



FUS/EWSR1::TFCP2-rearranged bone and soft tissue tumors: evidence of gene promiscuity

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Received: 18 March 2026 / Revised: 13 August 2026 / Accepted: 18 August 2026
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Abstract

The *FET(FUS/EWSR1)::TFCP2* fusion defines a distinct molecular subtype of spindle cell/sclerosing rhabdomyosarcoma (RMS), typically involving the mandible and maxilla. These tumors demonstrate myogenic differentiation and are clinically aggressive. In addition to the characteristic fusion, they often show features of homologous recombination deficiency (HRD), genomic instability, and expression of *ALK* and *TERT* truncated variants. This study presents a cohort of 25 patients with *FET::TFCP2* fusion, representing the largest single-center series to date. While most tumors involve bones, 8 cases are soft tissue tumors, including the cheek (2 cases), epididymis, bladder, abdominal wall, neck, temporal part, and scalp. Genomically, approximately 40% of cases had additional genomic amplifications involving receptor tyrosine kinase pathways and cell cycle/proliferation-related genes. Immunohistochemically, 23 of 25 tumors expressed myogenic markers (Desmin, MyoD1 and Myogenin); however, two lacked myogenic differentiation. These two cases had distinct histopathologic features characterized by round blue cell morphology and dense myxoid stroma, rather than the typical spindle and epithelioid cell morphology of *FET::TFCP2*-rearranged sarcomas. Methylation profiling further revealed that neither case clustered with canonical *FET::TFCP2*-rearranged sarcomas; one grouped with Ewing sarcoma, and the other did not overlap with any known soft tissue tumor class. These findings expand the morphologic, immunophenotypic and epigenetic spectrum of *FET::TFCP2*-rearranged tumors and suggest that a subset of these tumors may extend beyond the conventional spindle cell/sclerosing rhabdomyosarcoma phenotype.

Keywords *FET::TFCP2* fusion · RMS · Histopathologic features · Myogenic differentiation

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Introduction

The *FET(FUS/EWSR1)::TFCP2* fusion defines a distinctive molecular subtype of spindle cell/sclerosing rhabdomyosarcoma (RMS), the intraosseous spindle cell rhabdomyosarcoma [1]. This entity was first described in 2018 by Watson et al. [2], who identified 3 sarcomas with the *FET(FUS/EWSR1)::TFCP2* rearrangement. These cases formed a unique group in transcriptome cluster analysis, separate from other *FET (FUS/EWSR1)*-rearranged tumors, and were enriched for the expression of genes related to muscle development. Based on their spindle cell and epithelioid morphology, and the expression of myogenic and epithelial differentiation markers, Watson et al. [2] defined this entity as epithelioid rhabdomyosarcoma.

Approximately 130 cases of this tumor type have been reported (Table S1). These tumors predominantly occur in young adults around the age of 30 s, with roughly equal male and female patients [3–5], and have a strong preference for craniofacial bone regions as the primary onset site [6]. Histologically, these tumors show an admixture of spindle and epithelioid cells with moderately abundant eosinophilic cytoplasm [6–8], high-grade features including visible mitotic activity and areas of necrosis [7], and generally lack distinct rhabdoid morphology [5, 6]. On immunohistochemistry (IHC), myogenic differentiation markers are consistently present, with MyoD1 (usually diffusely positive) being more sensitive than Myogenin (usually focally positive) [8, 9]. Additionally, epithelial differentiation markers, such as Pan-CK, are observed in 60–85% of cases [4–6], and almost all tumors are positive for ALK expression with a diffuse cytoplasmic overexpression pattern.

Transcriptome analysis has shown that the *TFCP2*-rearranged sarcomas often have high mRNA expression of *ALK* and *TERT* [2, 9, 10]. The upregulation of *ALK* is primarily enriched in sequences 3' end to exon 17 [9], which is associated with short transcripts generated by somatic *ALK* long-in-frame deletion [10]. This molecular alteration explains the common ALK immunohistochemical positivity in these tumors, as commercial ALK antibodies typically bind the C-terminus of ALK. Additionally, these tumors express specific *TERT* Δe1-2 variants that lack exons 1 and 2, resulting from the *FET::TFCP2* fusion protein binding to intron 2, which leads to high expression of truncated TERT proteins [10]. These tumors also correlate with an overall complex genomic profile, characterized by highly rearranged genomes and associated with homologous recombination repair deficiency (HRD) [10]. Other recurrent genomic alterations include homozygous deletions of *CDKN2A* and its neighboring gene *MTAP*, and mutations of *IGFBP5* and *PAPPA2* [10].

In this report, we present 25 cases of the *TFCP2*-rearranged tumors from China, largely confirming the previously described clinical, pathological, and genomic features of this tumor type. Notably, we also describe two cases that were morphologically distinct from classic *TFCP2*-rearranged sarcomas. These tumors both exhibited round blue cell morphology with densely myxoid stroma. Importantly, both lacked expression of myogenic markers. Methylation profiling showed that both tumors clustered separately from the classic *TFCP2*-rearranged sarcomas. These findings further highlight the phenotypic heterogeneity of *TFCP2*-rearranged tumors, and their relationship to spindle cell/sclerosing rhabdomyosarcoma remains to be fully clarified.

Materials and methods

Patients

This retrospective study included 25 consultation cases of tumors with *EWSR1/FUS-TFCP2* fusion, which were reviewed at the Department of Pathology, Foshan Hospital of Traditional Chinese Medicine, between January 2021 and February 2025. The specimens were originally obtained from biopsies or surgical resections from various hospitals in China. For histopathological studies, hematoxylin and eosin (H&E) and IHC slides were obtained from the primary institutions and reviewed in our department. Additionally, 20 unstained slides were newly sectioned from the original tissue blocks and used for additional H&E staining, IHC, and molecular analysis. Clinical, radiological, and follow-up data were collected through direct communication (telephone or online) with the patients or their next of kin and from medical records provided by them.

IHC

IHC analysis was performed on 4 μm formalin-fixed, paraffin-embedded (FFPE) sections. Following deparaffinization and rehydration, antigen retrieval was carried out in citrate buffer (pH 6.0). The sections were blocked and then incubated overnight at 4 °C with primary antibodies. Staining was detected using an HRP-conjugated secondary antibody system and visualized with 3,3'-diaminobenzidine (DAB), followed by counterstaining with hematoxylin. Both primary and secondary antibodies were obtained from Gene Tech (Shanghai) Co., Ltd. Appropriate negative and positive controls were included for all staining procedures. The following primary antibodies were used: Myogenin (Cat. No. MM-0161), MyoD1 (Cat. No. MRM-0151), Pan-Cytokeratin (Cat. No. CM-0641), ALK (Cat. No. ARM-0081), Desmin (Cat. No. DRM-0021), and CD99 (Cat. No.

Table 1 The clinical and pathological features of 25 *TFCP2*-rearranged tumor cases from 2021 to 2025

Case#	Age	Gender	Location	Size	Gene fusions	Confirmed by	Cytological features	IHC		Other genomic changes		
								ALK	MyoD1	Myogenin	Desmin	CK
1	21	Female	Left cheek	9*6.5*8.6 cm	<i>FUS::TFCP2</i>	FISH	Spindle and epithelioid	Cyto- plasmic diffuse+	+	-	+	N/A
2	36	Male	Mandible	N/A	<i>FUS::TFCP2</i>	FISH	Spindle and epithelioid	Cyto- plasmic diffuse+	+	-	+	N/A
3	55	Female	Mandible	N/A	<i>EWSR1::TFCP2</i>	FISH	Spindle and epithelioid	Cyto- plasmic diffuse+	+	-	+	N/A
4	31	Male	Epididymis	7*5.6*4.5 cm	<i>EWSR1::TFCP2</i>	FISH	Epithelioid and rhabdoid	Cyto- plasmic focal+	+	+	+	N/A
5	11	Female	Mandible	11*7.5*7 cm	<i>FUS::TFCP2</i>	FISH	Spindle and partial epithelioid	Cyto- plasmic partial+	+	focal +	+	partial+
6	25	Female	Acetabulum	14*16.3*18.9 cm	<i>FUS::TFCP2</i>	FISH	Spindle and epithelioid	Cyto- plasmic focal	+	+	+	N/A
7	29	Female	Left temporomandibular region	6*4.5*3 cm	<i>FUS::TFCP2</i>	FISH (DNA NGS was performed but did not cover the fusion)	Spindle and epithelioid	Cyto- plasmic diffuse+	+	-	+	SNV: <i>ALK</i> LongInDel(E2-E17) (30%) CNV: Gains of 5p13.1, 7p22.3 → p14.1(<i>RAC1/ETV1</i> amplification, 6.4/6.9) and chr19; losses of 1p13.2 → p12, chr3, 5q, 6q15 → q26, chr8, 9p(including <i>CDKN2A/B</i> , 0/0), chr10, 12q13.13 → q24.33, chr13, chr14, chr15 and 16q CNV: Gains of 17q21.32 → q25.3, 19p13.2(regional amplification), 19p13.11 and Xp(<i>EIF1AX</i> amplification, 5.3); losses of 3p25.2 → p13, 8p23.1 → p12, 9p24.1 → p21.2(including <i>CDKN2A/B</i> , 0.1/0.6), 12p, 15q15.1 → q15.3, 19p13.12
8	25	Male	Mandible	7*4.5*3 cm	<i>FUS::TFCP2</i>	FISH (DNA NGS was performed but did not cover the fusion)	Spindle and rhabdoid	Cyto- plasmic diffuse+	+	-	+	partial+

