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Case reports in molecular and cellular oncology 2022

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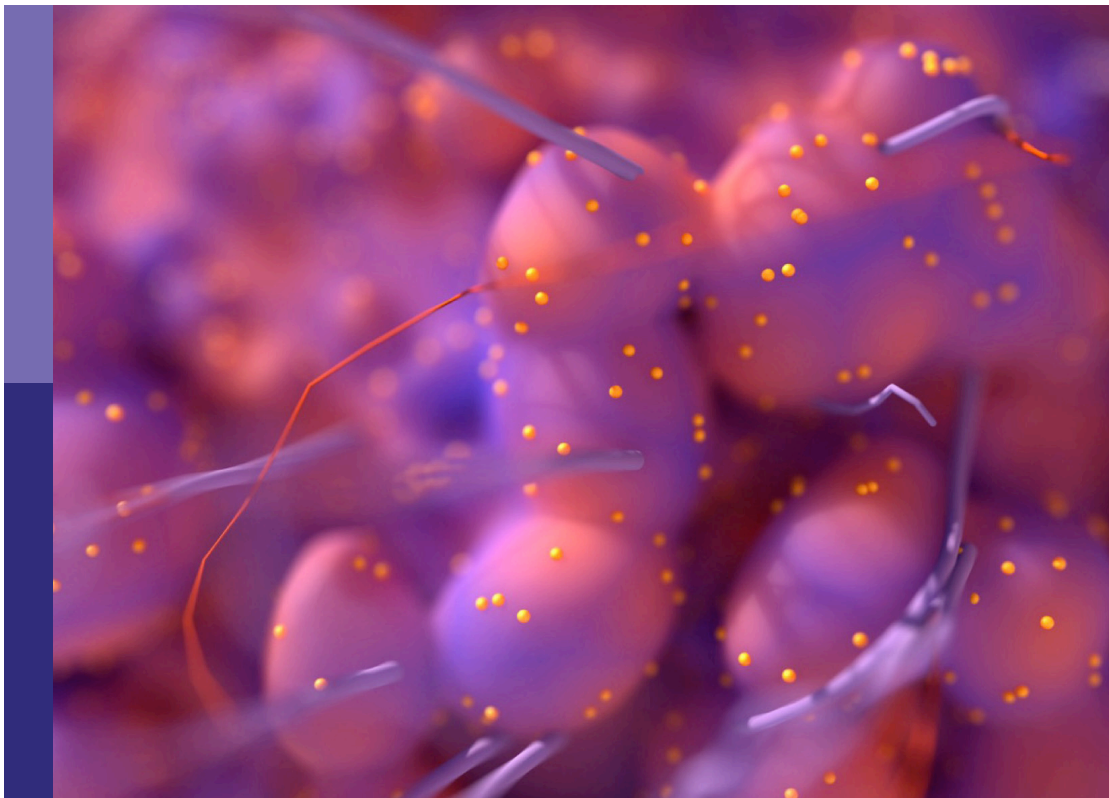

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Case reports in molecular and cellular oncology: 2022

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Editorial: Case reports in molecular and cellular oncology: 2022

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KEYWORDS

targeted therapy, molecular marker, radiation, lung cancer, personalized medicine

Editorial on the Research Topic
Case reports in molecular and cellular oncology: 2022

Large scale clinical trials clearly demonstrate overall survival benefits of targeted therapies in many human malignancies. This Research Topic in Molecular and Cellular Oncology demonstrated the unique challenges related with targeted therapy when disease evolves under selection pressure from targeted therapies. Li et al. reports an interesting evolutionary process of EGFR alteration patterns when a stage IV lung adenocarcinoma patient is under selective pressure while on a TKI. The resistance mechanism involves enrichment of differentially mutated or abnormally amplified EGFR. The patient has relatively extended stable disease periods when a TKI is tailored based on specific EGFR alterations from biopsies at different stages of disease progression. Accumulation of novel mutations during the disease course may exhaust options of targeted therapies. Case reports in this Research Topic show off-label use of TKIs for novel EGFR mutations may have unexpected therapeutic benefits as demonstrated by other cohort studies (Li et al., 1).

Safety and efficacy of combined treatment of targeted therapy and radiotherapy is not well reported in clinical trials. This Research Topic showed several cases with good outcomes when concurrent or sequential treatment of radiation and targeted therapeutic agents (Li et al.; Sun et al.). For example, a radiographic complete response was achieved with concurrent chemo-/radio-therapy and crizotinib for a rare locally-advanced gingival sarcomatoid squamous cell carcinoma patient with MET exon 14 skipping mutation (Sun et al.). Interestingly, Li et al. reported management of a intracranial lesion for an EGFR-mutated NSCLC patient with radiation followed by adaptive targeted therapies. They reported the patient had unexpectedly good clinical response with intracranial disease control of 23 months.

This series of articles in Molecular and Cellular Oncology highlights the gaps in understanding how targeting a single mutation alters the cellular microenvironment and implications of this as patients are living longer on these therapies. Further studies are needed to better understand genetic alterations in tumor response to novel targeted therapies and their interactions with chemo-/radio-therapies.

Author contributions

SZ drafted the editorial; TT edited and revised the editorial. All authors contributed to the article and approved the submitted version.

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.

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CASE REPORT
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Case Report: Prompt Response to Savolitinib in a Case of Advanced Gastric Cancer With Bone Marrow Invasion and *MET* Abnormalities

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Gastric cancer is one of the most common malignant tumors and patients show a short survival, those combined with bone marrow invasion have a median survival of only 37 days. Here we reported the treatment of a 47-year-old male with advanced gastric cancer and complicated with bone marrow invasion and extensive metastases, who did not tolerate chemotherapy, under monotherapy with savolitinib, a MET receptor tyrosine kinase inhibitor. Before treatment, the patient was in severe pain and presented with thrombocytopenia and hemorrhagic anemia. Savolitinib was given based on amplification and rearrangement of the *MET* gene in his tumor. After savolitinib treatment, the patient's condition promptly improved, efficacy evaluation indicated partial remission, and the patient was alive and remained progression-free at 15 weeks at the time of reporting. No obvious adverse reactions occurred. Besides, another case of a female gastric cancer patient with *MET* amplification who received savolitinib monotherapy as a third-line treatment that remained progression-free at 12 weeks was also reported. This report provides a new reference for understanding *MET* abnormalities in gastric cancer and offers a possibility for future application of MET tyrosine kinase inhibitors in the therapy of gastric cancer with *MET* abnormalities. Also, it suggests that sequencing of *MET* can be considered a routine target in advanced gastric cancer patients.

Keywords: savolitinib, *MET* gene, advanced gastric cancer, bone marrow invasion, case report

INTRODUCTION

Gastric cancer (GC) is the fifth most common cancer and ranks third in cancer-related deaths (1). The median overall survival (OS) of patients with advanced GC is less than one year with combination chemotherapy (2) but if bone marrow invasion is present, the median OS is shortened to 37 days (3).

The *MET* gene-encoded protein c-mesenchymal-epithelial transition factor (MET, also known as hepatocyte growth factor (HGF) receptor) is a tyrosine kinase receptor that modulates cell proliferation, growth, survival, apoptosis, and epithelial-mesenchymal transition. Abnormal

activation of MET, which promotes tumor cell proliferation and metastasis, involves *MET* exon 14 skipping mutation, *MET* amplification, and MET protein overexpression (4). Multiple studies have shown a positive relation between *MET* amplification and overexpression, and the median OS is shortened in GC patients with these abnormalities, which are considered poor prognostic factors (5–8). Savolitinib is a selective MET tyrosine kinase inhibitor (TKI) being developed for the treatment of multiple cancers. In June 2021, savolitinib was approved in China to treat progressive or intolerant local advanced or metastatic non-small cell lung cancer (NSCLC) with *MET* exon 14 skipping mutation after platinum-containing chemotherapy (9).

In this study, we reported an advanced GC patient with bone marrow invasion and extensive metastasis. Next-generation sequencing revealed that his tumor had *MET* amplification and rearrangement. After savolitinib treatment, his clinical symptoms promptly improved, no obvious adverse reactions occurred, and he was alive and remained progression-free at 15 weeks at the time of reporting. Meanwhile, another case of a female GC patient with *MET* amplification who received savolitinib monotherapy as a third-line treatment that remained progression-free at 12 weeks was also reported here. Our report demonstrated the effectiveness of savolitinib in the therapy of advanced GC with *MET* abnormalities.

CASE REPORT

A 47-year-old male was admitted for "low back pain and weight loss for 20 days". He had a six-year history of type 2 diabetes mellitus and no other significant past or family medical histories. He was unable to walk at admission, with a Karnofsky performance status (KPS) score of 60 points and a pain number rating scale (NRS) score (0 to 10 points) of 8 points. Physical examination showed systemic mucosal pallor, gingival bleeding, a few ecchymoses on the right lower abdomen skin where insulin was injected, and lumbosacral vertebral tenderness. Blood test indicated severe low platelets (14×10^9/L), hemoglobin (66 g/L), and normal leukocytes (6.14×10^9/L). Tumor markers evaluation showed elevated CEA (103.30 μg/L), CA125 (36.00 U/mL), and CA19-9 (1321.73 U/mL). Enhanced computed tomography (CT) evidenced that the gastric fundus and body were thickened (**Figure 1A**), and multiple lymph node metastases were found in perigastric, liver hilar, retroperitoneal, mediastinal, and bilateral lung hilar regions (**Figure 1B**). Extensive mixed bone metastases throughout the body (supraorbital margin of right frontal bone, bilateral clavicles, bilateral scapulae, multiple ribs, thoracic, lumbar and sacral vertebrae, pelvic bones, right humerus, and bilateral upper femurs) were observed (**Figure 1B**). Pathological biopsy with gastroscopy (**Figure 1C**) indicated poorly differentiated adenocarcinoma (**Figure 1D**), and immunohistochemistry

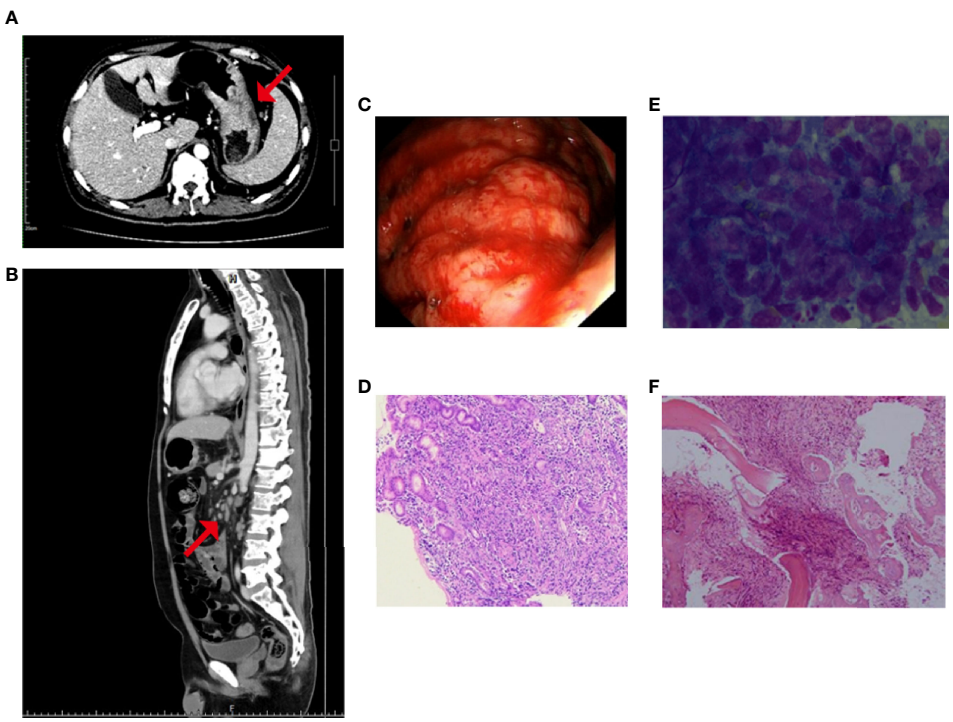

FIGURE 1 | Before treatment, enhanced CT showed **(A)** thickening of gastric wall and **(B)** multiple enlarged abdominal lymph nodes, as well as mixed bone metastasis of the vertebras; the red arrow represents the location of the lesion; **(C)** Gastroscopy showed the lesion was found in the greater curvature of upper gastric body, about 5 cm × 8 cm in size, with uneven surface and bloody substances; and Hematoxylin-eosin staining of **(D)** the gastric biopsy, **(E)** the bone marrow smear, and **(F)** the bone marrow biopsy.

(IHC) of cancer cells showed HER2 negative, PD-L1 combined positive score (CPS) = 3 (Dako 22C3 antibody). Fluorescence *in-situ* hybridization showed negative Epstein Barr Virus ambiguous. A bone marrow smear (of the left posterior superior iliac spine) showed a large number of metastatic cancer cells distributed in pile, absence of megakaryocytes, and few platelets (**Figure 1E**), and a bone marrow biopsy showed cancer cells striped or scattered between bone trabeculae (**Figure 1F**). A tumor next-generation sequencing (by Geneseeq Technology Inc.) showed *MET* amplification (copies in biopsy and plasma: 19.7 and 18.1 respectively), and *MET-suppression of tumorigenicity 7* (*ST7*) rearrangement (*MET*: exon 14 - *ST7*: exon 2, in 0.8% biopsy and 5.1% plasma respectively; **Figures 2A, B** and **Supplementary Figure 1**), microsatellite stable, tumor mutation burden 6.3 mutations/Mb. Clinical diagnosis of this patient indicated stage IVB GC (complicated with bone marrow invasion, extensive bone and lymph node metastases, with *MET* amplification and rearrangement at cT4aN3M1).

After admission, our patient received repeated transfusions of erythrocyte, platelets, and drugs for promoting platelet production (recombinant human thrombopoietin, interleukin-11, and avatrombopag) plus supportive treatments. His hemoglobin and platelets slightly and transiently increased after blood transfusion but rapidly decreased. Eleven days after admission, the patient received one dose of 240 mg nivolumab. However, the transfusion effect declined after repeated platelet transfusion probably due to the positive anti-platelet antibodies. To block the effect of platelet antibodies, intravenous immunoglobulin (IVIG, 0.5 g/kg) was infused several times before subsequent platelet transfusion. Sixteen days after admission, induction chemotherapy with low-dose tegafur-gimeracil-oteracil potassium (*i.e.*, S-1, 40 mg, twice daily) was started when no bleeding symptoms were shown. However, three days later (nineteen days after admission), dark stools and nasal mucosal bleeding appeared, and platelet count dropped to 12×10^9/L, thus S-1 was stopped. Twenty-one days after admission, the patient further developed fever, pulmonary infection, and acute left heart failure, which represented an extremely critical condition. Considering his intolerance of traditional chemotherapy as well as the significant abnormalities of the amplification and rearrangement of *MET* in his tumor, savolitinib was given to the patient (400 mg/d × 4 d, followed by 600 mg/d orally to date) along with antibiotics and supportive treatments. After savolitinib treatment for four days, platelets recovered to 27×10^9/L. Bone pain symptoms were significantly relieved and the dosage of analgesics was reduced. Eighteen days after savolitinib treatment, platelets recovered to 81×10^9/L, the thrombopoietic agents were stopped, then the patient was discharged. Fifty-five days after savolitinib treatment, the patient returned for a follow-up session. He was able to walk, had a KPS score of 80 points, and a pain NRS score of one point. Physical examination showed no signs of skin petechiae, ecchymoses, and lumbosacral vertebral tenderness. Blood test indicated normal platelets (188×10^9/L), hemoglobin (111 g/L), and leukocytes (**Figures 3A, B**). Tumor markers significantly decreased (CEA 2.71 μg/L, CA125 17.2 U/mL, and CA19-9 35.04 U/mL). CT re-examination showed that gastric fundus and body wall were thinner (**Figure 3C**), and partial lymph node metastases were shrunk (**Figure 3D**). Bone marrow smear re-examination showed the absence of metastatic cancer cells (**Figure 3E**), and bone marrow biopsies showed interstitial fibrous hyperplasia with foam cell infiltration, suggesting post-treatment changes (**Figure 3F**). No obvious adverse reaction was shown. The patient achieved partial remission (PR) based on Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1. At the time of reporting, the patient was alive and remained progression-free at 15 weeks.

During the preparation of this manuscript, in our department, there was another 39-year-old female patient with advanced GC was given savolitinib. At initial admission, her pathological biopsy with gastroscopy indicated poorly differentiated adenocarcinoma, and IHC of cancer cells showed HER2 and PD-L1 CPS negative. A tumor next-generation sequencing showed *MET* amplification (9.1 copies in biopsy),

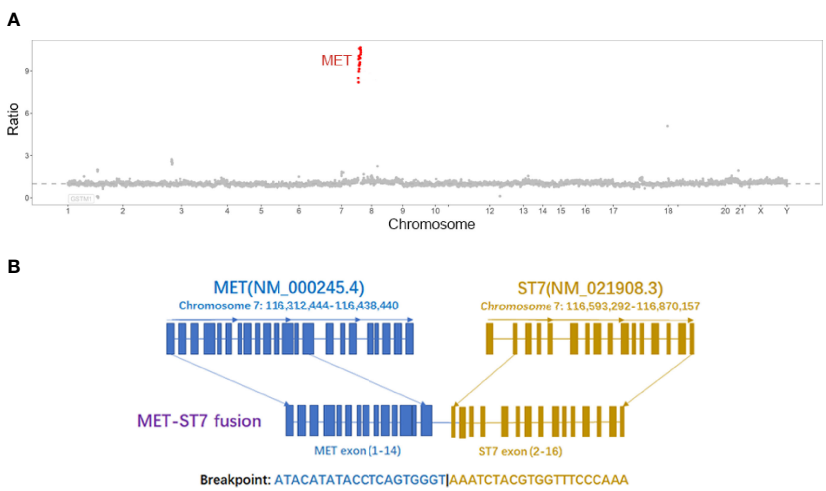

FIGURE 2 | **(A)** The copy number ratio of *MET* to centromere of chromosome 7 in tissues, each red point represents an exon of *MET*; **(B)** *MET-ST7* fusion diagram.

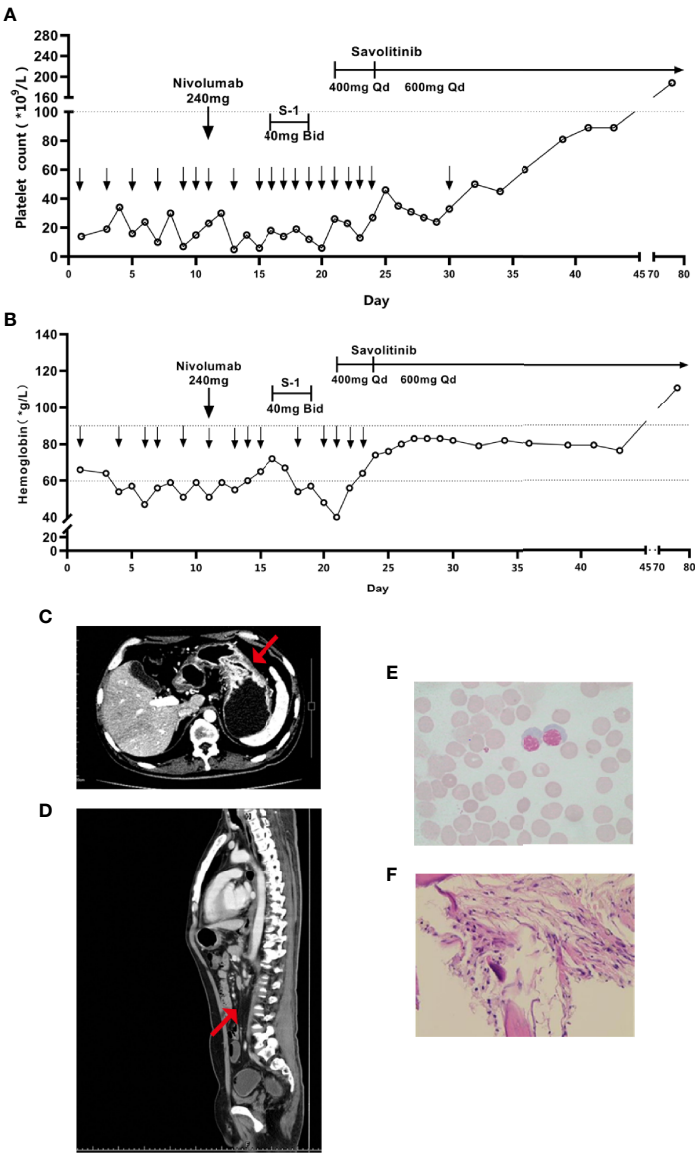

FIGURE 3 | Variation trends of **(A)** platelet and **(B)** hemoglobin since admission, the lower downward arrow represents the date of platelet and hemoglobin transfusion, respectively. After savolitinib treatment, enhanced CT showed that **(C)** the thickness of gastric wall was thinner and **(D)** the abdominal lymph nodes were shrunk and fewer, the red arrow represents the location of the lesion; and post-treatment Hematoxylin-eosin staining of **(E)** the bone marrow smear and **(F)** the bone marrow biopsy.

microsatellite stable, and tumor mutation burden 3.95 mutations/Mb. She was diagnosed as cT4aN3M1 stage IVB GC (complicated with bilateral ovarian metastases (Krukenburg tumors), extensive lymph node metastases in perigastric, retroperitoneal, and mesenteric regions, massive abdominal-pelvic ascites, with *MET* amplification). After an 8-month first-line therapy of nivolumab plus S-1 and oxaliplatin (5-month for oxaliplatin), and a 4-month second-line chemotherapy of paclitaxel-albumin, she had a KPS score of 70 points and a pain NRS score of 4 points (abdominal pain), her primary tumor lesion and metastases markedly progressed both in size and quantity, with multiple new metastases in bones. She could not tolerate continued chemotherapy. Considering that *MET* amplification was detected in her tumor (9.1 copies), she received savolitinib monotherapy as a third-line treatment. By the eighth week, she had a KPS score of 90 points, abdominal pain relieved (NRS score of 0 points), and gained 10 kg in body weight. CA19-9 dropped from > 12000 U/ml pretreatment to 2704.83 U/ml, CEA from 26.27 ug/L to 6.73 ug/L. CT re-examination showed PR (**Supplementary Figure 2**), no obvious adverse reaction was shown. At the time of reporting, she was alive and remained progression-free at 12 weeks.

