Pan-Trk Immunohistochemistry Identifies NTRK Rearrangements in Pediatric Mesenchymal Tumors

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汇 报 人：张微晨

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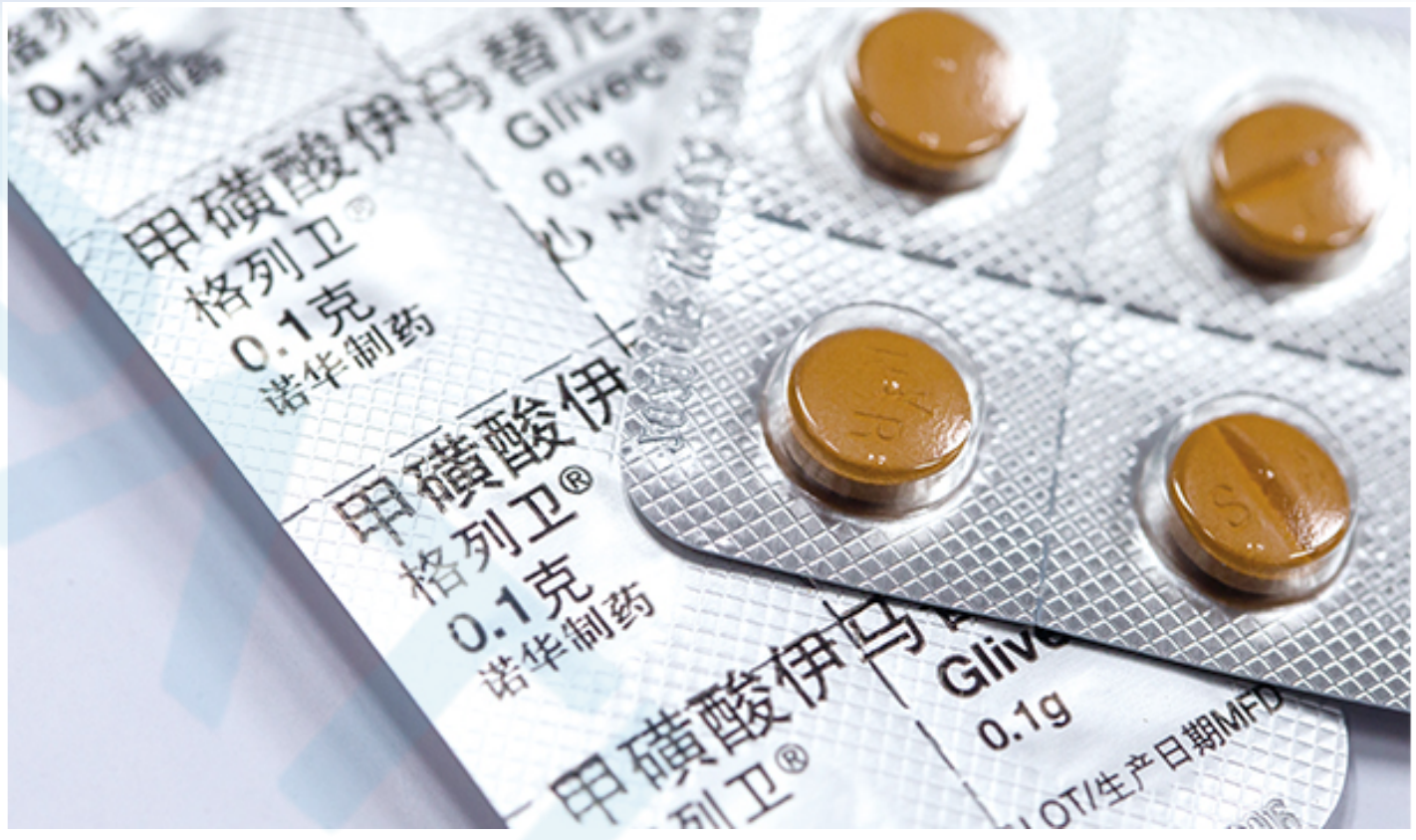
宁浩 徐峥 文牧野
我不是药神
DYING TO SURVIVE
2018.7.6