Table 1 (continued)

Case#	Age	Gender	Location	Size	Gene fusions	Con- firmed by	Cyto- logical features	IHC				Other genomic changes	
								ALK	MyoD1	Myogenin	Desmin		CK
9	23	Male	Right fron- totemporal region	N/A	<i>FUS::TFCP2</i>	FISH (DNA NGS was per- formed but did not cover the fusion)	Spindle and rhabdoid	Cyto- plasmic partial +	+	partial +	+	+	SNV: <i>KMT2B</i> p.P731S(20.34%); CNV: <i>FYN</i> amplification(copy number 5), <i>RUNX1T1</i> amplification(4), <i>TSC1</i> amplification(4), <i>FRS2</i> amplification(4), <i>CALR</i> amplification(7), <i>B4BAM1</i> amplification(7), <i>JAK3</i> amplification(7), <i>UPF1</i> amplification(6), <i>BCL2L1</i> amplification(4) CNV: 16p13 loss, 1p31 → 1p36 amplification
10	65	Female	Mandible	N/A	<i>FUS(E6)::TFCP2(E2)</i>	FISH and NGS	Spindle and rhabdoid	Cyto- plasmic diffuse +	+	-	+	+	
11	33	Male	Maxilla	Primary: 2.1*2*0.5 Recurrent: 5*4*3 cm	<i>FUS(E6)::TFCP2(E2)</i>	FISH and NGS (took NGS test twice, one for the pri- mary site and another for the recur- rent site after two years)	Spindle and epithelioid	Cyto- plasmic partial weakly +	+	-	+	-	•Primary: CNV: <i>CDKN2A</i> loss(copy number 1.1) •Recurrent: SNV: <i>APC</i> p.V7A (61.93%), <i>IDH1</i> p.A141P (36.39%), <i>JAK3</i> p.E571G (40.31%); CNV: <i>MET</i> gain(4.85), <i>CDKN2A</i> loss(0.23), <i>PTE</i> <i>N</i> loss(1.38), <i>RBI</i> loss(0.79)
12	8	Female	Bladder	5*4.5*4 cm	<i>EWSR1(E5)::TFCP2(E2)</i>	NGS	Spindle and epithelioid	-	+	-	Scattered +	-	SNV: <i>ALK</i> LongInDel(E2- E18 del and E2-E17 del), <i>NOTCH2</i> p.R2400X CNV: <i>CDKN2A/B</i> loss, <i>NF2</i> loss, <i>MTAP</i> loss
13	11	Male	Left orbit	7*5*1 cm	<i>EWSR1(E5)::TFCP2(E3)</i>	NGS	Spindle and epithelioid	Cyto- plasmic partial +	+	partial +	partial +	+	CNV: <i>CDKN2A/B</i> loss(copy number 0/0), <i>MTAP</i> loss(0)

Table 1 (continued)

Case#	Age	Gender	Location	Size	Gene fusions	Con- firmed by	Cyto- logical features	IHC		Other genomic changes			
								ALK	MyoD1	Myogenin	Desmin	CK	
14	32	Male	Left neck	N/A	<i>FUS</i> (E6):: <i>TFCP2</i> (E2)	NGS	Spindle and epithelioid	Cyto- plasmic diffuse+	+	-	+	+	CNV: <i>CDKN2A/B</i> loss(copy number 0/0), <i>EPHA3</i> amplification(6.8), <i>CLDN18</i> amplification(9.8), <i>PIK3CB</i> amplification(9.8) SNV: <i>SMARCA4</i> p.R1445Q (10%); CNV: <i>TCF3</i> amplification(copy number 4.2), <i>DOT1L</i> amplification(4.2), <i>GNAT1</i> amplification(4.2), <i>ARHGAP4</i> amplification(9.5), <i>MEK2</i> exon4_exon11 amplification(4.2) SNV: <i>RAD17</i> p.G346Rfs*20 (19%), <i>NOTCH3</i> p.P805T(16%); CNV: <i>CDKN2A/B</i> loss(copy number 0/0), <i>MTAP</i> loss(0), <i>EPH6</i> E6-E18 del(0), <i>MLLT3</i> loss(0), <i>CRKL</i> amplification(7.6), <i>BCL2</i> amplification(9.2), <i>CCNE1</i> amplification(11.9), <i>CD79A</i> amplification (5.3), <i>DGCR8</i> amplification(7.6), <i>ERCC1</i> amplification(9.7), <i>PHOX2B</i> amplification(29.7), <i>PIK3C3</i> amplification(6.4), <i>SERPINB3/4</i> amplification(6.3/6.3), <i>SETBP1</i> amplification(5.1), <i>SLC34A2</i> amplification(5.8), <i>CRBN</i> amplification(6), <i>CEACAM3</i> amplification(5.3), <i>CIC</i> amplification(7.2), <i>ERCC2</i> amplification(9.7), <i>LZTR1</i> amplification(10.1)
15	24	Male	Pubic bone	11*9*6.5 cm	<i>FUS</i> (E7):: <i>TFCP2</i> (E1)	NGS	Spindle	Cyto- plasmic diffuse+	partia+	-	partial+	-	
16	12	Female	Mandible	N/A	<i>FUS</i> (E6):: <i>TFCP2</i> (E2)	NGS	Spindle	Cyto- plasmic diffuse+	partia+	+	partial+	-	

Table 1 (continued)