DISCUSSION

At present, systemic chemotherapy (including platinum and/or fluorouracil, paclitaxel, irinotecan, *etc*), immunotherapy, and targeted therapy are mainly used to treat advanced GC (10). This provided theoretical evidence for nivolumab and S-1 induction chemotherapy, which were initially selected for the male patient reported in this study. However, nivolumab efficacy was probably not achieved because of repeated infusions of IVIG.

IVIG, which contains large amounts of gamma immunoglobulin (IgG) antibodies, is often needed for the therapy of autoimmune diseases based on its contribution to anti-inflammatory and immunomodulatory activities (11). In clinical practices regarding immunotherapy-related adverse events, some patients require immunosuppressive agents including IVIG besides steroids to reduce inflammatory reactions (12–16). One of its regulation mechanisms is that IVIG can compete with pathological autoantibodies for binding the neonatal Fc receptor (FcRn). The FcRn binds serum IgG that has been endocytosed by myeloid cells or endothelial cells, and recycles the IgG to the cell surface, avoiding IgG catabolism by the lysosome (11, 17, 18). In the absence of FcRn, the half-life of IgG is reduced (19, 20). In this reported case, the transfusion effect declined after repeated platelet transfusion, which was an unfavorable condition for the patient, and we detected positive anti-platelet antibodies in the serum. We used repeated IVIG to accelerate clearance of anti-platelet antibodies. However, IVIG also could compete with nivolumab, a kind of monoclonal IgG antibody that blocks PD-1 receptors of T cells to unleash anti-tumor effect, for binding FcRn, probably resulting in the accelerated clearance of nivolumab with a shortened half-life. It is unclear at present to what extent the use of IVIG contributes to the reduction of anti-tumor efficacy of PD-1 antibodies.

Pharmacokinetics of a single dose of S-1 showed that the mean half-life of its active components are between 1.9-13.1 hours (21). Our patient received 40 mg of S-1 twice daily, which was lower than the regular dose of 60 mg according to his body surface area. Moreover, the patient suffered from thrombocytopenia, acute gastrointestinal bleeding, pulmonary infection, and heart failure. He could not tolerate continued chemotherapy after S-1 treatment for only three days.

When no standard treatments were available, the significant abnormal *MET* in the tumor of this patient attracted our attention. *MET* is located on 7q21-31. Since the 1980s, *MET* exon 14 skipping mutation, *MET* amplification, and MET protein overexpression have been associated with tumor cell proliferation and metastasis (4). In recent years, great progress has been made in research on small molecule selective MET-tyrosine kinase inhibitors (TKI), represented by savolitinib, capmatinib, and tepotinib (9, 22, 23).

Savolitinib, which showed good antitumor activities in the human glioma xenograft model, was firstly reported in 2014 as one of the inventions of a series of novel MET inhibitors (24). Being a type I small-molecule MET-TKI, savolitinib efficiently binds to the active protein kinase conformation (Asp-Phe-Gly (DFG)-in) of MET, leading to the inhibition of MET phosphorylation and downstream signaling (25). Gavine et al. reported that in a panel of GC patient-derived tumor xenograft models, savolitinib demonstrated significant anti-tumor efficacy only in MET-amplified models through the inhibition of phospho-MET and downstream signaling *via* ERK and AKT pathways (26).

To date, therapy targeting MET is mostly reported in the treatment of lung cancer, whose overall incidence of *MET* exon 14 skipping mutation as a primary driver mutation is 3% to 4%, whereas acquired *MET* amplification is detected after 5% to 20% epidermal growth factor receptor (EGFR)-TKI resistance (27). In a study conducted in 70 cases of advanced NSCLC patients with *MET* exon 14 skipping mutation, savolitinib as second-line treatment achieved an overall response rate (ORR) of 42.9%, a median progression-free survival (PFS) of 6.8 months, and a median OS of 12.5 months (28). The phase 1b TATTON study showed that combined therapy of osimertinib (an EGFR-TKI) and savolitinib, in patients with *MET* amplification secondary to *EGFR* mutation following EGFR-TKI treatment, achieved an ORR of 48% to 64% (29).

In addition to lung cancer, savolitinib is also applied for the treatment of papillary renal cell carcinoma (PRCC) and GC which is related to MET abnormalities. In a single-arm phase II study (NCT02127710) of PRCC patients treated with savolitinib, patients in the MET-driven subgroup (defined as chromosome 7 copy gain, focal *MET* or *HGF* gene amplification ≥ 6 copies, or MET kinase domain mutations > 5% allele frequency) had significant longer median PFS and higher ORR than the MET-independent subgroup (6.2 vs 1.4 months and 18% vs 0%, respectively) (30). A phase III randomized study (SAVOIR) designed solely for metastatic MET-driven PRCC patients favored savolitinib over sunitinib with a numerically higher response rate (27% vs 7%) (31). However, in the phase II SWOG1500 study of PRCC patients unselected for MET status, patients treated with savolitinib showed an increased risk of progression compared to those with sunitinib (32). These indicated that savolitinib exerts its highly selective antitumor activity through a MET-dependent manner.

MET amplification is present 1% to 10% GC (27). Previous studies revealed that *MET* amplification is involved in invasion, metastasis, advanced stage, and poor prognosis of GC. For instance, An et al. reported that the median PFS and OS of patients carrying *MET* amplification were 3.6 and 5.7 months, respectively, whereas those of patients without *MET* amplification were 6.9 and 15.5 months (33). Lee et al. found that the survival rate of GC patients with *MET* copy number greater than 4 (incidence 21.1%) was distinctly lower than that of patients with a lower copy number (8).

The VIKTORY phase II clinical trial of advanced GC confirmed that the ORR of patients with *MET* copy number greater than 10 (incidence 3.5%) treated by savolitinib monotherapy was 50%, and their PFS were 4-6 months (34). In a phase I study of capmatinib monotherapy for multiple tumors with MET overexpression or gene amplification, 2 of 9 patients with GC achieved stable disease (SD) (35). In a phase I

clinical trial of tepotinib monotherapy in the treatment of multiple tumors (no limitation to *MET* status), 1 of 2 patients with GC achieved SD (36). At present, a single-arm phase II clinical trial (NCT04923932) of savolitinib for treating locally advanced or metastatic gastric cancer and esophagogastric junction adenocarcinoma with *MET* gene amplifications is ongoing.

Our male patient also had a *MET-ST7* rearrangement. *ST7* locates at 7q31, which is close to *MET*. *ST7* is often present with loss of heterozygosity (LOH), rather than point mutation in multiple tumors, and is believed to be a tumor suppressor gene (37–39). In a study carried out in *ALK*-rearranged positive NSCLC patients receiving ALK-TKI treatment, it was found that 3% exhibited secondary *MET-ST7* rearrangement (*MET* exons 2-21-*ST7* exon 1; the fusion site was different from that of our patient). Introducing the rearranged gene to ALK-TKI-sensitive cell line induced resistance to ALK inhibitors, and this resistance was reversed by combining treatment with MET-TKIs (crizotinib, capmatinib, or savolitinib) (40). These findings indicate that *MET-ST7* rearrangement may be pivotal in the development of cancer.

The male case reported in this article is an advanced GC patient with bone marrow invasion and extensive metastases, whose *MET* was highly amplified and co-existed with *MET-ST7* rearrangement. In the situation that routine chemotherapy was not well tolerated and the patient's condition was extremely critical, considering the significant abnormalities of his *MET* gene as well as the published clinical data and drug accessibility, savolitinib monotherapy was selected. After savolitinib treatment, the patient's condition promptly improved, his platelets returned to normal, anemia and bone marrow invasion disappeared. Tumor efficacy evaluation indicated PR and maintained over 15 weeks, no obvious adverse reactions occurred. We cannot completely rule out the possibility that the treatment effect is the result of a combination of all three treatments (nivolumab, S-1, and savolitinib), this is the limitation of our report. But taking into account that the patient received only one dose of nivolumab and 3-day low-dose S-1 before savolitinib treatment, accompanied by repeated IVIG which could accelerate nivolumab's clearance, his condition was still rapidly deteriorating, we believe that the anti-tumor activity of savolitinib played a major role in his treatment strategy.

In general, this report of savolitinib monotherapy validated the prominent efficacy of savolitinib in the treatment of advanced GC with *MET* amplification, providing a potential and effective treatment option for such patients.

CONCLUSION

Here we report the treatment of aggressive advanced GC patients with *MET* abnormalities, who responded to savolitinib monotherapy promptly and positively. It provides a new reference for understanding *MET* abnormalities in GC, and offers a possibility for future application of MET-TKIs in GC patients. Also, it suggests that sequencing of *MET* can be considered a routine target in advanced GC patients.

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.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

WY, LS, ZZ, and MD contributed to the implement of the treatment. WY and LH contributed to the collection, analysis and interpretation of data, drafting and revision of the manuscript. SY contributed to the conception of the treatment, revision and approval of the final manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.868654/full#supplementary-material

Supplementary Figure 1 | Base map of *MET-ST7* fusion site.

Supplementary Figure 2 | (A) Gastroscopy showed an ulcer lesion (black arrow) with uneven surface was found in the lesser curvature of gastric fundus, about 2.5 cm × 2 cm in size; a submucosal bulge (white arrow, proved to be poorly differentiated adenocarcinoma as well) was found at the junction of gastric fundus and body, about 1.5 cm in diameter; **(B)** Hematoxylin-eosin staining of the gastric biopsy from the ulcer lesion; Enhanced CT images of the gastric wall **(C)** at diagnosis, **(D)** progression after two lines of chemotherapy, and **(E)** after savolitinib treatment for 8 weeks. The red arrow represents the location of the lesion.

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Case Report: Dacomitinib is effective in lung adenocarcinoma with rare EGFR mutation L747P and brain metastases

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Due to the low incidence of rare EGFR mutation, its response to EGFR-TKI has not been fully investigated. L747P is a rare EGFR mutation in EGFR exon 19. Previous case reports showed that patients with EGFR L747P mutation responded to afatinib treatment. However, we encountered a patient with EGFR L747P who was resistant to afatinib but responded to dacomitinib. It is the first case report of the effective application of dacomitinib in a patient with L747P mutation and BMS, and the efficacy of BMS achieved PR.

KEYWORDS

Dacomitinib (PubMed CID: 11511120), lung cancer, brain metastasis, EGFR, L747P mutation

Introduction

Predictive biomarkers in advanced non-small-cell lung cancer (NSCLC) include sensitizing epidermal growth factor receptor (EGFR) mutations, ALK rearrangements, ROS1 rearrangements, BRAF V600E point mutations, METex14 skipping mutations, NTRK1/2/3 gene fusions, and RET rearrangements (1). The presence of EGFR exon 19 deletion or exon 21 L858R mutation suggests a potential benefit from EGFR tyrosine kinase inhibitor (EGFR-TKI) therapy; thus, these mutations are referred to as sensitizing EGFR mutations. About 47.6% of NSCLC in Chinese populations harbor somatic mutations in the tyrosine kinase domain of the EGFR gene, mostly consisting of in-frame deletions of exon 19 (36.7%) and L858R substitutions in exon 21 (33.4%) (2). However, because of the low incidence of rare EGFR mutation, its response to EGFR-TKI has not been fully investigated. L747P is a rare EGFR mutation in EGFR

exon 19. In previous case reports, patients with the EGFR L747P mutation responded to afatinib treatment (3, 4). Incidentally, we encountered a patient with EGFR L747P who was resistant to afatinib. The patient ultimately responded well to dacomitinib and had a significant clinical benefit on developed BMS.

Case presentation

In April 2020, a 66-year-old Chinese woman with no smoking history was found to have a pulmonary mass in the right lower lobe of her lung by chest computed tomography (CT) scan during physical examination. The patient was subsequently hospitalized in the Department of Thoracic Surgery. Enhanced CT scan of the chest revealed a nodular lesion on the lower lobe of the right lung, the boundary is unclear, and the range of the central plane is about 5.0 cm × 4.2 cm; enhanced CT of the abdomen, emission CT bone scan, and brain magnetic resonance imaging (MRI) showed that no distant metastasis was found. After eliminating surgical contraindications by perfecting preoperative examinations, on 12 May 2020, the patient underwent thoracoscopic right lower lobectomy and systemic lymph node dissection. Postoperative pathology prompted invasive adenocarcinoma and postoperative staging is stage pT2bN2M0 IIIA. Next-generation sequencing (NGS) was performed with panels covering nine lung cancer-related genes (namely, ALK, BRAF, EGFR, ERBB2, KRAS, MET, NTRK, RET, and ROS1), and PD-L1 (Ventana SP263) expression detection in tumor tissue paraffin section (30% neoplastic cell) found EGFR exon 19 L747P mutation (EGFR NM_005228.3 Exon19 c.2239_2240del TTinsCC p.L747P) (Figure 1) with a mutation abundance of 14.21% and PD-L1 expression was greater than 10% in tumor cells (TCs). After the operation, the patient did not receive any antitumor therapy due to personal refusal.

On 14 July 2020, a CT scan was taken after the seizure of the patient, and the result revealed brain metastases (BMS) in the right frontal lobe of the brain, and then she accepted brain radiotherapy, which started from 20 July 2020, with a dose of 500 cGy/f, once a day, five times a week, and a planned target volume tumor absorbed dose (PTV DT) was 5000 cGy/10f. Subsequently, the patient started oral afatinib 40 mg daily as first-line therapy on 4 August 2020 (Figure 2A), and achieved stable disease for 7 months until January 2021 (Figure 2B).

After experiencing dizziness and headache, the patient was reexamined by brain MRI on 19 January 2021, which revealed a new brain metastasis in the left occipital lobe (Figure 3A). The patient refused radiotherapy, chemotherapy, and immunotherapy, and started oral dacomitinib treatment with a dose of 30 mg daily. After several days, the patient's neurological symptoms were significantly relieved. The patient underwent MRI 2 months later, and found that the lesion was significantly reduced, the efficacy achieved partial response (PR) (Figure 3B), and follow-up MRI at 4 and 6 months showed that the lesion continued to shrink and almost disappeared (Figures 3C, D). The patient continued to receive dacomitinib until new right parietal lobe brain metastases were identified on 23 June 2022 (the cutoff day, Figures 4A, B), and the progression-free survival (PFS) was 17 months (Figure 5). No obvious adverse reactions have been observed during the patient's medication.

Discussion

The L747P mutation in exon 19 of EGFR is rarely reported. One reason may be that the PCR kits are commonly used in clinical practice in most hospitals, and the results are used to

FIGURE 1
NGS panel results showed EGFR mutation L747P (2239-2240 TT>CC) in exon 19.

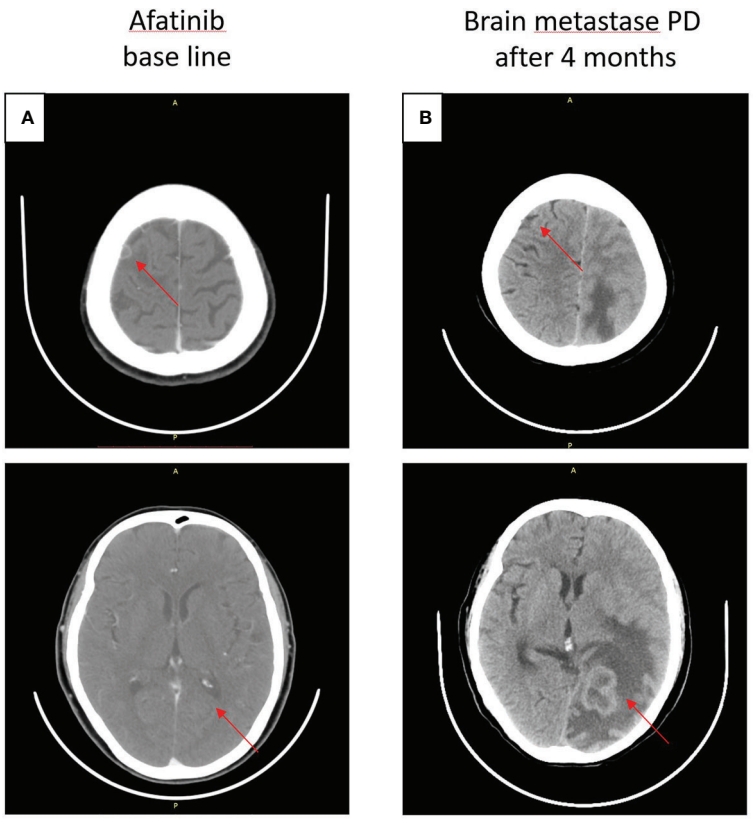

FIGURE 2
Craniocerebral CT about clinical response to afatinib therapy at different times. **(A)** Baseline before afatinib treatment. **(B)** Brain metastases progressive disease (PD) after 4 months.

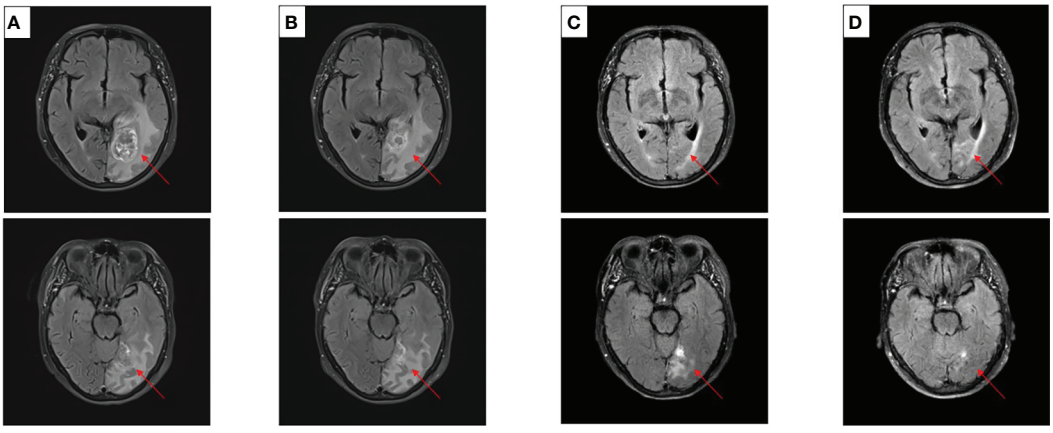

FIGURE 3
Craniocerebral MRI about clinical response to dacomitinib therapy at different times. **(A)** Baseline before dacomitinib treatment. **(B)** Brain metastases partial response (PR) after 2 months. **(C)** Brain metastases PR after 4 months. **(D)** Brain metastases PR after 6 months.

