Journal of Cancer Research and Practice

journal homepage: www.ejcrp.org

Case Report

Parapharyngeal Inflammatory Myofibroblastic Tumor Harboring Fibronectin 1-ROS Protooncogene 1 Fusion Responded to Crizotinib

Yu-Ju Kuo[1], Jen-Chieh Lee[2], Chun-Nan Chen[3], Tom Wei-Wu Chen[1]*

[1]Department of Oncology, National Taiwan University Hospital, Taipei, Taiwan
[2]Department of Pathology, National Taiwan University Hospital, Taipei, Taiwan
[3]Department of Otolaryngology, National Taiwan University Hospital, Taipei, Taiwan

Abstract

Inflammatory myofibroblastic tumor (IMT) is a rare tumor type usually arising in the thoracic or abdominal cavity. Despite its rarity, IMT commonly harbors driver gene rearrangements involving anaplastic lymphoma kinase (*ALK*), ROS proto-oncogene 1 (*ROS1)*, and neurotrophic tropomyosin-related kinase. We present a rare case of the parapharyngeal IMT with convoluted diagnostic test results in determining driver gene rearrangement. The immunohistochemical stains were *ALK*-negative and *ROS1* positive, but the result of *ROS1* fluorescence *in situ* hybridization was equivocal. Amplicon-based targeted next-generation sequencing (NGS) did not detect any *ROS1* rearrangement, but hybridization capture-based NGS revealed a rare fibronectin 1 (*FN1)-ROS1* fusion. Eventually, the patient started crizotinib and had a tumor response with tolerable toxicity. This case highlights the importance of appropriate molecular testing of IMTs to guide the proper targeted therapy.

Keywords: Case report, crizotinib, fibronectin 1-ROS protooncogene 1, inflammatory myofibroblastic tumor

Introduction

Inflammatory myofibroblastic tumor (IMT) is a rare mesenchymal tumor of borderline malignancy. Only 150–200 cases are diagnosed in the United States annually. IMT usually affects children and adolescents, and frequently involves sites, including the lung, abdomen, and retroperitoneal spaces.[1] The head-and-neck IMTs only account for 15% of all IMTs and are more prevalent among adults. Symptoms of the head-and-neck IMTs are related to the primary site from which they arise. The larynx, pharynx, sinonasal area, skull base, salivary glands, trachea, and orbit have been reported to be primary sites of IMT.[2]

Submitted: 30-Jul-2020 **Revised:** 28-Sep-2020
Accepted: 29-Sep-2020 **Published:** 01-Dec-2020

Supplementary material available online

Access this article online

Quick Response Code:

Website: www.ejcrp.org

DOI: 10.4103/JCRP.JCRP_25_20

Address for correspondence: Dr. Tom Wei-Wu Chen, Department of Oncology, National Taiwan University Hospital, No. 7, Chung Shan S. Rd., Zhongzheng Dist., Taipei City 10002, Taiwan.
E-mail: tomwchen@ntuh.gov.tw

How to cite this article: Kuo YJ, Lee JC, Chen CN, Chen TW. Parapharyngeal inflammatory myofibroblastic tumor harboring fibronectin 1- ROS protooncogene 1 fusion responded to crizotinib. J Cancer Res Pract 2020;7:179-83.

More than half of IMTs harbor anaplastic lymphoma kinase (*ALK*) rearrangements and respond to *ALK* inhibitors such as crizotinib. For IMTs without *ALK* rearrangements, Lovly *et al*. first reported ROS proto-oncogene 1 (*ROS1*) as well as platelet-derived growth factor receptor B (*PDGFRB*) fusions in 2014.[3] Other genetic alterations such as RET protooncogene (*RET*) and neurotrophic tropomyosin-related kinase (*NTRK*) have also subsequently been reported.[4,5] *ROS1* fusions have been reported in 7%–13% of IMTs, and most of them were TRK-fused gene (*TFG)-ROS1* fusion.[3-15] Here, we present a case of parapharyngeal IMT with a unique initial presentation and rare fibronectin 1 (*FN1)-ROS1* fusion.