甲磺酸伊马替尼
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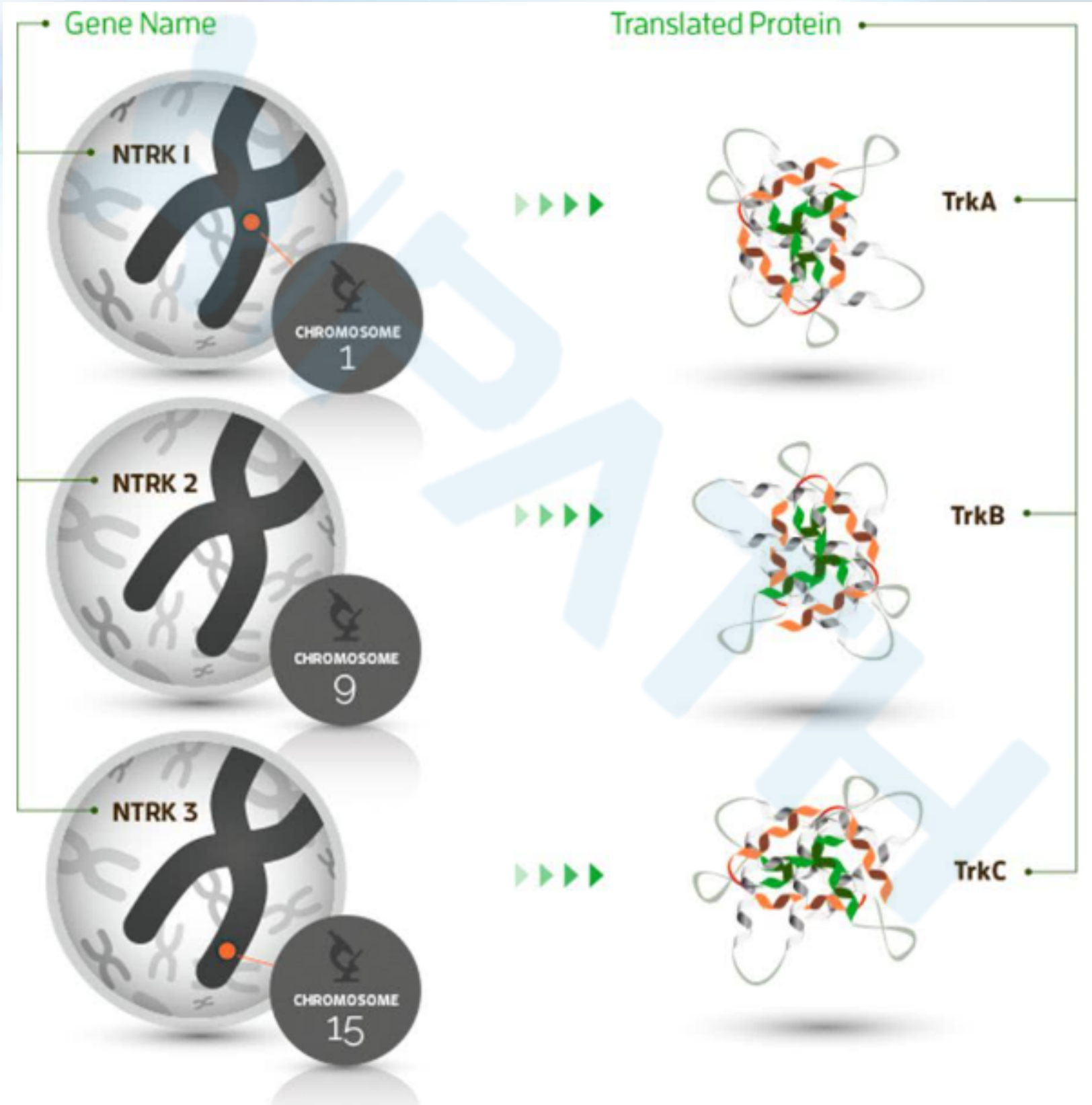
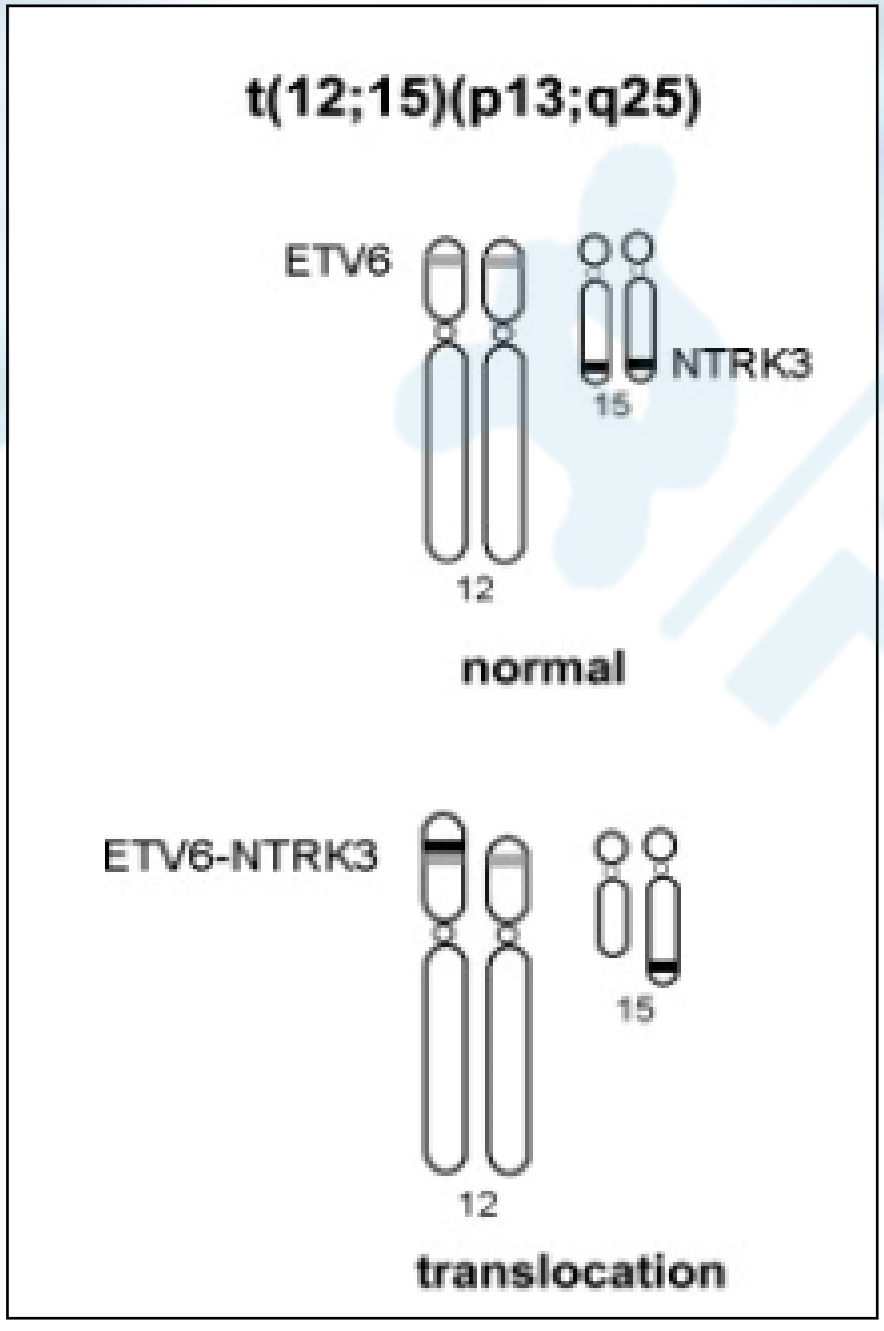
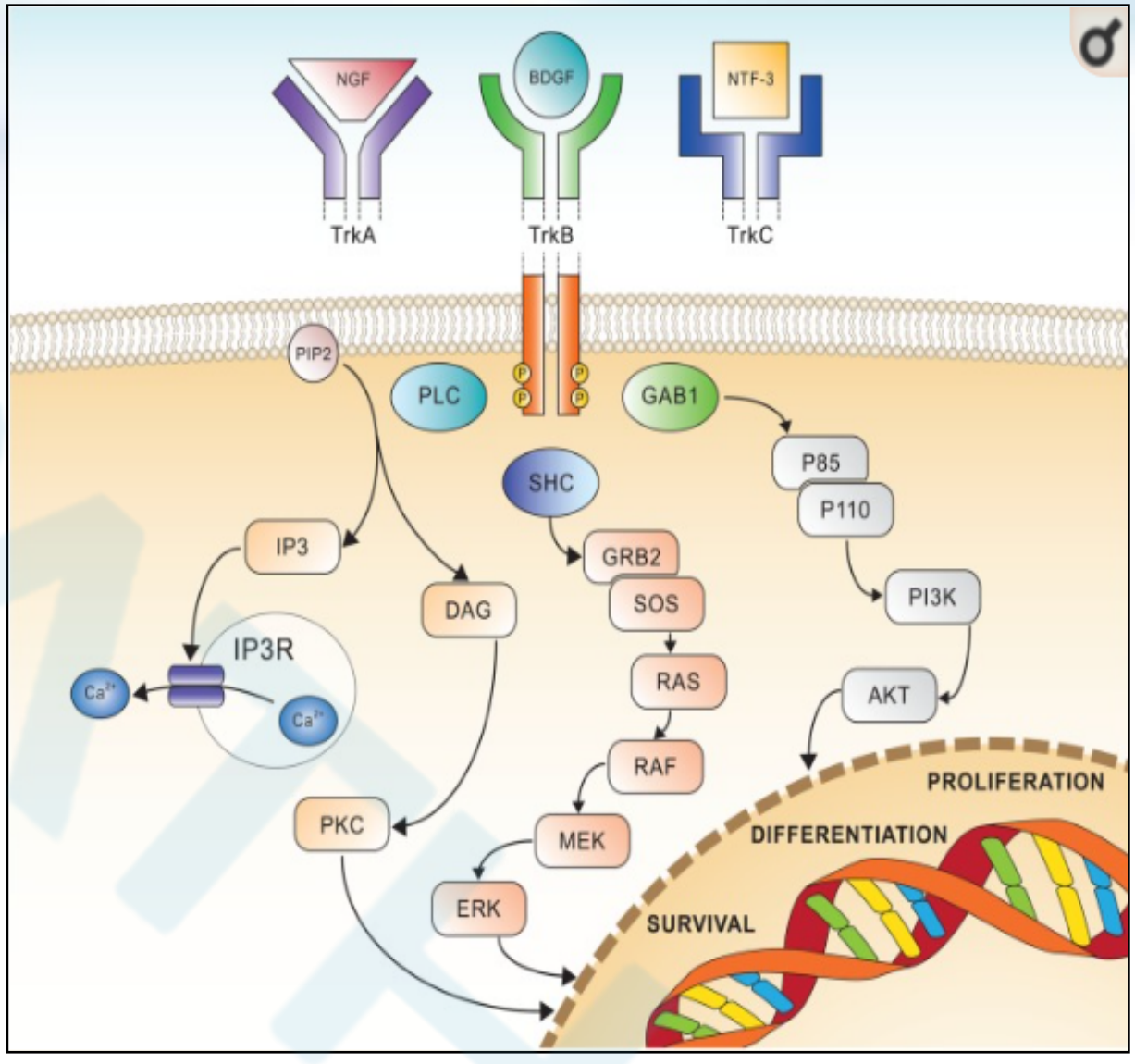
NTRK **TRK**

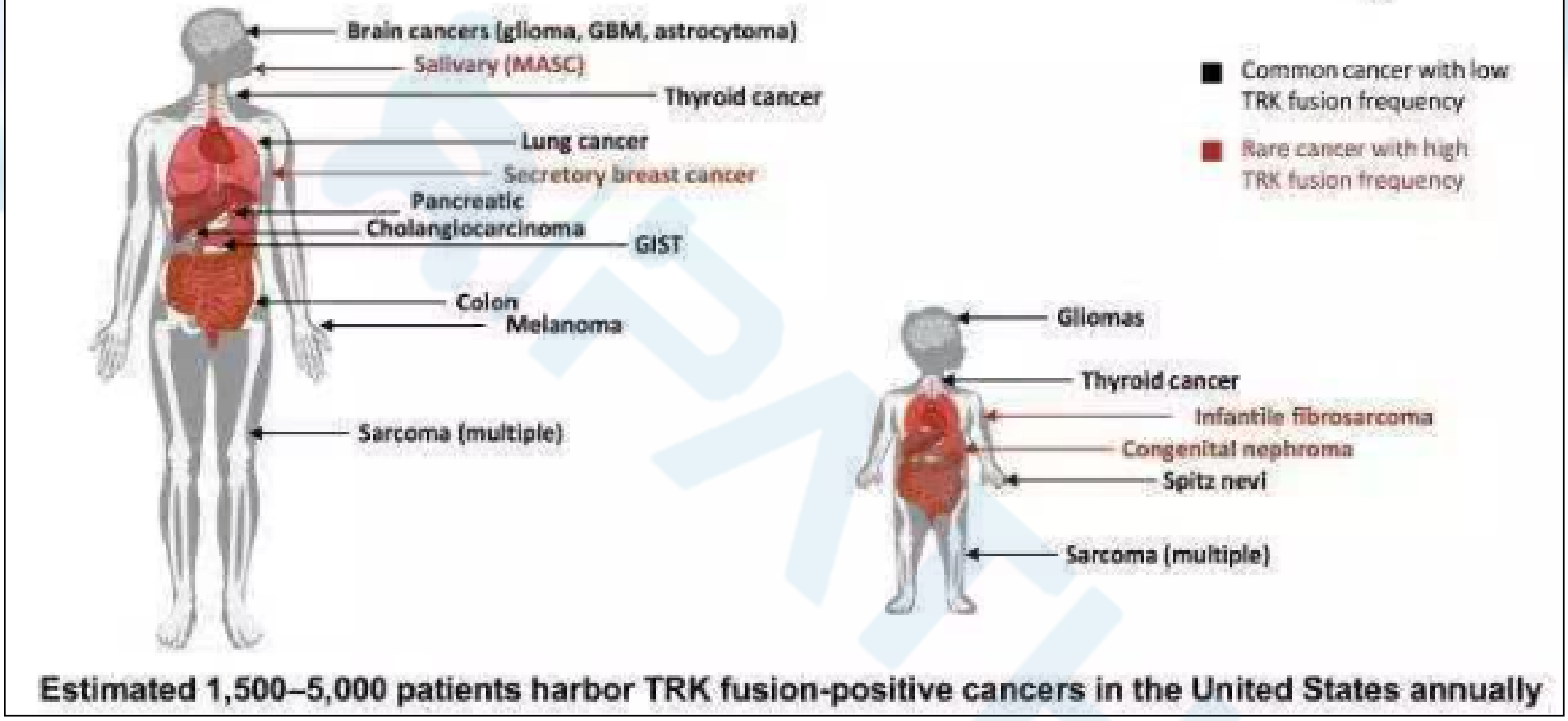
(neurotrophic tyrosine receptor kinase) （Tropomyosin Receptor Kinase）