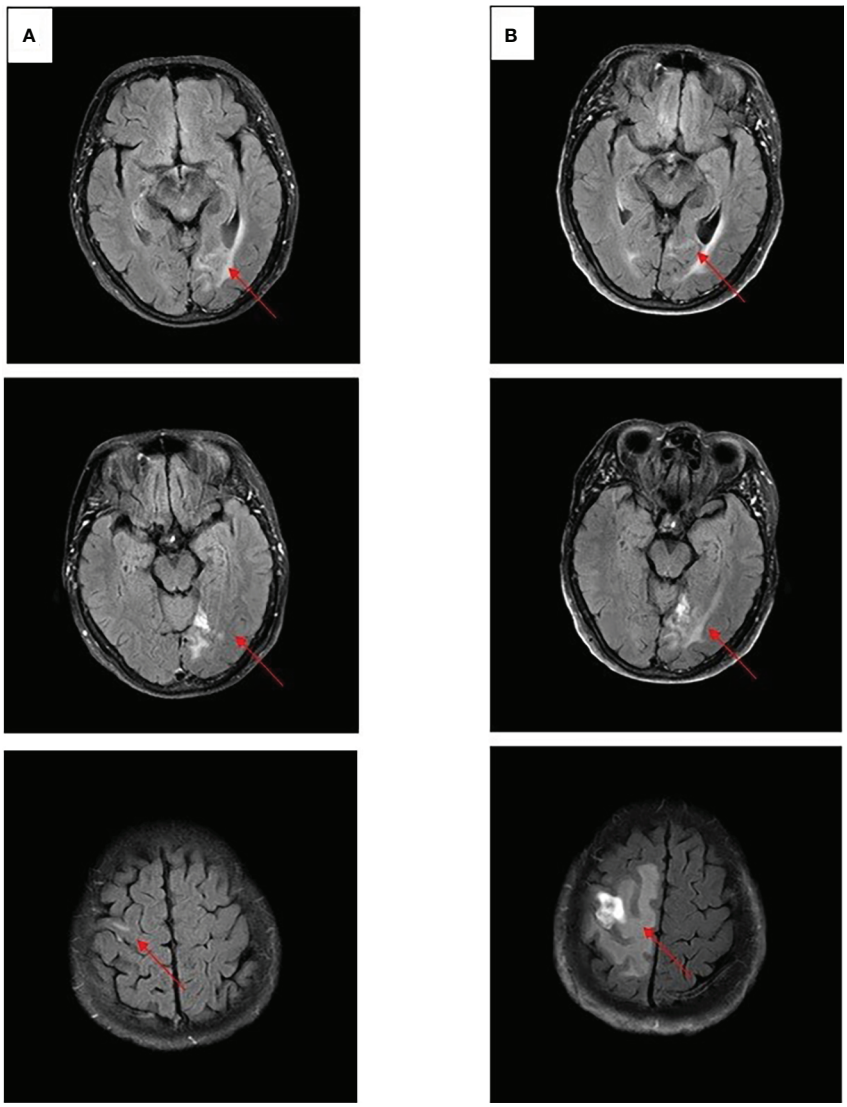

FIGURE 4
Craniocerebral MRI about clinical response to dacomitinib therapy at different times. **(A)** Brain metastases still PR after 12 months. **(B)** Brain metastases PD after 17 months.

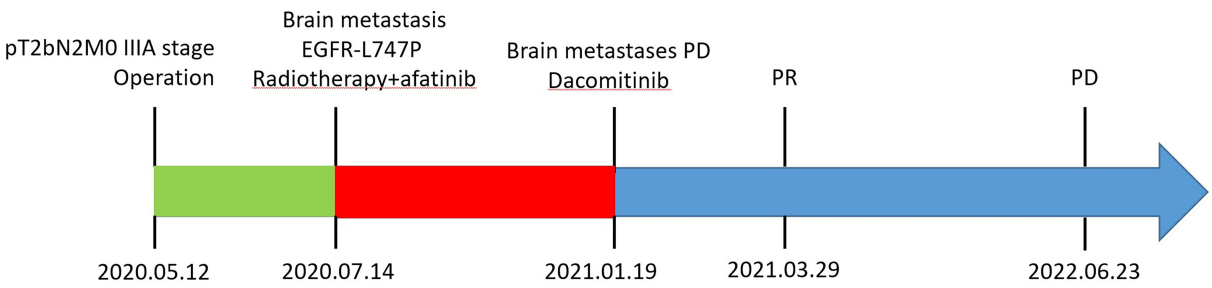

FIGURE 5
Treatment history and genomic results during the patient's clinical course.

guide doctors in treating patients. However, by using these methods, the L747P mutation may be incorrectly identified as 19del or false-negative as wild type, resulting in incorrect information for the guidance of clinical management (5, 6). Compared with the PCR detection method, NGS detection can more accurately identify the patient's EGFR mutation, reduce the misidentification of rare mutations, and provide more information to guide the clinical treatment strategies.

Most EGFR mutations are highly sensitive to EGFR-TKI, but some rare EGFR mutations, such as the L747P mutation, reduce sensitivity to some EGFR-TKI due to their special conformation. Yoshizawa's study (7) use binding free energy calculations and microsecond-timescale molecular dynamics (MD) simulations, revealing that the L747P mutation considerably stabilizes the active conformation through a salt-bridge formation between K745 and E762, and markedly decreased the van der Waals interaction between EGFR tyrosine kinase and gefitinib, resulting in resistance.

In the previous case report, patients with the uncommon EGFR mutations L747P were resistant to both first-generation (1G) and third-generation (3G) EGFR-TKI (7–11). Second-generation (2G) TKI afatinib shows better antitumor activity and achieved numerically longer PFS than that with 1G or 3G TKIs (3, 4, 11). In the binding affinity to EGFR TKIs by kinetic simulations, 1G TKIs (gefitinib, erlotinib, and icotinib) showed the worst binding affinity to the p.L747P mutation, and 3G TKI osimertinib showed moderate binding affinity. In contrast, the 2G TKIs (afatinib and dacomitinib) conferred the best binding affinity. In the subsequent cellular kinase inhibition assay and mice xenograft experiment, dacomitinib also achieved similar results to afatinib. 2G TKI (afatinib and dacomitinib) showed a lower IC_{50} value of Ba/F3 cell and A431 cell expressing p.L747P compared with 1G or 3G TKIs (Table 1), indicating that dacomitinib is likely to be as effective against L747P as afatinib (7, 11).

Moreover, compared with afatinib, dacomitinib has potent efficacy for the brain metastases. In the case series study of 14 central nervous system (CNS) metastasis in EGFR-positive NSCLC patients, the objective response rate (ORR) was 92.9% and the disease control rate (DCR) was 100% (12).. Another study also achieved 100% DCR in eight CNS metastasis patients (13). Therefore, dacomitinib may be more effective than afatinib for CNS metastases in patients with L747P.

TABLE 1 Kinase inhibition activity of diverse EGFR TKIs against Ba/F3 and A431 p.L747P cell lines (7, 11).

IC_{50} (nmol)	A431 p.L747P	Ba/F3 p.L747P
Gefitinib	147.3	45.3
Erlotinib	167.3	67
Afatinib	6.7	0.8
Dacomitinib	5.2	1.8
Osimertinib	80.9	12.6

In few case reports (14, 15), osimertinib may have a certain efficacy against EGFR L747P mutations, but in these cases, osimertinib is only effective in first-line use, and there is no clinical evidence that osimertinib can be effective after afatinib resistance. Therefore, although osimertinib can also achieve significant exposure in the brain, the treatment for L747P mutation should still be based on 2G EGFR TKIs as far as the existing conclusions are concerned.

In the present case, the patient had an EGFR L747P mutation, and she paid more attention to the quality of life and feared antitumor therapy, so she refused to accept chemotherapy and radiotherapy for BMS and was only willing to accept oral targeted therapy. We found that she was resistant to afatinib but responded to dacomitinib and achieved PR on brain metastases for 17 months.

Fortunately, dacomitinib worked well. This is the first case report of the effective application of dacomitinib to patients with L747P mutation, and also the first case report of the use of EGFR-TKI to achieve PR efficacy in L747P mutation patients with brain metastases.

Therefore, for L747P mutation patients with brain metastases, we can consider applying the second-generation EGFR-TKI with better effects on brain metastases like dacomitinib or using local treatment such as radiotherapy. By the way, the patient still refused chemotherapy but prepared for radiotherapy, and future treatment options remain to be further discussed.

Conclusion

In summary, this case demonstrates that a regimen of dacomitinib can achieve significant effects in patients with EGFR L747P mutation after progression on afatinib, especially in those who have brain metastases. This provides a new therapeutic strategy for these patients.

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 authors.

Author contributions

YL, WG, and BJ are lead authors who participated in data collection, manuscript drafting, table/figure creation, and manuscript revision. CH are senior authors who aided in drafting the manuscript and manuscript revision. FY and JW are the corresponding authors who initially developed the concept and drafted and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Publisher's note

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Heterogeneity of resistant mechanisms in an *EGFR*-TKI relapsed patient with *EGFR* amplification and response to nimotuzumab: A case report

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EGFR mutations are the most important drivers of gene alterations in lung adenocarcinomas and are sensitive to *EGFR*-TKIs. However, resistance to *EGFR*-TKIs is inevitable in the majority of *EGFR*-mutated lung cancer patients. Numerous resistant mechanisms have been revealed to date, and more are still under investigation. Owing to the selective pressure, intratumoral heterogeneity may exist after resistance, especially in patients after multiple lines of treatment. For those patients, it is important to choose therapies focused on the trunk/major clone of the tumor in order to achieve optimal clinical benefit. Here, we will report an *EGFR*-mutated lung adenocarcinoma patient with heterogeneity of resistant mechanisms including *EGFR* amplification, large fragment deletion of *RB1*, and histological transformations after targeted treatments. In our case, *EGFR* amplification seemed to be the major clone of the resistant mechanism according to the next-generation sequencing (NGS) results of both liquid biopsy monitoring and tissue biopsies. In consideration of the high *EGFR* amplification level, the patient was administered by combination treatment with *EGFR*-TKI plus nimotuzumab, an anti-*EGFR* monoclonal antibody (mAb), and achieved a certain degree of clinical benefit. Our case sheds light on the treatment of *EGFR*-mutant patients with *EGFR* amplification and indicates that a combination of *EGFR*-TKI with anti-*EGFR* mAb might be one of the possible treatment options based on genetic tests. Moreover, the decision on therapeutic approaches should focus on the major clone of the tumor and should make timely adjustments according to the dynamic changes of genetic characteristics during treatment.

KEYWORDS

case report, resistance, heterogeneity, EGFR amplification, nimotuzumab

Introduction

EGFR mutations are the most important driver of gene alterations in lung adenocarcinoma (LUAD) patients, especially in Asian non-smoking women (1). *EGFR*-TKIs are normally highly effective in patients with *EGFR* mutations. However, acquired resistance would eventually occur in the majority of patients. Among multiple mechanisms leading to *EGFR*-TKI resistance, the T790M mutation is most commonly found in subsequent first- and second-generation *EGFR*-TKIs (2, 3). Other resistant mechanisms, such as bypass activation and histological transformations, have also been reported (2, 4–6). Moreover, individual reports have also indicated that *EGFR* amplification contributes to acquired resistance to *EGFR*-TKIs (7–9). According to the AURA study, *EGFR* amplification occurred in 5.3% (1/19) of patients who developed resistance to first-line osimertinib (10). *EGFR* amplification was found in 42.9% (3/7) of T790M-positive tissues after resistance to third-generation tyrosine kinase inhibitor (TKI) (11).

Owing to the selective pressure, intratumoral heterogeneity may exist after resistance, especially in patients after multiple lines of treatment. Overcoming tumor heterogeneity is a major challenge for cancer treatment. Several studies have demonstrated that higher levels of heterogeneity in lung cancer predict inferior responses to anticancer therapies, including targeted therapy and immunotherapy (12, 13). For patients with intratumoral heterogeneity, it is important to choose therapies focused on the trunk/major clone in the tumor to achieve optimal clinical benefit. Herein, we report a LUAD patient with heterogeneity of resistant mechanisms to *EGFR*-TKIs, mainly based on *EGFR* amplification, who was successfully treated with a combination treatment containing nimotuzumab and *EGFR*-TKIs subsequently. We will present the following case in accordance with the CARE reporting checklist.

Case description

A 53-year-old Chinese man was found to have an occupying lesion in the upper lobe of the right lung during a routine checkup in March 2017. Positron emission tomography–computed tomography (PET-CT) showed multiple metastatic nodules in the hilum of the lung, lymph nodes, bone, and brain. The patient underwent tissue biopsy through a bronchoscope and was diagnosed with stage IV LUAD (Figure 1A). Next-generation sequencing (NGS) was performed using the tissue for driver gene alteration testing and revealed mutations of *EGFR* L858R and *TP53* R280T. Physical examination in this patient suggested no significant abnormalities except that the Karnofsky performance status (KPS) was 70. Laboratory findings were within the normal range, except for the carcinoembryonic antigen (CEA) level of 10.87 ng/ml (normal range, 0 to 5 ng/ml) in the serum. Other exams showed no positive signs at diagnosis.

The patients started with erlotinib 150 mg once daily as first-line therapy. After 16 months of erlotinib treatment, CT scans showed progressed disease (PD) due to the enlargement of lung metastatic nodules (Figure 1B). Liquid biopsy using plasma detected T790M along with L858R mutation, as well as *EGFR* amplification (Figure 1C). The patient started osimertinib treatment at 80 mg once daily as second-line therapy, and liquid biopsy monitoring only detected L858R and *EGFR* amplification 2 months after osimertinib, with no T790M retained. Osimertinib treatment lasted for 7 months before the resistance occurred (Figure 1B). Liquid biopsy after osimertinib resistance showed L858R mutation with high *EGFR* amplification. Moreover, *AKT1* E17K and a large fragment deletion of *RB1* (exon18–27) were also detected (Figure 1C). The patient then received pemetrexed plus platinum for six cycles. CT scans showed rapid growth of the primary lesion in September 2019 (Figure 1B). Taking the high level of *EGFR* amplification into consideration, the patient was administered by combination treatment with afatinib plus nimotuzumab and had stable disease (SD) with a slight shrinking of all the tumor sites including the primary and metastatic lesions. The combination treatment of afatinib and nimotuzumab lasted for 3.7 months, and then the patient underwent a re-biopsy in November 2019. Tissue biopsy guided by CT of metastatic lung lesion showed LUAD with sarcomatoid differentiation (Figure 1A). Then the patient asked for chemotherapy plus immunotherapy (bevacizumab + Abraxane + atezolizumab), and the therapy lasted for 10.4 months. In November 2020, a new liver metastatic lesion was found and was confirmed to be adenocarcinoma with neuroendocrine differentiation *via* tissue biopsy (Figure 1A). The NGS test showed different results in the lung and liver metastatic lesions: only the liver lesion was detected with *AKT1* E17K, while both harboring L858R, *TP53* R280T, *EGFR* amplification, and a large fragment deletion of *RB1* (exon18–27). Since then, the patient received a combination treatment of irinotecan, nimotuzumab, and dacomitinib with optimal efficacy of SD. The combination therapy has caused grade 3 hepatic disorders, represented as increased alanine aminotransferase (ALT), aspartate aminotransferase (ALP), and aspartate aminotransferase (AST) compared to baseline examination before the treatment, based on the Common Terminology Criteria for Adverse Events (CTCAE; version 5.0).

EGFR amplification was validated by fluorescence *in situ* hybridization (FISH) *via* tissue biopsies, including pre-treatment biopsies from primary tumors and post-treatment re-biopsies from lung and liver metastasis lesions. FISH signal accounts (copy number) were recorded for a total of 50 nuclei, and the tumor was considered *EGFR* amplification when *EGFR*/*CEP7* ratio was greater than or equal to 2 in 15% of recorded cells (14). Figure 1A shows that FISH revealed *EGFR* amplification in the

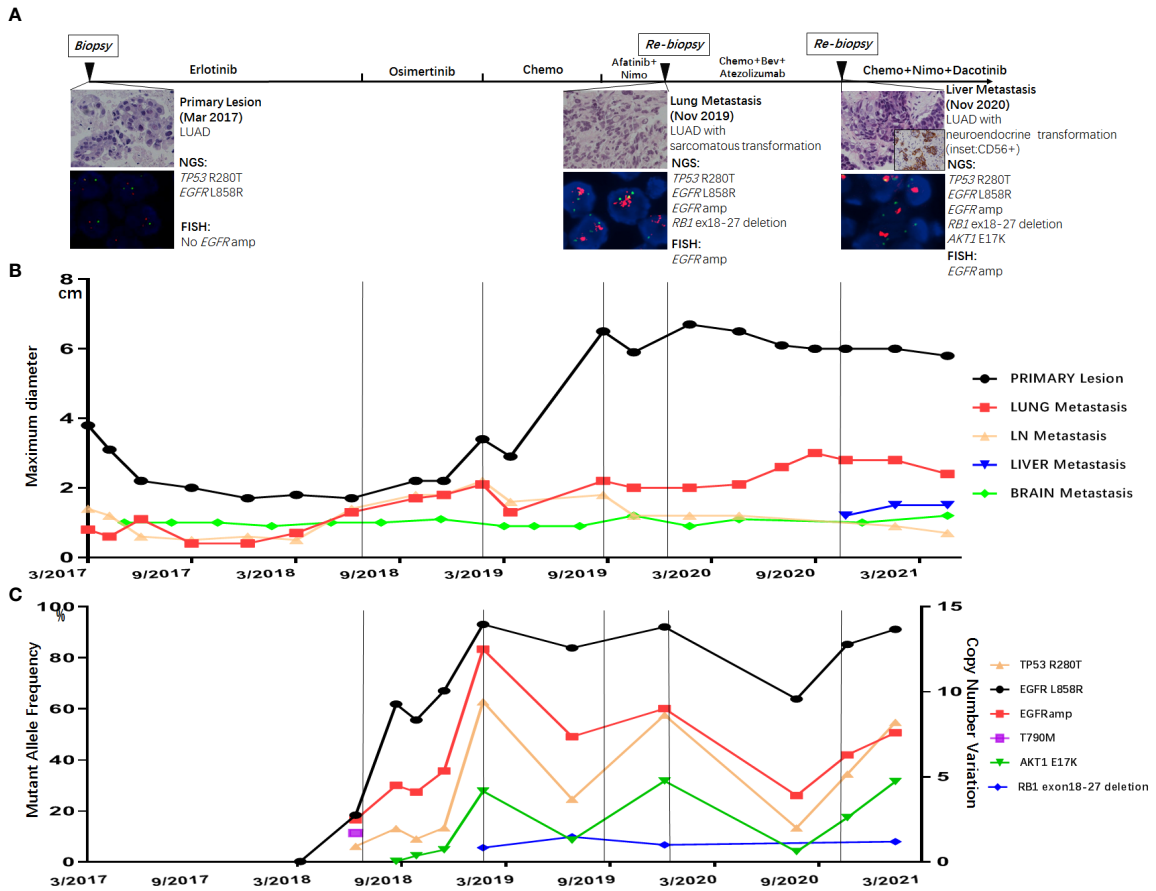

FIGURE 1
(A) Treatment timeline of the patient, histological morphology, and genetic results of three tissue biopsies (primary lesion in March 2017, lung metastasis in November 2019, and liver metastasis in November 2020). Dynamic monitoring of radiological results **(B)** and circulating tumor DNA using next-generation sequencing (NGS) **(C)**.

metastatic lesions of both lung and liver, while no *EGFR* amplification was observed in the primary lesion. Dynamic monitoring of radiological results and liquid biopsy NGS results are shown in Figures 1B, C. The study was approved by the institutional review board of the Cancer Hospital, Chinese Academy of Cancer Science (CAMS). Written informed consent was signed by the patient. The images of chest CT scans at different timepoints are shown in Figure 2.

The combination treatment of irinotecan, nimotuzumab, and dacomitinib remained for 8.6 months with continued disease control before the patient died in July 2021 due to tumor progression and complications. The PFS of different treatments is listed in Table S1.

Discussion

Resistance to *EGFR*-TKIs is inevitable, and numerous mechanisms have been discovered to date. Previously studies have described heterogeneity of resistant mechanisms after *EGFR*-TKI treatment. Roper et al. reported that 73% of osimertinib relapsed patients harbored at least two co-existing resistant mechanisms (15). Chabon et al. reported that intra-patient heterogeneity was observed in 46% of patients after first-line *EGFR*-TKI and 33% of patients after osimertinib treatment, according to circulating tumor DNA analysis (12). Our case emphasized the tumor spatial and temporal heterogeneity (Figure 3) by adequate investigation of tissue biopsies and liquid biopsy monitoring. Although the resistant mechanisms showed heterogeneity in our patients, *EGFR* amplification seemed to be the major clone of the resistant mechanism. Since *EGFR* amplification was observed early in liquid biopsy monitoring and all tissue biopsies after resistance harbored *EGFR* amplification, we speculated that other resistant mechanisms, such as large fragment deletion of *RB1* and histological transformation, were all divergent propagation of subclones from *EGFR* amplification. To achieve the optimal clinical benefit, it is important to choose therapies focused on

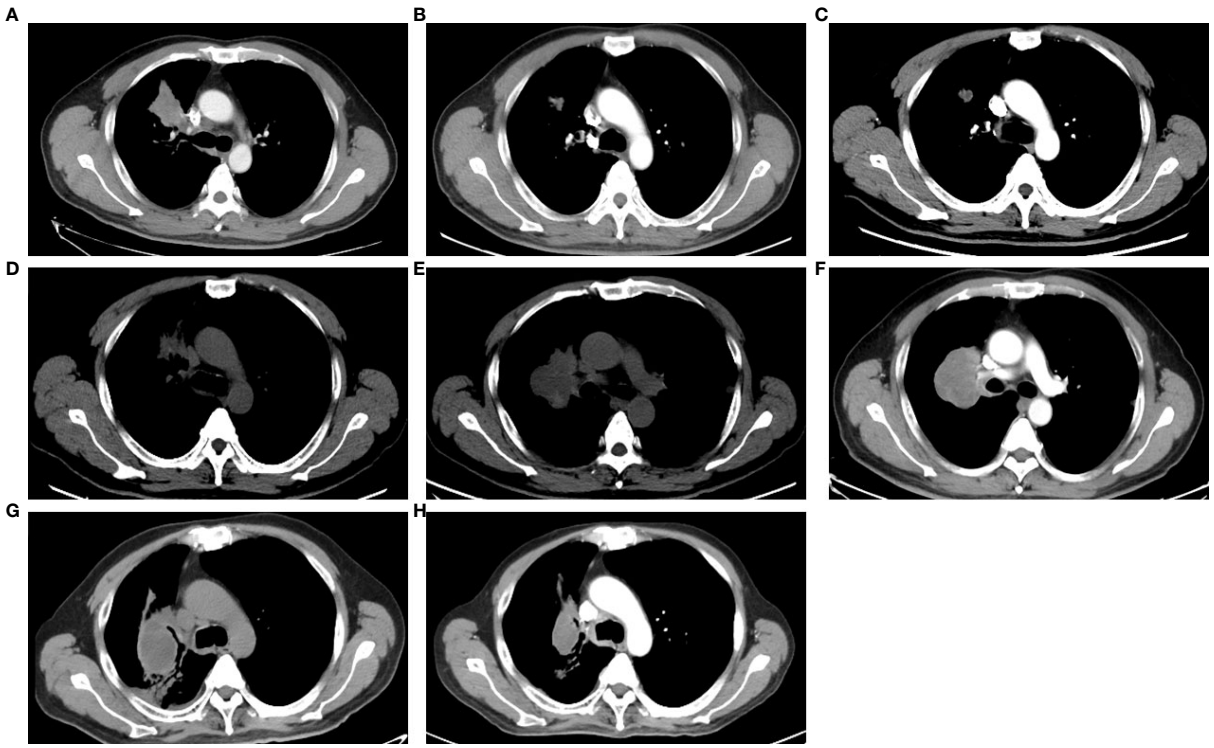

FIGURE 2
The images of CT scans with different treatments. **(A)** CT scan of basic examination before first-line erlotinib. **(B)** CT scan during the first-line erlotinib treatment. **(C)** CT scan at the progression of first-line erlotinib. **(D)** CT scan at progression of second-line osimertinib. **(E)** CT scan at the progression of chemotherapy. **(F)** CT scan at progression of afatinib plus nimotuzumab. **(G)** CT scan at the progression of immunotherapy. **(H)** CT scan during combination therapy of irinotecan, nimotuzumab, and dacomitinib.

