Recurrent *BRAF* Gene Fusions in a Subset of Pediatric Spindle Cell Sarcomas

Expanding the Genetic Spectrum of Tumors With Overlapping Features With Infantile Fibrosarcoma

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纤维母细胞性/肌纤维母细胞性肿瘤(2013)

- **中间性（局部侵袭性）**

浅表纤维瘤病（掌/跖）

韧带样型纤维瘤病

脂肪纤维瘤病

- **中间性（偶见转移型）**

孤立性纤维性肿瘤

炎症性肌纤维母细胞性肿瘤

低度恶性肌纤维母细胞肉瘤

黏液炎症性纤维母细胞肉瘤

婴儿型纤维肉瘤

- **恶性**

成人型纤维肉瘤

黏液纤维肉瘤

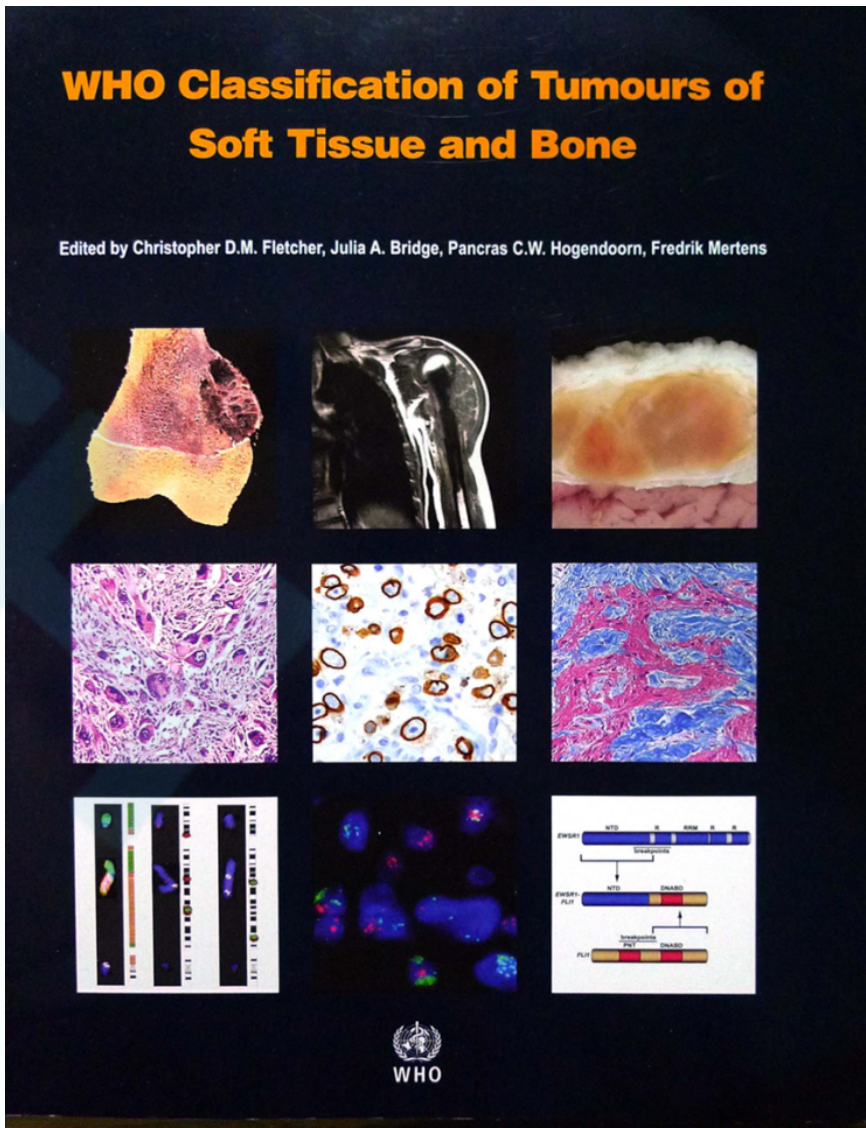
低度恶性纤维黏液样肉瘤

玻璃样变梭形细胞肿瘤

硬化性上皮样纤维肉瘤

KEY WOEDS

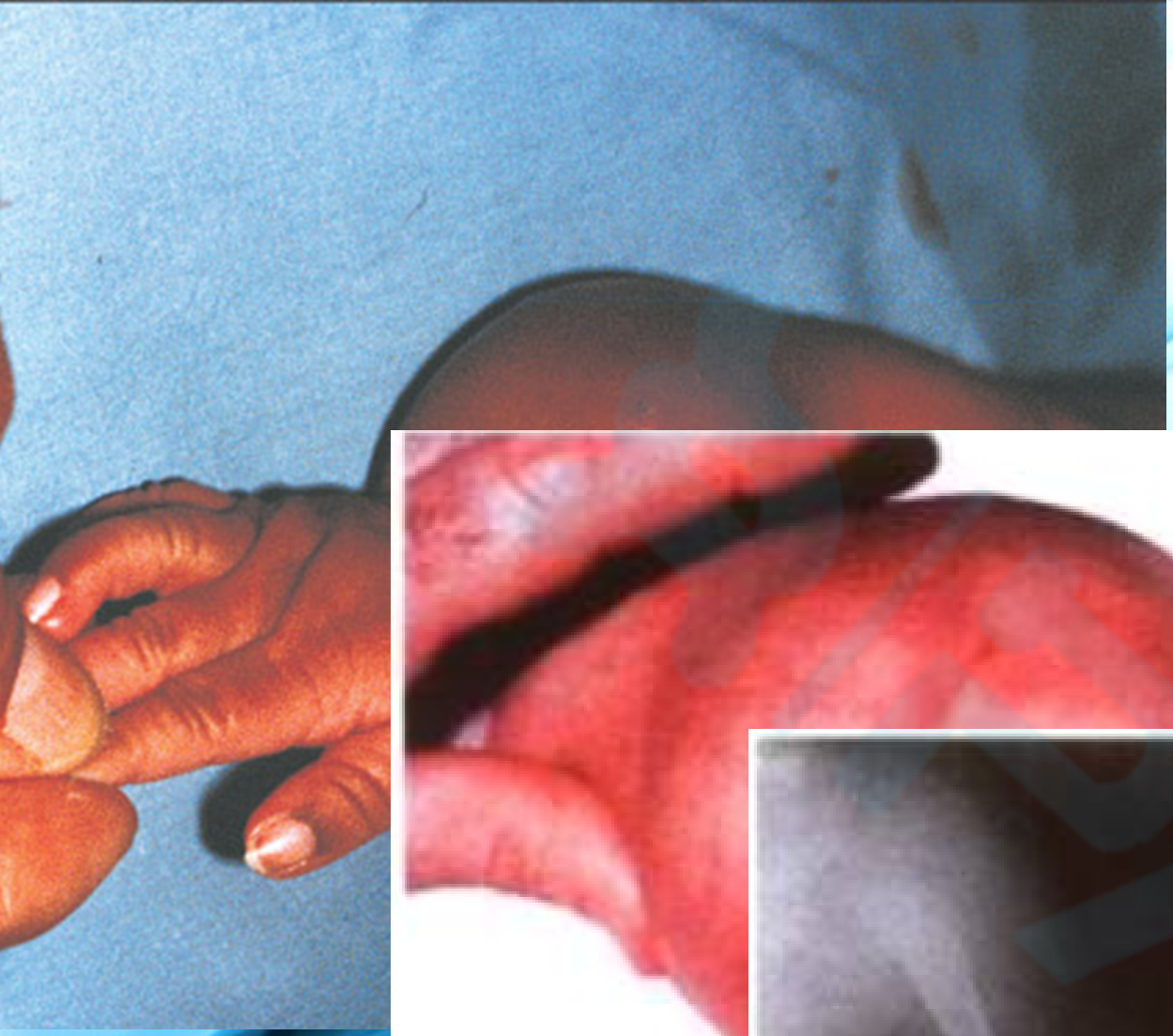
- fibrosarcoma
- infantile fibrosarcoma
- BRAF
- NTRK1, NTRK3, fusions



BACKGROUND

先天性/婴儿纤维肉瘤
Congenital/Infantile Fibrosarcoma

- 临床特征：大部分病例发生在1岁以前，1/3出生时即出现
- 主要部位是四肢，躯干和头颈部较少见
- 惰性肿瘤，预后良好，远隔转移率低，5年生存率89%，大部分可通过肿瘤扩大切除治愈



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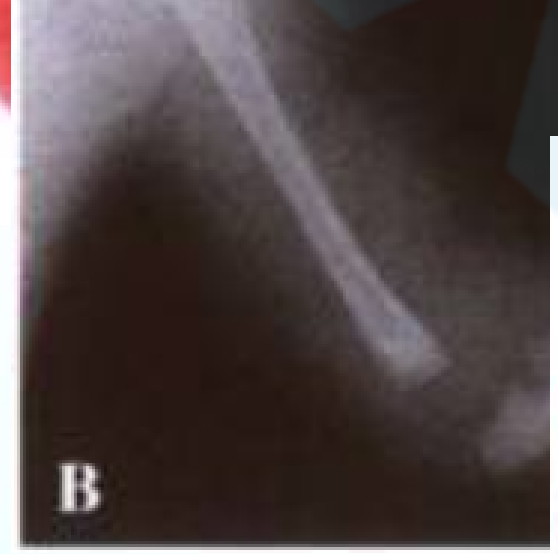

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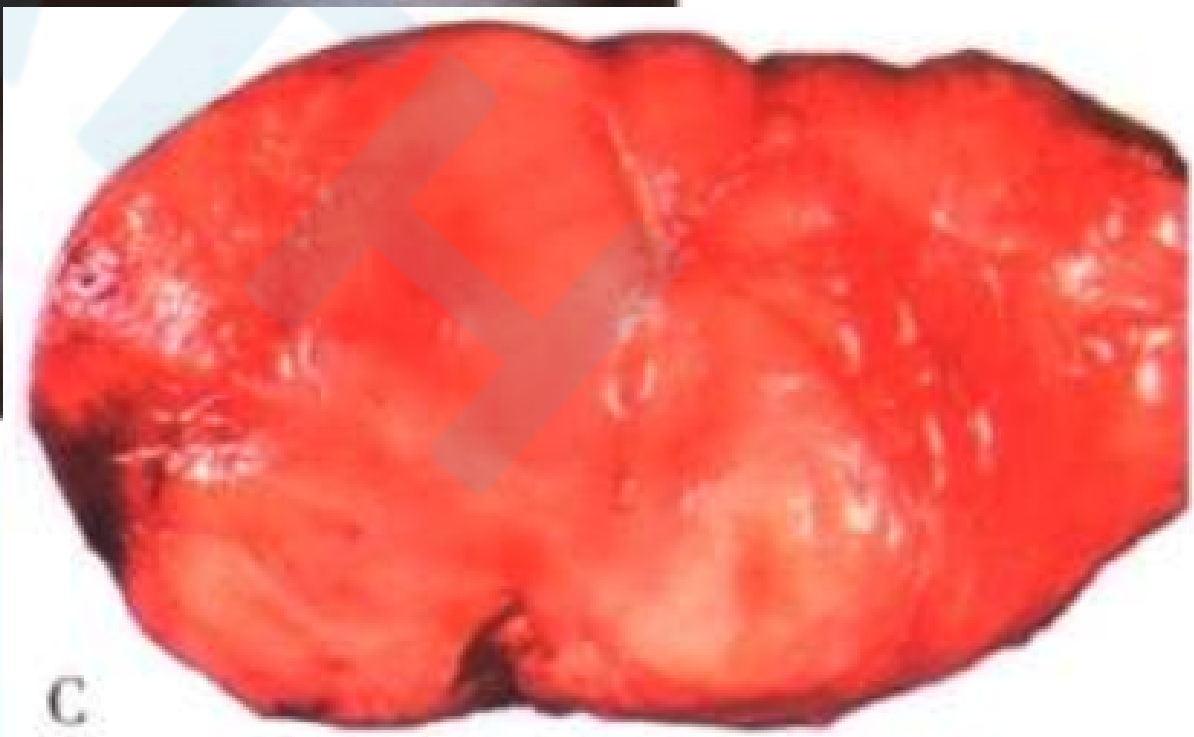


