

BRIEF REPORT

A Novel *ACVR2A::RAF1* Fusion in Spindle Cell Sarcoma

Anfeng Jiang¹ | Huan Li^{2,3} | Dongbing Li^{2,3} | Huafei Chen^{2,3} | Lina Zhao^{2,3} | Ying Zhang^{2,3} | Yangyang Li^{2,3} | Rong Rong⁴ | Bin Li¹ | Sheng Xiao⁵

¹Department of Oncology, Xiangya Hospital, Central South University, Changsha, Hunan Province, China | ²Advanced Molecular Pathology Institute of Soochow University and SANO, Suzhou, China | ³Suzhou Sano Precision Medicine Ltd, Suzhou, China | ⁴Department of Biological Sciences, Xi An Jiaotong-Liverpool University, Suzhou, China | ⁵Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

Correspondence: Bin Li (binsuxy@csu.edu.cn) | Sheng Xiao (sxiao@partners.org)

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ABSTRACT

Background: Kinase-rearranged spindle cell sarcomas are characterized by unique molecular features. The advent of next-generation sequencing (NGS) has enabled the detection of a multitude of kinase fusions, thereby contributing to the accurate categorization of these tumors.

Case Presentation: A 37-year-old woman experienced the fourth recurrence of a cranial base tumor 25 years following the initial surgery and radiation therapy. Histological analysis disclosed spindle-shaped and oval tumor cells, along with a high number of mitotic figures. Immunohistochemistry showed a null immunophenotype, negative for pan-TRK, S-100, CD34, pan-CK, GFAP, and Olig2. Molecular analysis of the tumor tissue identified a novel *ACVR2A::RAF1* fusion, comprising the first four exons of *ACVR2A* and the last nine exons of *RAF1*. The resulting fusion protein retains the extracellular and transmembrane domains of *ACVR2A*, while its kinase domain is replaced by the kinase domain of *RAF1*. This hybrid protein likely contributes to tumorigenesis by activating *RAF1* signaling in response to *ACVR2A* ligands from the TGF- β superfamily.

Treatment and Outcome: The patient was treated with the MEK1 inhibitor Trametinib, 2 mg per time and once a day. One month later, MRI showed significant tumor shrinkage and pain relief.

Conclusion: The *ACVR2A::RAF1* fusion represents a novel genomic profile in *RAF1*-rearranged spindle cell sarcoma, offering a rational basis for targeted therapy. This case highlights the importance of molecular diagnostics in identifying actionable targets and guiding treatment, potentially leading to significant clinical benefits.

1 | Introduction

Tyrosine kinase-rearranged spindle cell tumor is a diverse group of diseases ranging from benign to malignant. To date, 10 tyrosine kinases have been involved in these tumors, including *RAF1*, *BRAF*, *RET*, *NTRK1/2/3*, *ROS1*, *ALK*, *EGFR*, and *MET*.

Due to the lack of diagnostic histological and immunophenotypic features, accurate diagnosis can be challenging. However, with the rapid advancements in next-generation sequencing (NGS), the identification of specific molecular alterations, such as kinase fusions, has greatly improved disease classification and facilitated the development of precisely targeted therapies.

Anfeng Jiang and Huan Li contributed equally to this study.

RAF1, also known as c-RAF, is a significant factor in numerous malignancies [1]. Its activation is intricately connected to the RAS/RAF/MAPK signaling pathway. This pathway is crucial for modulating cell proliferation, differentiation, and survival. The formation of RAF1-BRAF heterodimers represents a key mechanism for their activation [2]. This interaction can also contribute to resistance against RAF inhibitors in specific types of tumors. RAF1 fusion gene has been detected in various tumors, including infantile fibrosarcoma like stromal tumors, melanoma, pancreatic acinar cell carcinoma, soft tissue sarcoma, and pleomorphic xanthoastrocytoma [1]. These fusion genes promote tumor cell proliferation and survival by constitutively activating the MAPK signaling pathway, providing a potential molecular basis for targeted therapy [3].