EGFR amplification for this patient. Combination approaches that target heterogeneous tumor clones have been proved to be successful in pre-clinical studies (16). Nimotuzumab, an anti-*EGFR* monoclonal antibody (mAb), was used to treat *EGFR* overexpression cancers including glioma (17), squamous cell carcinomas of the head and neck (18), non-small cell lung cancer (NSCLC) (19), and other tumors of epithelial origin (20). It was also reported to have superior antitumor activity when combined with *EGFR*-TKI such as afatinib (21) in *EGFR*-mutant lung cancer. In our case, combination treatment of *EGFR*-TKI (afatinib or dacomitinib) plus nimotuzumab showed efficacy to some extent and significantly inhibited the rapid growth of both primary and metastasis lesions (Figure 1B). Other *EGFR* mAbs have been also evaluated in lung cancer for a long time, and *EGFR* amplification may be a predictive factor of *EGFR* mAb such as cetuximab and necitumumab plus chemotherapy (22, 23). In gastrointestinal cancers such as colorectal cancer, panitumumab, another *EGFR* mAb, has also been proved to be effective for patients with *EGFR* amplification (24, 25). However, evidence of the combination treatment of EGFR mAb plus EGFR-TKI for patients with both *EGFR* mutation and amplification has been scarce.

It is noticed that patients underwent chemotherapy plus immunotherapy (bevacizumab + Abraxane + atezolizumab) for 10.4 months subsequent to standard treatment and received stable disease. The relationship between immunotherapy and driver mutations has long been a research hotspot. A previous meta-analysis study showed that immunotherapy could not enhance overall survival (OS) or progression-free survival (PFS) in *EGFR*-mutant patients (26). At the same time, IMpower 150 reported a prolonged OS in *EGFR*-mutated patients with atezolizumab plus chemotherapy and bevacizumab (27). In our study, the *EGFR*-mutant patient did benefit from immunotherapy after multiple lines of treatments. Thus, further studies are needed to effectively evaluate the efficacy of immunotherapy for NSCLC individuals with *EGFR* mutations.

Our case sheds light on the treatment of *EGFR*-mutant patients with *EGFR* amplification and indicates that a combination of *EGFR*-TKI with anti-*EGFR* mAb might be one

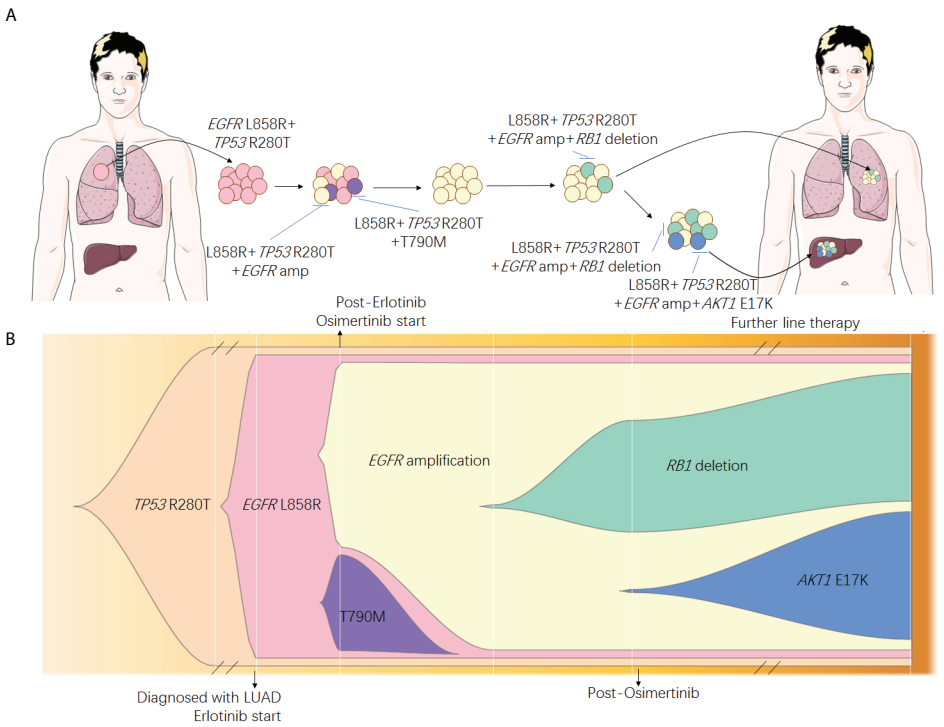

FIGURE 3
Speculated spatial heterogeneity **(A)** and evolutionary spectrum **(B)** of the patient.

of the possible treatment options. Moreover, as there are more and more treatment options for LUAD, patients could survive with tumors for a longer time. It brings up another problem that intratumoral heterogeneity might be more complicated after multi-line treatments. Thus, importance should be attached to therapeutic approaches to the major clone of the tumor, and these approaches should be multidimensional and dynamic with timely adjustments according to the patients' genetic characteristics.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by institutional review board of the Cancer Hospital, CAMS. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JL was the patient's physician, proposed the conception, collected the data for case presentation. YL conducted the literature search, performed the NGS tests for tissues and liquid biospies, and prepared the first draft of the manuscript. JY contributed to the conceptualization and contributed with final manuscript drafting. ZX conducted the follow-up and the revision of manuscript. TX was responsible for data analysis and figure drawing. PX contributed with final manuscript drafting. All authors contributed to the article and approved the submitted version.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.937282/full#supplementary-material

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Case report: Prompt response to radiotherapy and chemotherapy combined with crizotinib in gingival sarcomatoid squamous cell carcinoma with MET 14 mutation

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Background: As a kind of squamous cell carcinoma of head and neck (HNSCC), gingival sarcomatoid squamous cell carcinoma (GSSCC) is a rare biphasic malignant neoplasm. To date, surgical resection was often utilized for gingival squamous cell carcinoma (GSCC), while for patients with advanced gingival carcinoma who cannot tolerate surgery, radiotherapy and chemotherapy can be regarded as a treatment strategy. Many molecular-targeted drugs were investigated and approved for the treatment of malignant diseases, including hematologic diseases and solid tumors. Although targeted therapies such as EGFR inhibitors have shown therapeutic efficacy in HNSCC, there are still some patients who cannot benefit from it. New therapeutic targets and strategies should be further explored.

Case presentation: An 83-year-old woman was referred to our hospital with left lower gingival mass for more than 1 month in June 2021. Pathologic diagnosis is sarcomatoid squamous cell carcinoma. Due to the large tumor at the time of diagnosis and poor quality of life, the patient was intolerant to surgery, so she was given radiotherapy (RT) combined with concurrent chemotherapy (CT) with albumin bound paclitaxel. According to next-generation sequencing (NGS) results (MET exon 14 skipping mutation-positive), she was treated with crizotinib, a tyrosine kinase inhibitor that targets MET. Through the comprehensive treatment, the patient's condition promptly improved, clinical complete remission (CR) was achieved in 2 months, and 9-month progression-free survival (PFS) was obtained. She finally died from non-cancer-related diseases.

Conclusion: Here we report the treatment of a GSSCC patient with MET mutation, who responded to crizotinib promptly and positively. It provides a new reference for understanding MET abnormalities in GSSCC and offers a new idea for the targeted treatment of gingival carcinoma.

KEYWORDS

gingival carcinoma, MET, crizotinib, radiotherapy, chemotherapy

Introduction

Gingival carcinoma is a relatively rare malignancy that accounts for 10% of all oral cancers in Europe and the United States. Gingival carcinoma has the second highest incidence of oral cancer after tongue carcinoma. Its common pathological type is squamous cell carcinoma (SCC), while sarcomatoid squamous cell carcinoma is very rare (1, 2). The main therapy for gingival carcinoma is surgery alone or a comprehensive treatment based on surgery. However, early GSCC diagnosis remains challenging. Roughly 70% of patients have inoperable or metastatic disease at diagnosis. According to data, cumulative 5-year survival after the diagnosis was 43%, while at 10 years it was 11% (3). Furthermore, surgery can cause not only a disfiguring and poor quality of life but also financial and psychological burden for patients. Concurrent chemoradiotherapy (CRT) is an important treatment for locally advanced HNSCC patients, which can well-preserve organ and function integrity and improve the local control rate. Targeted therapy for gingival carcinoma is still in the exploratory stage. This case report is the first reported case of a patient with advanced gingival squamous cell carcinoma sarcomatoid lesions with MET gene mutation that achieved clinical complete remission after treatment with crizotinib targeted therapy combined with CRT.

Case presentation

An 83-year-old woman was referred to our hospital with left lower gingival mass for more than 1 month in June 2021. The patient presented with progressive enlargement of gingival swelling and discomfort such as swollen and painful gums and difficulty in opening the mouth for 1 month. The patient was first diagnosed in our hospital, with a healthy past and no special family history. Computed tomography (CT) scan revealed an oval-shaped mass in the left lower gingival area with adjacent mandibular bone destruction and multiple enlarged lymph nodes in the neck and left submandibular area, and an occupying lesion in the upper lobe of the right lung (Figure 1). PET/CT scan showed no other metastases. Physical examination revealed a mass of about 6 cm located in the left lower gingival area, causing difficulty in opening the mouth. Multiple enlarged lymph nodes were palpable in the left neck. According to laboratory testing, red blood cell count was 2.77×10^{12}/L, hemoglobin 77 g/L, albumin 32.2 g/L, and the results of the biomarkers CA153, CEA, CA199, CA125, and SCC showed no abnormalities. Histopathological examination revealed an actively growing spindle cell neoplasm, which tends to be a poorly differentiated sarcomatoid variant of squamous cell carcinoma. The pathology of the right pulmonary nodule was

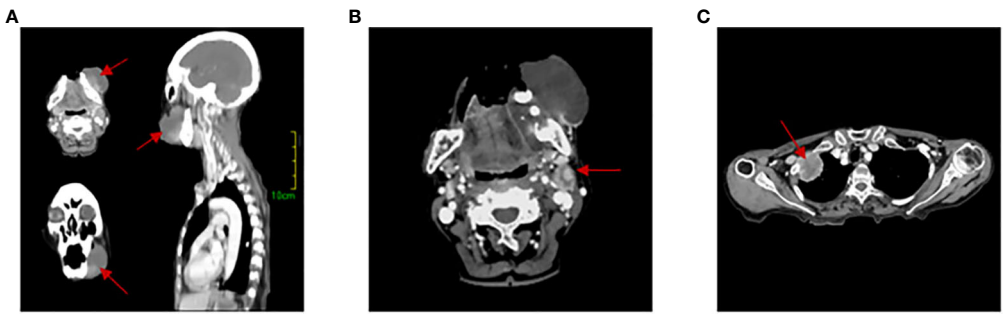

FIGURE 1
Computed tomography (CT) scans. **(A)** 3D image of gingival tumor. **(B)** Lymph node metastasis area. **(C)** Lung metastases lesion. The red arrow indicates the location of the lesion.

confirmed to be metastasis. Immunohistochemistry (Figure 2) outcomes were presented as follows: AE1/AE3 (+), P63 (+), EMA (+), CKpan (+), Vimentin (+), Ki67 (80%), EGFR (70%), S100 (-), CK5/6 (-), P40 (-), CK7 (-), and PD-L1 (DAKO 22C3) (CPS: 50). The patient was clinically diagnosed with cT4aN2bM1 stage IVB gingival carcinoma (according to the AJCC).

The patient poorly tolerated and refused surgery and chose CRT as the primary treatment. The patient started intensity modulated radiation therapy (IMRT) along with albumin-bound paclitaxel. Concurrent chemoradiotherapy began on 5 July 2021 and ended on 18 August 2021. The details of the regimen are as follows. Radiotherapy was given five times a week (from Monday to Friday). Radiotherapy planning: PGTV (gingival mass and metastatic lymph node) 66Gy/33F/7W, 2Gy/F; PGTV2 (lung metastases lesion): 66Gy/33F/7W 2Gy/F; PTV (lymphatic drainage area): 59.4Gy/33F/7W 1.8Gy/F. Chemotherapy regimen: albumin-bound paclitaxel (200 mg/m^2, d1, Q3W), a total of two cycles of single-agent chemotherapy. In order to search for targetable therapeutic drugs, we performed NGS on the patient. The NGS of the gingival biopsy specimen indicated a MET exon 14 skipping mutation (c.3028+3A>G), with an abundance of 49.67%, which can potentially cause alternative splicing of the MET protein. There was no significant change in the tumor volume during the first week of CRT. According to the results of the NGS, the patient was treated with crizotinib (250 mg, po qd) on 15 July 2021. The tumor volume decreased significantly after 1 week of crizotinib combined with CRT. During the treatment, two times of tissue cleanings were given due to the large amount of tumor necrosis tissue, and the target area was redrawn after each cleaning. In order to protect the surrounding normal tissues and avoid toxic reaction such as radiation-induced mandibular inflammation, CT simulation and target delineation of radiotherapy was re-performed on 26 July after cleaning up the necrotic tissues. The plan was reevaluation to ensure a high dose in the tumor site (Figure 3). This patient responded positively to crizotinib-targeted therapy, and no serious adverse effects of CRT were observed during treatment. Chemotherapy was only synchronized with radiotherapy for two cycles. After the end of CRT, the patient refused to continue chemotherapy and received oral crizotinib treatment at home. Two months later, reevaluation by CT scans showed complete remission (CR) in the gingival area. Followed up by telephone, the patient took crizotinib orally for 8 months, after which it was interrupted due to financial reasons. One month later, she died of pneumonia caused by a cold. The infection has been ruled out to be related to radiation pneumonitis and crizotinib treatment.

Discussion

Sarcomatoid squamous cell carcinoma (SSCC), also known as spindle cell squamous cell carcinoma, is a rare and peculiar biphasic malignant neoplasm that occurs mainly in the larynx, but also in other mucosal areas such as the gingiva, tongue, pharynx, and nasal cavity, accounting for 0.4%–4% of HNSCC (4). GSCC accounts for less than 1% of all tumors in the oral region, and GSSCC is even rarer. In the existing reports, SSCC is more difficult to treat than SCC, and the prognosis is worse (5). The National Comprehensive Cancer Network Guidelines

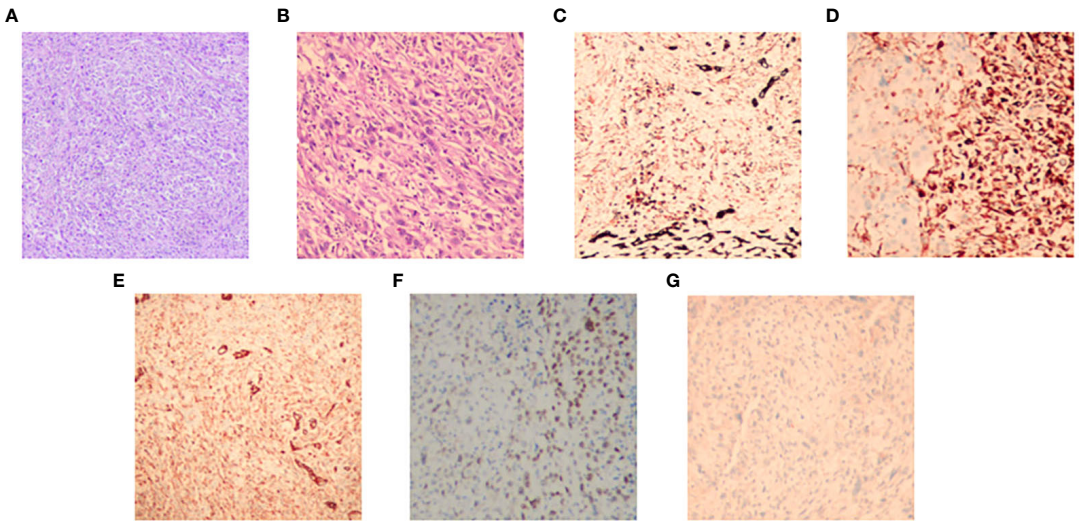

FIGURE 2
The hematoxylin–eosin (H&E) and immunohistochemical pictures of the tumor. **(A)** H&E, original magnification, ×100. **(B)** H&E, original magnification, ×400. Immunohistochemistry: CKpan **(C)**, Vementin **(D)**, EMA **(E)**, P63 **(F)**, and S100 **(G)**.

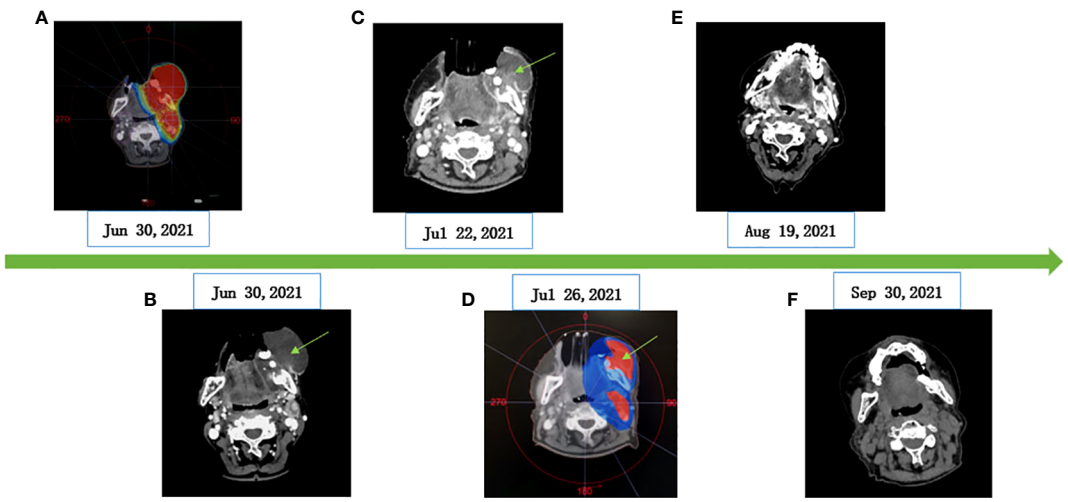

FIGURE 3
CT imaging of changes of gingival masses. **(A)** RT dose distribution image. **(B)** Before CRT (62 mm×53 mm×64 mm). **(C)** CRT and crizotinib for 1 week (50 mm×32 mm×42 mm). **(D)** Adjust the radiotherapy area (gingival mass: 8mm×27mm×46mm). **(E)** CRT and crizotinib for 1 month (undetectable tumor size). **(F)** Oral crizotinib for 2 months (efficacy evaluation as CR).

(NCCN) and previous reports recommend surgery as the primary treatment for GSCC. Moreover, radical neck dissection is the standard treatment for metastatic lymph nodes. For advanced GSCC patients who decline or cannot tolerate surgery, concurrent or sequential chemoradiotherapy is an alternative (6, 7).

With the development of precision medicine, the activation of the epidermal growth factor receptor (EGFR) is observed in HNSCC. The EGFR becomes one of the promising molecular targets for treating solid tumors including it. Cetuximab is an EGFR-targeted monoclonal antibody that improved the OS of HNSCC patients in both locally advanced and recurrent/metastatic settings, which was approved to be used as the standard treatment option in in the USA and Japan (8). The overexpression of the vascular endothelial growth factor (VEGF) in HNSCC also attracts people's attention, while most studies did not show clinical benefit of angiogenesis inhibitors such as bevacizumab, tyrosine kinase inhibitors, and endostatin (9).