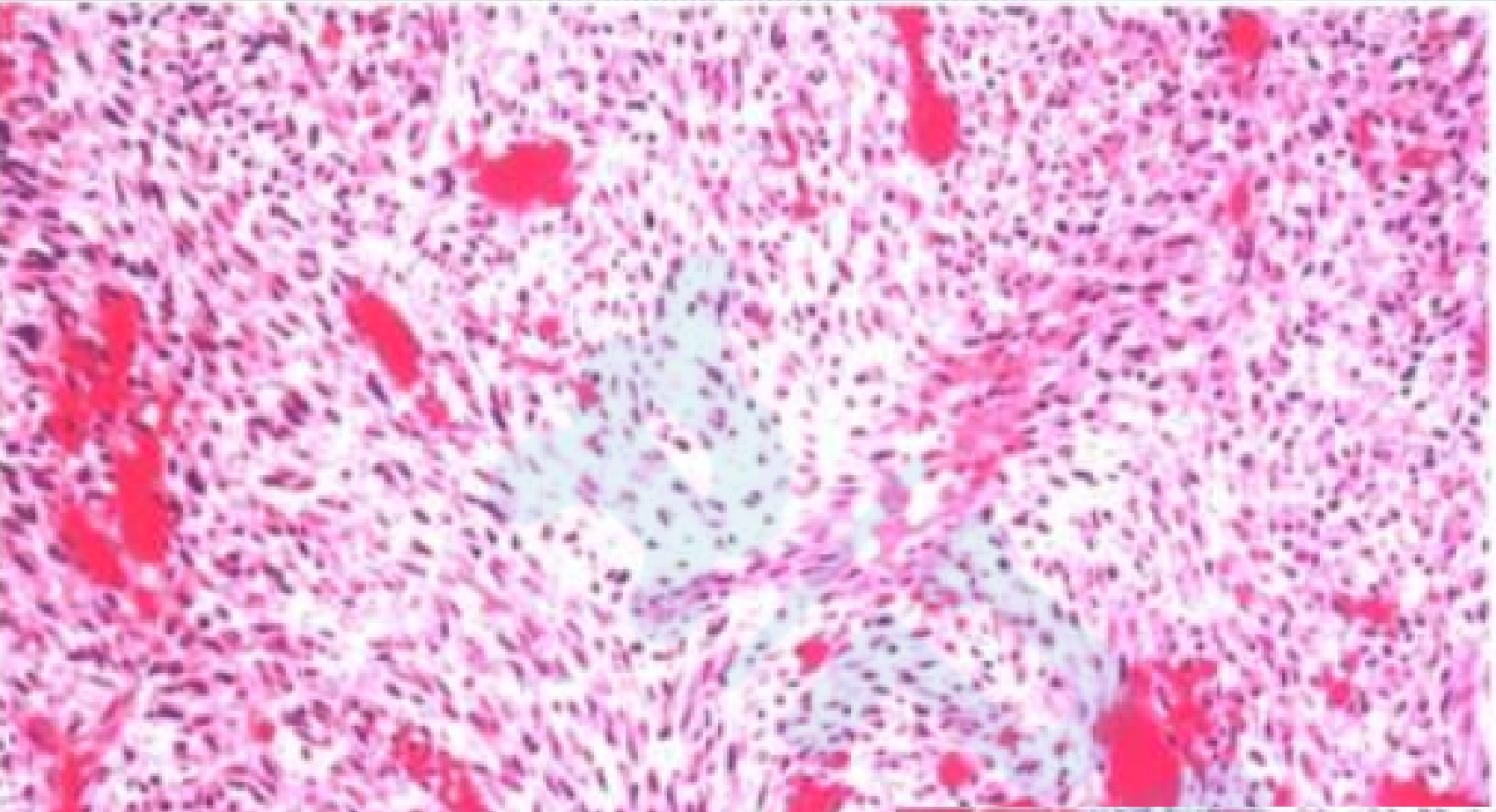
C

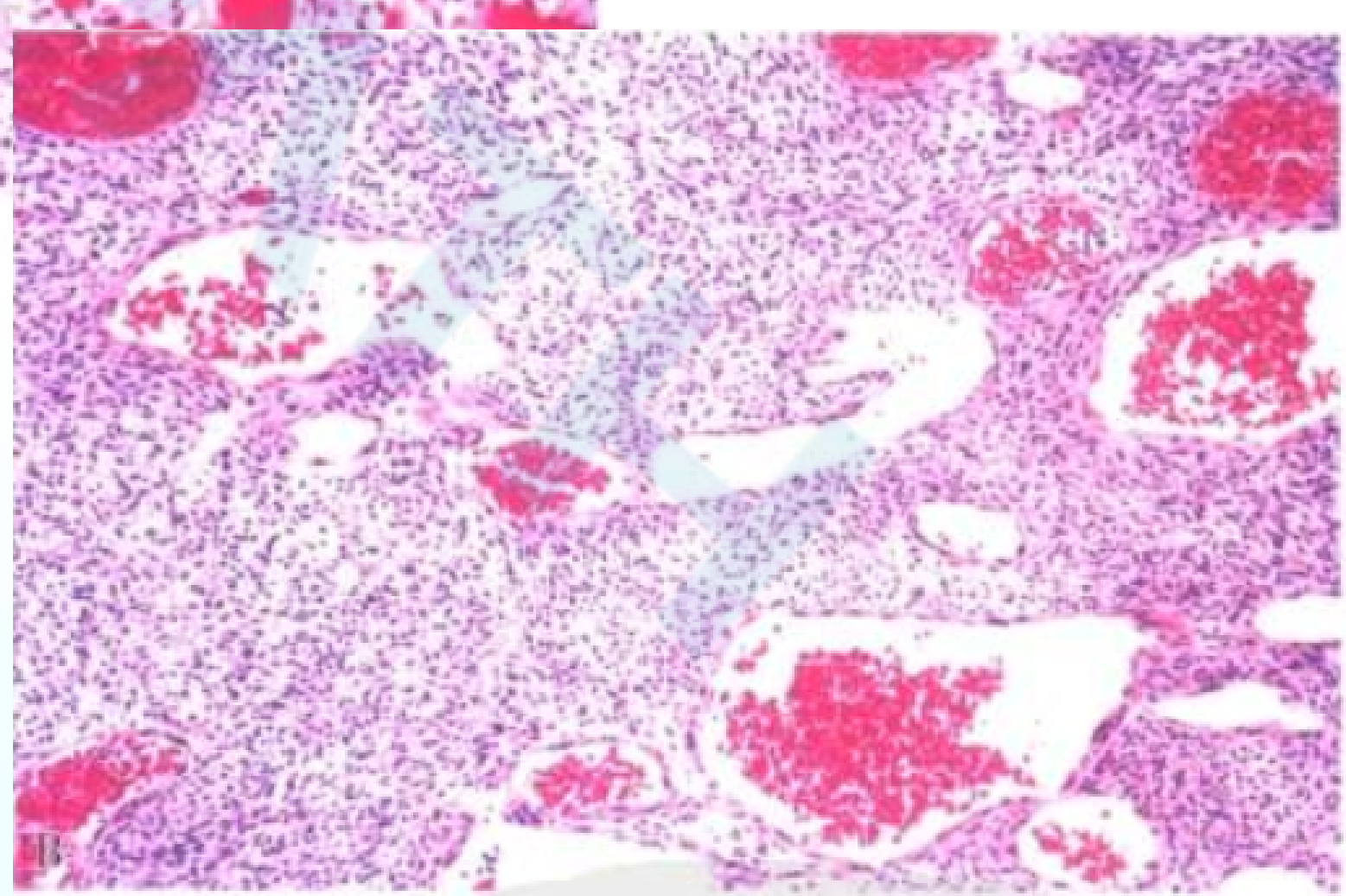
BACKGROUND

病理特征

- 形态单一、胞质稀少的梭形细胞密集排列成束状，呈**鱼骨样外观**
- 细胞核多形性不明显，**核分裂象活跃**
- 可出现较多胶原纤维的区域
- 其他改变：不规则血窦和裂隙状血管形成**血管外皮细胞瘤样结构**；多少不等的慢性炎性浸润、髓外造血
- 少见：多核巨细胞；圆形细胞区域