Case#	Age	Gender	Location	Size	Gene fusions	Con- firmed by	Cyto- logical features	IHC			Other genomic changes		
								ALK	MyoD1	Myogenin	Desmin	CK	
17	32	Female	Mandible	5.5*4 cm	<i>EWSR1</i> (E5):: <i>TFCP2</i> (E2)	FISH and NGS	Spindle and epithelioid	Cyto- plasmic diffuse+	+	-	+	+	SNV: <i>NF2</i> c.1122+2 T>C (59.4%), <i>A2M</i> p.C972R (33.2%), <i>AXIN1</i> p.p.A76I(19.3%), <i>NEK11</i> p.S14Qfs*25 (24.3%), <i>PIK3CD</i> p.A77P (18%), <i>PRKDC</i> p.N869S (17.8%) SNV: <i>SPTA1</i> p.Y653H(29%), <i>NSD2</i> p.C1259R(34%); CNV: <i>MDM2</i> amplification(copy number 4.5), <i>MTAP</i> E2-E8 del(0); Others: <i>MDM2</i> intergenic rearrangement
18	34	Female	Abdominal wall	5*3 cm	<i>FUS</i> (E6):: <i>TFCP2</i> (E2)	FISH and NGS	Spindle and epithelioid	Cyto- plasmic focal+	+	+	+	+	amplification(copy number 4.5), <i>MTAP</i> E2-E8 del(0); Others: <i>MDM2</i> intergenic rearrangement
19	29	Female	Paranasal sinus	1.8*1.5*0.4 cm	<i>EWSR1</i> (E7):: <i>TFCP2</i> (E2)	FISH and NGS	Polygo- nal and epithelioid	Cyto- plasmic partial+	+	partial+	+	-	SNV: <i>TP53</i> p.Q331H(56.79%) CNV: <i>CDKN2A/B</i> loss(copy number 0.7/0.7), <i>MTAP</i> loss(0.7), <i>CRKL</i> amplification(8.2) CNV: <i>CDKN2A/B</i> loss(copy number 0/0), <i>MDM2</i> amplification(16.9), <i>AKT3</i> amplification(5.8), <i>CUL4A</i> amplification(4.7), <i>DGCR8</i> amplification(5.8), <i>FGF18</i> amplification(5.3), <i>FRS2</i> amplification(16.9), <i>INPP4B</i> amplification(4.9), <i>TRIO</i> amplification(8.2), <i>RGS7</i> amplification(5.8), <i>FH</i> amplification(5.8), <i>NPM1</i> amplification(5.3) SNV: <i>ALK</i> LongInDel(E2-E16) (9%); CNV: <i>NET1</i> amplification(copy number 4.5)
20	31	Female	Ilium	5.9*5*9.1 cm	<i>FUS</i> (E6):: <i>TFCP2</i> (E2)	NGS	Spindle and epithelioid	Cyto- plasmic diffuse+	+	diffuse+	+	weakly+	SNV: <i>TP53</i> p.Q331H(56.79%) CNV: <i>CDKN2A/B</i> loss(copy number 0/0), <i>MDM2</i> amplification(16.9), <i>AKT3</i> amplification(5.8), <i>CUL4A</i> amplification(4.7), <i>DGCR8</i> amplification(5.8), <i>FGF18</i> amplification(5.3), <i>FRS2</i> amplification(16.9), <i>INPP4B</i> amplification(4.9), <i>TRIO</i> amplification(8.2), <i>RGS7</i> amplification(5.8), <i>FH</i> amplification(5.8), <i>NPM1</i> amplification(5.3) SNV: <i>ALK</i> LongInDel(E2-E16) (9%); CNV: <i>NET1</i> amplification(copy number 4.5)
21	6	Male	Right tem- poral region	7.5*7.3*1.9 cm	<i>FUS</i> (E7):: <i>TFCP2</i> (E2)	FISH and NGS	Spindle and epithelioid	-	+	-	+	-	SNV: <i>TP53</i> p.Q331H(56.79%) CNV: <i>CDKN2A/B</i> loss(copy number 0/0), <i>MDM2</i> amplification(16.9), <i>AKT3</i> amplification(5.8), <i>CUL4A</i> amplification(4.7), <i>DGCR8</i> amplification(5.8), <i>FGF18</i> amplification(5.3), <i>FRS2</i> amplification(16.9), <i>INPP4B</i> amplification(4.9), <i>TRIO</i> amplification(8.2), <i>RGS7</i> amplification(5.8), <i>FH</i> amplification(5.8), <i>NPM1</i> amplification(5.3) SNV: <i>ALK</i> LongInDel(E2-E16) (9%); CNV: <i>NET1</i> amplification(copy number 4.5)
22	19	Male	Left cheek	N/A	<i>EWSR1</i> (E6):: <i>TFCP2</i> (E2)	NGS	Spindle and epithelioid	Cyto- plasmic partial+	+	-	+	+	SNV: <i>TP53</i> p.Q331H(56.79%) CNV: <i>CDKN2A/B</i> loss(copy number 0/0), <i>MDM2</i> amplification(16.9), <i>AKT3</i> amplification(5.8), <i>CUL4A</i> amplification(4.7), <i>DGCR8</i> amplification(5.8), <i>FGF18</i> amplification(5.3), <i>FRS2</i> amplification(16.9), <i>INPP4B</i> amplification(4.9), <i>TRIO</i> amplification(8.2), <i>RGS7</i> amplification(5.8), <i>FH</i> amplification(5.8), <i>NPM1</i> amplification(5.3) SNV: <i>ALK</i> LongInDel(E2-E16) (9%); CNV: <i>NET1</i> amplification(copy number 4.5)

Table 1 (continued)

Case#	Age	Gender	Location	Size	Gene fusions	Con- firmed by	Cyto- logical features	IHC		Other genomic changes		
								ALK	MyoD1	Myogenin	Desmin	CK
23	18	Female	Mandible	N/A	<i>FUS</i> (E6):: <i>TFCP2</i> (E2)	NGS	Spindle and epithelioid	Cyto- plasmic partial +	partia +	-	partial +	weakly +
24	58	Male	Under the scalp	N/A	<i>FUS</i> (E6):: <i>TFCP2</i> (E2)	FISH and NGS	Undif-ferentiated small round blue cells	-	-	-	-	-
25	32	Male	Thoracic vertebra	N/A	<i>EWSR1</i> (E6):: <i>TFCP2</i> (E2)	FISH and NGS	Round blue cells	Cyto- plasmic diffuse +	-	-	-	partial +

N/A not applicable or available, *SNV* single nucleotide variation, *CNV* copy number variation

CRM-0411). All antibodies were obtained from Shanghai Jiehao Biotechnology Co., Ltd and were used according to the manufacturer's instructions.

Fluorescence in situ hybridization (FISH)

FISH analysis was performed on 4 µm FFPE sections using dual-color, break-apart probes for *EWSR1*, *FUS*, and *TFCP2* (Anbiping Pharmaceutical Technology Co., Ltd., Guangzhou, China) according to the manufacturer's instructions. Slides were evaluated under a fluorescence microscope, and signals from 200 tumor nuclei were counted. A case was considered positive for gene rearrangement if >20% of cells displayed a break-apart signal pattern, defined as one fusion signal (F) and one separated red signal (R) and one separated green signal (G), referred to as the 1F1R1G pattern.

DNA/RNA next-generation sequencing (NGS)

A total of 19 cases underwent targeted NGS for comprehensive genomic and transcriptomic profiling using formalin-fixed, paraffin-embedded (FFPE) tumor tissue. Due to the retrospective nature of the study, testing was performed at eight different certified molecular diagnostic laboratories. In all instances, a hybrid-capture-based approach targeting a large panel of cancer-related genes was performed for both DNA and RNA sequencing. Briefly, DNA and RNA were co-extracted from FFPE sections. Sequencing libraries were prepared according to each institution's validated protocols and subsequently sequenced on an Illumina platform. The sequencing data was analyzed through institution-specific, validated bioinformatics pipelines. The DNA sequencing analysis was designed to detect single nucleotide variants (SNVs), small insertions and deletions (indels), and copy number variations (CNVs). The RNA sequencing data were analyzed primarily for the detection of gene fusions.

Genome-wide DNA methylation

Genomic DNA from FFPE samples was analyzed for its methylation profile using the Infinium® MethylationEPIC v2.0 BeadChip (Illumina, San Diego, CA, USA) targeting around 930 K unique methylation sites of the human methylome. The DNA underwent a restoration step using the Infinium™ HD FFPE Restore Kit (Illumina) followed by bisulfite conversion with the EZ DNA Methylation™ Kit (Zymo Research, Irvine, CA, USA), per the manufacturer's instructions. Following hybridization, the BeadChips were scanned on an iScan® System (Illumina). Raw data were then interpreted using the Heidelberg Epignostix Sarcoma Classifier v12.3 (v0.4) to assign a methylation-based diagnosis.

Results

Clinicopathologic features of *FET(FUS/EWSR1)::TFCP2*-rearranged tumors

During the period 2021–2025, our department received 25 consultation cases of *FET(FUS/EWSR1)::TFCP2*-rearranged tumors. Of these, 17 tumors involved various bones including the mandible, pelvic bone, maxilla, temporomandibular joint, orbit, pubic bone, paranasal sinus, and thoracic vertebra. The remaining 8 cases occurred in soft tissues, including the cheek (2 cases), epididymis, bladder, abdominal wall, neck, temporal region, and scalp (Table 1). The patients ranged in age from 6 to 65 years with a median age of 29 years and showed no gender preference with a male-to-female ratio of 12:13. Tumor size varied considerably with an average diameter of 7.0 cm (range 1.8–18.9 cm).

Morphologically, most cases were composed of varying proportions of spindle and epithelioid cells. These cells were arranged in sheet-like, fascicular, storiform, and whorled patterns (Fig. 1A, B). Other morphologies included a purely spindle cell pattern, a rhabdoid pattern, and an undifferentiated round blue cell pattern (cases 24 and 25, described in details below). Immunohistochemically, all cases except cases 24 and 25 expressed MyoD1 (Fig. 1C), a highly consistent and critical diagnostic marker. In contrast, Myogenin expression was focally positive in 9 of 25 cases and negative in the remaining 17 cases (Fig. 1D), a feature that may help differentiate these tumors from conventional rhabdomyosarcomas, which typically show diffuse and strong Myogenin positivity. Desmin expression was observed in 23 of 25 cases (except for Cases 24 and 25). Pan-CK expression was observed in 14 of 25 cases (Fig. 1E). ALK expression was detected in 22 of 25 cases (Fig. 1F). The staining pattern was predominantly cytoplasmic and most frequently diffuse (12/25), whereas the remaining positive cases showed partial or focal cytoplasmic reactivity of variable intensity.