The MET proto-oncogene encodes for receptor kinase c-Met and binds to MET's ligand hepatocyte growth factor. Binding of the ligand to the receptor induces dimerization and activates the receptor, and recruits proteins involved in signal transduction like Grb2, Shc, Src, and phosphoinositide 3' kinase. This, in turn, leads to downstream cascades in pathways involved in cell apoptosis and cellular motility and invasion. Aberrant MET signaling drives the tumor growth through increased cell proliferation, survival, invasion, and metastasis (10, 11). Abnormal activation of MET involves MET exon 14 skipping mutation, MET amplification, and MET protein overexpression. Point mutations, deletions, and insertions might lead to exon 14 skipping mutation (12).

Several small-molecule TKI inhibitors targeting MET are being evaluated for the treatment of patients who

TABLE 1 The excellent response to crizotinib in some rare tumor with MET mutation.

Case	Gender	Age(years)	Tumor	MET mutation	OP	CT	RT	Achieve PR/CR(months)
1 (17)	M	53	Tongue Squamous-Cell carcinoma	exon 14-altered	YES	YES	YES	CR,1
2 (18)	F	41	Intrahepatic cholangiocarcinoma	EHBP1-MET Fusion	NO	NO	NO	PR,1
3 (19)	F	66	Sinonasal undifferentiated carcinoma	amplification	YES	YES	YES	PR,1
4 (20)	M	51	papillary renal carcinoma	amplification	YES	NO	YES	PR,3
5 (21)	F	19	Histiocytic sarcoma	exon 14-altered	NO	YES	YES	PR,1
6 (22)	M	51	Gastric cancer	amplification	NO	YES	NO	CR,2
7 (23)	F	59	Carcinoma of unknown primary	amplification	NO	YES	NO	PR,7
8 (24)	F	55	Gallbladder cancer	amplification	YES	YES	NO	PR,2

Op, operation; CT, chemotherapy; RT, radiotherapy; PR, partial remission; CR, complete remission.

have MET exon 14 skipping mutations. These drugs include nonselective type 1a inhibitors (e.g., crizotinib) and selective type 1b inhibitors (e.g., tepotinib, savolitinib, and capmatinib). Tepotinib has durable antitumor activity in patients with advanced NSCLC with MET exon 14 skipping mutations (13). Savolitinib yielded promising activity and had an acceptable safety profile in patients with pulmonary sarcomatoid carcinoma and other NSCLC subtypes positive for METex14 skipping alterations (14). Crizotinib is approved for the treatment of ALK- or ROS1-rearranged advanced NSCLCs. Apart from its activity against ALK and ROS1, it has potent activity against MET and low nanomolar potency in cell lines that harbor MET exon 14 alterations (15). A study showed that crizotinib is active in advanced NSCLC patients with MET exon 14-altered (16). According to the literatures of the past 10 years (Table 1), the excellent response to crizotinib in some rare tumor with MET mutation provides a basis for treatment of this patient.

In this case, the elderly patient with an advanced sarcomatoid variant of gingival squamous cell carcinoma and lung metastases was intolerant to surgery. Considering the rare tumor pathological type, NGS of the tumor tissue was performed. The result was unexpected, which revealed the presence of oncogenic mutation c.3028+3A>G in the MET gene, exon 14. Therefore, we combined crizotinib targeted therapy with concurrent chemoradiotherapy. After crizotinib treatment, her clinical symptoms promptly improved without obvious adverse reactions.

At the first visit, the patient had a high tumor load, which seriously affected oral intake. An Eastern Cooperative Oncology Group performance status of 3, a Nutrition Risk Screening 2002 score of 5, and a body mass index of 17.5 kg/m^2 indicated nutritional risk. During the treatment, we administered enteral nutritional support to the patient with nasal feeding tubes and ensured oral hygiene during radiotherapy to avoid secondary infection caused by necrotic tissue. The patient's nutrition index improved significantly that she could tolerate chemoradiation. There was significant tumor shrinkage and necrosis through the course of the treatment. The patient needed assurances about the effectiveness and remission of the treatment at this point. To avoid affecting the biological dose effect in the target area and protecting normal tissues as much as possible due to lack of oxygen and the occupying effect of necrotic tissues, we closely observed the changes of tumor tissues during the treatment, cleaned up the necrotic tissues in time, and repositioned and outlined the target area to make the plan, thus ensuring the treatment effect.

The patient reached CR in 2 months through comprehensive treatment. This case provides strong evidence for the application of crizotinib-targeted therapy for sarcomatoid lesions of advanced gingival squamous cell carcinoma with MET gene mutations, and provides new diagnostic ideas and inspiration for the comprehensive treatment of advanced gingival cancer. For patients with clinically rare tumors and large tumor loads and elderly patients who cannot tolerate surgery and whose surgery may lead to aesthetic impairment, precision testing to find therapeutic targets combined with simultaneous radiotherapy and chemotherapy and other comprehensive treatments may provide patients with improved quality of life and survival benefits.

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.

Ethics statement

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

ZS and BX contributed equally to this work and share first authorship. MZ conceived and designed the research. ZS, BX, SX, YM, and XZ performed the research and/or analyzed data. ZS, BX, and MZ wrote the paper. All authors contributed to the article and approved the submitted version.

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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

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Case report: A novel reciprocal ROS1-CD74 fusion in a NSCLC patient partially benefited from sequential tyrosine kinase inhibitors treatment

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Background: The clinical significance of majority oncogenic novel fusions is still unknown due to scarcity. Reciprocal *ROS1* translocation is a rare form of *ROS1* fusion and has not yet been clearly analyzed.

Case presentation: A 44-year-old Chinese woman with a large dimension in the left lobe of the lung was admitted to the hospital with IVB lung adenocarcinoma. It was discovered that intron 28 of *ROS1* and intron 6 of *CD74* produced a unique reciprocal *ROS1* rearrangement. In addition, the dual *CD74-ROS1* fusions were discovered using the RNA next-generation sequencing (NGS) findings. Although benefiting from crizotinib and lorlatinib sequential treatment, the overall prognosis of the patient was relatively poor, whose progression-free survival was 4 and 5 months for crizotinib treatment and lorlatinib treatment, respectively.

Conclusion: In summary, a novel *ROS1-CD74* fusion identified by DNA NGS was translated into dual *CD74-ROS1* transcripts. Furthermore, this patient with non–small cell lung cancer benefited from consecutive tyrosine kinase inhibitor therapy. Our discovery broadened the range of targetable ROS1 fusions and underlined the importance of sequential DNA and RNA sequencing in identifying uncommon but beneficial fusions, which eventually bring benefits to the patients.

KEYWORDS

ROS1, sequencing, inhibitor, sequential detection, NGS

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Introduction

Approximately 2% of East Asian patients with non–small cell lung cancer (NSCLC) have *ROS1* rearrangement, which has defined a unique molecular subtype (1). The U.S. Food and Drug Administration had authorized a number of targetable medications that target this kind of aberration. For instance, crizotinib, lorlatinib, and entrectinib are granted in patients with NSCLC carrying *ROS1* fusions (2–4). The clinical application of these targeted drugs offers a significant improvement in both survival and quality of life of patients with cancer. With the development of precision medicine, DNA NGS is quickly replacing fluorescence *in situ* hybridization (FISH) and Immunohistochemistry (IHC) as the primary method for identifying oncogenic fusions and may offer more detailed information. More than 40 *ROS1* partners have been found by NGS thus far (5).

Despite the individuals with *ROS1* rearrangements responding dramatically to crizotinib therapy, a prior study showed that the response time differs among patients with different clinical and genetic features (6). To choose the appropriate therapeutic approaches, it is crucial to recognize druggable ROS1 fusions. Previous research rarely reported single reciprocal ROS1 fusions, which kept ROS1 as the 5′ fusion gene without their counterpart of the 3′ ROS1 fusion variant (7). Although it occurs very rarely, research on this uncommon ROS1 fusion is vital to broaden the genetic landscape of usable ROS1 fusions and to provide personalized precision medicine.

In this study, for the first time, a non-smoker female patient with NSCLC with a novel reciprocal ROS1-CD74 fusion without tyrosine kinase domain was discovered in the DNA NGS, which could be transcribed into dual CD74-ROS1 fusions at transcription level. Furthermore, the patient benefited from sequential crizotinib and lorlatinib therapy.

Case presentation

This case report was approved by the Ethics Committee of the Anning First People's Hospital 2022-008(others)-01. A 44-year-old Chinese female patient was admitted to the hospital with paroxysmal abdominal pain in October 2020. Chest computed tomography (CT) revealed the shadow of a mass in the left lobe of lung. IVB lung adenocarcinoma was diagnosed on the basis of the combination of pathological results of biopsy specimen and CT (Figures 1A, B).

To explore the therapeutic opportunities, a comprehensive molecular profiling of tumor biopsy specimen from the lung was submitted for NGS with an 825 cancer-related gene DNA panel (Onco Panscan^{TM}) at Genetron Health, Inc. (Beijing, China). A novel reciprocal *ROS1* rearrangement generated by intron 28 of *ROS1* and intron 6 of *CD74* was identified by sequencing (Figure 2A). At the same time, three gene alterations co-existent with the novel *ROS1* fusion were also detected in primary tumor tissue samples. They were *SETD2* p.I1476Lfs*7 (variant allele frequency (VAF), 20.08%), *TP63* p.H247R (variant allele frequency (VAF), 19.49%), and *ACVR2A* p.V433del (variant allele frequency (VAF), 16.38%). The gene fusion was confirmed by FISH with *ROS1* break-apart probe (Figure 2B). Interestingly, dual functional fusions of *CD74-ROS1* (E6:E32) and *CD74-ROS1* (E6:E35) were presented at the transcriptional level by targeted RNA sequencing (Fusioncapture^{TM}, Genetron Health) (Figure 2C), which was confirmed by Sanger's sequencing (Figure 2D).

Crizotinib was firstly administrated on the basis of the positive *ROS1* fusion result by sequencing. Compared with the naive period, CT scan revealed a significant decrease in lung focus after 2 months treatment (16 × 16 m to 11 × 11 mm). However, the patient developed resistance to crizotinib after 4 months reflected by bone metastasis. Because of her own physical condition and other reasons, the patient did not take

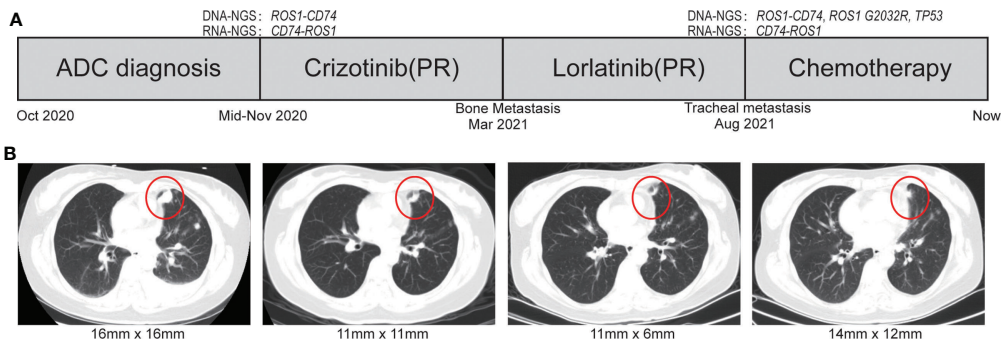

FIGURE 1
The treatment scheme and representative CT scan images during treatment. **(A)** Treatment scheme. **(B)** The representative CT scan of during treatment courses. ADC, adenocarcinoma; SD, stable disease; PR, partial response.

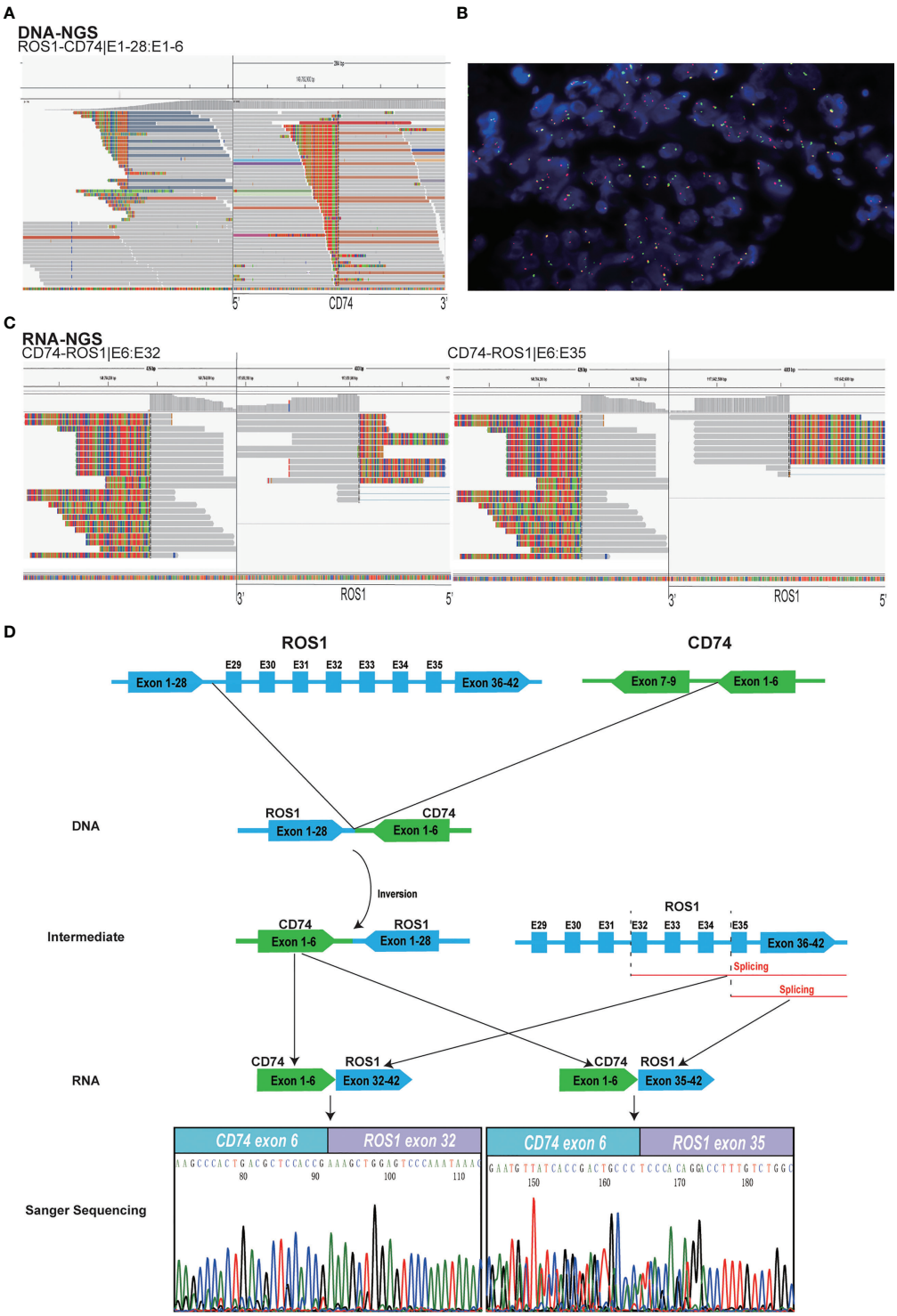

FIGURE 2
Identification of a novel ROS1 fusion. **(A)** DNA NGS analysis of the genomic ROS1-CD74 novel fusion. **(B)** FISH analysis showed fused yellow signals (negative signal), single green signals (positive signal), and single red signals (positive signal) in the patient's specimen. **(C)** RNA NGS analysis of the dual CD74-ROS1 fusions at transcript level. **(D)** Possible schematic diagram of ROS1-CD74 fusion detected by DNA-based NGS, but CD74-ROS1 fusions were identified by RNA-based NGS. NGS, next-generation sequencing; TKI, tyrosine kinase inhibitor; FISH, fluorescence *in situ* hybridization.

a puncture or liquid biopsy sample for genetic testing to determine the mechanism of resistance of crizotinib. At the patient's request, the patient immediately voluntarily received the third-generation *ROS1* tyrosine kinase inhibitor (TKI) lorlatinib treatment. The great treatment outcome was reflected by the significant decrement of the tumor size (16 × 16 mm to 11 × 6 mm). Unfortunately, the lorlatinib treatment had to be discontinued due to the trachea metastasis after 5 months therapy (Figures 1A, B). DNA NGS was performed on the metastasis tumor specimen after resistance to the lorlatinib, and the novel reciprocal *ROS1-CD74* fusion was detected again, which was also confirmed by FISH result (Figures 1A, 2B). In addition, except the three gene variations in the primary tissue of the tumor that were still detected, two other mutations, *TP53* p.R248W (12.3%) and *ROS1* p.G2032R (4.5%), were newly discovered in the puncture samples after lorlatinib resistance. Among which, *ROS1* G2032R has been reported to be a potential drug resistance site of crizotinib and lorlatinib. The patient was subsequently treated with chemotherapy but sadly died of tumor progression in May 2022.

Here, a novel reciprocal *ROS1-CD74* fusion identified by DNA NGS, which transcribed into dual *CD74-ROS1* fusions (E6:E32; E6:E35) at the RNA level, showed robust but unsustainable response to sequential crizotinib and lorlatinib administration. To our knowledge, this is the first report of a reciprocal *ROS1-CD74* fusion that did not have an intact kinase domain in DNA NGS that could benefit from *ROS1* inhibitor; the mechanism of the drug response to this rare fusion has also been elucidated.

It is frequently believed that the presence of an intact kinase domain is necessary for kinase inhibitors' treatment identified by DNA sequencing. Reciprocal fusions with incomplete kinase domain were occasionally seen alone in patients with NSCLC and usually considered as non-functional (8). Xu et al. reported that a patient with lung adenocarcinoma carried a *ROS1-ADGRG6* fusion at the DNA level and responded to crizotinib (9). However, this fusion was generated after *EGFR*-TKI resistance and had not been verified by RNA NGS; the mechanism of drug response also had not been studied. For the first time, we described the detection of a previously unreported reciprocal fusion of *ROS1-CD74* as a driver event in a patient with lung cancer. In this patient, exons 1–28 of *ROS1* were fused to exons 1–6 of *CD74* in the opposite direction at the chromosomal level. The raw data were queried, and none of the reads supporting the classical form of *CD74-ROS1* fusion was confirmed. Interestingly, this reciprocal form fusion could be transcribed into rare form of dual *CD74-ROS1* (E6:E32; E6:E35) fusions. Our result proved that the novel reciprocal fusion may be functional and should not be ignored during DNA NGS detection. This study also emphasized that sequential DNA and RNA sequencing could provide more precise evidence and optimize the targeted therapy strategy.

Although different *ROS1* fusion partners have been already identified, few data are currently addressed on how different fusion partners affect the efficacy of *ROS1*-TKIs. A previous study proved that the patients carried single non–*CD74-ROS1* fusions could get better prognostic outcomes than those with *CD74-ROS1* fusions during crizotinib treatment (6), whereas the studies on the efficacy of *ROS1*-TKI in patients with two *ROS1* fusions are rare and the conclusions are conflicting. Lan et al. identified a patient with NSCLC with non-reciprocal/reciprocal *ROS1* rearrangement (*ROS1-FBXL17* fusion co-existing with *CD74-ROS1* fusion), who obtained an excellent response to crizotinib, and the progression-free survival (PFS) was up to 17 months (10). However, in another study, a patient with lung adenocarcinoma harboring complex *ROS1* fusions (*GK-ROS1* and *SDC4-ROS1*) showed resistance only 8 months after crizotinib therapy (7). These inconsistent findings suggest that further investigations are needed to explore the efficacy of *ROS1* inhibitors in dual *ROS1* fusion. Furthermore, the reciprocal genomic ROS1-CD74 fusion was reported before in the NSCLC; however, whether it could transcribe into functional transcript and be targeted by TKI was unknown (11). In this case, we found a patient with NSCLC with novel dual reciprocal *ROS1* fusions (*CD74-ROS1*, E6:E32; *CD74-ROS1*, E6:E35), which is different from the non-reciprocal/reciprocal form of double *ROS1* fusions reported in other reports. The patient presented observably response to different *ROS1*-TKIs, but the effective duration was significantly shorter than the median PFS in patients with NSCLC (crizotinib, 4 months *vs.* 12.6 months; and lorlatinib, 5 months *vs.* 8.5 months, respectively). The reason of inferior response in our case may partially attribute to the tumor heterogeneity. A previous study demonstrated that a single tumor cell could only contain one RNA fusion isoform and that patients with multiple isoforms had worse prognostic outcomes (12). The co-existence of *CD74-ROS1* (E6:E32) and *CD74-ROS1* (E6:E35) fusion in the patient represented the complicated tumor heterogeneity, which may acted as a poor predictive marker with *ROS1* translocations similar to non-reciprocal/reciprocal *ALK* translocation in patients with NSCLC (13).

In addition, the clinical significance of *SETD2* p.I1476Lfs*7 that co-mutated with *CD74-ROS1* translocations in this patient was unclear. Previous studies have shown that concomitant mutations can also lead to relatively poor response to crizotinib in patients carrying *CD74-ROS1* translocations. This may be one of the other reasons why our patient was relatively ineffective in receiving *ROS1*-TKI treatment. The efficacy of *ROS1* inhibitors in patients with dual *ROS1* fusions should be explored in the future.