部分内容参考王哲主任课件





BACKGROUND

免疫表型：缺乏特异性

- Vim 阳性
- 局灶SMA、MSA阳性

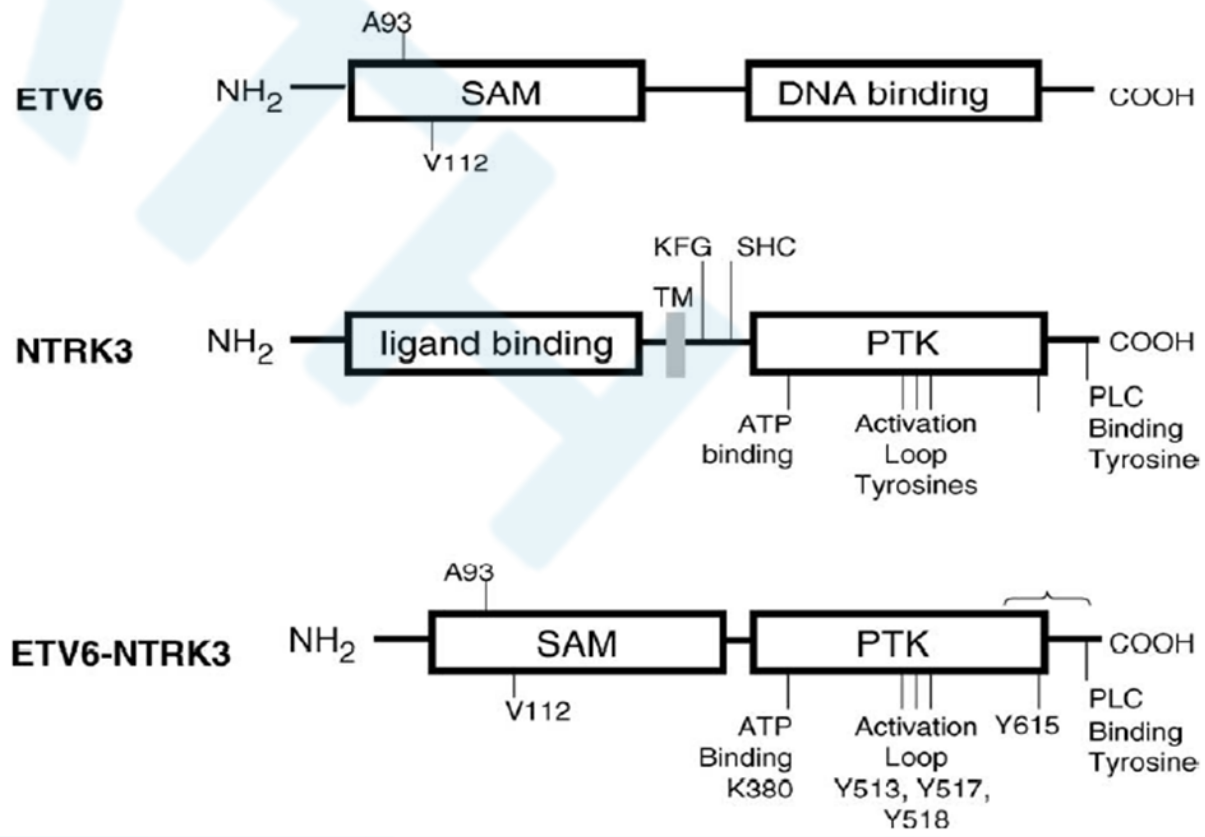
遗传学改变：

- 大部分先天性病例出现t(12;15)(p13;q25)→ **ETV6与NTRK3基因融合**

ETV6-NTRK3融合基因（E-N）

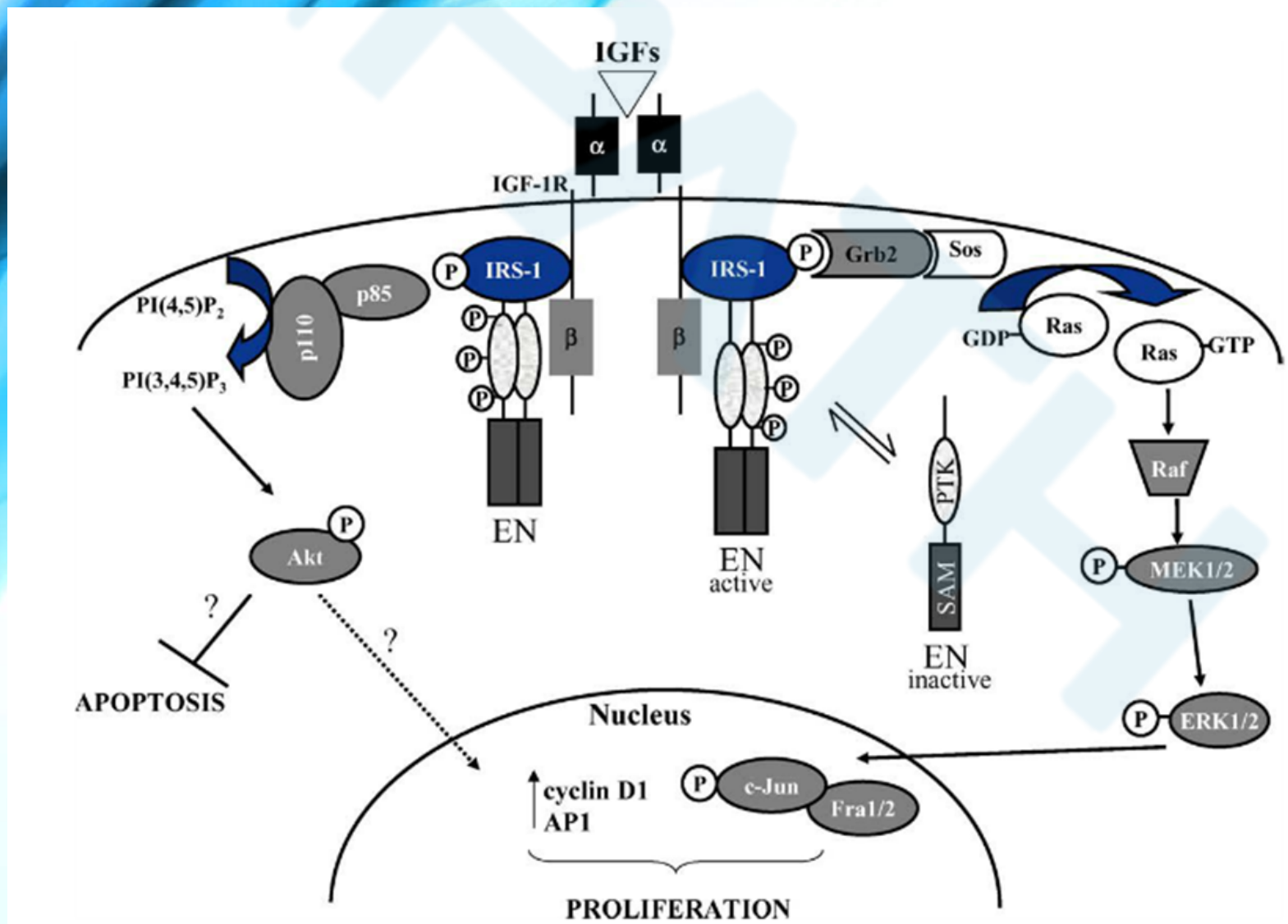
ETV6（ETS variant gene 6）：定位于12p13。编码的蛋白质是ETS转录因子家族中一种序列特异性的转录抑制因子，通过Fli-1结合抑制其活性，在早期造血和血管生成中起着重要作用。

NTRK3（neurotrophic tyrosine kinase，receptor type 3）：定位于15q25。编码神经营养因子-3（NT-3）的跨膜受体，在神经系统的生长发育中起着重要作用。



ETV6-NTRK3信号转导途径

激活RAS-RAF1-MEK-ERK1/2和PI3K-Akt两条酪氨酸途径引起细胞增殖和分化失控，从而引发肿瘤。



ETV6-NTRK3融合基因在肿瘤组织中的表达

- **先天性/婴儿型纤维肉瘤（CFS）**：几乎所有患者年龄<两岁，E-N融合基因只发生于CFS，在成人纤维肉瘤、婴儿纤维瘤、肌纤维瘤等组织学相似的梭形细胞肿瘤中不表达
- **富于细胞型先天性中胚层肾瘤（CMN）**：患者发病年龄轻，预后相对较好，可以和CFS并存
- **急性髓系白血病**：较少见或只表达在某些特定亚型
- **分泌型乳腺癌（SBC）**：有学者研究202例乳腺癌标本，5例SBC4例表达E-N融合基因，其他类型乳腺癌皆不表达

BACKGROUND

Therapy

IFS with ETV6-NTRK3 gene fusions

significant sensitivity to cytotoxic chemotherapy and more recently a promising response to NTRK inhibitors (crizotinib)

病例讨论(Index Case)

- 女性，16岁
- 腹膜后肿瘤，合并破裂和腹腔内出血
- 术中显示腹膜受累

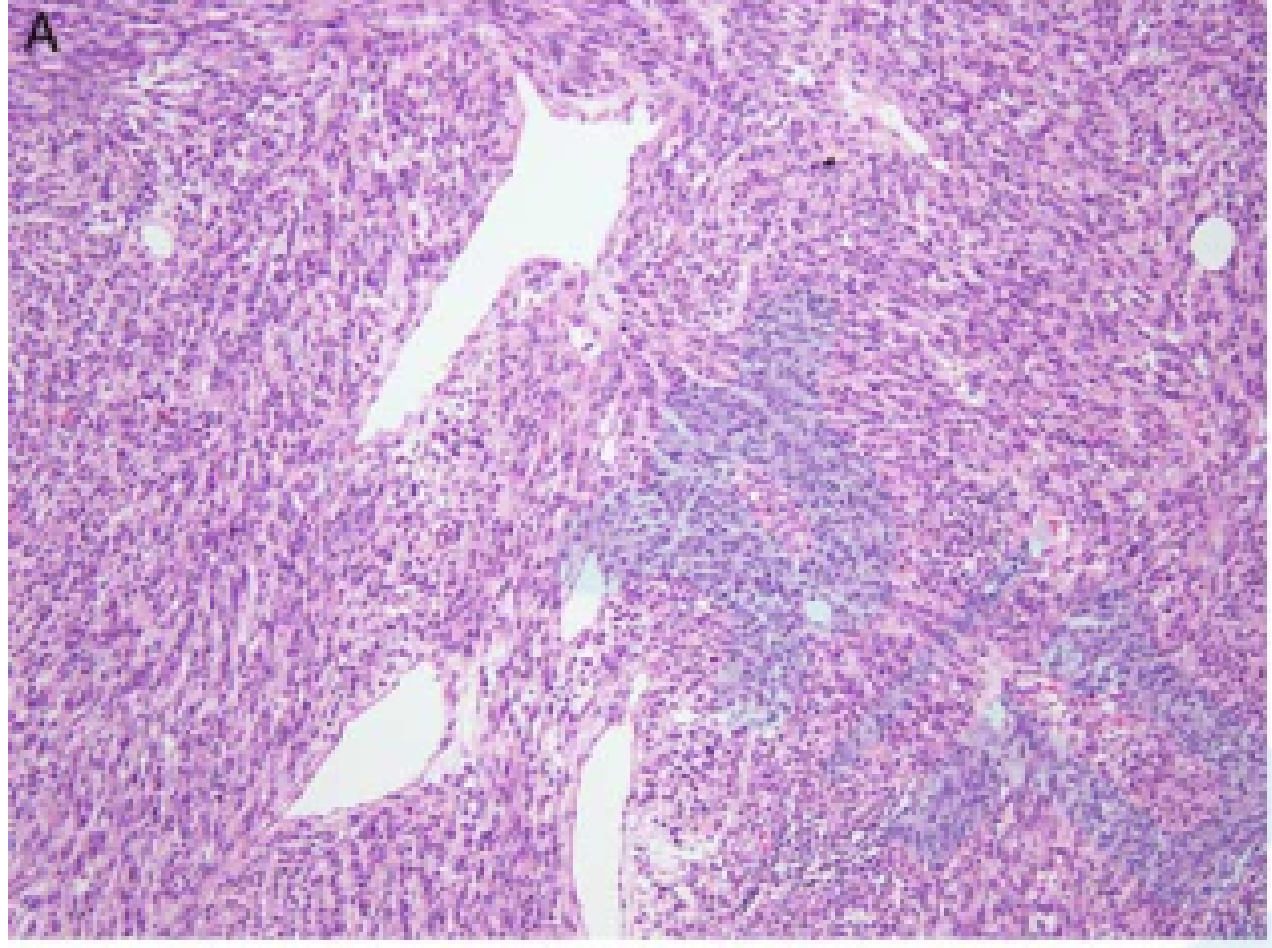
病例讨论(Index Case)

- H&E：疏松的间质中梭形细胞呈束状排列，细胞染色质细，细胞核不典型，可见淋巴细胞、浆细胞浸润及扩张的血管，核分裂象高达6/10HPF

- IHC：

On the basis of these findings, a diagnosis of IFS was assigned.