The *FET(FUS/EWSR1)::TFCP2* rearrangement was detected by NGS or both NGS and FISH in 16 cases, and by FISH alone in 9 cases, in which 7 cases were confirmed by both *FUS/EWSR1* and *TFCP2* probes (representative *TFCP2* FISH images are provided in Supplementary Fig. 1), while case 4 and 5 were only confirmed by *FUS/EWSR1* probe (histopathological information of these two cases is provided in the supplementary Fig. 1). Of them, 17 cases harbored *FUS::TFCP2* fusions, while 8 had *EWSR1::TFCP2*. Clinically and histopathologically, no significant differences were observed between sarcomas harboring *EWSR1::TFCP2* and *FUS::TFCP2* fusions. Interestingly, we noted that the fusion partner (*FUS* or *EWSR1*) appeared to influence the tumor's site predilection. The

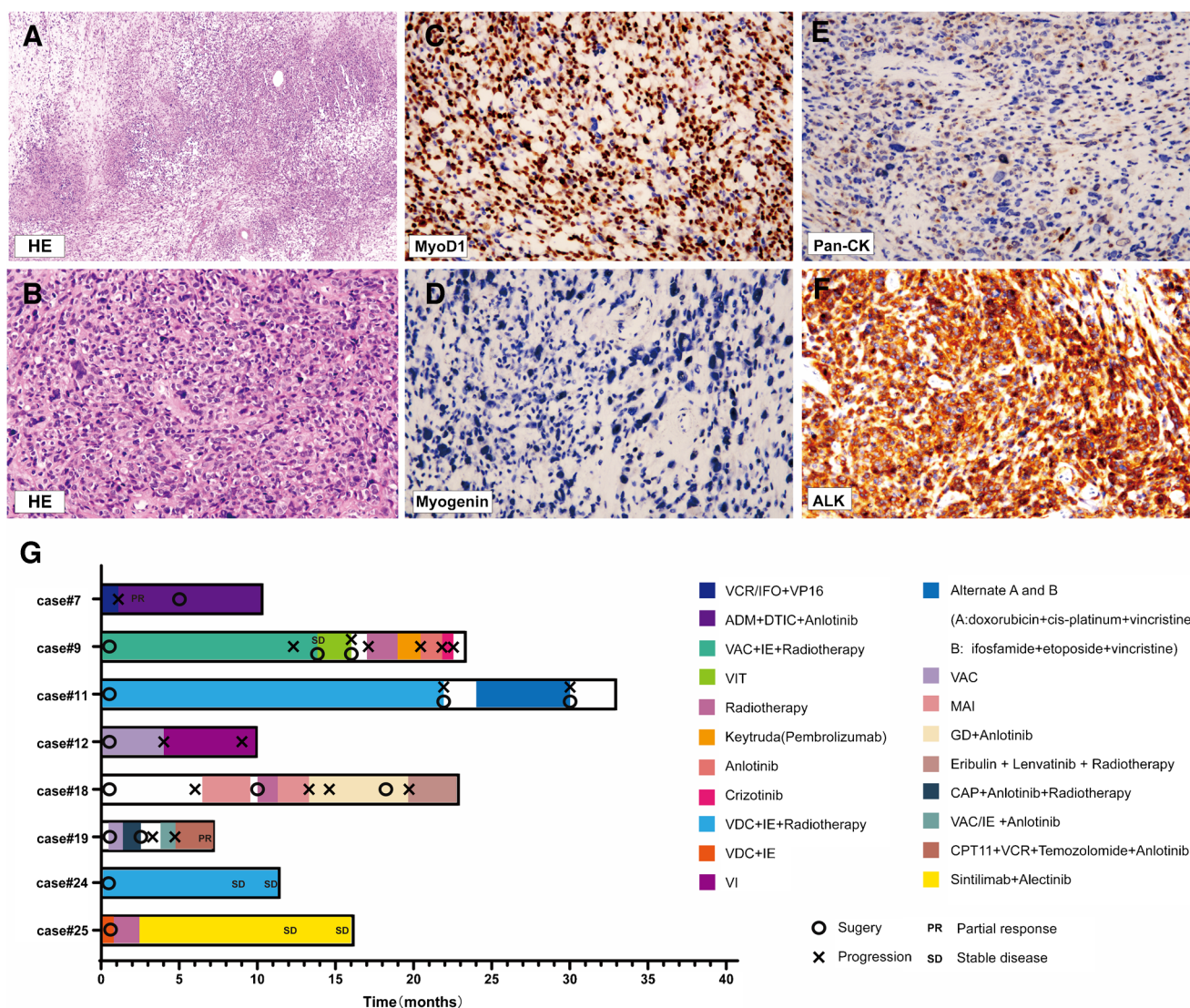


Fig. 1 Histopathological features and clinical follow-up of the classic *TFCP2*-rearranged sarcomas. Classical histopathological features of *TFCP2*-rearranged sarcomas was shown by case#7, H&E staining **A, B** revealed that spindle and epithelioid tumor cells arranged in sheet-like and fascicular pattern. Immunohistochemistry (IHC) demonstrated strongly positive for MyoD1 **C**, negative for Myogenin **D**, focal positive for Pan-CK **E**, and diffuse positive for ALK **F**. Swimmer's plot **G** showing timeline of indicated treatments for 8 out of 25 patients for which treatment data were available. (ADM+DTIC: Adriamycin (Doxorubicin)+Dacarbazine; CAP: Cyclophosphamide+Adriamycin (Doxorubicin)+Cisplatin; CP-T11+VCR+Temozolomide: Irinotecan (CPT-11)+Vincristine (VCR)+Temozolomide; GD: Gemcitabine+Dacarbazine; IE: Ifosfamide+Etoposide; MAI: Doxorubicin (Adriamycin)+Ifosfamide; VAC: Vincristine+Actinomycin+Cyclophosphamide; VDC: Vincristine+Doxorubicin+Cyclophosphamide; VCR/IFO+VP16: Vincristine (VCR)+Ifosfamide (IFO)+Etoposide (VP-16); VI: Vincristine+Irinotecan; VIT: Vincristine+Irinotecan+Temozolomide; PD: progression disease; PR: partial response; SD: stable disease.)

FUS::TFCP2 fusion is typically associated with osseous sarcomas, whereas the *EWSR1::TFCP2* fusion demonstrates a broader tissue distribution, with primary sites reported in bone, soft tissue, and visceral organs. This pattern is consistent with findings from a recent retrospective review of the literature [11].

Among the 19 cases that underwent genomic testing, the vast majority had genomic instability with numerous chromosomal rearrangements and copy number alterations.

CDKN2A loss was detected in 12 cases, while *MTAP* loss was detected in 7 cases. 11 cases had one or more amplified genomic regions, with the most recurrent amplification regions being 12q14-q15 (*MDM2/FRS2*) and 22q11.21 (*CRKL/DGCR8*). Five cases carried *ALK* long in-frame deletion (LongInDel). Single nucleotide variants (SNVs) were generally rare or absent, with no recurrent point mutations identified. Of the 16 cases had detailed breakpoint information, the *TFCP2* breakpoint was highly conserved,

predominantly occurring within exon 2 (14/16 cases). *FUS* breakpoints were enriched at exon 6 (8/10 cases), whereas *EWSR1* breakpoints were more variable, involving exon 5 (3/6), exon 6 (2/6), and exon 7 (1/6).

Genomic data were obtained from primary and relapsed tumors in case 11. The primary tumor had a heterozygous *CDKN2A* loss in addition to the *FUS::TFCP2* and displayed bland histology. In contrast, the relapsed tumor, analyzed two years post-surgery, showed complex genomic alterations, including homozygous *CDKN2A* loss, *MET* amplification, heterozygous *PTEN* loss, homozygous *RBI* loss, and three single nucleotide variants: *APC* p.V7A (variant allele frequency, VAF 61.93%), *IDH1* p.A141P (36.39%), and *JAK3* p.E571G (40.31%) (Table 1). These findings suggest that the *FUS::TFCP2* fusion may drive genomic instability, contributing to disease progression.

Treatment and follow-up data were available for 8 patients (Table 2 and Fig. 1G). All patients underwent surgical resection and received multimodal treatment. Responses to first-line therapy were generally poor, with a median progression-free survival (PFS) of 5.5 months (range, 1–22 months), and most patients subsequently developed recurrent or progressive disease requiring additional therapies. Despite aggressive multimodal management, outcomes remained unfavorable, indicating the highly aggressive clinical behavior and poor prognosis of this tumor type.

Undifferentiated tumors with *TFCP2* rearrangement

Of the 25 tumors with *FET(FUS/EWSR1)::TFCP2* rearrangements, two lacked expressions of myogenic markers (negative for MyoD1, Myogenin, and Desmin). These tumors displayed distinctive morphological, immunophenotypic, and genetic profile features.