Several limitations need to discuss. First, the tumor specimen after resistance to crizotinib was unavailable; therefore, the occurrence time of *ROS1* G2032R mutation is undetermined. In addition, although the concomitant mutations such as *TP53* R248W were detected in samples of patients resistant to loratinib treatment, the reason for the loratinib resistant mechanism is unknown. Given the limitation of patient number, the clinical

function of the dual *ROS1* fusions in cancer should be confirmed by further research.

In summary, a patient with NSCLC with novel reciprocal *ROS1-CD74* fusion, which could be transcribed into dual *CD74-ROS1* fusions was first identified and benefited from the sequential crizotinib and lorlatinib therapy. Detection of dual *CD74-ROS1* fusion may be associated with poor survival outcomes with *ROS1*-TKIs. Moreover, sequential DNA and RNA sequencing is essential to promote the fusion detection precision and to predict the benefit to targeted therapy.

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 authors.

Ethics statement

The studies involving human participants were reviewed and approved by Anning First People's Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

XZ and BW contributed to the experiment performing and manuscript writing. CW and CL participated to the data analysis. SW and RC provided the clinical samples and relevant information. TM and KW designed and optimized the experiment. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Authors BW, CW and TM are employed by Genetron Health Beijing Co. Ltd.

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Case report: Dramatic response to alectinib in a lung adenosquamous carcinoma patient harbouring a novel CPE-ALK fusion

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Lung Adenosquamous carcinoma (ASC) is a rare histological subtype of lung cancer accounting for 0.4%–4% of all lung cancers. ASC is generally considered to be an aggressive cancer with poor prognosis. There is no specific standard treatment for ASC, and current treatment of ASC is relied on the guideline for non-small cell lung cancer (NSCLC). To date, only sporadic canonical EML4-ALK fusions have been reported in ASC patients, and the efficiency of ALK-TKI is still unclear in non-canonical ALK fusion positive ASC patients. Here we describe the case of a stage IV ASC patient harboring a novel CPE-ALK fusion detected *via* 74 genes panel analysis. Interestingly, the TP53 was wild-type and no another somatic mutation was found within 74 genes. In addition, immunohistochemical staining (IHC) also supports an oncogenic role for the CPE-ALK fusion. Based on these findings, the patient received alectinib 600 mg twice daily. After 4 months on treatment the patients achieved a radiological partial response (PR) and his symptoms were significantly relieved. Imaging showed that lesions of the patient were reduced, and the clinical evaluation was partial response (PR). To the best of our knowledge, this is the first report of a dramatic tumor response to alectinib in a patient with ASC harboring a CPE-ALK fusion. In addition, targeted NGS analysis may improve detection of ALK fusion in routine practice.

KEYWORDS

lung adenosquamous carcinoma, NGS, CPE-ALK, alectinib, IHC

Abbreviations: ASC, Adenosquamous carcinoma; NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; PR, partial response; LUAD, lung adenocarcinoma; LUSC, squamous cell carcinoma; TKI, tyrosine kinase inhibitors; CT, Computed tomography; WHO, World Health Organization; IHC, immunochemistry; CEA, carcinoembryonic antigen; CA125, carbohydrate antigen 125.

Introduction

Adenosquamous carcinoma (ASC), a rare biphasic malignancy, consists of two morphologically distinct components, including Lung adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC) (1). Although ASC has worse prognosis than LUAD and LUSC, the standard treatment for ASC is currently not well defined, and therapeutic decisions are made according to the treatment guidelines of non-small cell lung cancer (NSCLC) (2). With the development of precision medicine, targeted therapies can be used as first-line therapy for advanced anaplastic lymphoma kinase (ALK) rearranged NSCLC, while there are limited data on the efficacy of ALK tyrosine kinase inhibitors (TKI) in ASC due to its rarity. Currently, only sporadic classical ALK fusion have been reported in ASC patients (3, 4). Although there are some ALK-positive cases and molecular profiling studies of ASC, ALK-TKI treatment for ASC patients with ALK rearrangement has been reported in only a handful of cases (5), and no non-classical ALK fusions have been reported in ASC. Here we report the case of a 71-year-old woman with stage IV ASC harboring a CPE-ALK fusion sensitive to alectinib, which highlights the importance of ALK-TKIs in ALK-positive ASC patients even in presence of non-canonical alterations.

Case presentation

A 71-year-old female was admitted to the hospital with a cough and sputum for more than two months. The patient had no history of smoking, drinking, hypertension or diabetes. A computed tomography (CT) scan showed that the middle lobe of the right lung was occupied by a mass near the hilum, with small nodules in the upper lobe of both lungs, accompanied by lymph node enlargement in area IV of the right neck (Figure 1A). Auxiliary examination of tumour-related markers showed the following: ferritin 180.7 ng/mL; carcinoembryonic antigen (CEA) 3.523 ng/mL; carbohydrate antigen 125 (CA125)106.341 U/mL. Lymph node puncture samples from area IV of the right neck were obtained for histopathological examination. Each slide was examined independently by two experienced pathologists based on the World Health Organization (WHO) classification criteria of lung cancer (1). The two components of adenocarcinoma and squamous cell carcinoma were both more than 10%. Pathology staining results showed TTF-1(+), Napsin A (+), CK5/6(+), CK7(+) and P63(-) (Figure 2). Based on pathological and imaging results, it was determined that the patient had stage IV (T1N3M1) lung adenosquamous carcinoma. Then, a 74 cancer-related gene NGS panel analysis was performed on the lymph node puncture sample by a CAP-certificated lab. The qualified DNA libraries were sequenced on Illumina NovaSeq6000 platform (Illumina, San Diego, CA) and generate 150 bp paired end reads. Base calls from Illumina NovaSeq6000 were conducted to FASTQ files. The software fastp (v.2.20.0) was used for adapter trimming and filtering of low-quality bases (6). The BWA-MEM (v.0.7.17) algorithm was performed to align to the reference genome (UCSC's hg19 GRCh37) (7). Duplicate reads from PCR were excluded using Dedup with Error Correct. SNVs/InDels were called and annotated *via* VarDict (v.1.5.7) (8) and InterVar (9), then the variants were filtered against the common SNPs in public database including 1000 Genome Project (Aug 2015) and Exome Aggregation Consortium (ExAC) Browser28 (v.0.3). CNVs and

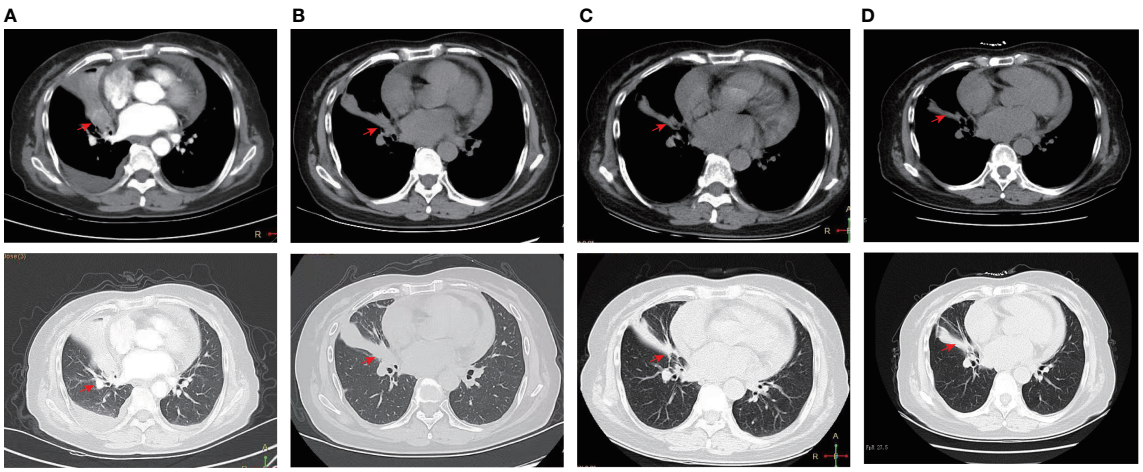

FIGURE 1
Dynamic imaging of lung lesions at different stages of treatment. **(A)** CT scan before alectnib treatment (March 1st, 2022); **(B)** CT scan after two months of alectinib treatment (April 22th, 2022); **(C)** CT scan after four months of alectinib treatment (June 29th, 2022); **(D)** CT scan after six months of alectinib treatment (June 29th, 2022); note: the red arrow marks the location of the lesion.

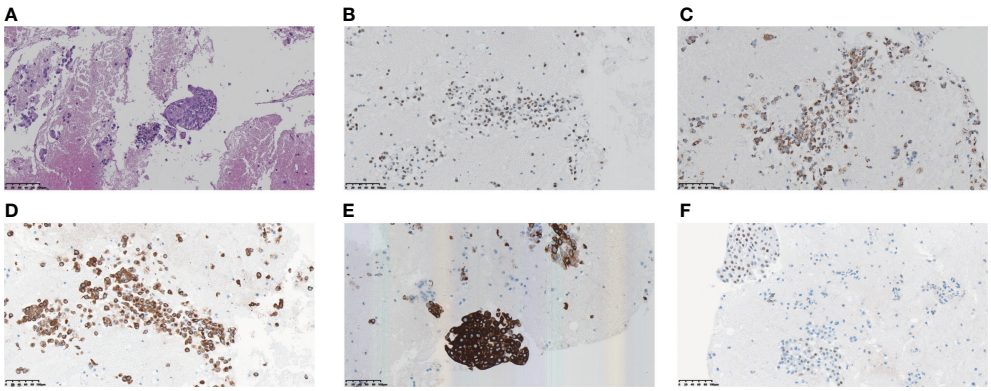

FIGURE 2
Morphology and immunohistochemistry confirmed the diagnosis of lung adenosquamous carcinoma by pathologists **(A)** H&E staining; **(B)** Positive staining of TTF-1 by immunochemistry; **(C)** Positive staining of Napsin A; **(D)** Positive staining of CK7; **(E)** Positive staining of CK5/6; **(F)** Positive staining of P63.

fusions were analyzed by CNVkit (dx1.1) (10) and factera (v1.4.4) (11), respectively. A novel CPE-ALK fusion was identified (Figure 3A), no other molecular alterations were found among the 74 genes analyzed (gene list was shown in Supplementary Table S1). The results revealed that a novel fusion was generated by the 1-6 exons of CPE (carboxypeptidase E) and exons 20-29 of ALK, this fusion retains the ALK kinase domain (Figure 3B). IHC also supports an oncogenic protein generation of the CPE-ALK fusion (Figure 3C). Based on the results above, the patient was started on alectinib 600 mg twice daily. Based on the above results, the patient was started on alectinib 600 mg twice daily from March 12th, 2022. After approximately 1 month of alectinib treatment on April 22th, 2022, a CT scan showed that the lesion in the middle lobe of the right lung had decreased from 2.9 cm to 2.3 cm, that the lymph nodes had decreased from 1.8 cm to 1.2 cm, but that the lesions in the upper lobe of the right lung had remained unchanged, at 0.3 cm (Figure 1B). CT scan on June 29th, 2022, which was after four months of alectinib treatment, showed that the lesions in the middle lobe of the right lung were reduced to 1.8 cm, the lymph nodes were reduced to 1.0 cm, but the lesions in the upper lobe of the right lung were still 0.3 cm; the cough and sputum symptoms of the patient had also improved significantly. The clinical evaluation was partial response (PR) (Figure 1C). The patient's condition was stable at the most recent follow-up on September 14, 2022. (Figure 1D). Besides that, there were no extreme drug-related side effects in this patient. Her treatment is ongoing, and we are continuing to follow up with the patient.

Discussion

With an estimated 2.2 million new cancer cases and 1.8 million deaths, lung cancer was the second most commonly diagnosed cancer and the leading cause of cancer death in 2020 (12). ASC is defined as combining both components of adenocarcinoma and squamous cell carcinoma, with each component composing more than 10% (1). ASC has more aggressive behaviour and a worse prognosis than adenocarcinoma or squamous cell carcinoma alone (13).

Currently, there is no unified treatment for ASC, and routine care options rely on NSCLC guidelines. Treatment of lung cancer has rapidly evolved as a result of the discovery of molecular targets and recent advances in tyrosine kinase inhibitors (TKIs) against EGFR mutations and ALK fusions (14). Because EGFR mutation is the most common genomic anomaly in ASC, some studies (4, 15) have focused on the efficiency of EGFR-TKIs in EGFR-positive ASC patients and found that ASC patients had similar efficacy to EGFR TKI compared with adenocarcinoma. Despite the presence of ALK-positive cases and molecular profiling studies of ASC (2, 4, 16, 17), only a small number of ALK-TKI therapies for EML4-ALK fusion ASC patients have been reported (5), no non-classical ALK fusions have been reported in ASC. Echinoderm microtubule-associated protein-like 4 (EML4)-ALK is the canonical and most common ALK gene arrangement found in NSCLC, by which multiple EML4 breakpoints fuse in frame with the kinase domain of ALK (18). By applying NGS, over 90 ALK fusion partners have been identified in NSCLC, some ALK fusions less commonly reported in NSCLC (i.e., noncanonical ALK fusions) include kinesin family member 5B (KIF5B)-ALK, TNIP2-ALK and so on (19, 20). Although ALK-TKIs have dramatically expanded the therapeutic landscape of ALK-positive NSCLC, it remains controversial whether patients with noncanonical ALK rearrangements benefit from targeted therapy as much as those with typical ALK rearrangements. For instance, the conclusions of two studies of survival analysis between patients with classical and nonclassical fusions are contradictory (21, 22). The substantial question, of whether noncanonical fusions can unequivocally produce the corresponding transcripts or response to ALK-TKI, is still uncertain (23). Based on the ALK

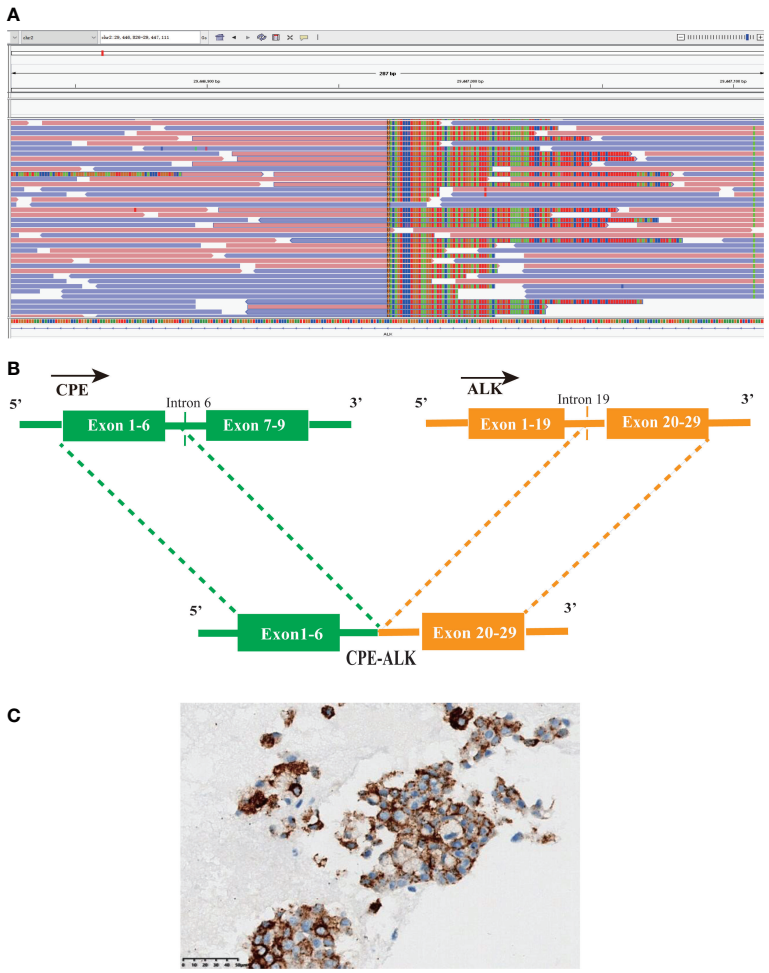

FIGURE 3
A novel CPE-ALK fusion was discovered in the ASC patient; **(A)** Sequencing reads of ALK were shown by the Integrative Genomics Viewer. **(B)** Illustration of CPE-ALK fusion. **(C)** IHC staining indicated the expression of ALK, the specimen was stained by IHC with the anti-ALK (D5F3).

rearrangements found by the DNA assay, plenty of the breakpoints for ALK are in intron 19 (22). Interestingly, we were able to find breakpoints at intron 19 of ALK and intron 6 of CPE at the DNA level in this patient, however, the CPE-ALK start in phase 1 of the first codon of exon 20 may result in the ALK gene being out of frame (Supplementary Figure S2). Furthermore, we were unable to guarantee that a fusion is transcribed due to the technical limitations of DNA sequencing. whereas RNA sequencing can accurately identify fusion transcripts, which can supplement fusion detection more effectively (24, 25). Due to insufficient samples, we were unable to perform RNA sequencing. However, the positive expression of ALK IHC and the same expression levels of EML4 and CPE in LUAD and LUSC indicate that CPE-ALK is oncogenic (Supplementary Figure S1). This is only a single-patient case report, and more cases are required to investigate the association between ALK fusion and survival benefit in ASC patients. ALK IHC and RNA-NGS (when available) are indispensable complements to DNA NGS for the precise molecular characterization of oncogenic fusions. Additional study and a larger sample size are required to appreciate the biological function of the CPE-ALK or non-classical ALK fusion gene.

The clinical course of ALK-positive NSCLC patients treated with chemotherapy versus ALK-inhibitors differs significantly (26, 27). This observation could be explained by genetic heterogeneity of ALK-positive tumors. The impact of co-mutations on the treatment of ALK-positive patients has been the focus of researchers. TP53, the most prevalent concomitant mutation, has also been shown to be a negative prognostic factor in EGFR mutation NSCLC patients (28). Likewise, ALK-rearranged NSCLC co-occurring TP53 mutations predict an unfavorable outcome of systemic therapy (29), it also tends to suggest that this patient may have a better prognosis.

In conclusion, To the best of our knowledge, this is the first description of a CPE-ALK fusion identified in a patient with

ASC who is sensitive to alectinib. this case also expands the spectrum of ALK fusions and provides valuable information on response to alectinib in ASC patients with CPE-ALK fusions, and further investigation is warranted. Overall, Targeted NGS analysis may improve detection of ALK fusions in routine practice.

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.

Author contributions

YQ, FL, YT and QQD prepared the manuscript and the literature search. YQ and FL reviewed and edited the manuscript. YQ treated and observed the patient. FL performed the histopathological, immunohistochemical examinations. All authors contributed to the article and approved the submitted version.

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Conflict of interest

YT, QD, and QZ are employed by Jiangsu Simcere Diagnostics Co., Ltd. YT, QD, and QZ are employed by Nanjing Simcere Medical Laboratory Science Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.998545/full#supplementary-material

SUPPLEMENTARY FIGURE 1
CPE and ALK expression level in LUAD and LUSC. A.CPE expression in LUAD and LUSC, T for tumor(red), N for normal(gray); B. EML4 expression in LUAD and LUSC, T for tumor(red), N for normal(gray)

SUPPLEMENTARY FIGURE 2
Exon 6 information in CPE **(A)** and exon 20 information in ALK **(B)**; Schematic of CPE-ALK may cause the ALK gene to be out of frame **(C)**.

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EWSR1::SMAD3-rearranged fibroblastic tumor: A case with twice recurrence and literature review

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EWSR1::SMAD3-rearranged fibroblastic tumor is a recently described entity that mostly occurs in acral locations. Only 15 cases have been reported in the English literature, with a wide age range and marked female predominance. The most common sites are the foot, followed by the hand and the distal lower leg. There are four cases that recurred locally during 5–120 months of follow-up, with no metastases to date. Herein, we presented a case of *EWSR1::SMAD3*-rearranged fibroblastic tumor that recurred twice in a 20-year-old man. The patient presented with a second recurrent painful nodule in the left plantar of the second toe. Grossly, the lesion was pale solid and well-defined, measuring 9 × 8 × 9 mm in size. Histological examination revealed a monomorphic spindle cell tumor composed of cellular fascicles of bland fibroblasts in a collagenous to myxoid stroma with low mitotic activity, which evoked a wide spectrum of differential diagnoses. Immunohistochemically, the tumor cells were diffusely and strongly positive for ERG while negative for S100, α-SMA, CD34, and other vascular markers. An unbalanced rearrangement of *EWSR1* was demonstrated by fluorescence *in situ* hybridization (FISH), and a gene fusion between *EWSR1 exon 7* and *SMAD3 exon 6* was confirmed by RT-PCR and Sanger sequencing. This case recurred twice within 6 years with no sign of further relapse and metastasis at another 9-month follow-up since the last surgery, indicating that this tumor was benign but prone to local recurrence. Nevertheless, more cases and further studies are needed to better interpret the biological behavior of this new entity.

KEYWORDS

***EWSR1*, *SMAD3*, fibroblastic tumor, ERG, case report**

Introduction

EWSR1::SMAD3-rearranged fibroblastic tumor is currently classified as an emerging entity in the fifth edition of the WHO classification of Tumors of Soft Tissue and Bone (1). Only 15 cases have been reported in the English literature (2–6). The tumor mostly occurs in superficial soft tissue at the extremities, with a marked female predominance. They appear to be benign tumors but are prone to local recurrence. The differential diagnoses are challenging, including a series of benign, borderline, and malignant fibroblastic lesions at acral sites. Herein, we present a male case of *EWSR1::SMAD3*-rearranged fibroblastic tumor with twice recurrence in the left plantar of the second toe. The clinical, histopathological, and molecular characteristics of this tumor along with differential diagnoses are summarized to increase awareness of this new entity among pathologists.