The Activin A receptor Type 2A (ACVR2A), which is a component of the TGF- β superfamily, is responsible for encoding a receptor that facilitates the function of activin [4]. It contains three structural domains: an extracellular receptor domain, a transmembrane domain, and a kinase domain. Upon ligand binding to the extracellular receptor domain, the kinase domain phosphorylates and activates the autophosphorylated type I receptor, further activating downstream SMAD and non-SMAD signaling pathways and initiating a series of intracellular signaling events [5].

This report presents a novel fusion, ACVR2A::RAF1, identified in a case of spindle cell sarcoma. The fusion protein retains the complete kinase domain of the RAF1 gene at the C-terminus, while the N-terminus is the ACVR2A. In this fusion, ACVR2A retains the extracellular receptor domain and transmembrane domain, which may contribute to the cellular localization of the ACVR2A::RAF1 fusion protein. However, the ACVR2A::RAF1 fusion is reported for the first time in spindle cell sarcomas, adding a new dimension to the molecular landscape of these tumors and potentially offering new therapeutic targets. The discovery of these novel fusions not only deepens our comprehension of the molecular underpinnings of spindle cell sarcoma but also paves the way for new targeted therapeutic approaches. This case report aims to highlight the significance of molecular diagnostics in identifying actionable genetic alterations and guiding personalized treatment strategies, ultimately improving patient outcomes.

2 | Case Presentation

2.1 | Clinical Findings

A 37-year-old female patient presented with the fourth recurrence of a cranial base tumor, 25 years after the initial surgery and radiation. She had undergone multiple surgeries and radiation therapy in the past, but the tumor recurred 3 months after the most recent surgery.

2.2 | Gross Examination

Gross examination revealed a large mass located in the nasal cavity, extensively invading the surrounding structures, middle cranial base, and infratemporal fossa, with the largest diameter measuring approximately 6.09 cm.

2.3 | Histology and Immunohistochemical Study

Microscopic observation showed spindle-shaped, or oval tumor cells arranged in short bundles, with easily visible mitotic figures (Figure 1A–C). Immunohistochemistry showed a null immunophenotype, negative for pan-TRK, CD34, S-100, and pan-CK (Figure 1D–F). As shown in Figure 1G, targeted DNA NGS analysis of tumor samples revealed complex genomic structure and numerical changes. A histopathologic diagnosis of spindle cell sarcoma was made.

2.4 | Molecular Analysis

Given the inconclusive results from morphology and immunohistochemistry, DNA and RNA next-generation sequencing (NGS) testing was performed. This testing revealed the ACVR2A::RAF1 fusion, consisting of the first four exons of ACVR2A and the last nine exons of RAF1. The resulting fusion protein retains the extracellular and transmembrane domains of ACVR2A, while its kinase domain is replaced by the kinase domain of RAF1 (Figure 2A). The ACVR2A::RAF1 fusion transcript was amplified by an RT-PCR assay using primers specific to ACVR2A (F_ACVR2A: 5'-AGTCACACAGCCCCTCAA-3'; F_ACVR2A nest: 5'-TGTGCATTTGGGTGTACAGG-3') and RAF1 (R_RAF1: 5'-CTCTCTGTCCACGAGGCCTAA-3'; R_RAF1 nest: 5'-TCCCAGATACTGGTGCCCG-3'), which produced a PCR product at the expected size. Sanger sequencing of the PCR product confirmed ACVR2A::RAF1 (Figure 2B,C). The DNA NGS testing also identified a likely pathogenic mutation in the CHEK2 gene and very complex genomic structural and numerical alteration. Homologous recombination repair deficiency (HRD) analysis showed evidence of HRD, with a genome instability score (GIS) of 91 (GIS cutoff ≥ 42).

2.5 | Targeted Therapy

The patient was treated with the MEK1 inhibitor Trametinib. One month later, MRI examination revealed significant shrinkage of the tumor mass (Figure 3A,B), and the patient experienced substantial pain relief.

