Case Report: iMDT Corner

Adjuvant crizotinib treatment selected by patient-derived organoids in a patient with stage IIIA adenocarcinoma with novel *LRRTM4-ALK* fusion: a case report

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Background: Over 90 different anaplastic lymphoma kinase (*ALK*) fusions have been reported, and patients with different ALK fusion partners exhibit different responses to targeted therapy. Patient-derived organoid (PDO), a kind of 3-dimensional culture, is a promising model for drug-sensitivity testing for personalized treatment decision-making. It further has the potential to provide treatment strategy for patients with novel mutations, rare mutations, and concomitant mutations, serving as a supplement to evidence-based medicine.

Case Description: We report a case in which a man with stage IIIA adenocarcinoma had pleural effusion 1 month after surgery. A novel leucine-rich repeat transmembrane neuronal protein 4 (*LRRTM4*)-*ALK* fusion was unveiled by next-generation sequencing (NGS), and PDOs were used in drug-sensitivity testing to select a proper adjuvant therapy for this patient. We chose crizotinib based on result of the test and drugs' availability in China and helped the patient achieve a more than 3-year-long disease-free survival (DFS). Higher variant allele frequencies (VAFs) of the driver mutation were also found in PDOs and their waste culture medium, indicating that the PDO model could filter out cells with driver genes or stemness and help us to identify the critical cancer cell colony in treatment decision-making.

Conclusions: For the first time, we report the case of a *LRRTM4-ALK* fusion. The patient achieved a more than 3-year long-term DFS under crizotinib treatment, which was selected by an emerging PDO drug-sensitivity test model. We also discovered the enrichment of a low-abundance driver mutation in PDO and its waste culture medium, providing a new direction for future research.

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Introduction

Anaplastic lymphoma kinase (*ALK*) fusion is one of the common driver mutations of non-small cell lung cancer (NSCLC), accounting for about 3–8% of all NSCLC cases (1). Over 90 fusion partners of the *ALK* gene have been reported, with 85% patients having *ALK* rearrangement with echinoderm microtubule-associated protein-like 4 (*EML4*)-*ALK* fusion (2,3). Although ALK tyrosine kinase inhibitors (TKIs) such as crizotinib, alectinib, and brigatinib have markedly improved the survival of patients with *ALK* fusion, most of the related clinical trials have been conducted in populations primarily constituted by patients with *EML4-ALK* fusion (4). Several retrospective studies have indicated that patients with different *ALK* fusion partners have varied responses to ALK-TKIs (5,6). Du *et al.* reported a case in which a patient with cap methyltransferase 1 (*CMTR1*)-*ALK* fusion had no response to crizotinib, reminding us that the determination of the *ALK* rearrangement mode prior to drug selection is important (7). Owing to the development of next-generation sequencing (NGS), more *ALK* fusion partners have been identified, and the *ALK*-fusion-mutated populations could be further differentiated according to the principle of precision medicine.

Patient-derived organoids (PDOs) are 3-dimensional culture derived from stem cells in specific tissue, such as the patient's tumor, and have been extensively used in drug screening experiments due to their histological and genetic similarity with the original tumor tissue (8). We here report a case of a patient with stage IIIA adenocarcinoma with an unreported novel leucine-rich repeat transmembrane neuronal protein 4 (*LRRTM4*)-*ALK* fusion who was treated with adjuvant crizotinib selected by PDO technology and who subsequently achieved long-term survival. We present this article in accordance with the CARE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-487/rc).

Highlight box

Key findings

- A novel leucine-rich repeat transmembrane neuronal protein 4 (*LRRTM4*)-anaplastic lymphoma kinase (*ALK*) fusion mutation was found in a patient with stage IIIA lung adenocarcinoma.
- Crizotinib was selected by patient-derived organoids (PDOs) and helped the patient achieve a more than 3-year-long disease-free survival (DFS).
- Enrichment of low abundance driver mutation was found in PDOs and their waste culture medium.

What is known and what is new?