Case description

The patient was a 20-year-old man of Chinese descent presenting with a painful nodule in the left plantar of the second toe for 1 month. For past history, the patient had surgery twice on the same site of the toe 6 years ago and relapsed 1 month later, and there was no other treatment except surgery. Unfortunately, the pathological data were not available, and the operating margin was not known. For this time, the nodule was subcutaneous and palpable. The third surgical resection was performed, and a diagnosis of "low-grade fibromyxoid sarcoma" was made in a local hospital. For further medical attention, the pathological materials were referred to our department for consultation. The clinical follow-up period was 9 months since the last surgery. The timeline is summarized in Figure 1.

Diagnostic assessment

Clinicopathological characteristics

Grossly, the lesion was pale solid, and well-defined measuring 9 × 8 × 9 mm in size. Histological examination revealed a relatively well-defined and nodular tumor without a capsule (Figure 2A). The tumor was composed of short fascicles of uniform spindle cells in a collagenous to myxoid stroma. The tumor cells had pale eosinophilic cytoplasm and elongated nuclei with finely dispersed chromatin and small inconspicuous nucleoli (Figures 2B, C). Mitotic figures were rare.

Immunohistochemistry

Immunohistochemical staining was performed on 4-μm formalin-fixed, paraffin-embedded sections, which were immersed in a 10-mM sodium citrate buffer (pH 6) for 20 min at 97°C for antigen retrieval. The following antibodies were used: pankeratin (AE1/AE3, 1:50; Dako, Glostrup, Denmark), epithelial membrane antigen (EMA) (E29, 1:200; Dako), bcl-2 (clone 124, 1:100; Dako), CD34 (QB End 10, 1:100; Dako), α-smooth muscle actin (α-SMA) (1A4, 1:200; Dako), desmin (clone D33, 1:200; Dako), S100 protein (polyclonal, 1:800; Dako), SOX10 (polyclonal, 1:200; Santa Cruz Biotechnology, Dallas, TX, USA), H3K27me3 (RM175, 1:2,000, RevMAb, South San Francisco, CA, USA), MUC4 (8G7, 1:100; Abcam, Cambridge, UK), ERG (EPR3864, prediluted; Roche, Basel, Switzerland), SATB2 (SATBA4B10, 1:100, ZSGB), and Ki67 (MIB1, 1:150; Dako).The positive, blank, and negative controls were also performed in parallel.

By immunohistochemistry, the spindle tumor cells showed diffusely and strongly nuclear positive for ERG (Figure 2D), while S100, α-SMA, CD34, desmin, SATB2, EMA, MUC4, and other spindle cell tumor and vascular markers were negative. Ki67 index was about 15% in tumors.

Molecular genetic studies

Fluorescence *in situ* hybridization

According to the manufacturer's instructions, fluorescence *in situ* hybridization (FISH) was performed on formalin-fixed, paraffin-embedded tissue sections. Unstained 4-μm sections were placed on electrostatically charged slides and then

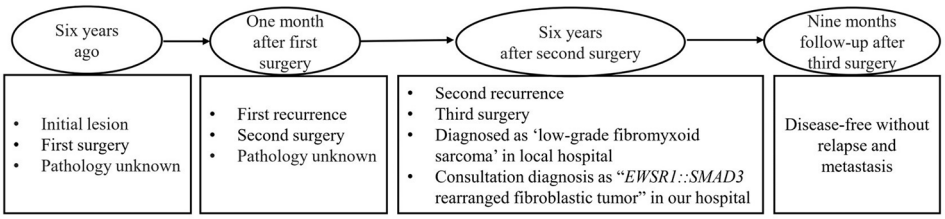

FIGURE 1
The timeline showed the clinicopathological process.

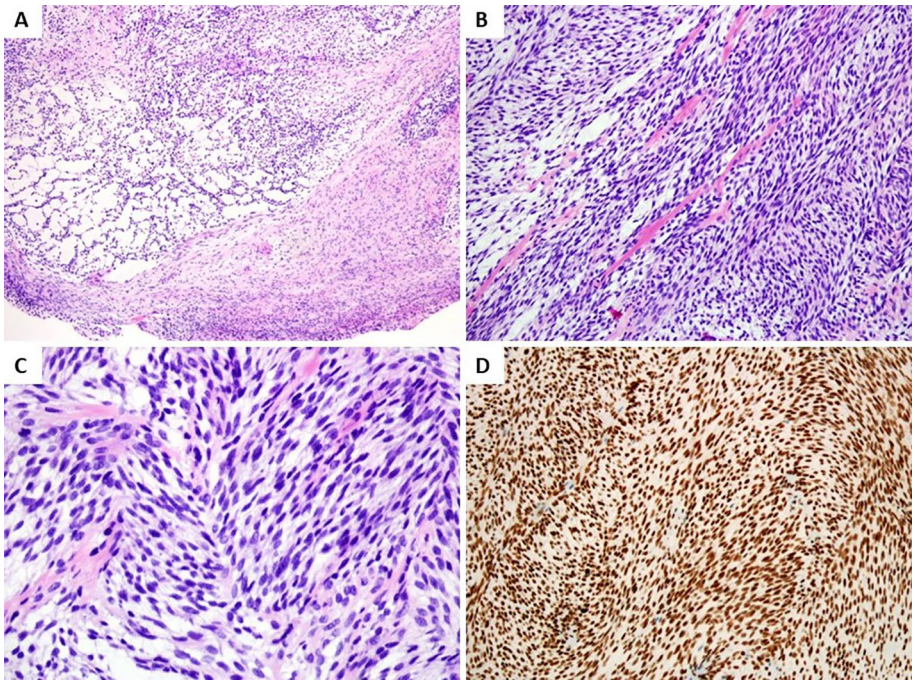

FIGURE 2
Histologic and immunohistochemical features. **(A)** Histological examination revealed a relatively well-defined, nodular tumor without capsule (H&E, low magnification). **(B)** Monomorphic spindle cell tumor composed of cellular fascicles of bland fibroblasts with hyalinization and mucoid degeneration (H&E, medium magnification). **(C)** Spindle cells showed elongated nuclei with finely dispersed chromatin and small inconspicuous nucleoli (H&E, high magnification). **(D)** Tumor cells showed diffuse nuclear staining for ERG (medium magnification).

evaluated using an *EWSR1* (22q12) rearrangement in a dual-color, break-apart probe and an *EWSR1* (22q12, green) and *SMAD3* (15q22, red) fusion probe (LBP, Guangzhou, China). Under fluorescence microscopy, signals from 200 non-overlapping nuclei were counted. The cutoff level for the score as positive was when at least 20% of the nuclei showed a break-apart signal and/or loss of telomeric part or fusion signal (yellow).

The FISH assessment demonstrated an unbalanced rearrangement of *EWSR1* with loss of the telomeric part (Figure 3A), combined with tumor cells, diffuse nuclear staining for ERG the diagnosis of *EWSR1::SMAD3*-rearranged fibroblastic tumor was considered. To identify the fusion partner, *EWSR1* and *SMAD3* fusion was detected by FISH (Figure 3B), and the fusion was confirmed by RT-PCR and Sanger sequencing between *exon 7* of *EWSR1* and *exon 6* of *SMAD3* (Figure 3C).

Follow-up study

Based on the morphology, immunohistochemistry, and gene analysis, the final diagnosis was *EWSR1::SMAD3*-rearranged fibroblastic tumor. Treatment of the tumor was wide excision. To date, the patient is disease-free without relapse and metastasis for 9 months after the last surgery.

Discussion

EWSR1::SMAD3-rearranged fibroblastic tumor is a new entity that mostly occurs at the extremities, initially reported in the foot of a 1-year-old infant, presenting with an ill-defined dermal and subcutaneous nodule (2). Thereafter, another series described similar cases and suggested the name *EWSR1::SMAD3*-rearranged fibroblastic tumor (3). However, only 15 cases have been reported in the English literature to our knowledge, of which the median age was 39 years (range 1–68 years) with a female predilection (10/15, 67%). The most common affected site was the foot (10 cases), and the other three cases were in the hand and two in the distal lower leg. Clinically, all tumors presented as a dermal or subcutaneous nodule or mass ranging in size from 1 to 2 cm (mean, 1.13 cm; median, 1.1 cm). Four cases recurred locally (4/6, 67%) during 5–120 months of follow-up mostly due to incomplete excision, but without metastases to date (2–6). Our case recurred twice within 6 years, indicating that a long-term follow-up needs to be warranted. The series of cases have long-term follow-up information in only seven cases, including what we report in this case, five cases of recurrence, and only two cases without relapse in the follow-up of 7 and 10 years later. The remaining six cases with follow-up information were newly reported, and

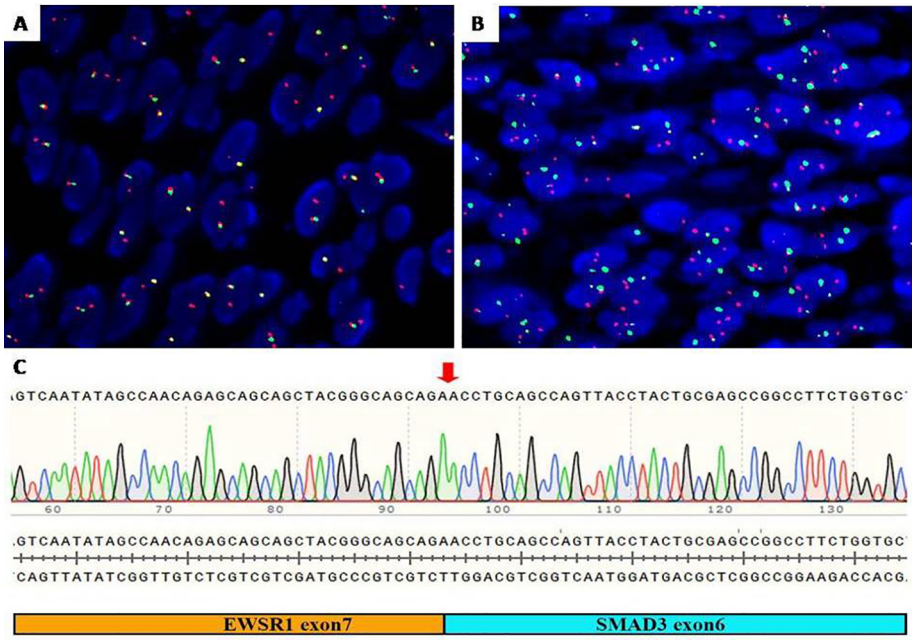

FIGURE 3
Molecular detection. **(A)** FISH assay showed an unbalanced rearrangement of *EWSR1* with loss of the telomeric part (green). **(B)** FISH assay showed *EWSR1* (green) and *SMAD3* (red) fusion signal (yellow). **(C)** RT-PCR identified *exon 7* of *EWSR1* and *exon 6* of *SMAD3* gene fusion. FISH, fluorescence *in situ* hybridization.

more cases of follow-up information need to be collected on the biological behavior of this group of tumors. The data are summarized in Table 1.

Recently, another *EWSR1*::*SMAD3*-rearranged fibroblastic tumor reported in the bone was supported by imaging and histopathological features (7). The tumor was relatively large (8 cm) with aggressive radiological features and initially diagnosed as an unusual cartilaginous tumor, raising the possibility of chondrosarcoma (possibly dedifferentiated or mesenchymal). Histologically, focal cytological atypia and necrosis were observed. Immunohistochemically, the tumor cells showed very focal ERG nuclear expression. The lesion was treated with complete excision and has shown no signs of relapse over a 7-year follow-up period. Although *EWSR1*::*SMAD3*-rearranged fibroblastic tumor is currently considered benign in soft tissue, the biological behavior of the bone lesion cannot be inferred from a single case (8).

The cellularity and fascicular morphology of *EWSR1*::*SMAD3*-rearranged fibroblastic tumor may lead to confusion with other histologic mimics of both benign and malignant soft tissue tumors (9), including fibromatosis, cellular schwannoma, low-grade malignant peripheral nerve sheath tumor (MPNST), monophasic synovial sarcoma, and low-grade fibromyxoid sarcoma. This neoplasm was composed of uniform fibroblastic spindle cells without nuclear atypia, pleomorphism, prominent nucleoli, and mitotic activity. In some cases, a distinctive zonation pattern with acellular central hyalinization and peripheral areas of cellular spindle cell fascicles was present, especially in adults. Stippled dystrophic calcification was found in one-part cases (2, 4). In addition, our case also showed an alternative myxoid change of the stroma, which could be misdiagnosed as a low-grade fibromyxoid sarcoma and low-grade MPNST. Immunohistochemically, the tumor characteristically showed diffuse and strong nuclear staining of ERG and focal and weak expression of SATB2 in a small number of cases but was usually negative for S100, SMA, CD34, and other vascular markers, excluding the endothelial, neurogenic, and smooth muscle differentiation of neoplastic cells. The ubiquitous staining of ERG in the tumor is considered to be attributed to the overexpression of ERG, which was revealed by mRNA expression that was significantly upregulated at even higher levels than vascular neoplasms (2, 10).

SMAD3 is an important signal transducer in the *TGF-β/SMAD* signaling pathway, which is involved in extracellular matrix synthesis by fibroblasts (2, 11). *EWSR1* gene rearrangement has been found in many bone and soft tissue tumors (12, 13), including Ewing or Ewing-like sarcoma, low-grade fibromyxoid sarcoma, and spindle cell rhabdomyosarcoma, and *EWSR1*::*SMAD3*-rearranged fibroblastic tumor should be added to the list. *EWSR1* gene rearrangement was most common in Ewing or Ewing-like sarcoma; the tumor cells were uniformly small and round and expressed CD99, ERG, NKX2.2,

TABLE 1 Clinical features of cases with *EWSR1::SMAD3*-rearranged fibroblastic tumor.

Authors and year	Case	Age (years)/ sex	Location	Depth	Size (cm)	Treatment method	Follow-up (time)
Kao et al., 2018 (2)	1	1/M	Heel	Dermis and subcutis	1.0	Surgical resection	LR (14 months)
	2	61/F	Foot	Subcutis	2.0	Surgical resection	NA
	3	58/F	Toe	Dermis and subcutis	1.1	Surgical resection	LR (5 months)
Michal et al., 2018 (3)	4	5/F and 15	Hand-palm	Subcutis	1.2/0.3	Surgical resection, including primary and recurrence	LR (10 years) 18 years
	5	68/F	Interphalangeal joint of the thumb	Subcutis	1.5 × 0.7 × 0.5	Surgical resection	ANED (10 years)
	6	39/F	Calf	Subcutis	1 × 0.5 × 0.5	Surgical resection	ANED (7 years)
	7	34/F	Left foot-dorsal metatarsal aspect	Subcutis	1.1 × 0.8 × 0.5	Surgical resection	Recent case*
Zhao et al., 2019 (4)	8	24/M	Dorsum of the right foot	Subcutis	1 × 0.8 × 0.5	Surgical resection, including primary and recurrence	LR (24 months)
Foot et al., 2020 (5)	9	28/F	The distal phalanx of 2nd toe	Subcutis	1.5	Surgical resection	NA
Habeeb et al., 2021 (6)	10	27/F	Left lower extremity	Mostly superficial dermis	1.7 × 1.3 × 0.8	Surgical resection	Recent case*
	11	35/F	Left 4th/5th finger web space	Deeper dermis	0.7 × 0.3 × 0.2	Surgical resection	Recent case*
	12	45/F	Right plantar forefoot	Mostly superficial and dermal	0.9 × 0. 7 × 0.2	Surgical resection	Recent case*
	13	46/F	Left 4th toe	Superficial dermis	0.3 × 0.2 × 0.2	Surgical resection	Recent case*
	14	54/M	Left foot	Superficial dermis	0.5 × 0.3 × 0.3	Surgical resection	Recent case*
	15	57/F	Left 5th toe	Deep dermis/ subcutis	1.4 × 1.2 × 0.6	Surgical resection	Recent case*
	Current case	20/M	Left 2nd toe	Subcutis	0.9 × 0.9 × 0.8	Surgical resection, including primary and recurrence	LR (1 month and 6 years), ANED (9 months)
De Noon et al., 2021 (6)	Bone case	M/44	Right proximal tibia	intramedullary lesion	8	Neoadjuvant chemotherapy and surgical resection	ANED (7 years)

M, male; F, female; LR, local recurrence; NA, not available; ANED, alive without evidence of disease.
*Newly diagnosed cases as of press time.

and most differently, the fusion partners with *EWSR1* including *FLI-1*, *ERG*, and other candidate genes. Low-grade fibromyxoid sarcoma may have been an *EWSRI-CREB3L1* fusion, but MUC4 is highly sensitive and specific immunohistochemistry. Spindle cell rhabdomyosarcoma sometimes appeared in *EWSR1-TFCP2* fusion, combined with immunohistochemical markers such as desmin, myogenin, and MyoD1; the diagnosis can be confirmed.

Identification of *EWSR1::SMAD3*-rearranged fibroblastic tumor is critical to provide prognostic information and guide appropriate therapeutic decisions and most importantly avoid overtreatment (9, 14, 15). When encountered with uniform spindle cells in a collagenous to myxoid stroma, diffuse and strong ERG expression will render a clue for *EWSR1::SMAD3*-rearranged fibroblastic tumor. *EWSR1* rearrangement by FISH would help to give a correct interpretation. The data on the morphology, immunohistochemistry, and molecular genetic characteristics are summarized in Table 2.

In conclusion, *EWSR1::SMAD3*-rearranged fibroblastic tumor should be taken into consideration for the differential diagnosis when encountered with spindle cell lesions in acral sites with strong ERG expression only, and further molecular studies should be employed to confirm the diagnosis. More cases and further studies are needed to further understand the nature of this tumor.

TABLE 2 Pathological and genetic features of cases with *EWSR1::SMAD3*-rearranged fibroblastic tumor.

Authors and year	Case	Histomorphology	Immunohistochemistry					FISH	*EWSR1::SMAD3* Gene fusion	Gene fusion sites
			ERG	CD34	SMA	S100	SATB2			
Kao et al., 2018 (2)	1	Fibroblastic spindle cell	+	–	–	–	NA	Unbalanced rearrangement of SMAD3 and EWSR1	NGS	NA
	2	Fibroblastic spindle cell, central zone collagenized, and focal calcification	+	–	–	–	NA	EWSR1 gene rearrangement	NGS	NA
	3	Fibroblastic spindle cell, central zone collagenized	+	–	–	–	NA	EWSR1 gene rearrangement	NGS	NA
Michal et al., 2018 (3)	4	Spindle cell component located at the periphery and central hyalinization	+	–	–	–	Focal+	NA	NGS	NA
	5	Spindled cells with randomly intermingled hyalinized component	+	–	–	–	Focal+	NA	NGS	NA
	6	Spindled cells with randomly intermingled hyalinized component	+	–	–	–	NA	NA	NA	NA
	7	Spindle cell component located at the periphery and central hyalinization	+	–	–	–	NA	Unbalanced rearrangement of EWSR1	NGS	NA
Zhao et al., 2019 (4)	8	Bland spindle cells, stromal hyalinization, focal stippled calcification	+	–	–	–	Focal+	Unbalanced rearrangement of EWSR1	NGS	EWSR1 exon 7 and SMAD3 exon 6
Foot et al., 2020 (5)	9	Bland spindle cells and collagenous stroma	+	–	Focal +	–	NA	EWSR1 gene rearrangement	NGS	EWSR1 exon 7 and SMAD3 exon 5
Habeeb et al., 2021 (6)	10	Uniform spindled cells in a variably collagenous to myxoid stroma	+	–	–	–	NA	NA	NGS	EWSR1 exon 7 and SMAD3 exon 6
	11	Uniform spindled cells and small hyalinized areas	+	–	Focal +	–	NA	NA	NA	NA
	12	Uniform spindled cells and collagenous to myxoid stroma	+	–	–	–	NA	EWSR1 gene rearrangement	NA	NA
	13	Uniform spindled cells and small collagenous nodules	+	–	–	–	NA	NA	NA	NA
	14	Uniform spindled cells and myopericytomatous pattern focally	+	–	–	–	NA	NA	NGS	EWSR1 exon 7 and SMAD3 exon 6
	15	Uniform spindled cells and central hyalinization	+	–	–	–	NA	NA	NGS	EWSR1 exon 7 and SMAD3 exon 5
	Current case	Bland spindle cells and collagenous or myxoid stroma	+	–	–	–	–	Unbalanced rearrangement of EWSR1	FISH fusion probe and RT-PCR	EWSR1 exon 7 and SMAD3 exon 6
De Noon et al., 2021 (7)	Bone case	Spindle to oval cells and myxo-hyaline to chondroid foci	scattered +	–	–	–	NA	EWSR1 gene rearrangement	Whole-genome sequencing	NA

NA, not available; FISH, fluorescence in situ hybridization; NGS, next-generation sequencing.