3 | Discussion

This study describes a novel ACVR2A::RAF1 fusion identified in a spindle cell sarcoma, significantly expanding the genomic profile of RAF1-rearranged tumors. This finding not only underscores the importance of molecular diagnostics in identifying actionable genetic alterations but also highlights the potential for targeted therapies to achieve substantial clinical benefits. The identification of this fusion prompts further investigation into its biological significance and oncogenic mechanisms.

The ACVR2A::RAF1 fusion represents a unique genetic alteration in spindle cell sarcomas. In this fusion, ACVR2A replaces the N-terminal self-inhibitory domain of RAF1, positioning the kinase domain closer to the plasma membrane. This structural change likely disrupts RAF1's intrinsic auto-inhibition, allowing for constitutive activation of the MAPK pathway independent

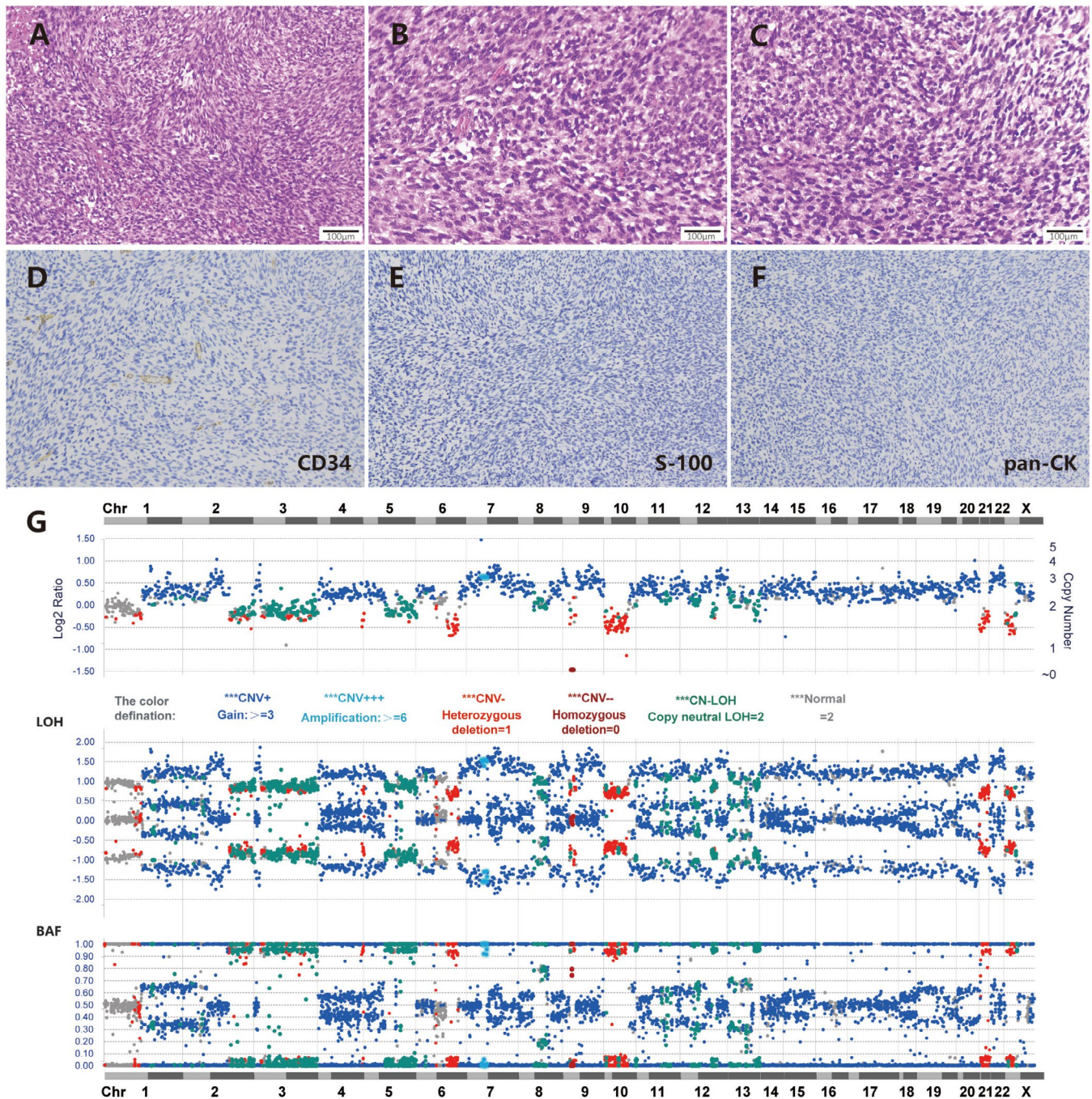


FIGURE 1 | Histological, immunohistochemical, and genomic results of the surgical sample. (A–C) Hematoxylin and eosin (H&E) staining demonstrated spindle cells arranged in fascicles, with mitosis frequently observed. Immunohistochemistry was negative for (D) CD34, (E) S-100, and (F) Pan-CK. (G) Targeted DNA NGS analysis of the tumor specimen showed complex genomic structural and numerical alterations.

of RAS activation. This mechanism is consistent with previous studies demonstrating that RAF1 fusions can drive tumorigenesis through aberrant activation of downstream signaling pathways.

RAF1 fusions have been identified in various malignancies, including melanoma, soft tissue sarcomas, and pancreatic acinar cell carcinomas. These fusions typically involve the replacement of RAF1's N-terminal regulatory domain with various fusion partners, resulting in constitutive activation of the kinase domain. The ACVR2A::RAF1 fusion identified in this study is unique in that it incorporates the extracellular receptor and

transmembrane domains of ACVR2A, potentially influencing cellular localization and signaling properties. This feature distinguishes it from previously reported RAF1 fusions and may contribute to its oncogenic potential.

RAF1 belongs to the RAF family, which also encompasses A-RAF and BRAF. The RAF1 gene encodes a serine/threonine kinase containing an N-terminal RAS-binding domain (RBD), a cysteine-rich domain (CRD), a serine/threonine-rich domain (CR2), and a C-terminal kinase domain. The self-inhibition of RAF1 is facilitated by the interaction between its N-terminal region and C-terminal kinase domain, as well as by the