Case #24 is a 58-year-old male who presented with a scalp mass. Magnetic resonance imaging (MRI) revealed an irregular, marked enhanced subcutaneous lesion in the left frontal and parietal regions (Fig. S2A, B). The tumor was surgically resected. H&E staining of the tumor tissue sections demonstrated a fibrously encapsulated neoplasm composed of tumor cells arranged in cord-like, reticular, and nested patterns. The stroma was predominantly myxoid, with focal areas of fibromyxoid matrix. The tumor cells were small round cells with scant cytoplasm and hyperchromatic nuclei. Mitotic figures were rare, and no evidence of necrosis was observed (Fig. 2A–C). IHC was positive for Vimentin, BCL2, Syn, and CD99 (Fig. 2D), and negative for myogenic markers including Myogenin (Fig. 2E), MyoD1 (Fig. 2F), and Desmin (Fig. 2G). Additional negative markers included SMA, Calponin, Pan-CK (Fig. 2H), AE1/AE3, SOX10, S-100, CD34, and ALK (Fig. 2I). The Ki-67 proliferation index was estimated at 15–20%.

Targeted DNA NGS of 638 cancer-related genes (Table S2) on FFPE tumor tissue found *ARID5B* p.A248Sfs*37 (VAF 43.3%) and *DDX3X* p.R46X (92.8%) mutations. Whole-genome chromosomal numerical and structural analysis detected gains of 1q, 5pter→q14.2, and chromosome 15, and losses of 9p21.3→p21.2 (including *CDKN2A/B*), 9q34.12→q34.3, and 11q14.2→qter (including *ATM* and *CHEK1*) (Fig. 2J). HRD analysis, which uses a combination of LOH (loss of heterozygosity), LST (large-scale state transition), and TAI (telomeric allelic imbalance), showed no evidence of HRD, with a genome instability score (GIS) of 13 (GIS cutoff ≥42 for HRD). Simultaneous targeted RNA NGS using probes for 60 fusion genes (Table S3) detected an in-frame *FUS(E6)::TFCP2(E2)* fusion transcript (Fig. 2K). Reverse transcription polymerase chain reaction (RT-PCR) analysis of tumor RNA, followed by Sanger sequencing, confirmed the presence of the *FUS(E6)::TFCP2(E2)* fusion transcript (Fig. 2K). Subsequent FISH showed *TFCP2* rearrangement in 64.5% of cells from tumor tissue sections (Fig. 2L).

To further characterize this diagnostically challenging case, genome-wide DNA methylation profiling was performed. Methylation-based t-SNE analysis showed that this tumor was classified with the "Ewing sarcoma" group (Fig. 2M, sample 1). In comparison, a representative case of classic *TFCP2*-rearranged sarcoma from our series (Case #11) was classified with "undifferentiated sarcoma (USARC)" (Fig. 2M, sample 3), as expected for this entity [19]. Given the small round cell morphology, diffuse CD99 positivity, and absence of myogenic differentiation, Ewing sarcoma/primitive neuroectodermal tumor (PNET) was initially considered in the differential diagnosis. However, the identification of a *FUS::TFCP2* fusion, together with the atypical DNA methylation profile and the lack of supporting molecular evidence for any currently recognized undifferentiated round cell sarcoma subtype, argued against this diagnosis. Accordingly, given the uncertain lineage and absence of myogenic differentiation, the tumor was provisionally classified as an undifferentiated tumor with *TFCP2* rearrangement.

The patient underwent extensive surgical resection of the tumor with wide margins. Postoperatively, the patient received radiotherapy combined with alternating VDC and IE (ifosfamide, etoposide) chemotherapy. The patient is currently on follow-up (11 months), remains tumor-free, and has a Karnofsky Performance Status (KPS) of 100. (Table 2 and Fig. 1G).

Case #25 is a 32-year-old male who presented with back pain. MRI and Computed Tomography (CT) scans revealed a discontinuity of the bone structure on the left side of the T4 vertebra, with localized bone destruction and an associated soft tissue density (Fig. S2C, D). The tumor

Table 2 Clinical follow-up of 8 *TFCP2*-rearranged tumor cases

	case#7	case#9	case#11	case#12	case#18	case#19	case#24	case#25
Clinical symptoms	Restricted mouth opening	Outer mass around the right eye socket, with pain in the surrounding area	/	Hematuries	A mass in the right abdomen, with redness, swelling and pain	Poor nasal ventilation	Head mass	Chest pain
Location	Left temporomandibular region	Right frontotemporal region	Maxilla	Bladder	Abdominal wall	Paranasal sinus	Under the scalp	Thoracic vertebra
Primary Size	6*4.5*3 cm	/	2.1*2*0.5 cm	5*4.5*4 cm	5*3 cm	1.8*1.5*0.4 cm	/	/
Locoregional extension	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Metastasis	N/A	Yes	No	Yes	Yes	Yes	No	No
Primary Surgery	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
1 st line	VCR/IFO+VP16	VAC/IE(16 cycles)+Radiotherapy	VDC/IE(12 cycles)+Radiotherapy apy(50 Gy/25Fx)	VAC	Considered Myofibroblastic tumor initially and no treatment	VAC(3 cycles), CAP+Anlotinib + Radiotherapy	VDC/IE(12 cycles)+Radiotherapy apy(50 Gy/25Fx)	VDC/IE(4 cycles), intolerance; Radiotherapy (25 times)
Outcomes	No response for 1 month	Metastasis after 14 months	Local recurrence after 22 months	Local recurrence after 4 months	Local recurrence after 6 months	Local recurrence after 5 months	SD for 11 months	SD for 16 months
2nd line	ADM+DTIC+Anlotinib	Surgery 2nd time, VIT	Surgery the 2nd time Regimen A: doxorubicin + cis-platinum + vincristine Regimen B: ifosfamide + etoposide + vincristine Took alternate chemotherapy with the AB regimen (6 alternate cycles)	VI	Surgery the 2nd time, MAI (5 cycles)	VAC/IE+Anlotinib 1 cycle, PD; CPT11+VCR+Temozolomide+Anlotinib	N/A	N/A
Outcomes	PR, the radical resection surgery was performed after 4 months of therapy	Local recurrence after 3 months	Local recurrence after 5 months	lung metastasis after 4 months	Local recurrence after 4 months	PR	N/A	N/A

Table 2 (continued)

	case#7	case#9	case#11	case#12	case#18	case#19	case#24	case#25
3rd line	N/A	Surgery the 3rd time Radiotherapy for 26 times and Keytruda (pembrolizumab) for 5 times Then used Anlotinib for 4 weeks, drug with- drawal for intolerance; Crizotinib (6 pills), discontinued due to intolerance	Surgery the 3rd time, N/A	N/A	Surgery the 3rd time Radiother- apy(5000 cGy/25Fx)	N/A	N/A	N/A
Outcomes	N/A	N/A	N/A	N/A	PD after 6 months	N/A	N/A	N/A
Posterior line	N/A	N/A	N/A	N/A	GD(6 cycles), Anlo- tinib, Denosumab Eribulin + Lenva- tinib + Radiotherapy	N/A	N/A	N/A
Follow-up duration	10 months	24 months	33 months	10 months	24 months	8 months	11 months	16 months

ADM+DTIC: Adriamycin (Doxorubicin)+Dacarbazine, CAP: Cyclophosphamide+Adriamycin (Doxorubicin)+Cisplatin, CPT11+VCR+Temozolomide: Irinotecan (CPT-11)+Vincristine (VCR)+Temozolomide, GD: Gemcitabine+Dacarbazine, IE: Ifosfamide+Etoposide, MAI: Mesna+Doxorubicin (Adriamycin)+Ifosfamide, VAC: Vincristine+Actinomycin+Cyclophosphamide, VDC: Vincristine+Doxorubicin+Cyclophosphamide, VCR/IFO+VP16: Vincristine (VCR)+Ifosfamide (IFO)+Etoposide (VP-16), VI: Vincristine+Irinotecan, VIT: Vincristine+Irinotecan+Temozolomide, PD: progression disease; PR: partial response; SD: stable disease