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 authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board Committee of Xijing Hospital. Written informed consent for participation was not required for this study in accordance with the institutional requirements.

Author contributions

LY and LF wrote the initial draft of the manuscript. ZY analyzed the clinical aspects of the case. HC and ZW co-designed the study, collected the histopathological and molecular studies, interpreted the findings, and edited the manuscript. YL conducted the FISH analysis and interpreted the findings. DZ performed RT-PCR/direct sequencing. All authors contributed to the article and approved the submitted version.

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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.

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Commentary: Case report: Primary intraosseous poorly differentiated synovial sarcoma of the femur

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A Commentary on

Case report: Primary intraosseous poorly differentiated synovial sarcoma of the femur

by Pang K, Guo X, Jiang Y, Xu L, Ling L and Li Z (2022) *Front. Oncol.* 12:754131. doi: 10.3389/fonc.2022.754131

1 Introduction

We read with great interest the article by Dr. Pang and colleagues entitled "Case Report: Primary Intraosseous Poorly Differentiated Synovial Sarcoma of the Femur," published in *Frontiers in Oncology* (1). The authors concluded that the primary site of poorly differentiated synovial sarcoma (SS) in this patient was not soft tissue, but bone, and that misdiagnosis between SS and Ewing sarcoma (ES) had occurred. However, we had some concerns regarding the interpretation of this unusual case, which we would like to discuss with the authors.

2 Primary site diagnosis

Our first concern regards whether the primary site of this tumor was the bone or soft tissue.

Case summary: Magnetic resonance imaging (MRI) showed two tumors; one was located in the intraosseous lesion and the other in the deep soft tissue between the adductor muscles of the thigh. The intensity of T1- and T2-weighted images of the intraosseous tumor was almost the same as in the soft tissue tumor. These tumors seemed not to be connected but adjacent because the tumor in the soft tissue was surrounded by muscle and fat. The size of both tumors was >5 cm. Positron emission tomography-computed tomography (CT) revealed slight uptake in the two tumors and a pathological fracture of the thigh. CT also revealed multiple nodules in the lung. The candidates for the primary site at diagnosis were the bone, soft tissue, and lung. Approximately 70% of SS occur in the deep soft tissue of the lower and upper extremities (2); in pediatric cases, 82% are located under the fascia and 70% in the lower extremities (3). The bone, lung, peripheral nerves, etc., have been reported as unusual primary sites (2). There are 13 cases reported as intraosseous SS confirmed by fluorescence *in situ* hybridization (FISH) or polymerase chain reaction (4–14). The ages of the patients were >20 years and the most frequently affected bone was the tibia (5 cases). Among the 10 cases in which metastasis at diagnosis was discussed, there were 8 with no metastasis and 2 with lung metastasis. Although the lung is well known as a metastasis site (2, 15, 16), there was a case with primary lung SS metastasized to bone (17); therefore, special caution is required when two separate tumors exist at diagnosis. In cases in which two tumors coexist, it is difficult to completely distinguish between the primary and metastatic sites using histopathological and imaging findings. The primary site of this case was determined with no metastasis at diagnosis. Although the authors stated that osseous metastases in soft tissue malignancy tend to be multiple, in the SS cases with bone metastasis, 33% are reported as solitary and 67% as multiple (16), suggesting that solitary bone metastasis of SS is not uncommon. The most common metastatic site of SS is the lung at 70–80%, followed by the bone and lymph nodes (2, 15, 16). Considering these findings, we strongly question whether the SS in the soft tissue had perhaps metastasized to the bone and lung.

3 Time for metastasis

Our second concern is that the authors state that the time for metastasis was short; in this case, the pain was derived from the bone lesion with the pathological fracture, suggesting that the duration of the skeletal-related event (SRE) was 7 months. There is a possibility that the soft tissue lesion was in existence prior to this because in pediatric cases, painlessness and lack of palpability are distinctive clinical features (3); in this case, both symptoms were present and there was no complaint of the soft tissue lesion. The median time to SRE in bone metastasis patients affected by soft tissue sarcoma is 1–9 months (average 4 months) and the time course is similar to this case (18). SS generally grows slowly and the duration of clinical symptoms is as long as 2–4 years (3, 15). Of course we will never know when these two tumors first occurred, but considering these findings, there is a chance that the soft tissue lesion might have occurred prior to the 7-month period.

4 Misdiagnosis as ES

Finally, one thing that occurred which might not be directly related to the primary site was the misdiagnosis as ES. Recently, in addition to Ewing family tumors, round cell sarcomas with EWSR1 gene fusion, BCOR-rearrangement, and CIC-rearrangement have been recognized as new entities; furthermore FISH and PCR are essential for the differential diagnosis between ES and Ewing family tumors (19). Even if misdiagnosis had not occurred, we imagine that there would have been little difference in prognosis because the case involved a tumor that was >5 cm in size, metastasis at diagnosis, the poorly differentiated variant were an unfavorable factor (15).

5 Conclusion

In conclusion, we believe that this typical SS originated from the deep soft tissue and metastasized to the bone and lung. In the case of tumors within multiple organs, it is often difficult to establish which is the primary site and careful analysis is required, especially when concluding rare events.

Author contributions

Conception/design: JI, HI, and TK. Provision of study material or patients: JI, HI, and TK. Data collection and analysis: all authors. Manuscript writing: JI, HI, and TK. All the authors read and approved the manuscript.

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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.

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Next-generation sequencing study on poorly differentiated carcinoma derived from a thirty-year-old epidermoid cyst: A case report

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Although epidermoid cysts are frequently seen as benign lesions, they are highly uncommon to develop into cancerous lesions. A 36-year-old man with a cystic mass present on his left flank since childhood presented to our department. Based on the patient's medical history and abdominal computed tomography scan, we excised the lesion under the suspicion of an epidermoid cyst. Histopathological evaluation revealed the presence of poorly differentiated carcinoma with squamoid and basaloid differentiation, which showed a strong possibility of carcinoma arising from an epidermal cyst. Next-generation sequencing using TruSight oncology 500 assay showed copy number variation of *ATM* and *CHEK1* genes.

KEYWORDS

epidermoid cyst, squamous epithelium, next-generation sequencing, DNA copy number variation, malignant transformation

Introduction

Epidermoid cysts are the most common benign skin lesion with a high prevalence, compromising about 85-90% of all excised subcutaneous cysts (1). However, malignant transformations of epidermoid cysts have been rarely reported, with the transformation rate to squamous cell carcinoma (SCC) from 0.011 to 0.045% (2). Due to the rarity of the phenomenon, some cases of malignant transformationa have been reported in the skin, the abdominal cavity, and the intracranial space; however, the etiology or the risk factors are not well known yet (3, 4). We report a case of poorly differentiated carcinoma with squamoid and basaloid differentiation arising from a suspected epidermoid cyst, diagnosed using immunohistochemistry and next-generation sequencing using TruSight™ Oncology 500 (TSO500). This study was approved beforehand by the Institutional Review Board of Seoul National University Bundang Hospital (IRB No. B-2208-773-702) and performed in

accordance with the principles of the Declaration of Helsinki. All patients provided written informed consent for publication and use of their images.

Case report

A 36-year-old male visited our clinic with a flank mass that has existed since childhood and has rapidly grown in size over six months. On the computed tomography (CT) scan, a 2.2cm cystic mass in the subcutaneous tissue was observed (Figure 1). An excisional biopsy was performed under the initial clinical impression of epidermoid cyst. Following the pathological diagnosis of malignancy, further wide excision was performed with a 2cm margin from the previous scar and deep to the deep fascia of the external oblique muscle to obtain clear surgical margins.

Microscopically, a malignant lesion with an infiltrative margin was observed. The cells had a high nuclear to cytoplasm ratio and often contained clear vacuoles in their cytoplasm. The nuclei showed severe nuclear atypia and partial basaloid features (Figures 2A, B). A lesion corresponding to squamous cell carcinoma *in situ* was also observed at the edge of the main lesion, but no connection with normal skin epithelium was observed (Figure 2C). In some areas, cells with clear squamous differentiation were observed and cells containing keratin pearl were also noted (Figure 2D). In immunohistochemical staining, the cells of the lesion showed diffuse strong positivity for p63 and bcl-2 while epithelial membrane antigen (EMA) was positive only in well differentiated squamous cells (Figure 3). Taken together, the pathological diagnosis was poorly differentiated carcinoma with squamous and basal differentiation.

As the tumor was situated in the subcutaneous tissue and no direct connection to overlying skin was observed on gross and histological findings, metastatic carcinoma could not be ruled out. Further systemic evaluation including whole-body fluorodeoxyglucose-positron emission tomography (FDG-PET), bronchoscopy, esophagogastroduodenoscopy, and colonoscopy did not reveal any other cancerous lesions. Also, since the flank mass was present since childhood, the possibility of the tumor being a metastatic lesion was very low. Considering the histopathological features, clinical examination results and past medical history, the final diagnosis was likely to be primary poorly differentiated carcinoma with squamous and basal cell differentiation arising from an epidermoid cyst.

Since TNM staging system is not available for the tumor, NGS using TSO500 assay was performed to determine if additional postoperative treatments such as adjuvant chemotherapy or immunotherapy were applicable (Supplementary Table 1). There was no detected translocation or genomic alteration on Tier I and II genes, but gene fold changes were identified as copy number variation in homologous recombination-related genes *ATM* and *CHEK1*. The total tumor mutation burden was 5.5/MB (Table 1). No additional postoperative treatments were performed. No recurrence or metastasis was observed until the two-year follow-up visit.

Discussion

Epidermoid cysts, commonly seen as benign intradermal or subcutaneous tumors, are defined as cutaneous cystic mass appearing on the whole body with epidermis-like epithelial walls (5). Though epidermoid cysts grow slowly and are mostly benign, malignant transformations have rarely been reported. Possible malignant tumors that can develop from epidermoid cysts include SCC, basal cell carcinoma, Paget's disease, Bowen's disease, mycosis fungoides, Merkel cell carcinoma, and malignant melanoma. Locations of previously reported malignant transformations include the skin, the intracranial, and the abdominal cavity (3, 4). SCC is the most commonly reported transformation among the abovementioned malignancies, with a rate of 0.011%–0.045% (2, 6).. A cohort study of 41 cases from 1976 to 2018 showed that the most common site of SCC transformation was head and neck (54.8%), and the mean time from occurrence to diagnosis was 92.6 months (7).

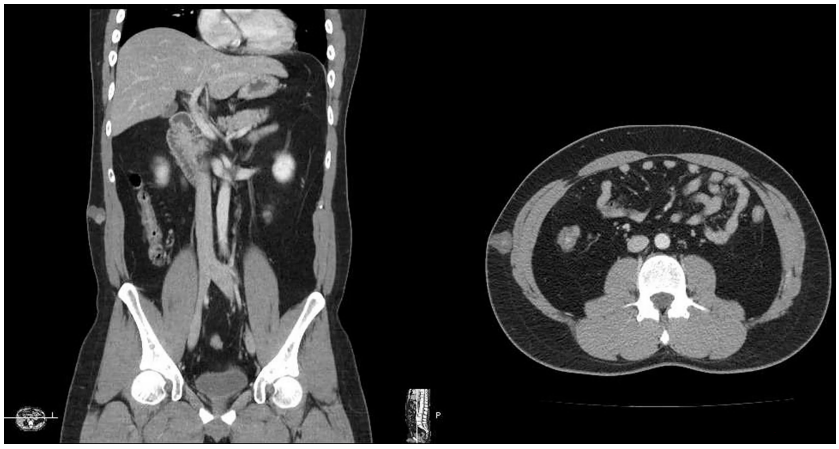

FIGURE 1
A 2.2 cm cystic lesion at the right flank isolated in the subcutaneous fat layer.

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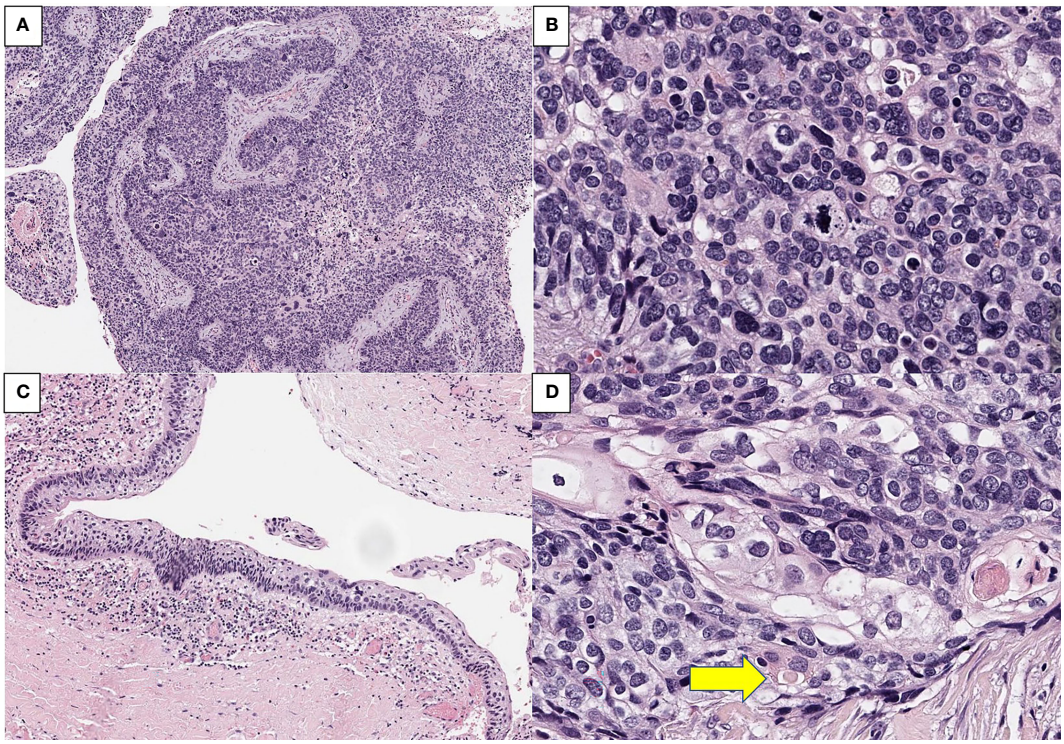

FIGURE 2
Representative H&E images of the tumor. **(A)** An infiltrative tumor with connection to cystic lining with high grade squamous intraepithelial lesion (x 100). **(B)** the tumor cells showed severe nuclear atypia and clear, vacuolar cytoplasm (x 800). **(C)** adjacent squamous cell carcinoma *in situ* lesion with no direct connection to normal skin epithelium (x 200). **(D)** cells with clear squamous differentiation and keratin pearl (arrow, x800).

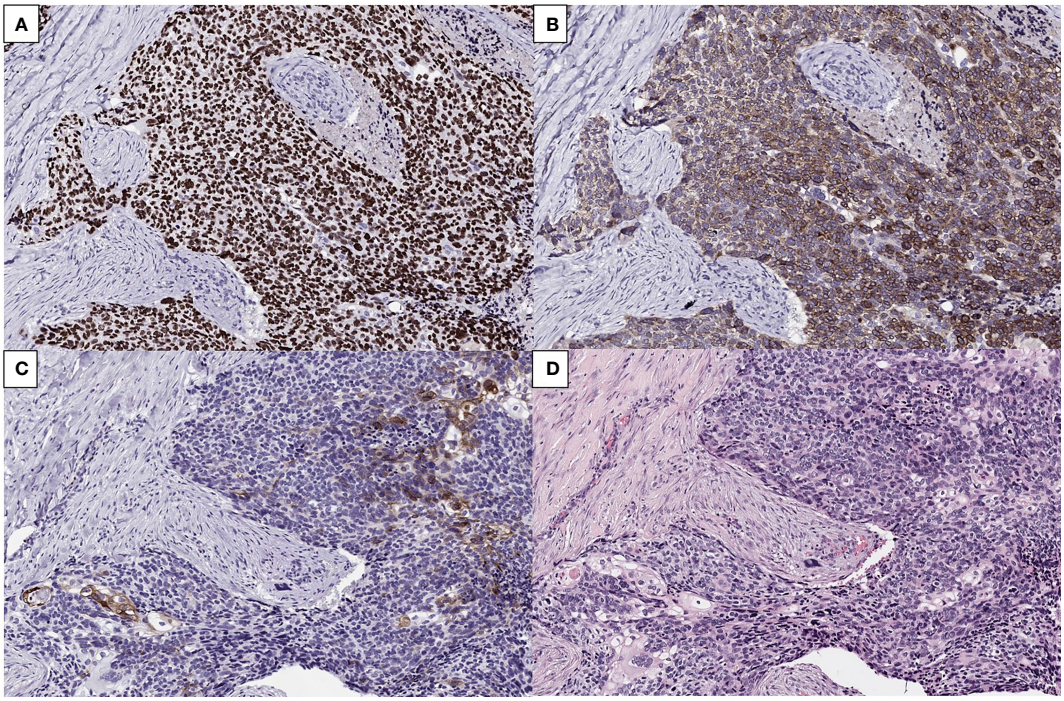

FIGURE 3
(A, B) Immunohistochemistry showing diffuse strong positivity of the tumor cells for p63 **(A)** and bcl-2 protein **(B)** (x200). **(C, D)** EMA stain revealing focal positivity in well differentiated cells **(C)** and corresponding H&E image **(D)** (x200).

TABLE 1 TruSight Oncology 500 assay result (Tier I and II only).

SNV/INDEL (Allele Frequency ≥ 2%)		No detected SNV/Indel in Tier I and II genes					
Fusion and splice variants (RNA)	Fusion (Fusion supporting Reads ≥ 3)	No detected translocation in Tier I and II genes					
	Splice variants	No detected translocation in Tier I and II genes					
Copy Number Alteration (Fold change ≥ 2.2)		No detected CNV in Tier I and II genes					
Homologous Recombination-related Genes	SNV/INDEL† (Allele Frequency ≥ 2% with pathogenic mutation, ≥ 25% with truncation mutation)	No detected genomic alteration in 25 homologous recombination-related genes included in *SNUBH pancancer TruSight Oncology 500 LVII					
	CNV‡ (Fold change ≤ 0.8)	Gene	Type	Locus	Fold change	Estimated copy number (purity-adjusted)	Tier
		ATM	LOSS	11q22.3	0.39	0.257	III
		CHEK1	LOSS	11q24.2	0.41	0.314	III
Microsatellite instability (MSI-H ≥ 20% unstable loci)		Unstable Loci	Passing Loci	Present Unstable MSI sites		Prediction of MSI Status	
		2	124	2		MSS/MSI-L	
Tumor Mutation Burden (N(var) x 10^6/ExonLength)		Number of Passing Eligible Variants		Coding Region Size in Megabases		Total TMB	
		7		1.27		5.5/MB	

*SNUBH pancancer TruSight Oncology 500 LVII (ATM, ATR, BAP1, BARD1, BLM, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, MRE11A, NBN, PALB2, POLD1, RAD50, RAD51, RAD51B, RAD51C, RAD51D, RAD52, RAD54L, PPP2R2A, XRCC2).

The etiology of malignant transformation of the epidermoid cyst has not yet been determined. The risk factors for cutaneous SCC, such as radiation, immunosuppression, scars, and chronic inflammation, are also considered risk factors for this transformation (6). Clinically, pain, fast growth, overlaying skin abnormalities, and continuous drainage are some warning signs of neoplastic progression (7).

In the present case, TSO500 assay was performed to characterize the tumor and assist in determining the need for adjuvant therapy. At our institution, SNV/Indel, fusion or splice variants (RNA), copy number variation(CNV), twenty-five homologous recombination-related genes, microsatellite instability, and tumor mutation burden (TMB) are reported. Interestingly, CNV with fold change below 0.8 of *ATM* and *CHEK1* homologous recombination-related genes were identified in our case.

The activation of ATR-Chk1 and ATM-Chk2 pathways are essential in DNA damage response and cell cycle checkpoints. The ATR-Chk1 pathway, in particular, manages a broad spectrum of DNA abnormalities (8, 9). Furthermore, ATM loss at the cellular level is associated with chromosomal instability and radiosensitivity (10). The activation of ATM and downstream checkpoint kinases Chk1 has a major role in eradicating precancerous cells. The mutations identified in the present case may have promoted tumor growth and cell proliferation (11–13).

Both ATM gene and CHEK1 gene mutations have been found to be related to various cancers through previous studies. ATM gene is one of the most frequently aberrant genes in sporadic cancers, especially hematologic malignancies, according to next-generation sequencing studies (14). Numerous ATM mutations have been identified and linked to a moderate risk of BC occurrence (15). In the case of CHEK1 gene, the association with endometrial and colorectal cancer is known (16), and the association with solid tumor is also being studied (17).

To the best of our knowledge, this is the first report of NGS on carcinoma originating from an epidermoid cyst. Due to the low incidence of such malignant transformations, the mechanism behind it has been difficult to research. With the increased availability of NGS and other sequencing modalities, more data can be collected to elucidate our understanding of these malignant transformations and to assist in finding new adjuvant immunotherapies for these entities.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

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

WC, JP, and BK contributed to conception and design of the study. WC wrote the first draft of the manuscript. WC and JP wrote sections of the manuscript. SS selected proper pathologic image and interpreting those images. All authors contributed to the article and approved the submitted version.

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.

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Supplementary material

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