Case Report

A 26-year-old woman presented with a headache for 1 month. The headaches were located at the left temporal area with radiation to the ear, neck, and upper shoulder, and she also complained of phonophobia, photophobia, and left ear fullness. She went to a neurology clinic and was tentatively treated for migraine. Nonsteroid anti-inflammatory drugs, muscle relaxants, and even antidepressants were tried, but her symptoms did not resolve at all.

Her headaches persisted and got worse in the following 6 months, with newly developed diplopia, hoarseness, dysphagia, and weight loss of around 8 kg. Contrast-enhanced of the head-and-neck magnetic resonance imaging (MRI) revealed a 6.3 cm necrotic tumor at the left carotid space with invasion to left C1 and skull base. The left internal carotid artery and the left internal jugular vein were encased [Figure 1]. Fiberoptic nasopharyngoscopy showed bulging of the left nasopharynx and oropharynx. Left vocal cord palsy was also noted.

An echo-guided core needle biopsy was done, which showed a spindle cell tumor with scattered inflammatory cells and focal myxoid stroma [Figure 2]. Immunohistochemically, the spindle cells showed focal weak staining for smooth muscle actin (SMA). Murine double minute 2 showed scattered staining, and a desmin stain was negative. These findings suggested an atypical myofibroblastic tumor. CD34 (marker for solitary fibrous tumor and epithelioid sarcoma) and S100 (marker for nerve sheath tumor) were negative. An *ALK* (D5F3) stain was negative, wheres a *ROS1* stain was diffusely and strongly positive with cytoplasmic staining. The myofibroblast-like cytomorphology and striking *ROS1* immunostaining suggest the likelihood of a *ROS1*-rearranged IMT. However, an informal ROS1 fluorescence *in situ* hybridization (FISH) study revealed an equivocal result. Therefore, a formalin-fixed paraffin-embedded sample was sent for an amplicon-based targeted next-generation sequencing (NGS) focusing on 31 fusion genes and 182 transcripts [Supplement 1].

While waiting for the results, crizotinib 250 mg twice daily was started. Grade 1 blurred vision was noted; otherwise, she tolerated the treatment well, and her headaches gradually resolved. Follow-up examinations at the otolaryngologist clinic after 3 weeks showed less left parapharyngeal swelling. A contrast-enhanced neck MRI after taking crizotinib for 1 month confirmed partial response and significantly smaller tumor.

The NGS study did not report a *ROS1* fusion, which could not explain the response to crizotinib. Therefore, a computed tomography (CT)-guided biopsy was done, and the tumor tissue was sent for another targeted-NGS study using a hybrid-capture method (FoundationOne® Heme) [Supplement 2], which identified a rare *FN1-ROS1* fusion with *FN1* exon 23 (NM_002026) fused with *ROS1* exon 32 (NM_002944) [Figure 3]. Other alterations identified included Tet methylcytosine dioxygenase 2 (*TET2)* mutation (mutation allele frequency [MAF]: 25.7%), neurofibromin 2 mutation (MAF: 15.5%), and lysine methyltransferase 2D exon 6 rearrangements, all of which are tumor suppressor genes found in hematologic or solid organ malignancies.

The patient has been on crizotinib for 6 months with a partial response and ongoing necrosis within the tumor according to follow-up MRI images [Figure 1].

Discussion

IMT is a mesenchymal myofibroblastic lesion of borderline malignancy, with frequent local recurrence but rare