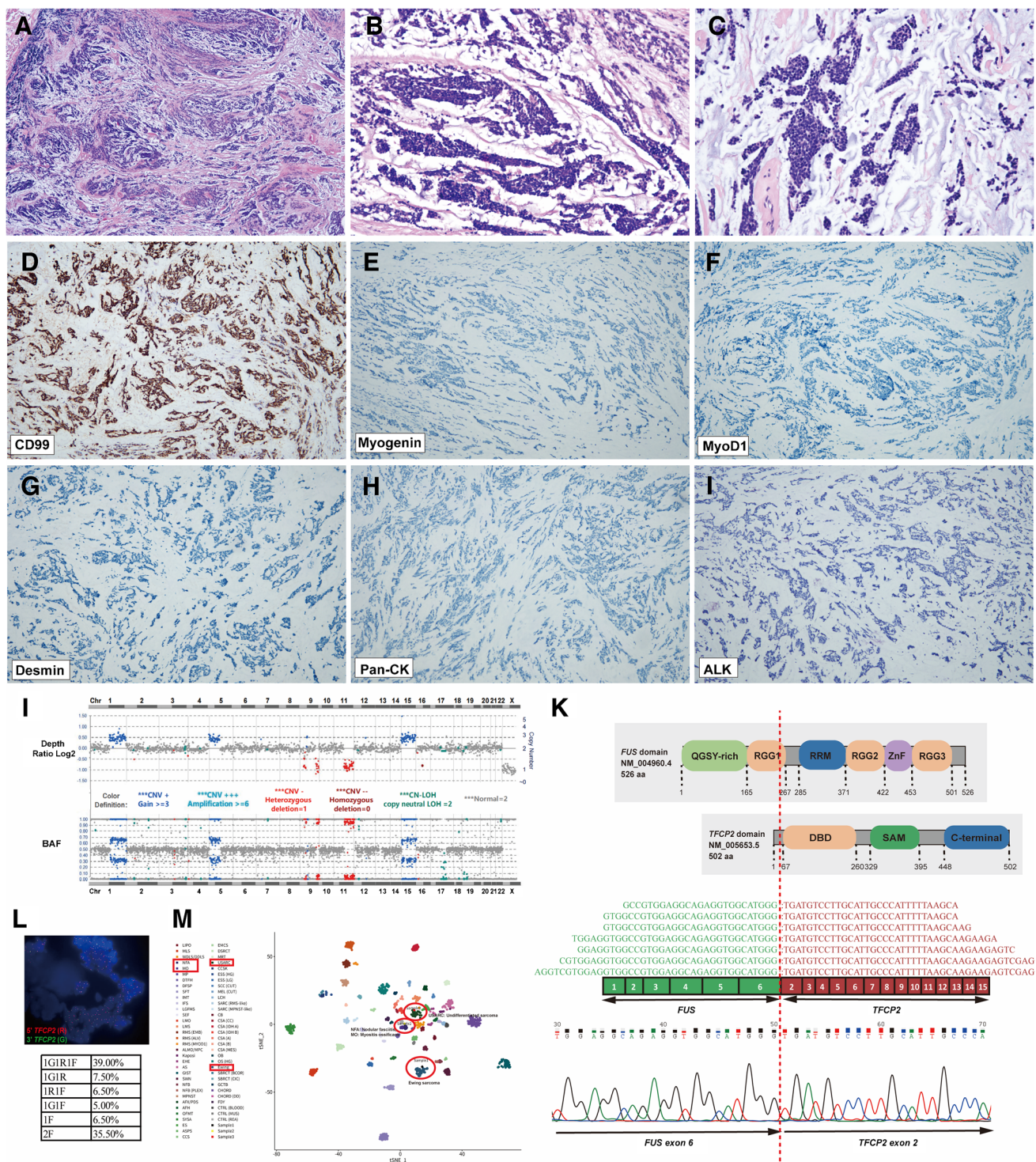


Fig. 2 Histopathological, immunohistochemistry (IHC) and genomic features of the case#24 tumor. **A** H&E staining revealed that tumor cells were arranged in cord-like and nested patterns within a myxoid stroma (H&E, low power). **B-C** High-power view showed small round cells with hyperchromatic nuclei and scant cytoplasm. IHC demonstrated that tumor cells showed strong positivity for CD99 **D**, and negative for Myogenin **E**, MyoD1 **F**, Desmin **G**, Pan-CK **H**, and ALK **I**. Genomically, **J** Whole-genome copy number analysis showed genomic structural and numerical alterations. **K** Targeted RNA NGS revealed an in-frame *FUS(E6)::TFCP2(E2)* fusion transcript. Sanger

was surgically resected. Microscopically, the tumor was unencapsulated with indistinct borders. Tumor cells were separated by fibrous connective tissue, forming variably sized nests, cord-like, and reticular patterns. The stroma was rich in myxoid, with focal areas of fibromyxoid matrix. The tumor cells were round or oval with moderate to scant eosinophilic cytoplasm, often located peripherally or at the poles of the nucleus. The nuclei were hyperchromatic, with inconspicuous nucleoli, and mitotic figures were rare (Fig. 3A–C). Focal areas of tumor necrosis were observed. IHC analysis was positive for ALK (Fig. 3D), pan-CK (Fig. 3E), S100 (Fig. 3F), EMA (focal), and CK8/18 (scattered), and negative for myogenic markers including Myogenin (Fig. 3G), MyoD1 (Fig. 3H), and Desmin (Fig. 3I). The Ki-67 proliferation index was estimated at 30–35%.

Targeted DNA NGS of 638 cancer-related genes (Table S2) identified an *ALK* Exon2-Exon17 LongInDel, and a near-haploid genome, with the loss of most chromosomes except chromosomes 3q, 5, 13, 17, 19, and 21 (Fig. 3J). Evaluation of HRD showed positive HRD, with a GIS of 53 (GIS cutoff ≥ 42 for HRD). Simultaneous targeted RNA NGS confirmed the in-frame *EWSR1(E6)::TFCP2(E2)* fusion transcript (Fig. 3K) and the *ALK* E1::E18 short transcript generated by intragenic deletion (Fig. 3L). RT-PCR analysis of tumor RNA, followed by Sanger sequencing, further validated the presence of the *EWSR1(E6)::TFCP2(E2)* fusion (Fig. 3K). Additional FISH confirmed *TFCP2* rearrangement in 87.5% of cells from tumor tissue sections (Fig. 3M). Additionally, a short *TERT* transcript lacking exons 1 and 2 (*TERT* Δ 1-2 variant) was detected by whole transcriptome RNA sequencing (Fig. 3N).

Methylation-based t-SNE analysis was performed to further characterize the lesion. The tumor was classified in a region unrelated to any established sarcoma methylation classes (Fig. 2M, sample 2), distinctly separated from both *TFCP2*-rearranged sarcoma and Ewing sarcoma. Although soft tissue myoepithelial carcinoma was briefly considered because of the co-expression of CK and S100, the identification of a *TFCP2* rearrangement and the absence of supporting morphologic and molecular features argued against this diagnosis. Given the absence of myogenic differentiation and the lack of definitive evidence for mesenchymal lineage, the lesion was provisionally diagnosed as an undifferentiated tumor with *TFCP2* rearrangement.