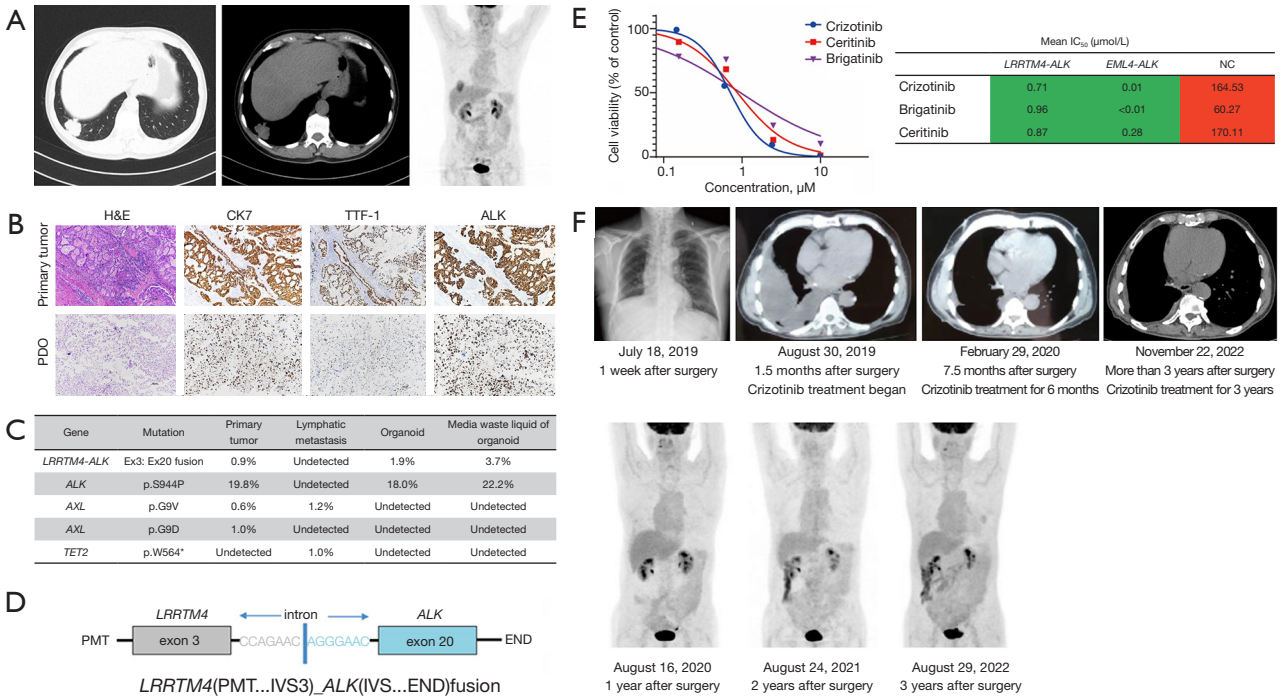
- Clinical trials of ALK-tyrosine kinase inhibitors (TKIs) have mainly been conducted in patients with echinoderm microtubule-associated protein-like 4 (*EML4*)-*ALK* fusion. However, patients with different *ALK* fusion partners have diverse responses to ALK-TKIs.
- We used an emerging model PDOs to select crizotinib as adjuvant therapy and helped the patient achieve a more than 3-year long-term DFS. Enrichment of a low-abundance driver mutation was also found in PDO and its waste culture medium.

What is the implication, and what should change now?

- Personalized precise medicine guided by PDO could serve as a complement to evidence-based medicine, especially when dealing with novel mutations, rare mutations, and concomitant mutations.

Case presentation

A 76-year-old male never-smoker patient was admitted to our hospital for a right-lower-lobe solid mass clinging to the pleura with a largest dimension of 3.1 cm and signs of lobulation; the mass had been revealed by computed tomography (CT) accidentally. The maximum standardized uptake value (SUV_{max}) of this mass was 4.3 according to ^{18}F-fluorodeoxyglucose positron emission tomography/CT (^{18}F-FDG-PET/CT) (*Figure 1A*). Pleural thickening was also found around this opacity. The patient then underwent video-assisted thoracoscopic right-lower lobectomy and systematic lymph node dissection. No pleural effusion or implant tumor nodules were discovered, but multiple swollen hilar and mediastinal lymph nodules were found during the operation. No evidence of parietal pleural