- FISH：未检测到ALK、SS18、ROS1及RET基因重排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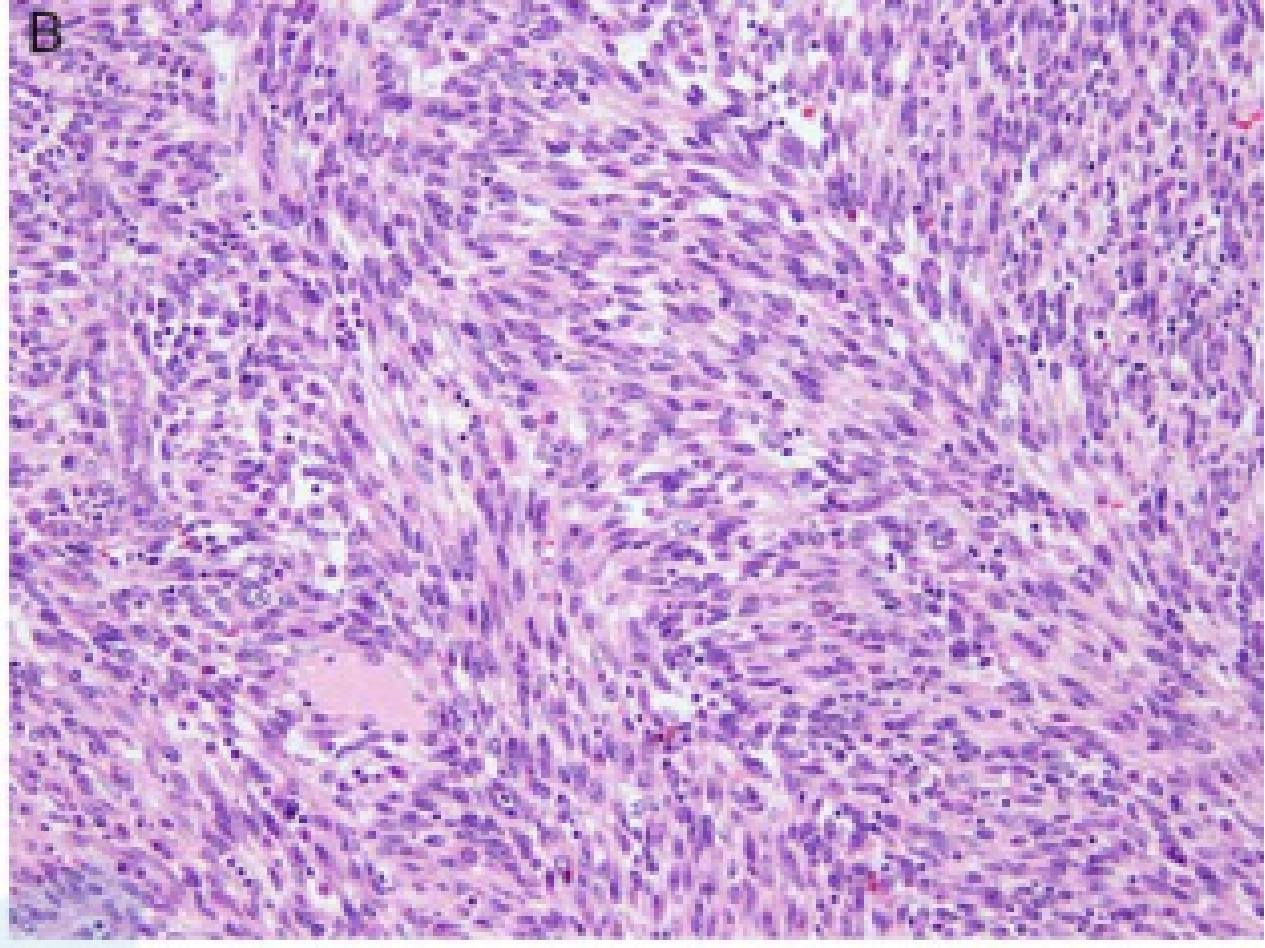
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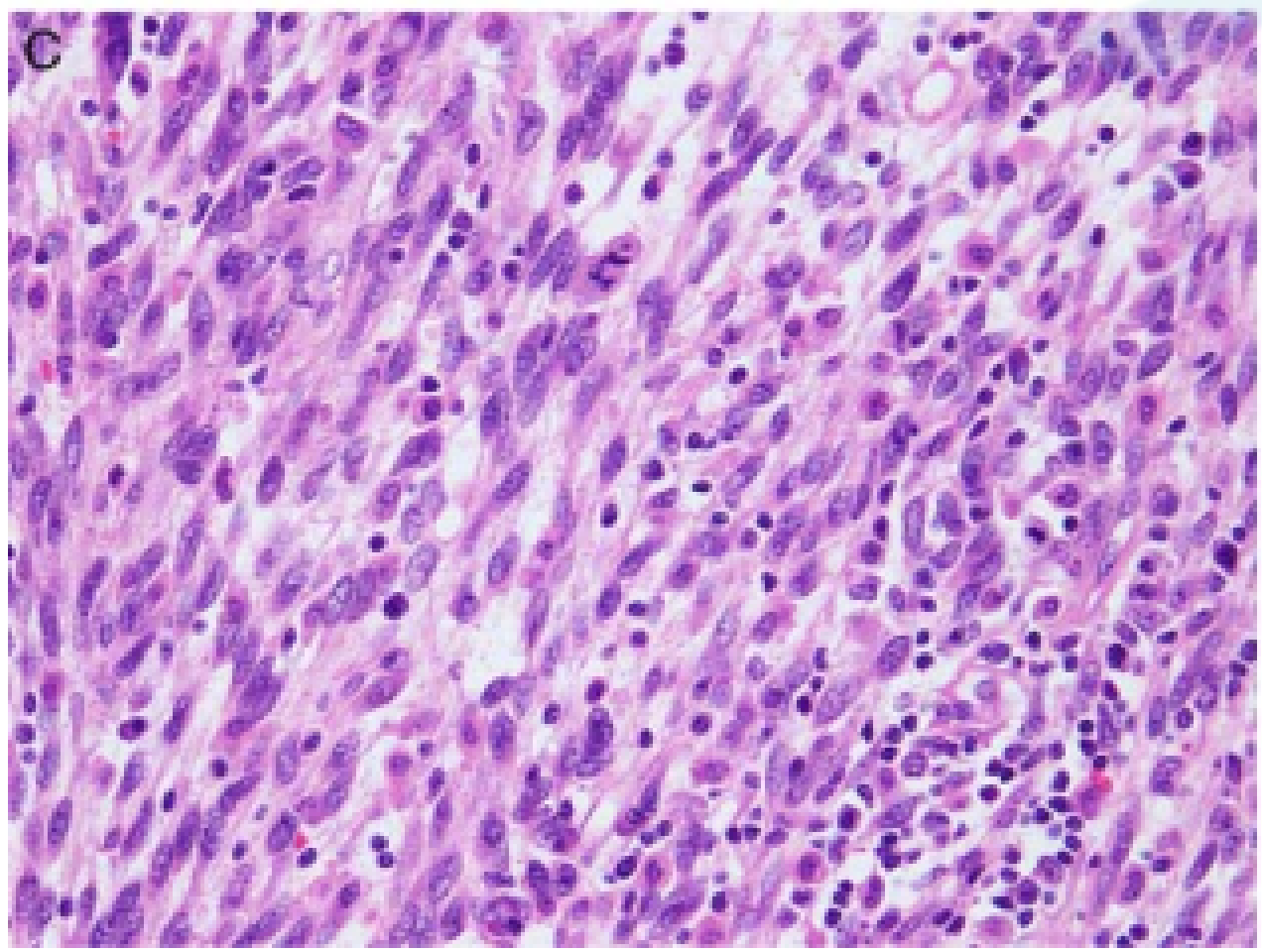
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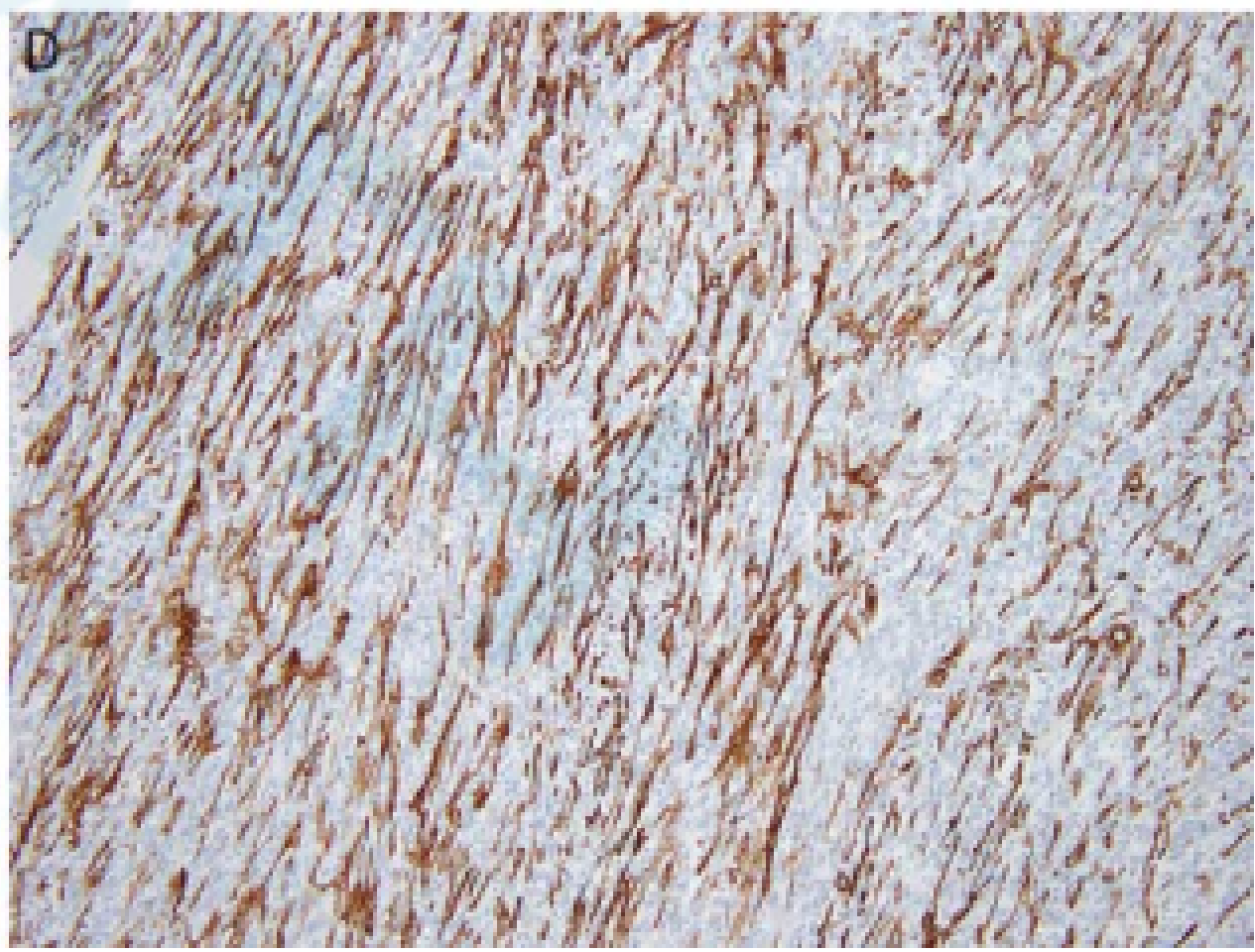
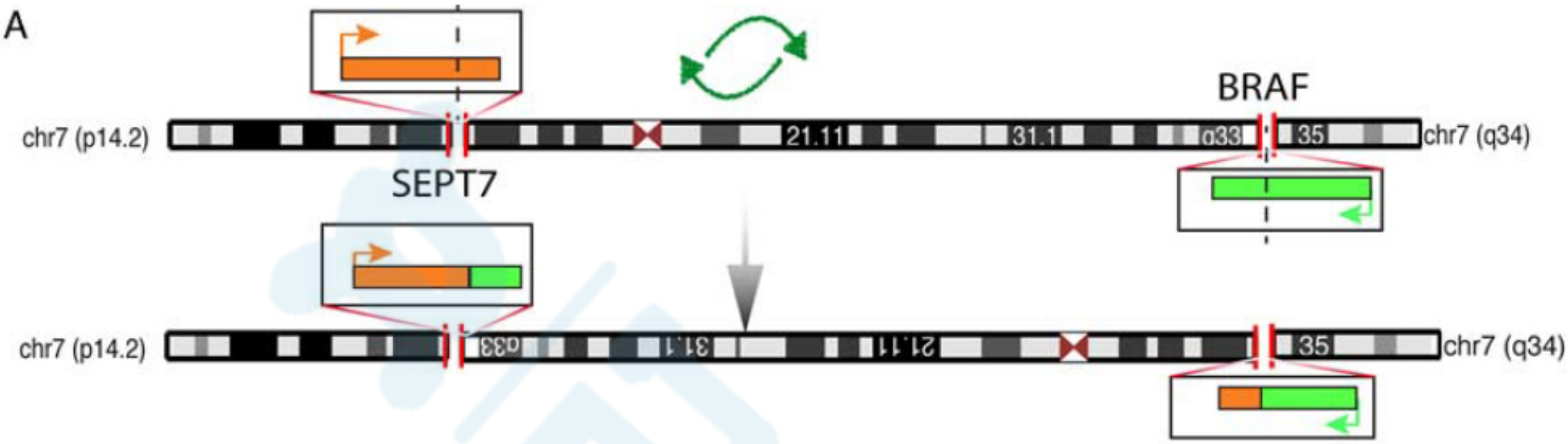
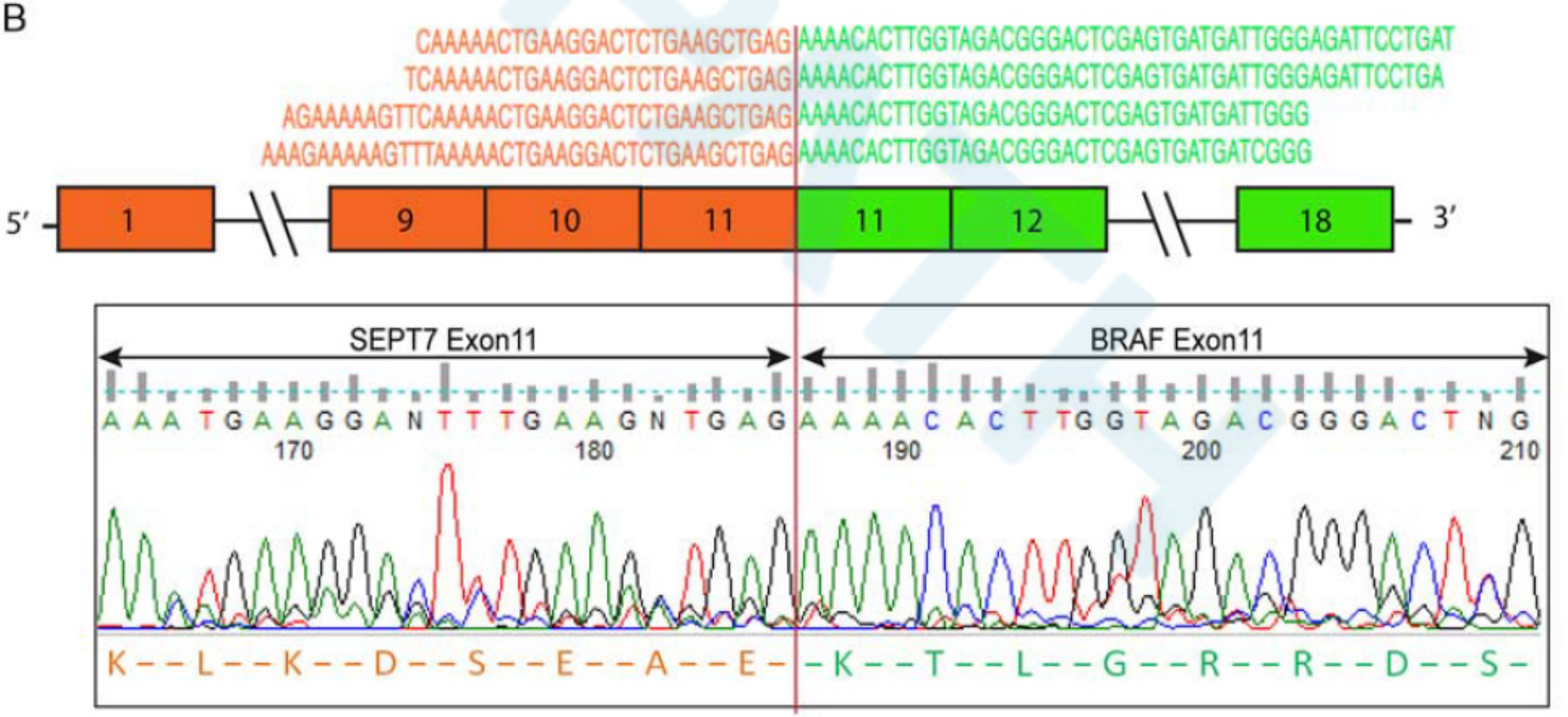