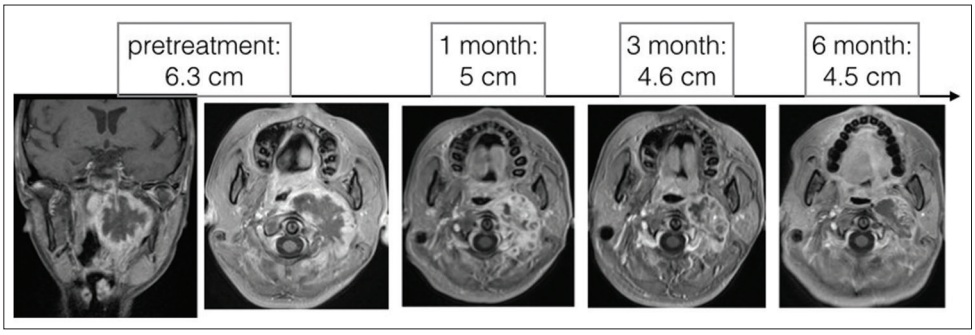

Figure 1: Neck and brain magnetic resonance imaging with contrast enhancement: (T1, fat-saturated phase). A 6.3 cm necrotic tumor at the left carotid space, with invasion to the left C1, occipital condyle, clivus, left skull base, left hypoglossal canal, and medial part of the jugular foramen. The left internal carotid artery and left internal jugular vein were encased. The tumor responded to crizotinib treatment and was smaller on serial follow-up images

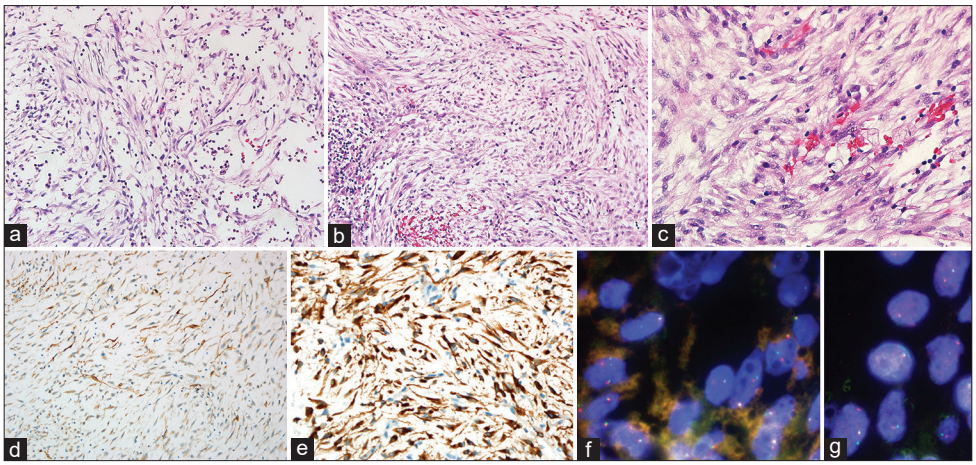

Figure 2: A spindle cell tumor arranged in fascicles. There were focal hypercellular areas comprising plump spindle cells with up to moderate nuclear atypia, increased mitoses, and scattered lymphocyte and plasma cells. Focal myxoid stroma was noted (a-c). Immunohistochemically, the spindle cells showed focal weak staining for smooth muscle actin (d). ROS1 stain was diffusely and strongly positive (e). ROS1 fluorescence *in situ* hybridization was equivocal (<10% tumor cells harboring split signals) (f), while fibronectin 1 fluorescence *in situ* hybridization was positive (g)

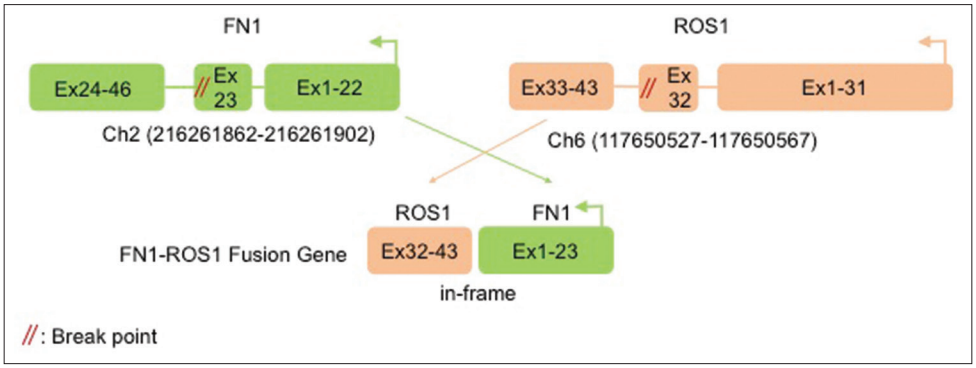

Figure 3: Illustration of fibronectin 1-*ROS1* fusion in this case

distant metastasis. The characteristic histology of IMT is plump or elongated myofibroblasts with inflammatory infiltration of lymphocytes, plasma cells, and eosinophils. Immunohistochemically, the majority of IMTs are positive for SMA. In addition, about 50% of IMTs are positive for *ALK* immunohistochemical (IHC) staining, which is correlated with *ALK* rearrangement and response to *ALK* inhibitors such as crizotinib. On the other hand, IMTs with negative *ALK* IHC stains have been reported to be more aggressive and to have a higher frequency of distant metastasis.[16]