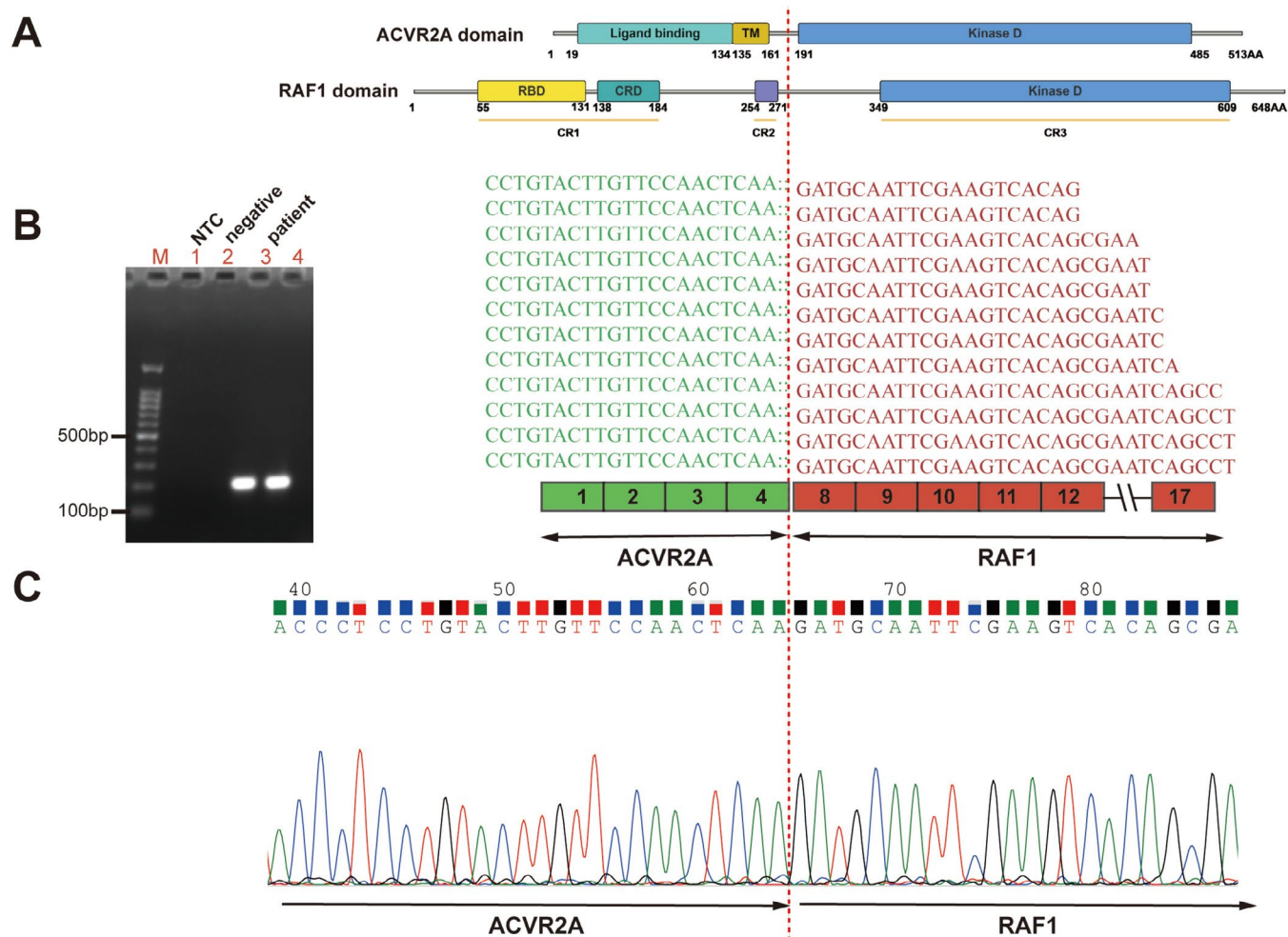


FIGURE 2 | Characterization of the *ACVR2A::RAF1* fusion. (A) Targeted RNA NGS found *ACVR2A::RAF1* fusion transcript, encoding the ligand domain and transmembrane domain (TM) from *ACVR2* and the kinase domain from *RAF1*. The vertical dotted line indicates the breakpoint. (B) RT-PCR using *ACVR2A* and *RAF1* gene-specific primers amplified a product of expected size from *ACVR2A::RAF1*. (C) Sanger sequencing of the PCR product confirmed the fusion.