D

A


BRAF
chr7 (p14.2)
21.11
31.1
q33
35
chr7 (q34)
SEPT7
chr7 (p14.2)
q33
31.1
21.11
35
chr7 (q34)

B


CAAAAACTGAAGGACTCTGAAGCTGAG|AAAACACTTGGTAGACGGGACTCGAGTGATGATTGGGAGATTCCTGAT
TCAAAAACTGAAGGACTCTGAAGCTGAG|AAAACACTTGGTAGACGGGACTCGAGTGATGATTGGGAGATTCCTGA
AGAAAAAGTTCAAAAACTGAAGGACTCTGAAGCTGAG|AAAACACTTGGTAGACGGGACTCGAGTGATGATTGGG
AAAGAAAAAGTTTAAAAACTGAAGGACTCTGAAGCTGAG|AAAACACTTGGTAGACGGGACTCGAGTGATGATCGGG
5′
1
9
10
11
11
12
18
3′
SEPT7 Exon11
BRAF Exon11
A A A T G A A G G A N T T T G A A G N T G A G A A A A C A C T T G G T A G A C G G G A C T N G
170
180
190
200
210
K – – L – – K – – D – – S – – E – – A – – E – – K – – T – – L – – G – – R – – R – – D – – S –

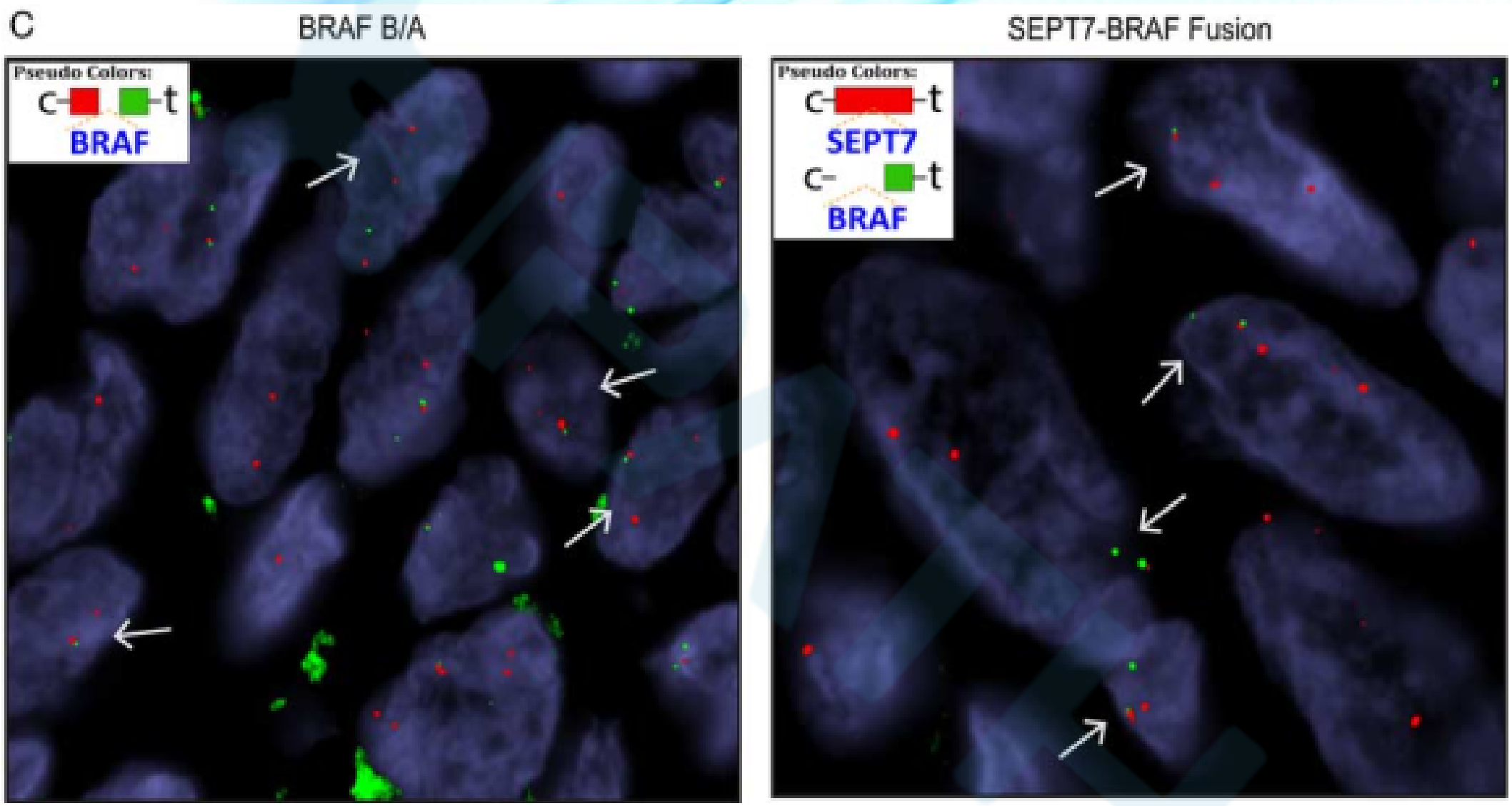


FIGURE 2. A *SEPT7-BRAF* gene fusion was identified in the index case by targeted RNA sequencing. A pericentric inversion of chromosome 7 resulted in the fusion of *SEPT7* (7p14.2) and *BRAF* (7q34) (A). RNA sequencing fusion junction reads and subsequent confirmatory RT-PCR showed *SEPT7* exon 11 fused in-frame to *BRAF* exon 11 (B). FISH further confirmed *BRAF* gene rearrangement with break-away centromeric (red) and telomeric (green) signals (C, left, arrows) as well as *SEPT7-BRAF* fusion with come-together *SEPT7* (red) and *BRAF* (green) signals (C, right, arrows).

Patient Selection

TABLE 1. Clinicopathologic and Genetic Findings of Pediatric Spindle Cell Sarcomas With Features Reminiscent of Infantile Fibrosarcoma

				IHC				
No.	**Age**	**Sex**	**Site**	**SMA**	**CD34**	**S100**	**Desmin**	**Gene Fusions**
1	16 y	F	RP	Patchy +	NA	–	–	*SEPT7-BRAF**,†
2	3 y	F	Pelvis	NA	NA	NA	NA	*BRAF* rearrangement†
3	6 mo	F	Pelvis	Focal+	–	–	–	*BRAF* rearrangement†
4	1.5 y	M	T6-12 spine (extradural)	NA	NA	–	–	*CUX1-BRAF*†,‡
5	2 d	M	Thigh	Focal+	–	–	Rare cells	*BRAF* rearrangement†
6	1 y	M	Foot	–	+	–	–	*EML4-NTRK3**,†
7	7 wk	M	RP	+	+	–	–	*TPM3-NTRK1*†,§
8	12 y	F	Forearm	NA	NA	NA	NA	Unknown
9	5 mo	F	Lumbar	+	–	–	–	Unknown
10	3 y	F	RP	–	NA	–	–	Unknown

*By targeted RNA sequencing.
†By FISH.
‡By FoundationOne, Foundation Medicine.
§By whole transcriptome sequencing.
F indicates female; IHC, immunohistochemistry; M, male; NA, not available; RP, retroperitoneum.