The application of NGS in oncology practice has led to the discovery of other druggable genetic alterations in patients with *ALK*-negative IMTs. Rearrangements of *ROS1, PDGFRB, NTRK,* and *RET* have all been reported in *ALK*-negative IMTs, of which *ROS1* fusion was the first reported and the most prevalent genetic alteration. In the reported case series, the *ROS1* fusion has been reported in 7%–13% of all IMT patients.[3-15] A positive of *ROS1* IHC stain can predict *ROS1* rearrangement in IMT, and the staining pattern can be cytoplasmic staining, nuclear staining, or both.[6] *ROS1* IHC has very high sensitivity (100%) but unsatisfactory specificity (84%) on detecting *ROS1* fusions in nonsmall-cell lung cancer (NSCLC). Therefore, *ROS1* FISH, reverse transcription-polymerase chain reaction (RT-PCR), or NGS is needed as a confirmatory test.[17] A total of 29 patients with *ROS1*-rearranged IMTs have been reported in the literature;[3-15] however, only one case with negative *ROS1* stain has been reported to have a *TFG-ROS1* fusion by RT-PCR.[11] Thus, *ROS1* IHC stain can be used as a screening tool, especially for *ALK*-negative IMTs.

FISH using the break-apart method is the gold standard for the detection of *ROS1* rearrangements in NSCLC.[18] However, our patient's FISH result was equivocal. Reviewing the literature, two other IMTs with *ROS1* fusions also had false-negative FISH results, which eventually proved to harbor *TFG-ROS1* fusions by RT-PCR. The equivocal *ROS1* FISH results may be due to a more complex mechanism of rearrangement instead of simple, balanced translocation of the partner gene.[5,10]

We performed two different NGS studies sequentially. The first used an amplicon-based library focusing on 31 fusion genes and 182 transcripts, and the result turned out to be a false negative. The other used hybrid-capture based library sequencing of both DNA (406 genes and selected introns of

31 genes involving rearrangements) and RNA (265 genes commonly involved in fusions), and detected a rare *FN1-ROS1* fusion. Both amplicon-base and hybrid-capture-based NGS methods have their pros and cons. Amplicon-based NGS needs less genomic material and hence is very sensitive to detect hotspot single-nucleotide variations. However, the sensitivity to detect gene rearrangements, especially those with different partners with variable breakpoints, is limited by the number of primers (amplicons). In contrast, hybrid capture-based NGS has an advantage in the detection of gene rearrangements because of the direct hybridization of the sequence of interest without the necessity of PCR. However, the higher demand for genomic material and longer turn-around time for library preparation are disadvantages that should be taken into consideration.[19]

To date, 29 *ROS1*-rearranged IMTs have been reported, of which 21 have been confirmed by RT-PCR or NGS, and *TFG-ROS1* was the most common fusion transcript (77%).[3-15] Our patient is the second reported case harboring an *FN1-ROS1* fusion in the medical literature. The other patient with an *FN1-ROS1* fusion IMT was reported by Lopez-Nunez *et al*. in 2020, but the *FN1* breakpoint was exon 41 (NM_212482) and the *ROS1* breakpoint was exon 32 (NM_002944).[13] Other fusion genes, such as *YWHAE-ROS1* and *TIMP3-ROS1,* have also been reported.[3,15] In terms of efficacy, the reported response rate of crizotinib was 100% (seven responders in seven cases) in IMT patients harboring *TFG-ROS1*.[3-15] Ceritinib and entrectinib have been reported to be effective in treating *ROS1*-rearranged IMTs as well.[8,12]