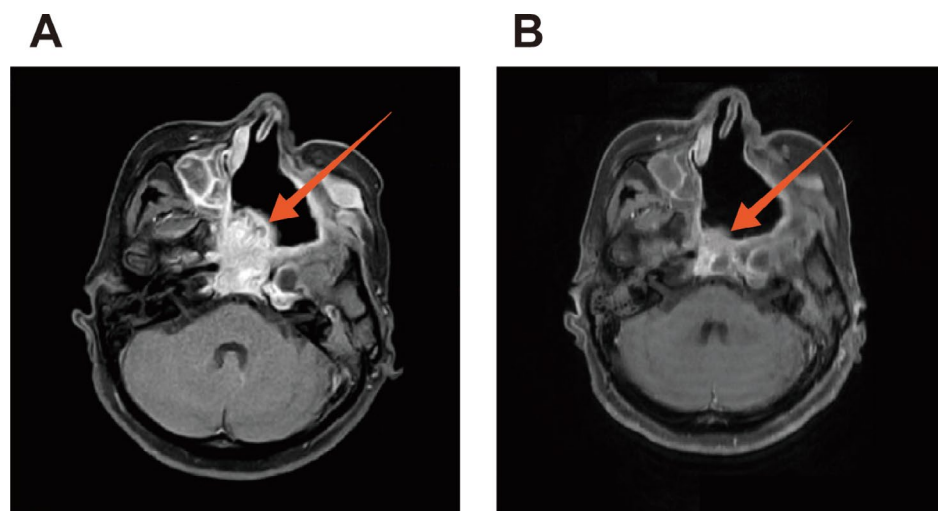


FIGURE 3 | MRI images of the patient's tumors before and after treatment. (A) Pre-treatment: A large mass located in the nasal cavity extensively invades the surrounding structures, middle cranial base, and infratemporal fossa, with the largest diameter of about 7 cm (illustrated by arrows). (B) One-month post-treatment: The mass significantly shrank, with the largest diameter of about 3 cm.

phosphorylation of RAF1 at Ser259 and Ser621 and its binding to 14-3-3 proteins [6, 7]. The activation of RAF1 is a complex process. Initially, when the RAS protein is activated, RAF1 is recruited to the plasma membrane through the interaction between RAS-RBD and RAS-GTP. This interaction leads to the relocalization of the RAF1-CRD and alleviates self-inhibition by phosphorylating its own activation site. Subsequently, the action of multiple kinases promotes the dimerization of the catalytic domain, thereby activating RAF1 [8].

To date, There are currently 19 reported RAF1 fusion genes, including ATG7, BCL6, CLIP1, EFN1, ESRP1, GATM, GOLGA4, HERPUD1, LMNA, LRCH3, LRRFIP2, KIF5B, MAP4, MTAP, PDZRN3, QKI, RUFY1, TLN2, and TRIM33 [1, 9–17]. The identification of actionable genetic alterations such as the ACVR2A::RAF1 fusion has significant implications for targeted therapies. In this case, treatment with the MEK1 inhibitor Trametinib resulted in marked tumor shrinkage and pain relief, highlighting the efficacy of targeting the MAPK pathway in RAF1 fusion-positive tumors. This finding is consistent with previous reports demonstrating the effectiveness of MEK inhibitors in treating RAF1 fusion-driven malignancies [18, 19]. Additionally, preclinical studies suggest that combining MEK inhibitors with mTOR inhibitors may enhance therapeutic efficacy by targeting multiple oncogenic pathways [20]. Future studies should explore the potential benefits of combination therapies in managing RAF1 fusion-positive spindle cell sarcoma.

The clinical management of spindle cell sarcoma remains challenging due to their aggressive nature and high propensity for recurrence and metastasis. The advent of molecular diagnostics has revolutionized the field by identifying novel genetic alterations that can guide personalized treatment strategies. The ACVR2A::RAF1 fusion identified in this study represents a new therapeutic target in this context. Further research is warranted to elucidate the biological functions of this fusion protein, its role in tumor initiation and progression, and the development of resistance mechanisms. Additionally, large-scale genomic studies are needed to determine the prevalence of this fusion in other spindle cell sarcoma and related malignancies.

In conclusion, the identification of the ACVR2A::RAF1 fusion in a spindle cell sarcoma expands our understanding of the molecular landscape of these tumors and provides a rational basis for targeted therapies. This case highlights the importance of molecular diagnostics in uncovering actionable genetic alterations and guiding personalized treatment strategies. Future studies should focus on exploring the biological significance of this fusion and its potential applications in cancer therapy.

Acknowledgments

The authors have nothing to report.

Ethics Statement

The authors have nothing to report.

Consent

Informed consent was obtained from the patient.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data and materials of this study will be available from the corresponding author [Bin Li] upon reasonable request.

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