NTRK基因与某些配体基因意外融合时，TRK可能被重新激活



NTRK基因融合在大多数肿瘤中只占据了0.5%-1%，在某些罕见肿瘤中占比较高，比如在唾液腺癌、乳腺分泌性癌、婴儿纤维肉瘤及先天性中胚叶肾瘤中，可达90%以上。NTRK融合在肿瘤发生早期就会出现，并在肿瘤生长和扩散过程中持续存在。

Efficacy of Larotrectinib in TRK Fusion–Positive Cancers in Adults and Children

Alexander Drilon, M.D., Theodore W. Laetsch, M.D., Shivaani Kummar, M.D., Steven G. DuBois, M.D., Ulrik N. Lassen, M.D., Ph.D., George D. Demetri, M.D., Michael Nathenson, M.D., Robert C. Doebele, M.D., Ph.D., Anna F. Farago, M.D., Ph.D., Alberto S. Pappo, M.D., Brian Turpin, D.O., Afshin Dowlati, M.D., et al.

罹患17种不同癌症的50例携带TRK融合基因的患者使用Lartrectinib（LOXO-101，高选择性TRK抑制剂），有效率达到76%。用药12个月后，79%的患者仍无疾病进展。

Abstr

BACKGRO
diverse c
highly se

METHOD
–positive
of three protocols: a phase 1 study involving adults, a phase 1–2 study involving children, or a phase 2 study involving adolescents and adults. The primary end point for the combined analysis was the overall response rate according to independent review. Secondary end points included duration of response, progression-free survival, and safety.

RESULTS A total of 55 patients, ranging in age from 4 months to 76 years, were enrolled and treated. Patients had 17 unique TRK fusion–positive tumor types. The overall response rate was 75% (95% confidence interval [CI], 61 to 85) according to independent review and 80% (95% CI, 67 to 90) according to investigator assessment. At 1 year, 71% of the responses were

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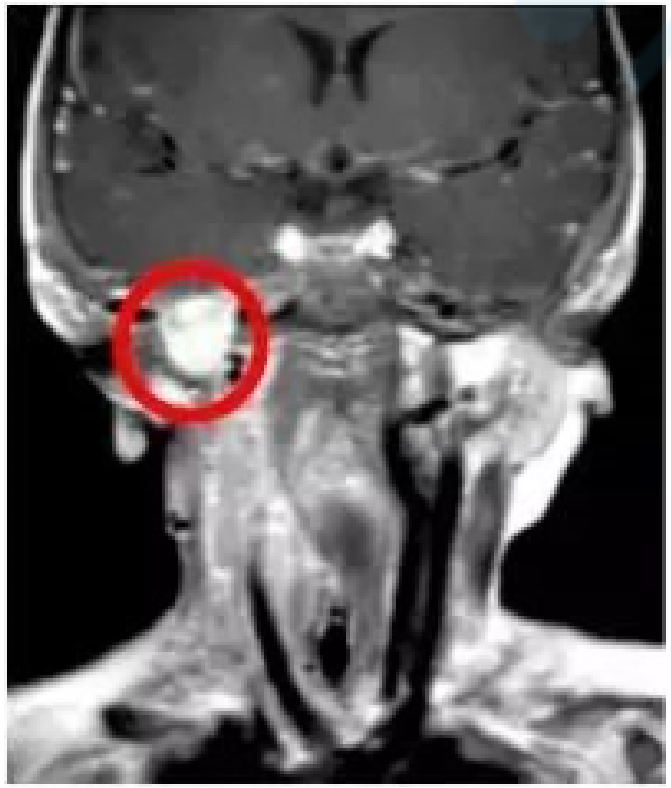
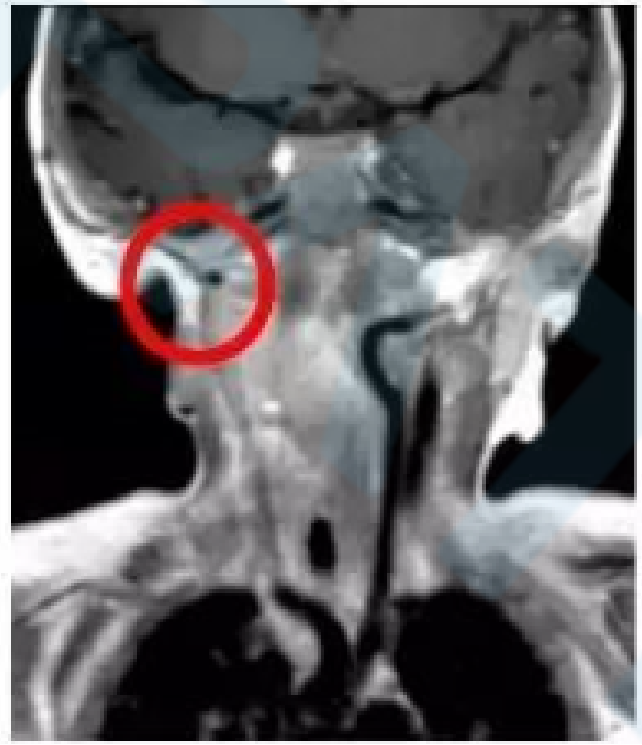
案例