The *FN1-ROS1* fusion found in our patient demonstrated a similar fusion pattern to other oncogenic *ROS1* fusion proteins, with a retained *ROS1* kinase domain at the 3' end and the junction point on *ROS1* occurring at the 5' end of exon 32. Although the *FN1-ROS1* fusion protein is rare, *FN1* has been reported to fuse with *ALK* on IMTs.[3] *In vitro* studies have demonstrated different oncogenic properties and different responses to tyrosine kinase inhibitors among different *ALK* fusion partners, and *FN1-ALK* fusion protein has been reported to have the greatest ability to form foci in agar compared to all other *ALK* fusions.[20] Whether the *FN1-ROS1* fusion has different oncogenicity or different efficacy to tyrosine kinase inhibitors compared with other *ROS1* fusions remains unknown.

Conclusion

Our patient demonstrated that *ROS1* IHC still plays an important role in *ALK*-negative IMT patients, as it is a cheap and fast diagnostic tool to guide further treatment. As NGS has become a popular choice of confirmatory test, different library preparation methods might have different sensitivity, especially when targeting oncogenic fusions with a variety of partner genes. Crizotinib for IMT patients with *FN1-ROS1* fusion is effective and tolerable.

Ethical approval and declaration of patient consent

This study was approved by the NTUH research ethics committee (project number: 202007045W).

The authors certify that they have obtained all appropriate patient consent forms. In the forms, the patient has given her consent for her images and other clinical information to be reported in the journal. The patient understands that her name and initials will not be published, and due efforts will be made to conceal her identity, but that anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Tothova Z, Wagner AJ. Anaplastic lymphoma kinase-directed therapy in inflammatory myofibroblastic tumors. Curr Opin Oncol 2012;24:409-13.
2. Baranov E, Hornick JL. Soft tissue special issue: Fibroblastic and myofibroblastic neoplasms of the head and neck. Head Neck Pathol 2020;14:43-58.
3. Lovly CM, Gupta A, Lipson D, Otto G, Brennan T, Chung CT, *et al*. Inflammatory myofibroblastic tumors harbor multiple potentially actionable kinase fusions. Cancer Discov 2014;4:889-95.
4. Antonescu CR, Suurmeijer AJ, Zhang L, Sung YS, Jungbluth AA, Travis WD, *et al*. Molecular characterization of inflammatory myofibroblastic tumors with frequent ALK and ROS1 gene fusions and rare novel RET rearrangement. Am J Surg Pathol 2015;39:957-67.
5. Yamamoto H, Yoshida A, Taguchi K, Kohashi K, Hatanaka Y, Yamashita A, *et al*. ALK, ROS1 and NTRK3 gene rearrangements in inflammatory myofibroblastic tumours. Histopathology 2016;69:72-83.
6. Hornick JL, Sholl LM, Dal Cin P, Childress MA, Lovly CM. Expression of ROS1 predicts ROS1 gene rearrangement in inflammatory myofibroblastic tumors. Mod Pathol 2015;28:732-9.
7. Fujita H, Yoshida A, Taniguchi H, Katai H, Sekine S. Adult-onset inflammatory myofibroblastic tumour of the stomach with a TFG-ROS1 fusion. Histopathology 2015;66:610-2.
8. Ambati SR, Slotkin EK, Chow-Maneval E, Basu EM. Entrectinib in Two Pediatric Patients With Inflammatory Myofibroblastic Tumors Harboring ROS1 or ALK Gene Fusions. JCO Precis Oncol. 2018;2018:https://ascopubs.org/doi/10.1200/PO.18.00095. Epub 2018 Sep 13. PMID: 31763577; PMCID: PMC6874363.
9. Taylor MS, Chougule A, MacLeay AR, Kurzawa P, Chebib I, Le L, *et al*. Morphologic overlap between inflammatory myofibroblastic tumor and IgG4-related disease: Lessons from next-generation sequencing. Am J Surg Pathol 2019;43:314-24.
10. Chang JC, Zhang L, Drilon AE, Chi P, Alaggio R, Borsu L, *et al*. Expanding the molecular characterization of thoracic inflammatory myofibroblastic tumors beyond ALK gene rearrangements. J Thorac Oncol 2019;14:825-34.
11. Mai S, Xiong G, Diao D, Wang W, Zhou Y, Cai R. Case report: Crizotinib is effective in a patient with ROS1-rearranged pulmonary inflammatory myofibroblastic tumor. Lung Cancer 2019;128:101-4.
12. Li Y, Chen X, Qu Y, Fan JM, Li Y, Peng H, *et al*. Partial response to ceritinib in a patient with abdominal inflammatory myofibroblastic tumor carrying a TFG-ROS1 fusion. J Natl Compr Canc Netw 2019;17:1459-62.
13. Lopez-Nunez O, John I, Panasiti RN, Ranganathan S, Santoro L, Grélaud D, *et al*. Infantile inflammatory myofibroblastic tumors: Clinicopathological and molecular characterization of 12 cases. Mod Pathol 2020;33:576-90.
14. Preobrazhenskaya EV, Iyevleva AG, Suleymanova AM, Tiurin VI, Mitiushkina NV, Bizin IV, *et al*. Gene rearrangements in consecutive