Gene	Mutation	Primary tumor	Lymphatic metastasis	Organoid	Media waste liquid of organoid
LRRTM4-ALK	Ex3: Ex20 fusion	0.9%	Undetected	1.9%	3.7%
ALK	p.S944P	19.8%	Undetected	18.0%	22.2%
AXL	p.G9V	0.6%	1.2%	Undetected	Undetected
AXL	p.G9D	1.0%	Undetected	Undetected	Undetected
TET2	p.W564*	Undetected	1.0%	Undetected	Undetected

Mean IC_{50} (μmol/L)	*LRRTM4-ALK*	*EML4-ALK*	NC
Crizotinib	0.71	0.01	164.53
Brigatinib	0.96	<0.01	60.27
Ceritinib	0.87	0.28	170.11

Figure 1 A patient with novel *LRRTM4-ALK* fusion was treated with adjuvant crizotinib therapy selected by drug-sensitivity test based on PDOs and achieved long-term survival. (A) The lung and mediastinal window images and ^{18}F-FDG-PET/CT of the patient's 3.1-cm mass. (B) H&E was performed to show similar cellular characteristics between the primary tumor (surgical specimen) and the PDOs. CK7 and TTF-1 were detected by IHC assays in paraffin section of the primary tumor and the PDOs to demonstrate their histological similarity. ALK protein expression was also detected via IHC assays with D5F3 antibody (VENTANA). The magnification of the microscope was 100 times. (C) Mutations detected in different samples. The data are VAFs of each mutation. (D) Sketch map of the structure of the *LRRTM4-ALK* fusion. (E) Drug-sensitivity test by PDOs. The left figure is the dose-response curve for crizotinib, ceritinib, and brigatinib in PDOs. An ATP-based assay was used to indicate the cell viability. The right table is the IC_{50} for organoids with the *LRRTM4-ALK* fusion, *EML4-ALK* fusion, and non-*ALK* fusion (NC). Green indicates IC_{50} <50 μmol/L, and red indicates IC_{50} >50 μmol/L. (F) Chest X-ray showed clear costal angles, indicating little pleural effusion 1 week after surgery. However, 1 month later, CT revealed suspected pleural effusion to be a sign of relapse. After 6 months' treatment with crizotinib selected by an organoid drug-sensitivity test, the pleural effusion did not recur. The three PET/CT scans showed no signs of metastasis after crizotinib treatment. *LRRTM4*, leucine-rich repeat transmembrane neuronal protein 4; *ALK*, anaplastic lymphoma kinase; PDOs, patient-derived organoids; ^{18}F-FDG-PET/CT, ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography; H&E, hematoxylin and eosin staining; CK 7, cytokeratin 7; TTF-1, thyroid transcription factor-1; IHC, immunohistochemistry; VAFs, variant allele frequencies; ATP, adenosine triphosphate; NC, negative control; IC_{50}, half maximal inhibitory concentration.

invasion was found during the surgery.

Pathological diagnosis of this mass was invasive adenocarcinoma, with metastases at station 11 and 7 mediastinal lymph nodes. The tumor did not invade the parietal or visceral pleura according to the pathology report and was postoperatively staged as T2aN2M0 (stage IIIA). Immunohistochemistry with the VENTANA anti-ALK D5F3 antibody showed strong, diffuse expression of the ALK protein (*Figure 1B*). Through NGS (1021-Gene Variant Assay, DNA-NGS, Geneplus-Beijing, Beijing, China), a previously unreported fusion gene, *LRRTM4-ALK*, was discovered, along with other clinically equivocal mutations (*Figure 1C*). *LRRTM4* is suspected to be involved in the regulation of synapse assembly and is expressed in the brain and lungs (9,10). The *LRRTM4-ALK* fusion mutation is produced by deletion within the short arm of chromosome 2, linking intron 3 of *LRRTM4* with intron 19 of *ALK* (*Figure 1D*).

The patient developed right pleural effusion 1 month after surgery along with pleural thickening revealed by CT.