MATERIALS AND METHODS

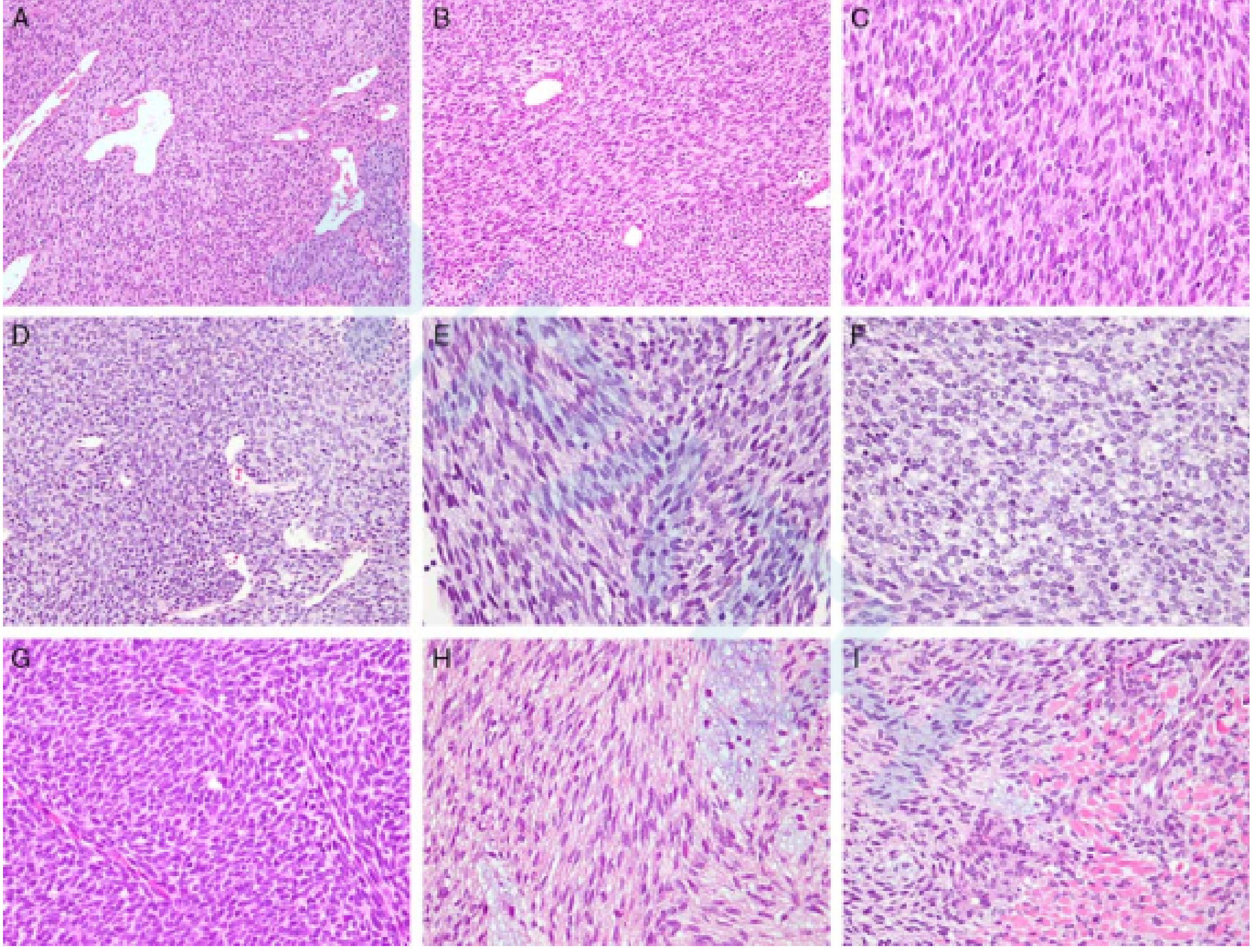
- **Targeted RNA Sequencing**(Index Case/#1 & case#6)
- **Whole Transcriptome Sequencing**(case#7)
- **FISH :**

✓ index case/#1: BRAF break-apart and SEPT7-BRAF fusion

✓ case#6: EML4 and NTRK3 break-apart

✓ case#7: TPM3-NTRK1 fusion

- **Reverse Transcription-Polymerase Chain Reaction Sequencing**(Index Case/#1 & case#7)


A
B
C
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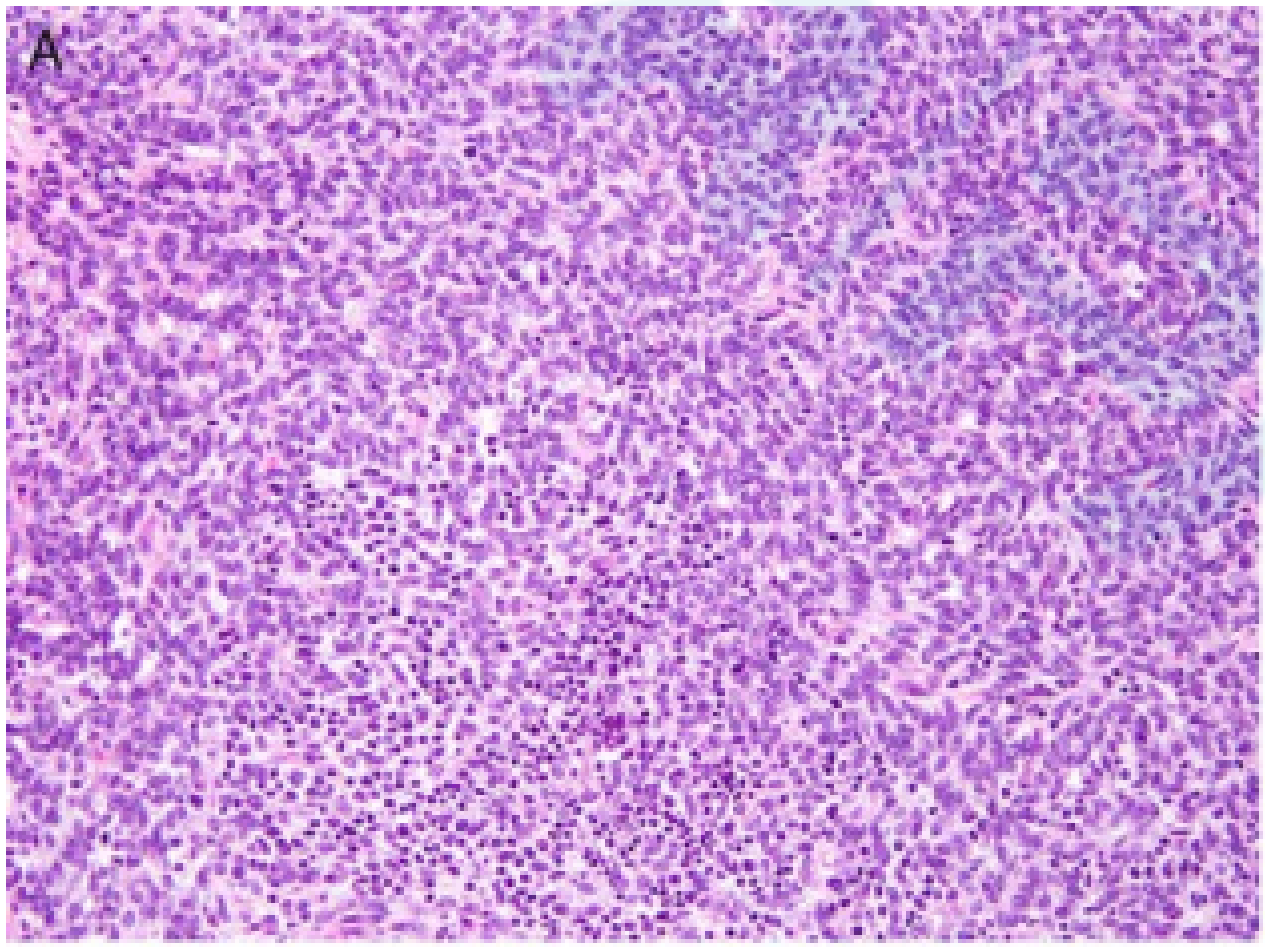
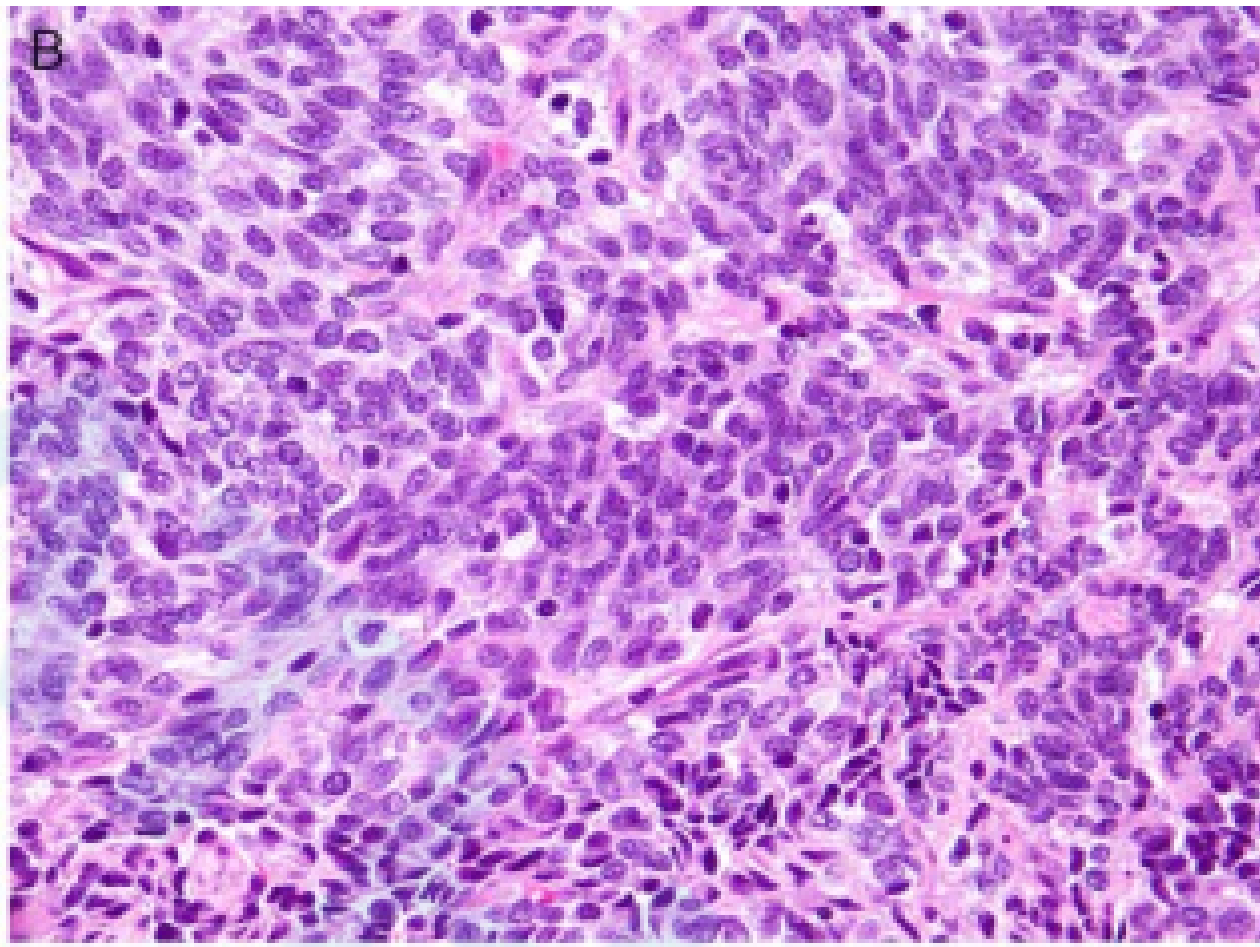
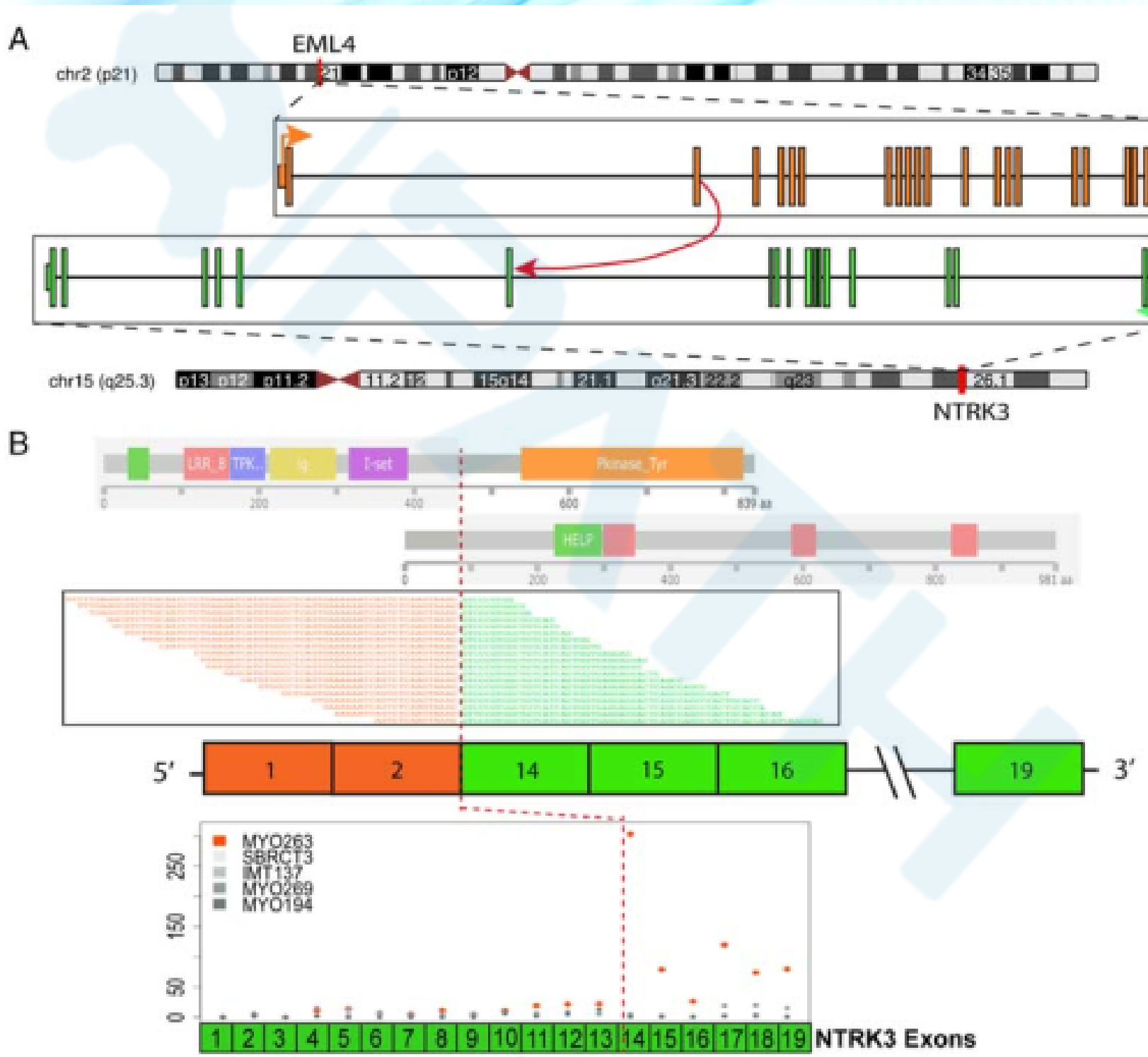
F
G
H
I