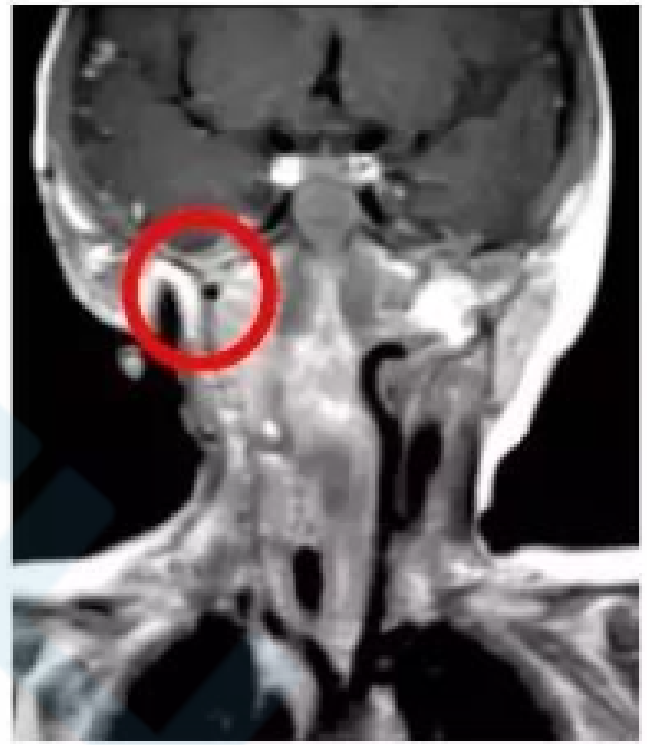
婴儿型纤维肉瘤，16个月，之前接受了3次手术及化疗



治疗前



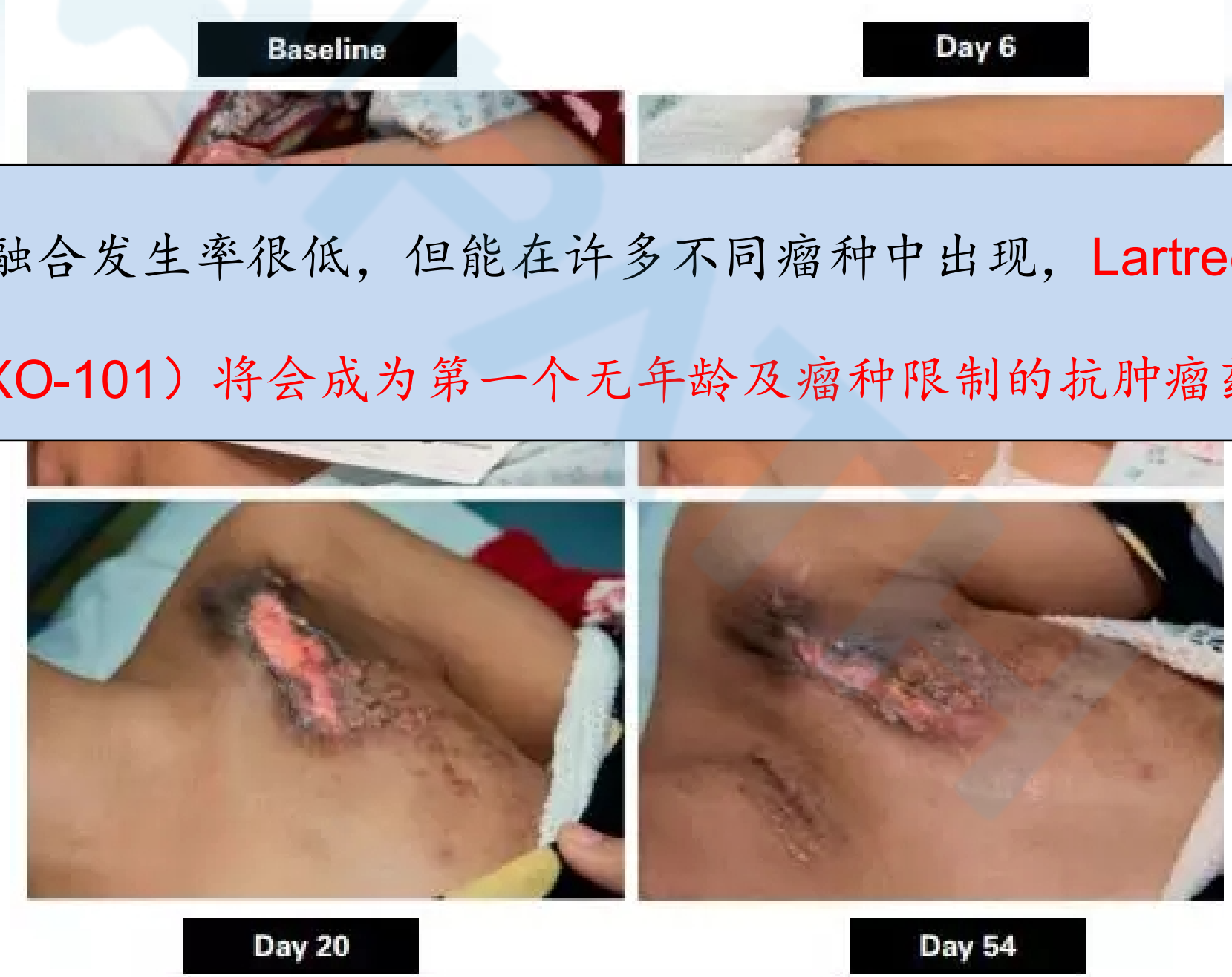
治疗2周期



治疗3周期

案例

乳腺癌病人，接受多次化疗及手术治疗，肿瘤复发



TRK融合发生率很低，但能在许多不同瘤种中出现，Lartrectinib（LOXO-101）将会成为第一个无年龄及瘤种限制的抗肿瘤药物。

检测NTRK基因融合的经典方法：二代测序（NGS）

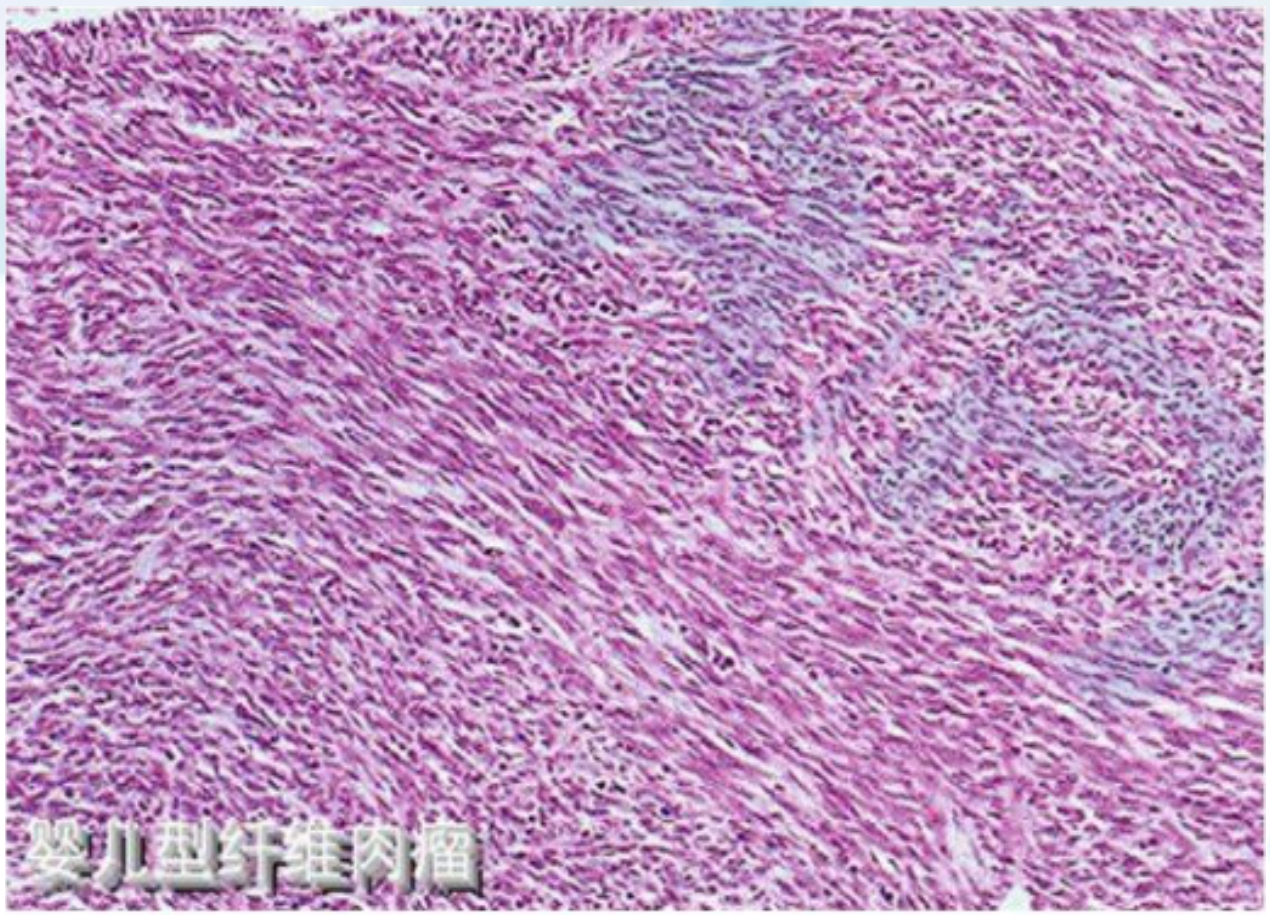
缺点：费时、需较多样本、费用较高。

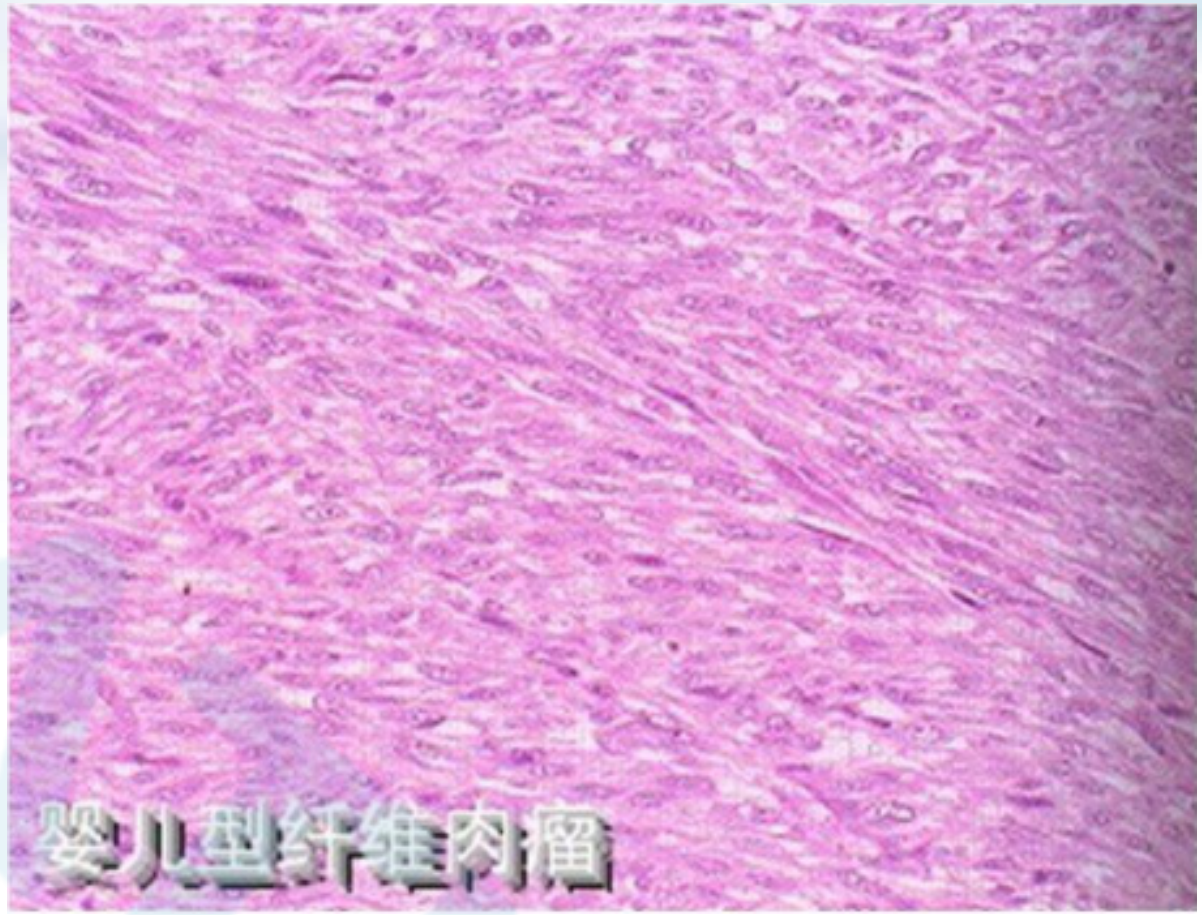
材料 & 方法

实验组：婴儿型纤维肉瘤、先天性中胚层肾瘤共31例，并通过FISH或RT-PCR证实存在NTRK基因融合或ETV6基因断裂。

对照组：50例证实无NTRK融合的其他间叶源性肿瘤（2例隆突、3例婴儿纤维错构瘤、1例脂肪母细胞瘤病、1例脂肪纤维瘤病、5例肌纤维瘤、1例肌纤维瘤病、5例硬纤维瘤、2例恶性外周神经鞘瘤、2例滑膜肉瘤、2例横纹肌肉瘤、3例神经鞘瘤、6例炎性肌纤维母细胞瘤、4例高级别肉瘤、1例神经纤维瘤病、1例后肾间质肿瘤、2例中胚叶肿瘤、1例未分化多形性肉瘤）