We conducted cytological examination, but no tumor cells were found in the pleural effusion. To identify a suitable adjuvant therapy for potential relapse, we used tumor tissue to construct a prespecified PDO for conducting a drug-sensitivity test right after the surgery (11). Histological features of the source tumor tissue were faithfully recapitulated in the PDOs (*Figure 1B*), and the PDOs comprehensively inherited the tumor's genetic characteristics, with the same *LRRTM4-ALK* fusion and passenger mutation (*ALK p.S944P*) detected (*Figure 1C*). Abundance of the suspicious driver gene, *LRRTM4-ALK* fusion, was increased in PDOs (0.9% in primary tumor and 1.9% in PDOs). Inspired by the detection of circulating tumor DNA (ctDNA) in blood and pleural effusion which was also known as 'liquid biopsy', we hypothesized that ctDNA will also be released into the culture medium in which organoids grow. Therefore, we collected the culture medium of the patient's organoid for ctDNA testing. Notably, the waste culture medium of organoids showed the highest abundance of *LRRTM4-ALK* fusion (3.7%) (*Figure 1C*). The half maximal inhibitory concentrations (IC_{50}) based on the viability of PDOs for ceritinib (0.87 μM), crizotinib (0.71 μM), and brigatinib (0.96 μM) were not significantly different as compared to the disparity between positive controls (*EML4-ALK* fusion organoids) and negative controls (non-*ALK*-fusion organoids), showing approximate and reasonable response against tumor cells (*Figure 1E*).

Considering the drug-sensitivity test results and the availability of drugs in China, crizotinib (250 mg, oral, twice daily) was chosen as adjuvant therapy 1 month after surgery. The pleural effusion was absorbed and did not reappear, and annual ^{18}FDG-PET/CT showed no dubious metastasis (*Figure 1F*). At the time of writing, the patient has achieved a more than 3-year-long disease-free survival (DFS) and has not experienced any disease recurrence events. Only grade 1 diarrhea occurred during the treatment of crizotinib according to Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, and the attitude of the patient towards treatment has been positive.

All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committees and with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

International multidisciplinary team (iMDT) discussion

Discussion among clinicians from Peking Union Medical College Hospital

We conducted multidisciplinary consultation of this case. Given that several studies have suggested that fusion partners of the *ALK* gene may influence the efficacy of ALK-TKIs, it would be worth exploring which drug would be most suitable for this patient.

Department of thoracic surgery

An adenocarcinoma patient was admitted to our hospital due to a 3.1-cm, right-lower-lobe solid mass and underwent surgery. The NGS of the mass was conducted, and a novel *LRRTM4-ALK* fusion was unveiled. Adjuvant crizotinib was selected by a PDO drug-sensitivity test, and the patient has achieved a more than 3-year DFS to date. To the best of our knowledge, we are the first to report this unknown *ALK* fusion form through NGS and prove its sensitivity to crizotinib.

Biological engineering laboratory

We explored constructing PDOs for treatment selection. This process consists of three parts. The first step is to obtain the tissue of the patient's primary tumor through surgery or biopsy, and utilize three-dimensional Matrigel to construct a collagen skeleton *in vitro* that is similar to the structure of connective tissue *in vivo*, allowing tumor cells to grow into organisms that can more realistically simulate the patient's tumor in a three-dimensional form. The second step is to demonstrate PDOs' histological and genetical similarity to the primary tumor. Finally, we can fit the drug-sensitivity curve by adding a drug with different concentrations to the organoids and calculate the IC_{50} value of this drug. Drug-sensitivity test for individual's treatment decision-making can subdivide the population in a manner which advanced clinical trials fail to do at the individual level. Thus, PDOs would play a distinguished role in this "black-box testing" for their higher similarity with original tissue than would cell lines and offer lower temporal and financial cost compared with patient-derived xenografts (PDXs) (8). In this case, the patient's tumor tissue was sent to the laboratory for cultivation within 4 hours after the resection. Ten days later, tumor cells expanded into organoids that can undergo drug sensitivity. We obtained the patient's drug sensitivity results approximately 3 weeks after the surgery. We believe that more novel mutations,

rare mutations, and concomitant mutations will be revealed as sequencing technology advances, and thus personalized precise medicine guided by PDOs will be a huge complement to evidence-based medicine.