series of pediatric inflammatory myofibroblastic tumors. Pediatr Blood Cancer 2020;67:e28220.
15. Schoolmeester JK, Minn K, Sukov WR, Halling KC, Clayton AC. Uterine inflammatory myofibroblastic tumor involving the decidua of the extraplacental membranes: Report of a case with a TIMP3-ROS1 gene fusion. Hum Pathol 2020;100:45-6.
16. Coffin CM, Hornick JL, Fletcher CD. Inflammatory myofibroblastic tumor: Comparison of clinicopathologic, histologic, and immunohistochemical features including ALK expression in atypical and aggressive cases. Am J Surg Pathol 2007;31:509-20.
17. Viola P, Maurya M, Croud J, Gazdova J, Suleman N, Lim E, *et al*. A validation study for the use of ROS1 immunohistochemical staining in screening for ROS1 translocations in lung cancer. J Thorac Oncol 2016;11:1029-39.
18. Rossi G, Jocollé G, Conti A, Tiseo M, Zito Marino F, Donati G, *et al*. Detection of ROS1 rearrangement in non-small cell lung cancer: Current and future perspectives. Lung Cancer (Auckl) 2017;8:45-55.
19. Sheikine Y, Kuo FC, Lindeman NI. Clinical and technical aspects of genomic diagnostics for precision oncology. J Clin Oncol 2017;35:929-33.
20. Childress MA, Himmelberg SM, Chen H, Deng W, Davies MA, Lovly CM. ALK fusion partners impact response to ALK inhizbition: Differential effects on sensitivity, cellular phenotypes, and biochemical properties. Mol Cancer Res 2018;16:1724-36.

Supplementary material available online

Supplement 1: List of selected fusion genes and transcripts included in the amplicon-based next-generation sequencing

Fusion genes:

ABL1, ALK, BCR, BRAF, CD74, ERG, ESR1, ETV1, ETV4, ETV5, ETV6, EZR, FGFR1, FGFR2, FGFR3, KMT2A, MET, NRG1, NTRK1, NTRK2, NTRK3, NUTM1, PDGFRA, PDGFRB, RARA, RET, ROS1, RSPO2, SDC4, SLC34A2, TMPRSS2

Transcripts for ROS1:

CCDC6-ROS1, CD74-ROS1, CLIP1-ROS1, CLTC-ROS1, ERC1-ROS1, EZR-ROS1, GOPC-ROS1, MS-ROS1, MYO5A-ROS1, PPFIBP1-ROS1, SDC4-ROS1, SLC34A2-ROS1, TFG-ROS1, TMEM106B-ROS1, TPM3-ROS1

Supplement 2: List of selected genes for rearrangement included in the hybrid capture-based next-generation sequencing:

ALK, BCL2, BCL6, BCR, BRAF, CCND1, CRLF2, EGFR, EPOR, ETV1, ETV4, ETV5, ETV6, EWSR1, FGFR2, IGH, IGK, IGL, JAK1, JAK2, KMT2A (MLL), MYC, NTRK1, PDGFRA, PDGFRB, RAF1, RARA, RET, ROS1, TMPRSS2, TRG