婴儿型纤维肉瘤

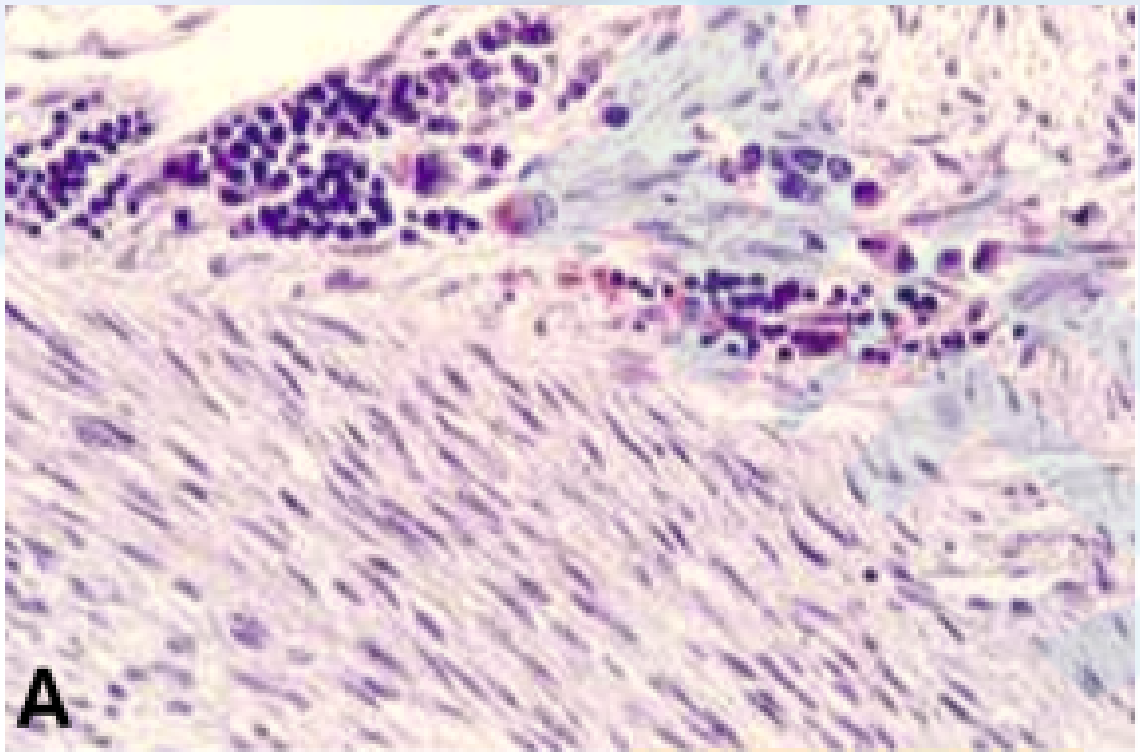


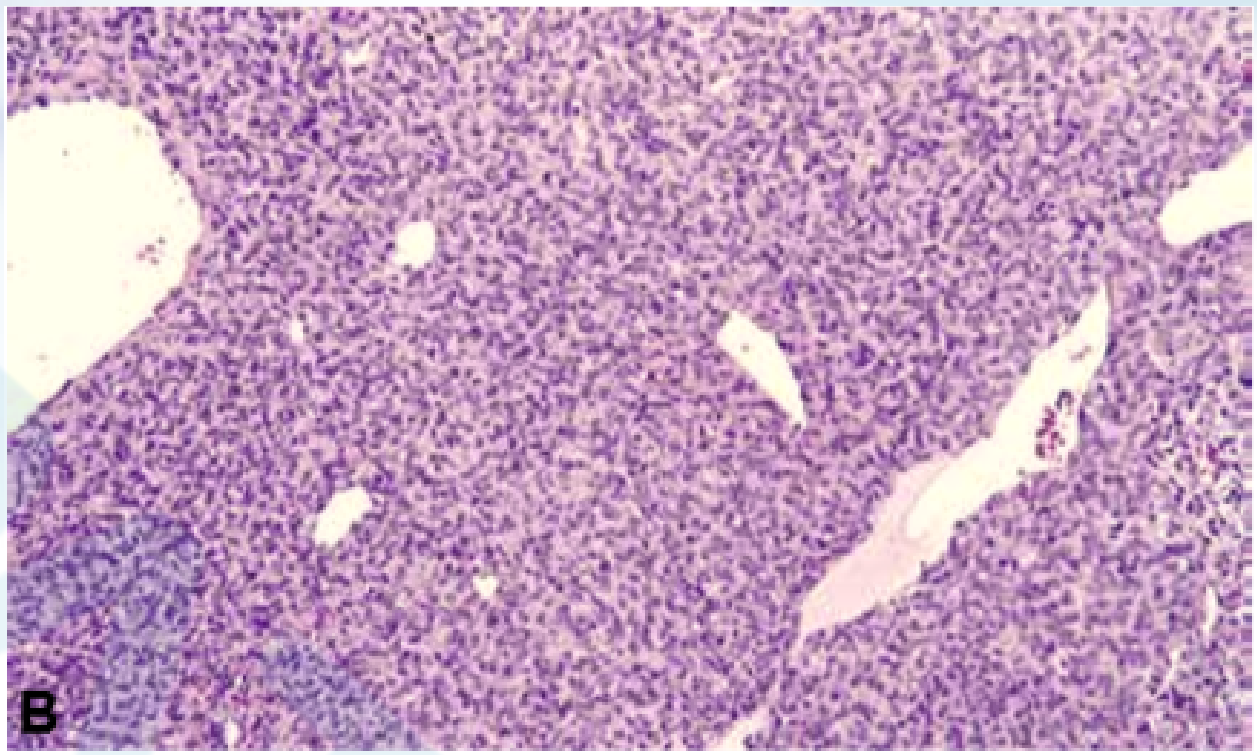


肿瘤界限不清，由致密的交织条束状或鱼骨样排列的梭形细胞组成，核分裂象易见。

IHC：无特殊，瘤细胞主要表达Vimentin、部分病例可局灶表达SMA和MSA。

先天性中胚层肾瘤





组织学表现：经典型和细胞型。前者表现为交错排列的梭形细胞，核分裂指数变化较大，肿瘤呈挤压或浸润式生长，累计周围肾组织；后者则表现为高细胞密度，细胞呈短梭形或卵圆形，富于核分裂，可伴囊性变，坏死和出血。