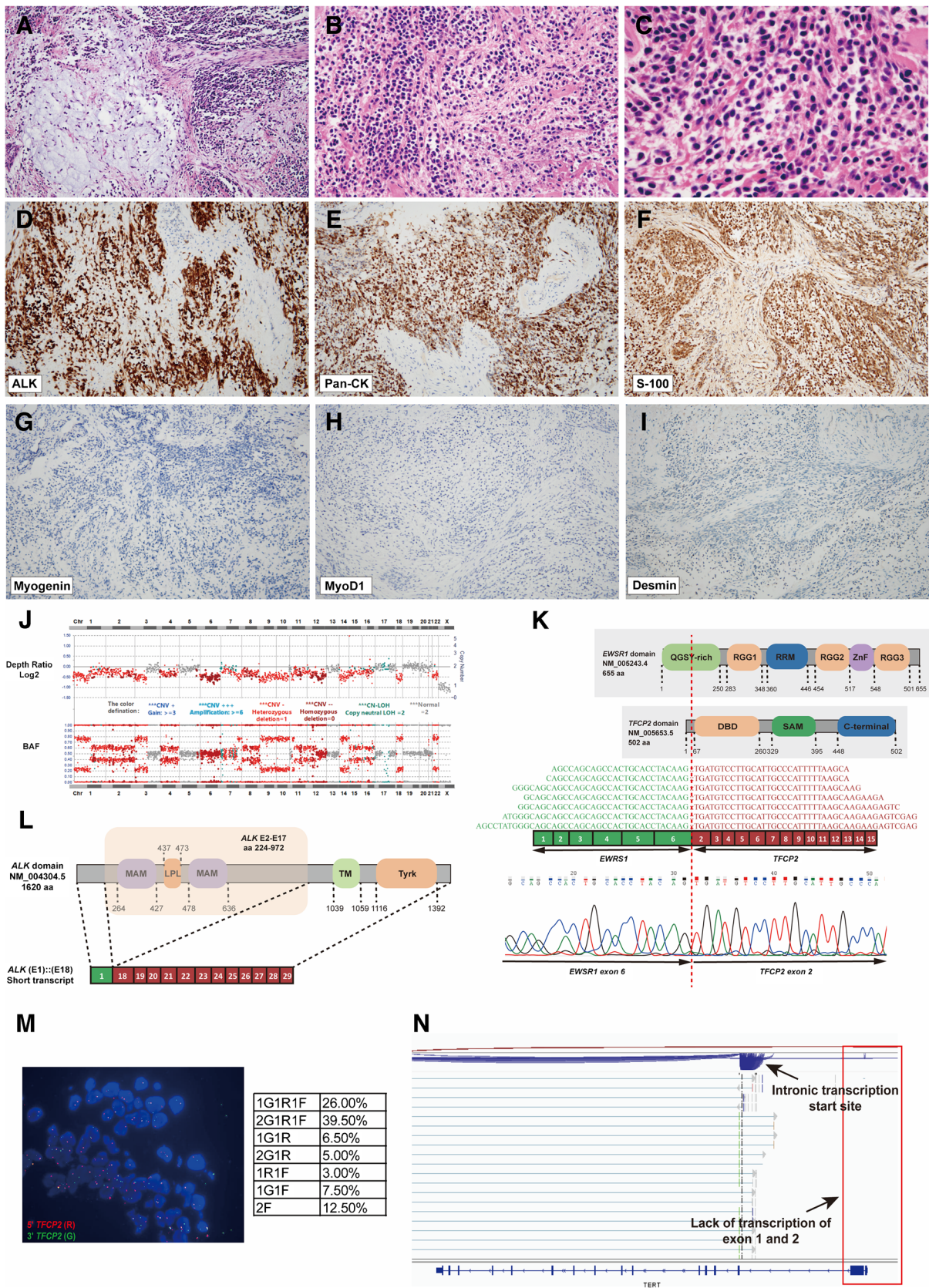
Clinically, the patient underwent surgical resection followed by adjuvant chemotherapy with alternating VDC and IE regimens. Due to treatment intolerance, the patient was transitioned to radiotherapy (TOMO, 25 fractions) in combination with immunotherapy (Sintilimab) and ALK-targeted therapy (Alectinib). The patient remains tumor-free on follow-up (16 Months) and currently has a KPS of 100 (Table 2 and Fig. 1G).

Fig. 3 Histopathological, immunohistochemistry (IHC) and genomic features of the case#25 tumor. **A** H&E staining revealed that tumor cells displayed a nested and cord-like architecture in a myxoid stroma (H&E, low power). **B–C** The tumor cells were round or oval with moderate to scant eosinophilic cytoplasm, often located peripherally or at the poles of the nucleus. The nuclei were hyperchromatic, with inconspicuous nucleoli, and mitotic figures were rare (H&E, high power). IHC demonstrated positivity for ALK **D**, Pan-CK **E** and S-100 **F**, while showing negativity of myogenic markers Myogenin **G**, MyoD1 **H** and Desmin **I**. Genomically, **J** Whole-genome copy number analysis showed genomic structural and numerical alterations, which revealed a near-haploid genome. **K** Targeted RNA NGS revealed an in-frame *EWSR1(E6)::TFCP2(E2)* fusion transcript which was further validated by RT-PCR and Sanger sequencing. **L** Targeted RNA NGS found the *ALK* E1::E18 short transcript generated by intragenic deletion (Exon2-Exon17 LongInDel). **M** FISH using a *TFCP2* break-apart probe showed split red (R) and green (G) signals, indicating *TFCP2* rearrangement. **N** Whole transcriptome RNA sequencing identified a short *TERT* transcript variant lacking exons 1 and 2 (*TERT* Δ 1-2)

Discussion

We present a cohort of 25 bone and soft tissue tumors harboring the *FET(FUS/EWSR1)::TFCP2* fusion. The majority of these cases had classic clinical features of *TFCP2*-rearranged sarcoma, including predominantly young adults. Notably, our series includes 4 pediatric patients, including a 6-year-old, the youngest patients reported to date within this tumor group. For the primary site, the mandible, maxilla, and axial skeleton remain the most common sites. We also identified soft tissue primary locations, including the cheek, epididymis, bladder, abdominal wall, neck and temporal region. Immunohistochemically, MyoD1 was consistently expressed, whereas Myogenin was detected in approximately half of the patients. Desmin was expressed in most cases (23/25), although expression was heterogeneous rather than diffuse. In contrast to conventional rhabdomyosarcoma, *TFCP2*-rearranged sarcomas often exhibit a distinctive immunophenotypic profile of Desmin, MyoD1, ALK, and epithelial markers, including cytokeratins and EMA [12, 13]. This unusual profile may create significant diagnostic overlap with a variety of epithelioid and round cell neoplasms, including carcinoma, epithelioid sarcoma, myoepithelial tumors, and other ALK-positive mesenchymal neoplasms. In this setting, Desmin is best regarded as a supportive marker of myogenic differentiation, particularly when interpreted together with MyoD1, Myogenin, ALK, and CK. This immunophenotype should prompt consideration of *TFCP2*-rearranged sarcomas and testing for *TFCP2* fusion events. Histologically, most tumors demonstrated a combination of spindle cell and epithelioid morphology. Clinically, these tumors typically showed poor response to chemotherapy and radiotherapy and often presented with local and/or distant metastases.

At the genomic level, all tumors in our cohort harbored the *FET::TFCP2* fusion. Consistent with previous findings



[10], *ALK* and *TERT* alterations were frequently observed. *ALK* dysregulation appears to be a recurrent molecular feature of *TFCP2*-rearranged sarcomas. Diffuse *ALK* protein expression has been reported in most cases, even in the absence of canonical *ALK* fusions, and is thought to result from aberrant transcriptional activation mediated by the *FET::TFCP2* fusion protein [10]. In our cohort, intragenic *ALK* deletion was identified in 5 of 25 cases, further supporting the recurrent nature of *ALK* alteration in this tumor family. Such deletions may generate truncated *ALK* transcripts that retain the kinase domain and exhibit oncogenic activity. Functional studies by Schöpf et al. [10] showed that these truncated *ALK* variants promote cellular proliferation and tumorigenesis and can be inhibited by *ALK* inhibitors such as ceritinib and crizotinib in preclinical models. *ALK*-targeted therapy may therefore represent a potential therapeutic option in selected cases, although its clinical efficacy remains variable [14, 15]. In the copy number change level, most tumors exhibited widespread genomic instability, with numerous structural rearrangements and copy number alterations, including frequent homozygous deletions of *CDKN2A/B* and *MTAP*. Notably, we identified recurrent gene amplifications in 11 of 19 cases with available DNA NGS data. These amplifications involved genes in the receptor tyrosine kinase (RTK) signaling pathways, including *MET*, *EPHA3*, *FGF18*, *FRS2*, *PIK3CB*, *AKT3*, *CRKL*, and *ERCC1*, as well as genes regulating the cell cycle and proliferation, including *CCNE1*, *CDK4*, *MDM2*, and *NET1*. Two recurrent amplification regions, 12q14-q15 (*MDM2/FRS2*) and 22q11.21 (*CRKL/DGCR8*), were observed in this series.

While the majority of tumors in our cohort closely resembled the clinicopathologic profile of previously reported *TFCP2*-rearranged sarcomas, two tumors exhibited atypical patterns (Cases 24 and 25). Although both tumors harbored the classic *FET::TFCP2* fusion, they completely lacked the expression of any myogenic markers (Myogenin, MyoD1, and Desmin), a hallmark of this group of tumors. Morphologically, these tumors were composed predominantly of small round-to-ovoid cells arranged in nested, cord-like, or reticular patterns within a myxoid stroma, rather than the spindle and epithelioid morphology typically described in *TFCP2*-rearranged sarcomas. These findings indicate that *TFCP2* fusions are not invariably associated with a skeletal muscle differentiation phenotype. DNA methylation findings further supported the biological divergence of these two tumors. Case 24 clustered within the Ewing sarcoma methylation cohort, whereas Case 25 failed to match any established sarcoma methylation class. However, these results should be interpreted cautiously. As highlighted

by Schöpf et al. [10], *TFCP2*-rearranged sarcomas themselves exhibit substantial epigenetic heterogeneity and do not form a single unified methylation class. Therefore, the methylation results are best regarded as supportive rather than diagnostic evidence. Notably, the two tumors were also distinct from one another. Case 24 showed greater overlap with undifferentiated small round cell sarcoma, including diffuse CD99 expression and proximity to the Ewing sarcoma methylation class, whereas Case 25 retained several molecular hallmarks of classic *TFCP2*-rearranged sarcomas, including diffuse *ALK* expression, *ALK* intragenic deletion and *TERT* alteration. Taken together, the available evidence does not support designation as a separate tumor type. Rather, given the absence of conventional myogenic differentiation markers and the unusual immunophenotypic features observed in these cases, we consider that a more conservative, lineage-neutral designation is appropriate at the current stage. Therefore, we favor the term ‘undifferentiated tumor with *TFCP2* rearrangement’ for these diagnostically challenging lesions, while acknowledging their close molecular relationship with the *TFCP2*-rearranged sarcoma spectrum. Further studies and additional cases will be required to better define the biological classification of these rare tumors. This phenotypic diversity is not unique to *TFCP2*-rearranged tumors but reflects a broader phenomenon in mesenchymal neoplasia, in which identical fusion genes may give rise to distinct tumor phenotypes depending on the cellular and epigenetic context.

