hongying Chen from the Core Facility, West China Hospital of Sichuan University for their assistance and guidance. We also thank Yang Yang and Xiaoting Chen from the Animal Experimental Center of West China Hospital for their suggestions.
Remon, J., Hendriks, L. E. L., Cardona, A. F., and Besse, B. (2020). EGFR Exon 20 Insertions in Advanced Non-small Cell Lung Cancer: A New History Begins. Cancer Treat. Rev. 90, 102105. doi:10.1016/j.ctrv.2020.102105

Riess, J. W., Gandara, D. R., Frampton, G. M., Madison, R., Peled, N., Buﬁll, J. A., et al. (2018). Diverse EGFR Exon 20 Insertions and Co-occurring Molecular Alterations Identiﬁed by Comprehensive Genomic Profiling of NSCLC. J. Thorac. Oncol. 13 (10), 1560–1568. doi:10.1016/j.jtho.2018.06.019

Shah, M. P., Aredo, J. V., Padda, S. K., Ramchandran, K. J., Wakelee, H. A., Das, M. S., et al. (2021). EGFR Exon 20 Insertion NSCLC and Response to Platinum-Based Chemotherapy. Clin. Lung Cancer 162, 140. doi:10.1016/j.clc.2021.07.001

Tsiambas, E., Lefas, A. Y., Georgiannos, S. N., Ragos, V., Fotiades, P. P., Grapsa, D., et al. (2016). EGFR Gene Deregulation Mechanisms in Lung Adenocarcinoma: A Molecular Review. Pathol. - Res. Pract. 212 (8), 672–677. doi:10.1016/j.prp.2016.06.005

Wang, B.-Y., Huang, J.-Y., Chen, H.-C., Lin, C.-H., Lin, S.-H., Hung, W.-H., et al. (2020). The Comparison between Adenocarcinoma and Squamous Cell Carcinoma in Lung Cancer Patients. J. Cancer Res. Clin. Oncol. 146 (1), 43–52. doi:10.1007/s00432-019-03079-8

Xie, C. Y., Wu, S. X., and Zhang, P. (2004). Efficacy of Combined Paclitaxel and Oxaliplatin Therapy in Patients with Pretreated Advanced Non-small Cell Lung Cancer. Ai Zheng 23 (8), 947–950.

Yang, G., Li, J., Xu, H., Yang, Y., Yang, L., Xu, F., et al. (2020). EGFR Exon 20 Insertion Mutations in Chinese Advanced Non-small Cell Lung Cancer Patients: Molecular Heterogeneity and Treatment Outcome from Nationwide Real-World Study. Lung Cancer 145, 186–194. doi:10.1016/j.lungcan.2020.03.014

Zhang, P., Wu, X., Tang, M., Nie, X., and Li, L. (2019). Detection of EGFR Gene Mutation Status from Pleural Effusions and Other Body Fluid Specimens in Patients with Lung Adenocarcinoma. Thorac. Cancer 10 (12), 2218–2224. doi:10.1111/1759-7714.13201

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or ﬁnancial relationships that could be construed as a potential conflict of interest.

**Publisher’s Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their afﬁliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Zhu, Liu, Gao, Liu, Zhou and Luo. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.