IHC：Vimentin、SMA弥漫强阳性。

材料 & 方法

方法：IHC

抗体：pan-TRK (EPR17341, Abcam）⟶ TrkA, TrkB, TrkC

TrkA (EP1058Y, Abcam）⟶ TrkA

阳性标准：细胞核或细胞浆着色，胞浆着色的肿瘤细胞数需＞50%

即为阳性。

实验结果

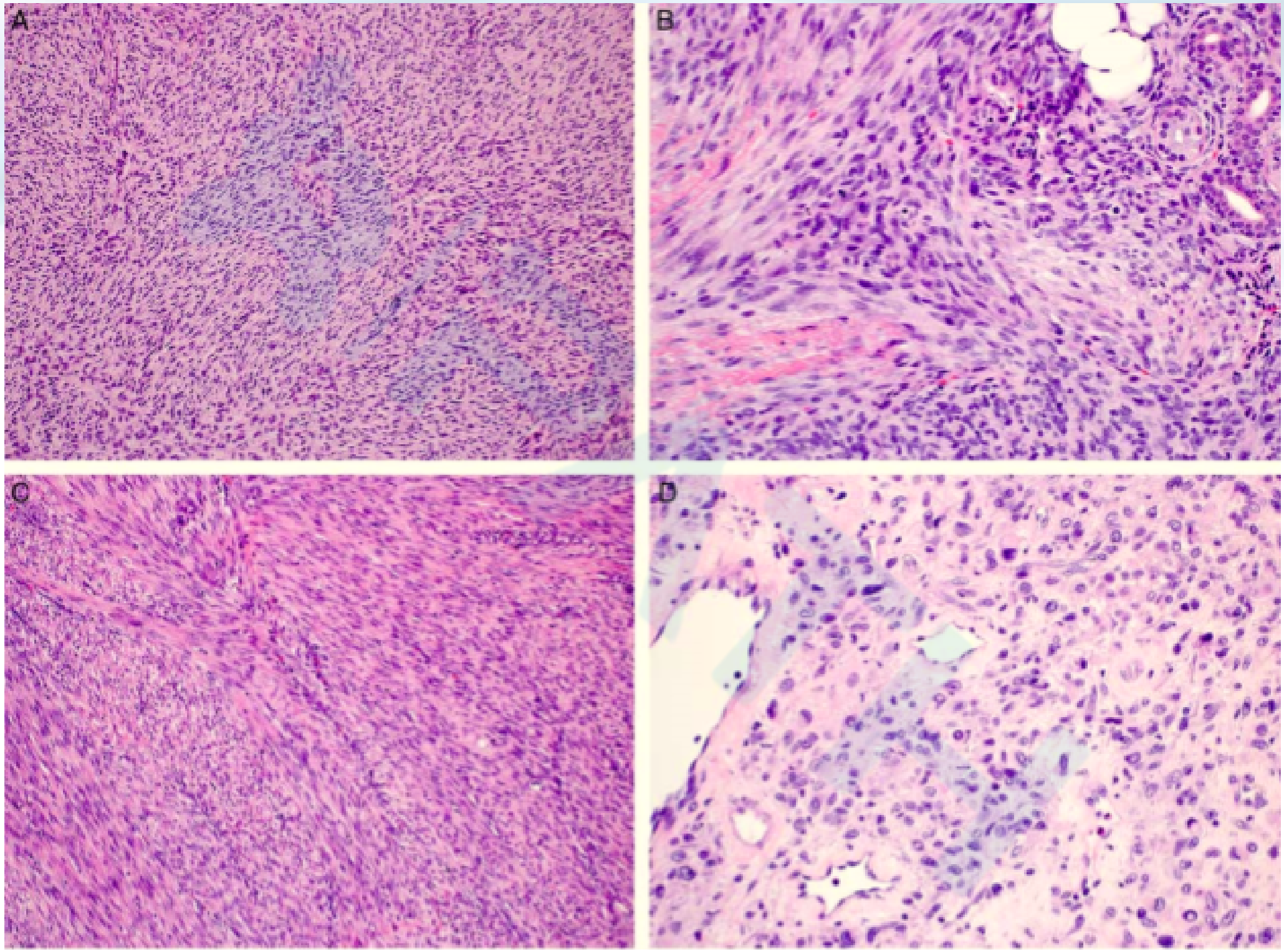


FIGURE 1. Representative H&E images of various cases with a variety of NTRK rearrangements include (A) case 9: LMNA-NTRK1 fusion; (B) case 3: TPM3-NTRK1 fusion; (C) case 13: NTRK1 amplification; (D) case 14: NTRK2-STRN fusion

实验结果

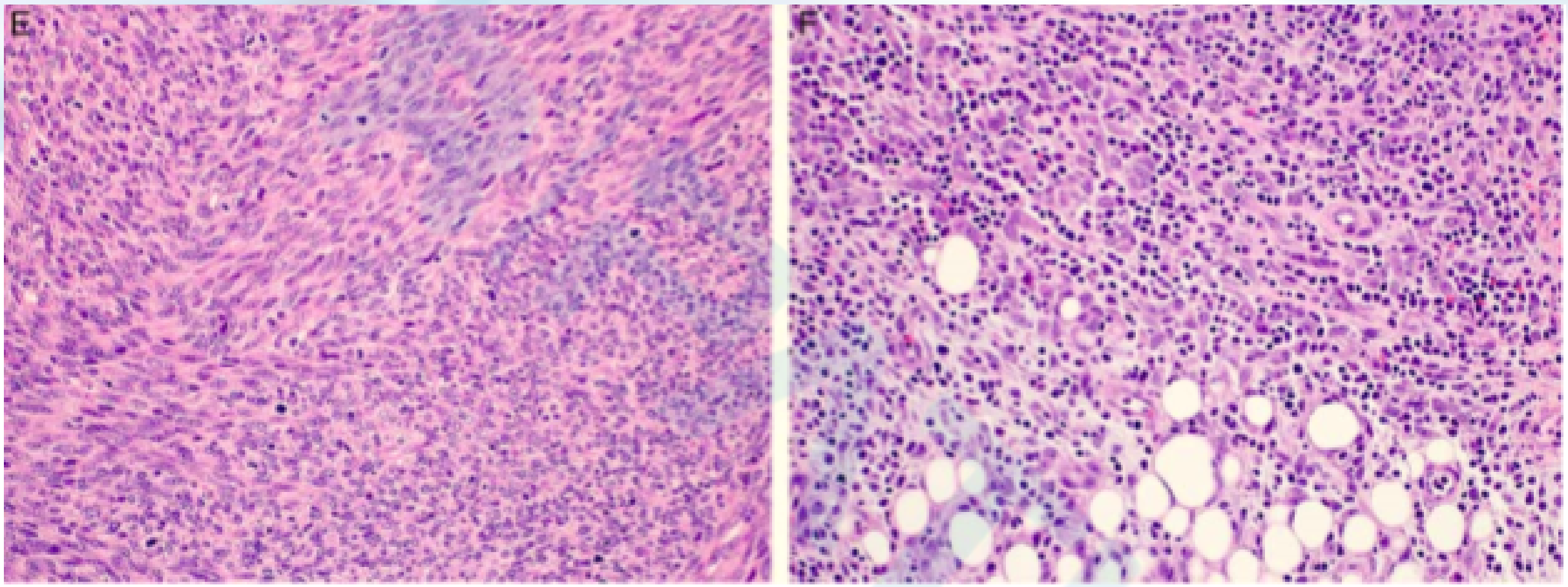


FIGURE 1. (E) case 17: ETV6-NTRK3 fusion; and (F) case 18: ETV6-NTRK3 fusion H&E indicates hematoxylin and eosin

实验结果

TABLE 1. Pan-Trk and TrkA IHC Characteristics for *NTRK*-rearranged Mesenchymal Tumors

Case	Site	NTRK Gene	Partner Gene	Staining Pattern			
				Pan-Trk		TrkA	
				Cytoplasmic	Nuclear	Cytoplasmic	Nuclear
1	ST	*NTRK1* intron 8	*TPM3* intron 7	+++	–	+++	–
2	ST	*NTRK1* intron 8	*TPM3* intron 7	+++	–	+++	–
3	ST	*NTRK1* intron 8	*TPM3* intron 7	+++	–	+++	–
4	ST	*NTRK1* intron 8	*TPM3* intron 7	++	–	+++	–
5	ST	*NTRK1* intron 8	*TPM3* intron 7	+++	–	+++	–
6	ST	*NTRK1*	*TPM3*	+++	–	ND	ND
7	ST (gastric)	*NTRK1* intron 8	*TPM3* intron 7	+++	–	ND	ND
8	ST	*NTRK1* intron 10	*LMNA* intron 3	++	–	+++	–
9	ST					+++	–
10	ST					++	–
11	ST					+++	–
12	ST					++	–
13	ST	*NTRK1* partial amplification (exons 7-17)	—	+	–	++	–
14	ST	*NTRK2* exon 4	*STRN* exon 15	++	–	++, Dot	–
15	ST	*NTRK3* intron 13	*EML4* intron 2	+	+	+, Dot	+
16	ST	*NTRK3* intron 13	*ETV6* intron 5	+	+++	++, Dot	++
17*	ST	*NTRK3* intron 14, intron 14†	*ETV6* intron 5, intron 5†	++	++	++	++
18*	ST	*NTRK3* intron 13	*ETV6* intron 5	+	+	++	+
19*	ST	*NTRK3* intron 14, intron 13	*ETV6* intron 5, intron 7	+	+	+, Dot	+
20*	ST	*NTRK3* intron 13	*ETV6* intron 5	+	+	+	–
21	ST (dura)	*NTRK3*	*ETV6*	+	++	ND	ND
22	ST	*NTRK3* intron 14	*ETV6* intron 5	+	++	++, Dot	+
23	ST		*ETV6+*	+	+	++, Dot	–
24	ST		*ETV6+*	–	+	++, Dot	–
25	ST		*ETV6+*	–	+	++	+
26	ST		*ETV6+*	+	++	++	++
27	ST		*ETV6+*	–	+	++	–
28*	Renal	*NTRK3* intron 13	*ETV6* intron 5	–	–	+++	–
29	Renal		*ETV6+*	+	+	ND	ND
30	Renal		*ETV6+*	+	+	++, Dot	–
31	ST	QNS	QNS	–	+	–	+

Pan-Trk共30例 TrkA共26例

*Original cytogenetic testing negative for ETV6-NTRK3 translocation (FISH, RT-PCR, or karyotype).
†Two distinct fusion breakpoints between ETV6 and NTRK3 were identified in this case.
FISH indicates fluorescent in situ hybridization; ND, not done; QNS, quantity not sufficient; RT-PCR, reverse-transcriptase polymerase chain reaction; STS, soft tissue.