RESULTS





RESULTS


A
EML4
chr2 (p21)
NTRK3
chr15 (q25.3)
B
LRR_8
TPK_
Ig
I-set
Pkinase_Tyr
HELP
5'
3'
1
2
14
15
16
19
MYO263
SBRCT3
IMT137
MYO269
MYO194
NTRK3 Exons

RESULTS

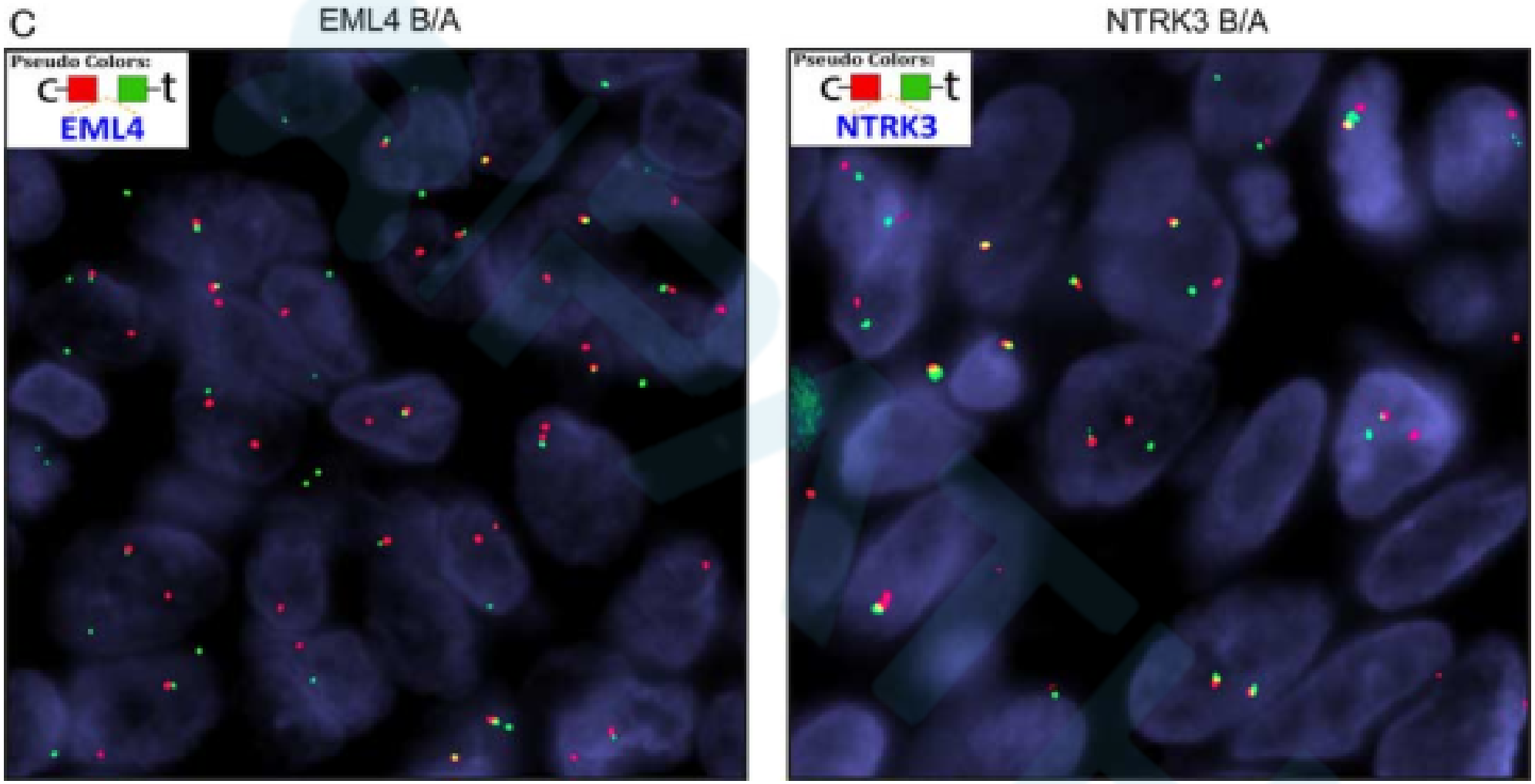
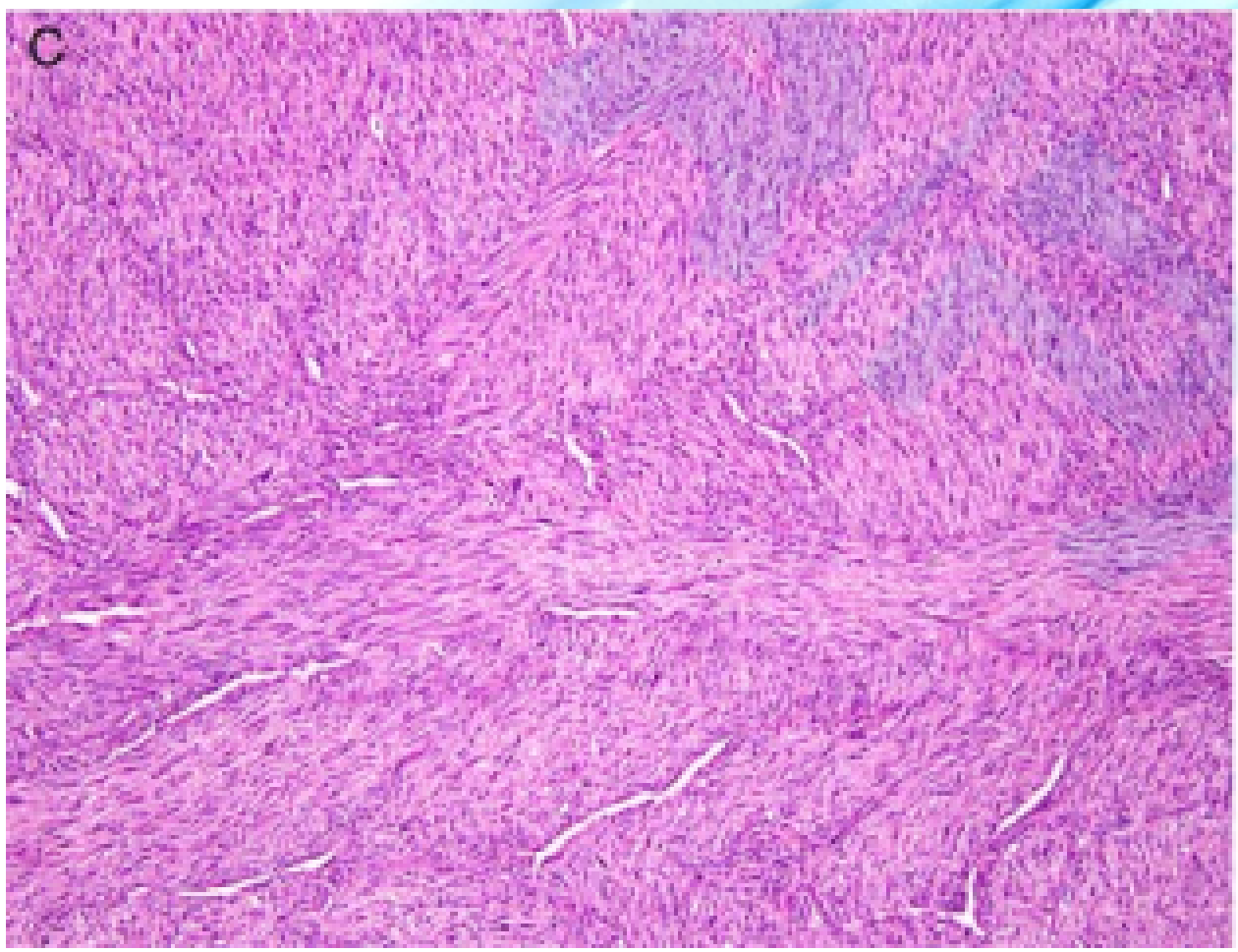
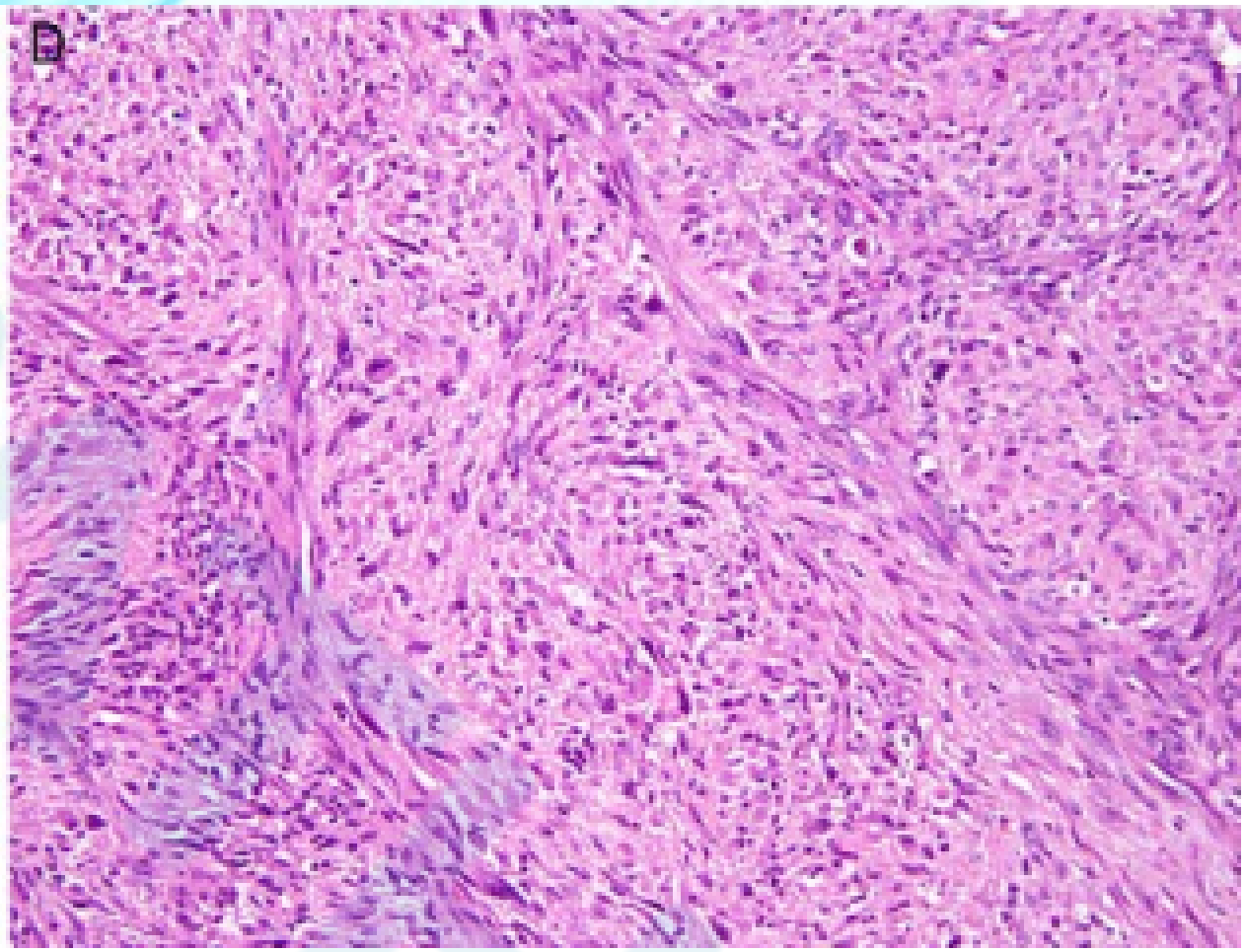