Gene promiscuity is common in bone and soft tissue tumors, and the *FET(FUS/EWSR1)* family of genes is notorious for its promiscuity, particularly *EWSR1*. This gene is involved not only in a long list of mesenchymal tumors, including Ewing sarcoma, myxoid liposarcoma, clear cell sarcoma, angiomatoid fibrous histiocytoma, desmoplastic small round cell tumor, myoepithelial tumors, and extraskelatal myxoid chondrosarcoma [16], but also, rarely, in epithelial carcinomas, including mesothelioma [17] and breast cancer [18]. The frequent rearrangement of *EWSR1* in tumors likely stems from two reasons: (1) Genomic fragility: *EWSR1* is located on chromosome 22q12.2, a fragile chromosomal site prone to structural rearrangements [19]. Additionally, the 5' end of *EWSR1* is rich in repetitive DNA sequences, making it susceptible to homologous or non-homologous recombination during DNA repair errors, thereby increasing the frequency of translocations in tumor cells; and (2) Functional potency: The N-terminal low-complexity domain (LCD) of *EWSR1* contains a potent transcriptional activation domain [20]. When fused to a DNA-binding domain from fusion partners, it hijacks the partner's specificity and activates a new set of target genes.

Given the large number of transcription factors in the human genome, this deregulation can lead to diverse malignant transformations. *EWSR1* has been reported to be fused with dozens of different partners, driving a spectrum of tumors with diverse phenotypes. Even identical fusion genes may give rise to different tumor entities. A classic example is the *EWSR1::FLI1* fusion, which not only defines classic Ewing sarcoma but also occurs in a rare adamantinoma-like Ewing sarcoma [21, 22]. Despite sharing the same driver fusion, the latter exhibits distinct epithelial differentiation features and a unique methylation profile [22]. Similarly, the atypical tumors in our cohort may represent an expanded phenotypic spectrum of *TFCP2*-rearranged tumors.

In summary, we present a large series of bone and soft tissue tumors carrying the *FET::TFCP2* fusion and identify morphological, immunophenotypic, genomic, and clinical heterogeneity in two unique cases. These findings highlight the promiscuity of *FET::TFCP2* rearrangements and their diverse oncogenic potential.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00428-026-04694-w>.

Author contributions R.M. and Y.Li contributed equally to this work. R.M. conceptualized the study and performed the histopathological review. Y.Li analyzed and evaluated all molecular data and wrote the initial draft of the manuscript. S.C. guided the methodology and edited the manuscript. Y.Y.L., H.L., and L.X. were responsible for data collection, clinical follow-up, and auxiliary histopathological review. J.F. performed the bioinformatic analysis of the NGS data. R.R. reviewed and edited the manuscript and contributed to data curation. B.L. provided clinical samples and curated the associated clinical and follow-up data. F.Y. contributed to data curation and manuscript proofreading. S.X. conceived and supervised the entire project and critically revised the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

Funding None.

Data availability All data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate This study was conducted on retrospectively collected, anonymized patient samples and associated clinical data. All procedures were carried out in accordance with institutional guidelines and the principles outlined in the Declaration of Helsinki. Identifiable personal information was not included in the analysis.

Consent for publication Written informed consent was obtained from the patients. No identifiable personal information is included in this manuscript.

Competing interests The authors report no relevant conflicts of interest.

References

- Dehner CA, Rudzinski ER, Davis JL (2024) Rhabdomyosarcoma: updates on classification and the necessity of molecular testing beyond immunohistochemistry. *Hum Pathol* 147:72–81
- Watson S et al (2018) Transcriptomic definition of molecular subgroups of small round cell sarcomas. *J Pathol* 245(1):29–40
- Haug L et al (2023) Epithelioid and spindle cell rhabdomyosarcoma with *EWSR1::TFCP2* fusion mimicking metastatic lung cancer: a case report and literature review. *Pathol Res Pract* 249:154779
- Sivakumar N et al (2023) Clinicopathological and molecular characteristics of intraosseous rhabdomyosarcoma involving head and neck region: a systematic review and meta-analysis. *Pediatr Dev Pathol* 26(3):299–309
- Fang Z et al (2024) Pediatric spindle cell/sclerosing rhabdomyosarcoma with *FUS-TFCP2* fusion: a case report and literature review. *Transl Pediatr* 13(1):178–191
- Bradová M et al (2024) Spindle cell rhabdomyosarcomas: with *TFCP2* rearrangements, and novel *EWSR1::ZBTB41* and *PLOD2::RBM6* gene fusions. A study of five cases and review of the literature. *Histopathology* 84(5):776–793
- Plotzke JM et al (2024) Primary intraosseous spindle cell rhabdomyosarcoma: a case report in an unusual location. *Pediatr Dev Pathol*. <https://doi.org/10.1177/10935266241257547>
- Si C, Wang Y, Zhu J (2025) A rare case report of intraosseous spindle and epithelioid rhabdomyosarcoma with *TFCP2* rearrangement: a pathological diagnostic conundrum and literature review. *Int J Surg Pathol*. <https://doi.org/10.1177/10668969241239676>
- Le Loarer F et al (2020) A subset of epithelioid and spindle cell rhabdomyosarcomas is associated with *TFCP2* fusions and common *ALK* upregulation. *Mod Pathol* 33(3):404–419
- Schöpf J et al (2024) Multi-omic and functional analysis for classification and treatment of sarcomas with *FUS-TFCP2* or *EWSR1-TFCP2* fusions. *Nat Commun* 15(1):51
- Ginn MP et al (2025) *TFCP2* fusion-positive rhabdomyosarcomas: a report of 10 cases and a review of the literature. *Cancers (Basel)*. <https://doi.org/10.3390/cancers17091441>
- Dehner CA et al (2023) Fusion-driven spindle cell rhabdomyosarcomas of bone and soft tissue: a clinicopathologic and molecular genetic study of 25 cases. *Mod Pathol* 36(10):100271
- Xu B et al (2021) Head and neck rhabdomyosarcoma with *TFCP2* fusions and *ALK* overexpression: a clinicopathological and molecular analysis of 11 cases. *Histopathology* 79(3):347–357
- Brunac AC et al (2020) The combination of radiotherapy and *ALK* inhibitors is effective in the treatment of intraosseous rhabdomyosarcoma with *FUS-TFCP2* fusion transcript. *Pediatr Blood Cancer* 67(5):e28185
- Lewin J et al (2019) Lack of clinical activity with crizotinib in a patient with *FUS* rearranged rhabdomyosarcoma with *ALK* protein overexpression. *Pathology* 51(6):655–657
- Wachtel M et al (2024) Functional classification of fusion proteins in sarcoma. *Cancers (Basel)*. <https://doi.org/10.3390/cancers16071355>
- Desmeules P et al (2017) A subset of malignant mesotheliomas in young adults are associated with recurrent *EWSR1/FUS-ATF1* fusions. *Am J Surg Pathol* 41(7):980–988
- Lei T et al (2023) A novel *EWSR1-MYB* fusion in an aggressive advanced breast adenoid cystic carcinoma with mixed classical and solid-basaloid components. *Virchows Arch* 483(5):717–722
- Riggi N et al (2007) Sarcomas: genetics, signalling, and cellular origins. Part 1: the fellowship of TET. *J Pathol* 213(1):4–20

20. Bianco JR et al (2024) EWSR1::ATF1 translocation: a common tumor driver of distinct human neoplasms. *Int J Mol Sci* 25(24):13693
21. Rooper LM, Bishop JA (2020) Soft tissue special issue: adamantinoma-like Ewing sarcoma of the head and neck: a practical review of a challenging emerging entity. *Head Neck Pathol* 14(1):59–69
22. Towery EA, Papke DJ (2024) EWSR1: the promiscuous king of mesenchymal neoplasia. *J Clin Pathol* 77(11):721–725

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