TABLE 2. Pan-Trk and TrkA IHC Characteristics for Non–*NTRK*-rearranged Mesenchymal Tumors

Case	Diagnosis	Expected Mutation Detected	Staining Pattern			
			Pan-Trk		TrkA	
			Cytoplasmic	Nuclear	Cytoplasmic	Nuclear
1	DFSP	*PDGFB*	–	–	–	–
2	DFSP	*PDGFB*	–	–	Dot	–
3	FHI	*EGFR* exon 20	–	–	Dot	–
4	FHI	*EGFR* exon 20		–	+, Dot	–
5	FHI	*EGFR* exon 20	–	–	+	–
6	Lipofibromatosis	None		–	–	–
7	Lipoblastomatosis	None	–	–	–	–
8	Myofibroma	ND	–	–	+, Dot	–
9	Myofibroma	ND	–	–	Dot	–
10	Myofibromatosis	*PDGFRB*	–	–	–	–
11	Cellular myofibroma	None	–	–	+, Dot	–
12	Cellular myofibroma	None	–	–	+	–
13	Cellular myofibroma	None	–	–	+	–
14	Desmoid	*APC*	–	–	–	–
15	Desmoid	ND	–	–	Dot	–
16	Desmoid	ND	–	–	+	–
17	Desmoid	ND	–	–	+	–
18	Desmoid	ND	–	–	+	–
19	MPNST	*NF1*	–	–	++	–
20	MPNST	ND	–	–	+	–
21						–
22						–
23						–
24						–
25						–
26	Classic schwannoma	ND	–	–	Dot	–
27	Classic schwannoma	ND	–	–	Dot	–
28	Cellular schwannoma	None	–	–	–	–
29	Neurofibroma	ND	–	–	Dot	–
30	IMT	None	–	–	Dot	–
31	IMT	None	–	–	+	–
32	IMT	ALK	–	–	+, Dot	–
33	IMT	ALK	–	–	+, Dot	–
34	IMT	ALK	–	–	+, Dot	–
35	IMT	ALK	–	–	++	–
36	*BCOR* rearranged sarcoma	CCNB3	–	–	–	–
37	Undifferentiated pleomorphic sarcoma	ND	–	–	–	–
38	High-grade sarcoma	None	–	–	–	–
39	High-grade sarcoma	None	–	–	–	–
40	High-grade sarcoma	None	–	–	–	–
41	High-grade sarcoma	None	–	–	–	–
42	Low-grade spindle cell lesion	None	ND	ND	–	–
43	Low-grade spindle cell lesion	None	+	–	++	–
44	Low-grade spindle cell lesion	None	–	–	–	–
45	Low-grade spindle cell lesion	None	ND	ND	–	–
46	Low-grade spindle cell lesion	None	–	–	–	–
47	Metanephric stromal tumor	*BRAF* V600E	–	–	–	–
48	Classic mesoblastic nephroma	None	–	–	Dot	–
49	Cellular mesoblastic nephroma	*EGFR* mutation	–	–	ND	ND
50	Clear cell sarcoma of the kidney	ND	–	–	+, Dot	–

DFSP indicates dermatofibrosarcoma protuberans; FHI, fibrous hamartoma of infancy; IMT, inflammatory myofibroblastic tumor; MPNST, malignant peripheral nerve sheath tumor; ND, not done; RMS, rhabdomyosarcoma.

Pan-Trk共48例 TrkA共49例

实验结果

Pan-Trk

存在NTRK基因突变的病例：29/30阳性

无NTRK基因突变的病例：47/48阴性

敏感性：96.7% 特异性：97.9%

TrkA

存在NTRK基因突变的病例：26/26阳性

无NTRK基因突变的病例：18/49阴性

敏感性：100% 特异性：63.3%

实验结果 (NTRK1)

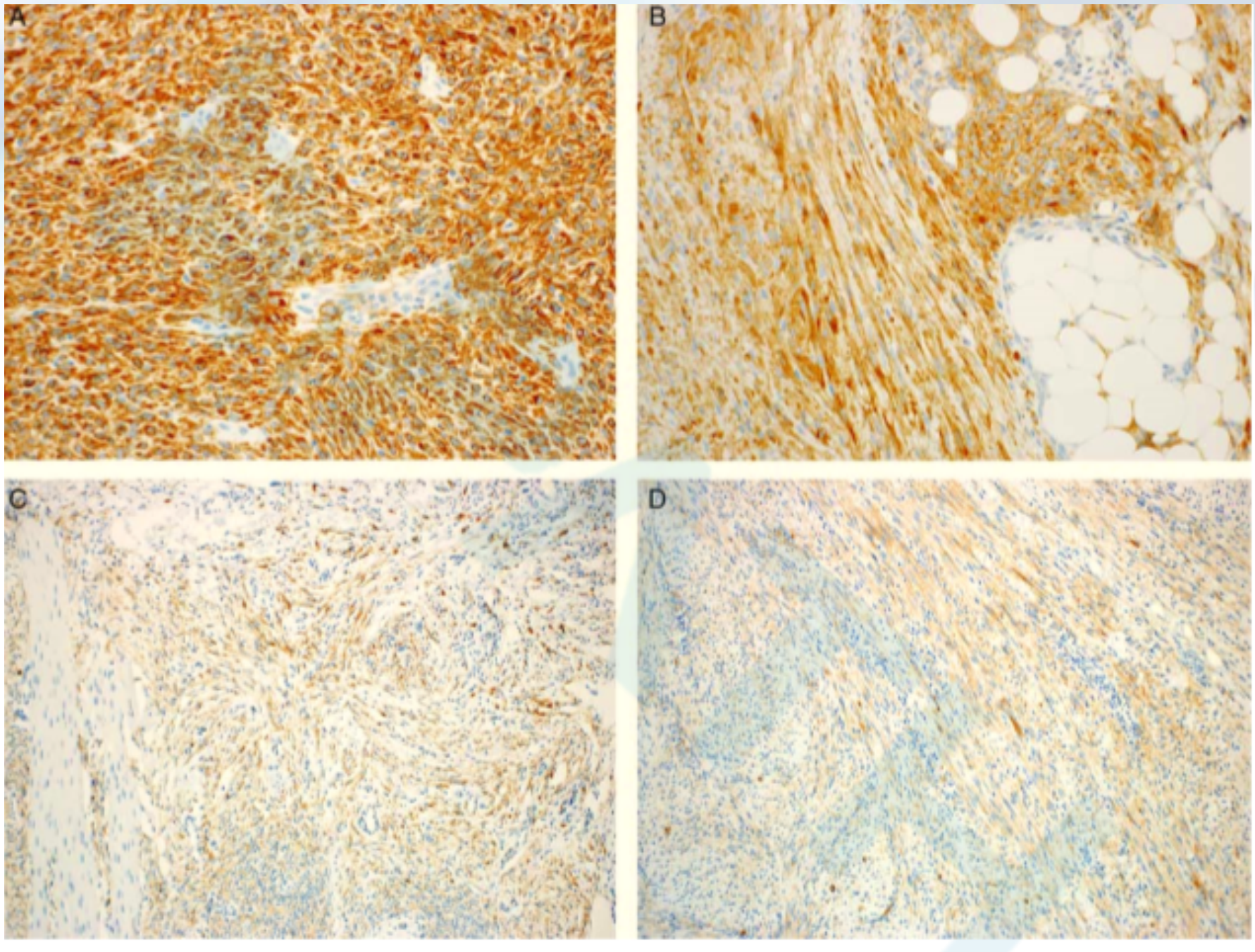


FIGURE 2. NTRK1 rearrangement. Strong diffuse cytoplasmic staining for pan-Trk was seen in most NTRK1-rearranged tumors (A, case 9: LMNA; B, case 3: TPM3). A few tumors showed moderate (C, case 8: LMNA) or weak (D, case 13: NTRK1 amp) but diffuse cytoplasmic staining.

实验结果 (NTRK2)