Institution of gene sequencing

Higher variant allele frequencies (VAFs) of *LRRTM4-ALK* fusion of PDOs compared with original tumor tissue revealed that cells with diver genes or stemness might have greater tendency in supporting the PDOs' construction, providing more mutation abundance for us to detect. For tumors with low mutation abundance, PDOs could help to identify cell populations with potent vigor and pathogenicity, which could guide the treatment prioritization. Additionally, with the popularization of the concept of liquid biopsy, we attempted to detect ctDNA in culture medium in which organoids grow. PDOs would release their genetic material and metabolites into the environment by means of exosomes or death (12). As a result, high abundance mutations could be detected in the culture medium, providing a new "liquid biopsy" method for tumors with minimal mutation abundance.

Although this case report presents several novel viewpoints, it is experience gained from the diagnosis and treatment of one patient. The scientific findings in this study came from a single case, and should not be considered as direct or circumstantial evidence for the applicability and/or effectiveness of organoid-related technologies on other patients. Due to limitations of organoid culture techniques back then, there might not be enough organoids to conduct repeated experiments. Also, we were unable to obtain some drugs for sensitivity testing in 2019, such as alectinib. More research should be carried out to demonstrate the ability of PDOs in drug selection.

Several issues

The efficacy of ALK-TKIs have been confirmed in clinical trials mainly in the population with *EML4-ALK* fusion. However, several studies have claimed that patients with different *ALK* fusion partners have diverse responses to ALK-TKIs. Thus, how should doctors select drug for patients with these so-called uncommon *ALK* fusion (such as *LRRTM4-ALK* fusion in our case)?

Andrea De Giglio: Targeted therapies against the fusion protein represent the standard of care for advanced *ALK*-positive NSCLC. The first-generation crizotinib improved survival outcomes and quality of life compared to standard platinum-based chemotherapy (13). Then, second-generation drugs such as alectinib, brigatinib, and ceritinib have proven their superiority to crizotinib (13), still representing the first-line standard of care in many countries. Lastly, the CROWN study demonstrated that upfront, third-generation, lorlatinib increased life expectancy compared with crizotinib, even if the tolerability profile showed some critical points (13). A sequential-generation strategy is the most suitable approach due to the better efficacy of next-generation TKI on single or compound resistance mutations occurring within the *ALK*-rearranged gene (13). Notably, no randomized controlled trials required NGS testing as inclusion criteria to enroll *ALK*-positive patients, but rather an immunohistochemistry or FISH testing (13). In this context, no data regarding fusion partners are available, and no efficacy analyses have been performed. The body of evidence on this matter is composed of retrospective experiences that predominantly confirmed the efficacy of ALK inhibitors on uncommon non-*EML4* variants (14,15). Remarkably, preliminary reports exploited the TKI resistance for specific gene partners (7,16). Considering that no prospective studies are available and only observational data exist for specific rare non-*EML4* variants, physicians should prefer a sequential approach using first or second-generation TKIs as the initial form of treatment.

Petros Christopoulos: An *ALK* fusion detected with RNA (instead of plain DNA) sequencing is certainly expressed and can be assumed to be oncogenic due to ALK autophosphorylation induced by oligomerization of the chimeric oncoproteins, which is facilitated by the fusion partner (17). Since the oncogenic signaling of *ALK* fusions is mediated by the ALK kinase, the relative efficacy of various ALK-TKIs against such a fusion is expected to be similar as the relative efficacy against the wild-type ALK in the order first < second < third generation ALK-TKI (4). The PDO technique, as nicely demonstrated in the current work, is an elegant *in vitro* method to verify TKI sensitivity in a personalized manner. However, if this method cannot be performed and no information about the TKI sensitivity of the given rare *ALK* fusion exists in the literature, I would aim for the most potent ALK-TKI available.

As an *ex vivo* drug sensitivity tool, can PDOs perform as well as *in vivo* drug sensitivity models, such as the PDX model, in terms of reflecting the effectiveness of drugs?