FIGURE 4. *EML4-NTRK3* fusion in an IFS involving the foot of a 21-month-old boy (case #6). Targeted RNA sequencing showed fusion of *EML4* exon 2 to *NTRK3* exon 14, resulting from a t(2;15) translocation (A, B). The chimeric protein contains tyrosine kinase domain from NTRK3 but no known functional domain from EML4 (B). The downstream exons after the breakpoint of *NTRK3* (exon 14) showed upregulated mRNA level in contrast to the 5′ *NTRK3* exons and other samples in the same data set (B, orange dots). FISH confirmed rearrangement of both *EML4* and *NTRK3* genes, with break-apart of centromeric (red) and telomeric (green) signals (C).

RESULTS





RESULTS

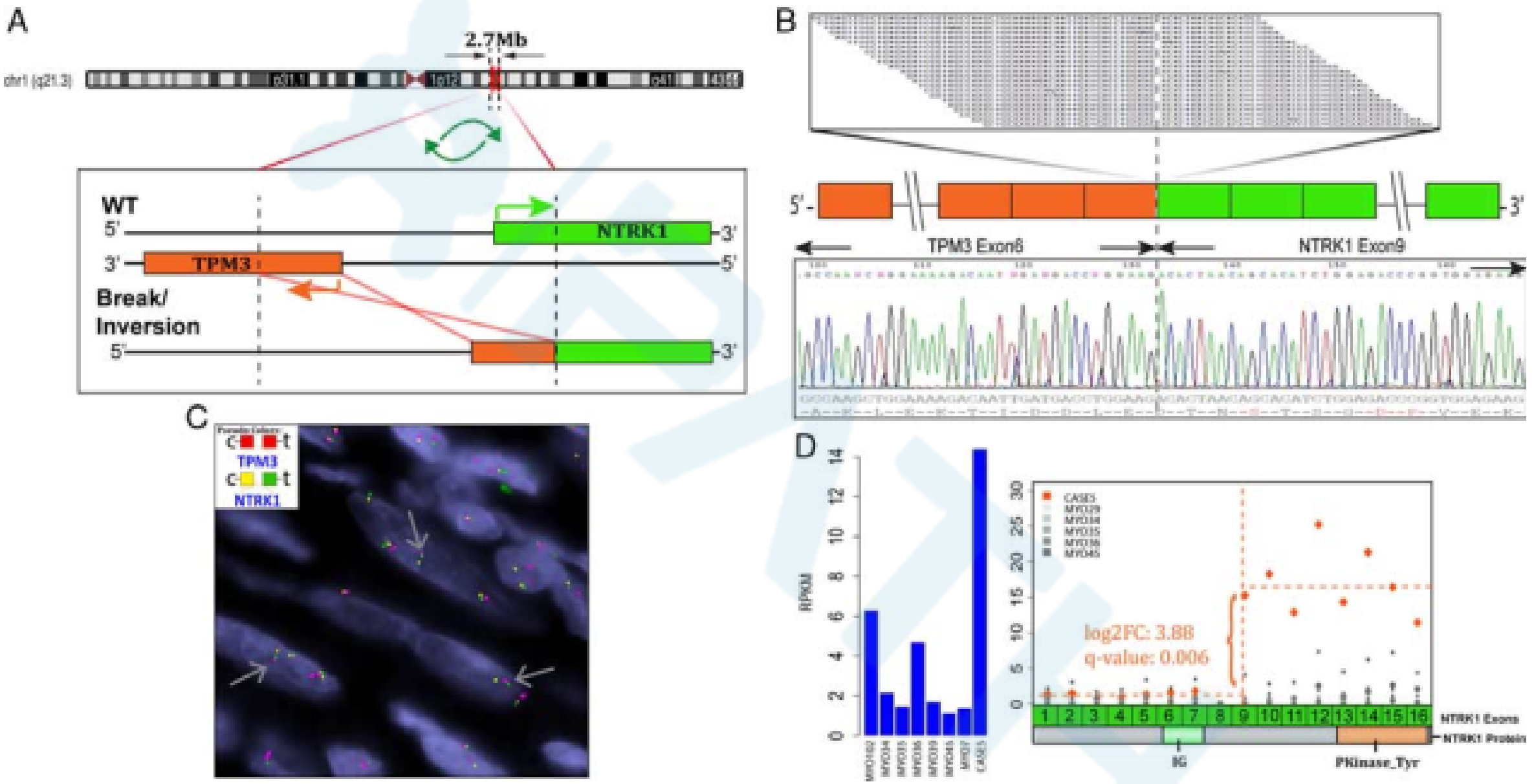


FIGURE 5. *TPM3-NTRK1* fusion in a 7-week-old infant retroperitoneal IFS (case #7). Whole transcriptome RNA sequencing identified a 2.7 Mb inversion-fusion at 1q21.3 locus, leading to a *TPM3-NTRK1* candidate gene fusion (A). The RT-PCR confirmed an in-frame fusion of *TPM3* exon 6 to *NTRK1* exon 9 (B). FISH also confirmed *NTRK1* rearrangement (split of green and yellow signals) and fusion of *TPM3* (red) with *NTRK1* (green, telomeric/3' of *NTRK1*) (arrows) (C). *NTRK1* mRNA expression was upregulated compared with other samples in the same platform (D, left). Exonic expression levels showed high expression of *NTRK1* exons downstream to the break in exon 9 (orange dots); the fusion retaining the entire tyrosine kinase domain in the chimeric protein (D, right).

DISCUSSION

BRAF mutations

- ✓ melanoma
- ✓ papillary thyroid carcinoma
- ✓ non-small cell lung cancer
- ✓ colorectal cancer
- ✓ hairy cell leukemia
- ✓ Langerhans cell histiocytosis
- ✓ ovarian tumors

BRAF related fusions

- **Gliomas, carcinomas, and melanocytic neoplasms through either intrachromosomal or interchromosomal translocations**
- **Soft tissue tumors**

✓ Only in a subset of myxoinflammatory fibroblastic sarcomas: characterized by superficial acral location and composed of alternating myxoid and solid areas, with a histiocytoid phenotype and Reed-Sternberg-like tumor cells

✓ 1 case of chest wall malignant spindle cell neoplasm: KIAA1549-BRAF fusion

EML4-NTRK3 fusion

2 cases (including our case)

- **located in the extremity**
- **A boy (9 month):** Composed of elongated spindle cells and CD34 (-); lung metastasis which responded to chemotherapy and radiation therapy
- **Our case:** a more primitive appearance comprised of round to short spindle cells and CD34 (+)
- **Both cases showed a brisk mitotic activity (>10/10HPF)**

NTRK1-related fusion

- **SQSTM1-NTRK1 (1 case): IFS**
- **LMNA-NTRK1 (1 case):**

✓ male ； infant

✓ developed local recurrence and metastasis to bilateral lungs and S5 vertebral body.

✓ refractory to chemotherapy but responded to crizotinib

- **TPM3-NTRK1: our case**
- Other soft tissue tumor types: lipofibromatosis-like neural tumors & a group of spindle cell sarcomas with prominent myopericytic/hemangiopericytic pattern

SEPTIN gene family

- **SEPT7:** has GTPase activity and is associated with cytoskeleton structure and organelle transport.
- **SEPT9:** previously reported in a case of T-cell prolymphocytic leukemia with SEPT9-ABL1 fusion, which was resistant to both imatinib and dasatinib therapy
- **Others (SEPT2, SEPT5, SEPT6, SEPT9, SEPT11):** involved as 3’ partners in hematological malignancies with MLL-SEPTIN genes fusions

CONCLUSION

- Our findings of recurrent BRAF gene fusions in a group of tumors with features reminiscent of IFS expand the spectrum of fusion-positive spindle cell sarcomas to also include older children and adolescents and predilection for intra-abdominal sites.
- Pediatric tumors with IFS-like phenotype, the molecular work-up should ideally include testing for abnormalities in other kinases, such as BRAF, NTRK1, MET, if the tumor lacks the canonical ETV6-NTRK3 fusion.

感谢聆听