Andrea De Giglio: The lack of literature regarding ALK inhibitors effectiveness on uncommon *EML4* or non-

EML4 variants may represent a burning issue for treating these patients. Unfortunately, the rarity of these patients limits the possibility of studying the *in vivo* efficacy of ALK-TKI extensively. The deductive approach for treating a large number of patients sharing the same genic alteration is unsuitable for rare tumors. For this reason, a preliminary study of the drug sensitivity on cell cultures or tridimensional PDX or PDO models may represent an excellent opportunity to select the best drug and personalize the treatment. PDX and PDO models can accurately reproduce the primary tissue's structure (18), conserving the genomic and histological features of the patient-derived tissue. PDX models confer a reliable reproduction of tumor microenvironment with a remarkable biological similarity (19), even if the prolonged turnaround time and the non-negligible failure rate compromise their applicability in a real-world context. Interestingly, the PDO models have quick distribution with a high success rate, thus showing a potential use in clinical practice to investigate drug sensitivity with a consequent decision-making impact (19).

Petros Christopoulos: Since PDO and PDX are based on the same patient-derived tumor cells, they will generally provide comparable results of drug sensitivity, especially for drugs whose pharmacologic activity mainly relies on tumor-cell-intrinsic mechanisms, as is the case with TKIs (20). On the other hand, for therapies depending on the tumor microenvironment, such as immunotherapy, suitable *in vivo* systems may offer important advantages over *in vitro* tools, because they allow for the study of all steps necessary for effective anti-tumor immune responses. Nonetheless, it should be noted that promising PDO are currently under development for precision cancer immunotherapy, as well (21), and that important advantages of *in vitro* over *in vivo* models include easier implementation, lower costs and faster results.

What treatment strategy should we choose as adjuvant therapy for postoperative patients with *ALK* fusions?

Andrea De Giglio: The perioperative treatment of early NSCLC has radically changed in the last few years, from standard platinum-based chemotherapy to a personalized approach, including novel therapies, such as immunotherapy or targeted therapy. Osimertinib is the first TKI approved for the adjuvant treatment of resected NSCLCs carrying EGFR mutations, with a remarkable benefit in DFS and overall survival (22). Two randomized phase III trials are ongoing to investigate the efficacy of adjuvant ALK inhibitors. The ALCHEMIST trial is currently randomizing stage IB-IIIA resected patients to receive crizotinib or observations for 2 years (23). The ALINA trial is a phase 3 trial investigating the efficacy of alectinib, given for 2 years after the resection, or standard platinum doublets (24). Interestingly, the ALNEO trial is a phase 2, single-arm study investigating the antitumor activity of 8 weeks of neoadjuvant alectinib treatment with the major pathological response as the primary endpoint (25). No data from ongoing prospective trials are currently available. Recently, an observational study reported the survival outcomes of 30 patients treated with 2-year adjuvant crizotinib compared to a control group of 29 patients treated with standard chemotherapy (26). Patients within the crizotinib group experienced a significantly improved median DFS (P<0.001) and median overall survival (P=0.021). In conclusion, ALK inhibitors may represent an excellent therapeutic strategy for the perioperative treatment of early, *ALK*-positive NSCLC.

Petros Christopoulos: Currently, the standard of care for patients with stage II–IIIA(/B), according to TNM8, *ALK*$^+$ NSCLC after complete resection is adjuvant chemotherapy, which typically comprises a cisplatin-based doublet administered over 4 cycles every three weeks and starting approximately 1 month after surgery. Whether the postoperative administration of ALK inhibitors can further improve the outcome of *ALK*$^+$ NSCLC, is still under investigation in ongoing prospective clinical trials. Based on the recently published significant overall survival benefit from adjuvant osimertinib after complete resection lung adenocarcinoma with classical *EGFR* mutations cancer in the ADAURA trial (22), ALK inhibitors, and particularly the more potent second-/third-generation drugs, appear to be very promising in this regard. However, the results of ongoing trials should be awaited before use of ALK-TKI in the adjuvant setting can be broadly recommended, because earlier adjuvant studies with older EGFR inhibitors had shown only a delay of relapse, but no long-term benefit and cure of patients (27).

Conclusions

In this case report, we demonstrated how to use the organoid as a favorable tool of precision medicine to make a decision on the adjuvant treatment for a patient with lung cancer carrying a novel mutation *LRRTM4-ALK* fusion. We hope that this experience can provide insights for clinical workers, especially when encountering novel mutations,

rare mutations, and concomitant mutations.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committees and with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

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