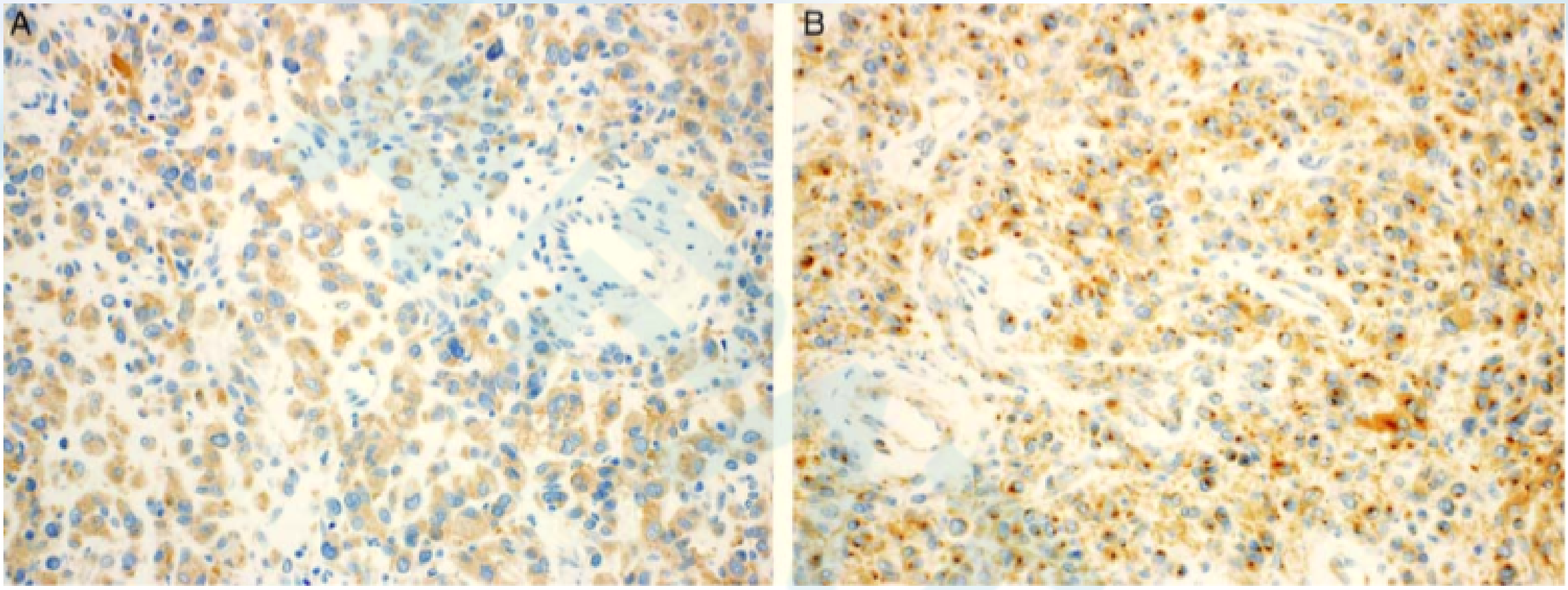


FIGURE 3. NTRK2 rearrangement. A, Pan-Trk IHC showed diffuse, moderate cytoplasmic staining in 1 case with an NTRK2-STRN fusion (case 14). B, TrkA IHC in this same case showed diffuse, moderate cytoplasmic staining with perinuclear dot accentuation (a nonspecific pattern).

实验结果 (NTRK3)

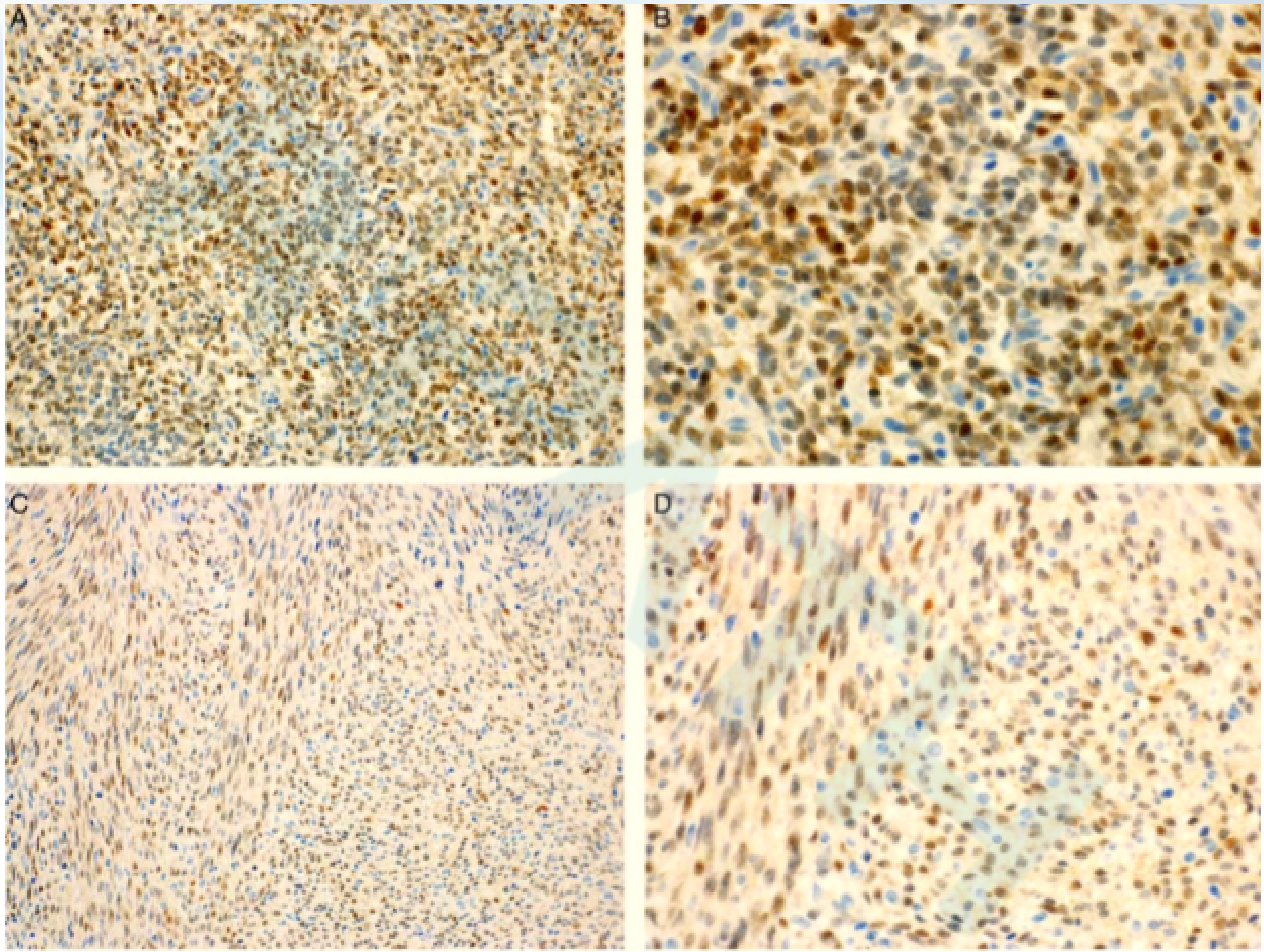


FIGURE 4. NTRK3 rearrangement. Nuclear staining in ETV6-NTRK3-rearranged tumors ranged from strong (case 16: A, B) to moderate (case 17: C, D) to weak (case 18: E, F).

实验结果 (NTRK3)

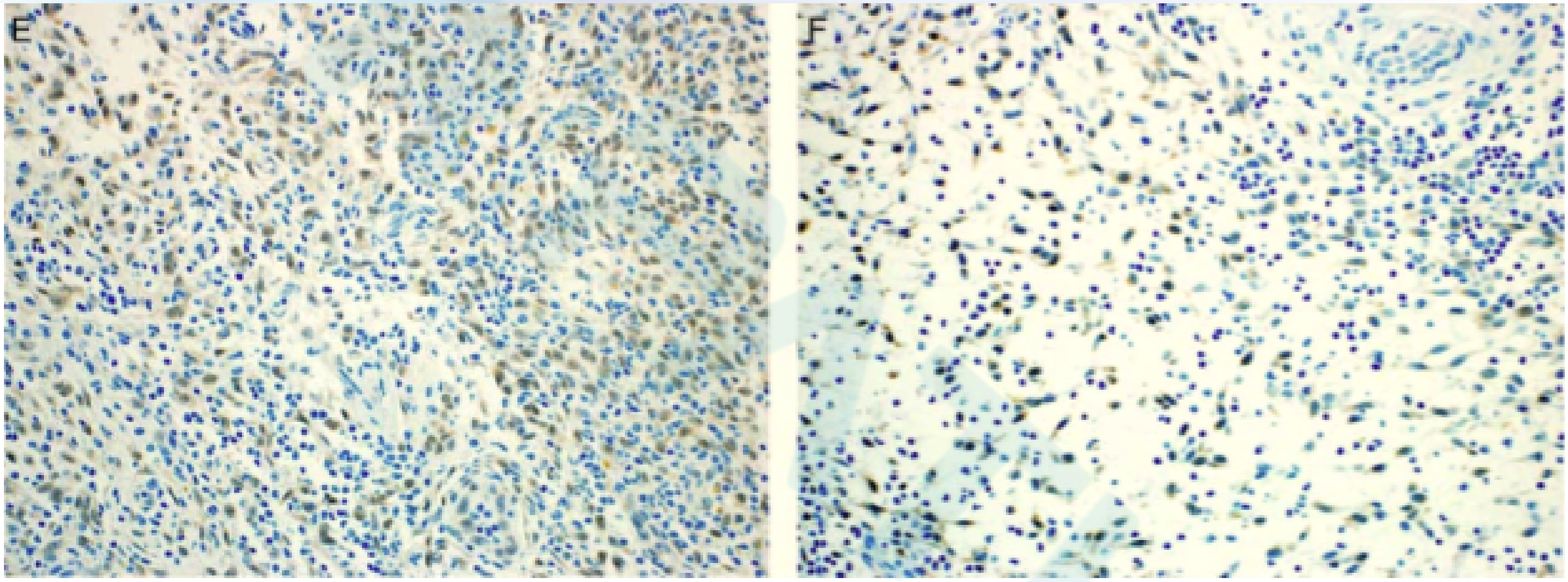


FIGURE 4. NTRK3 rearrangement. Nuclear staining in ETV6-NTRK3-rearranged tumors ranged from strong (case 16: A, B) to moderate (case 17: C, D) to weak (case 18: E, F).

实验结果 (NTRK阴性)

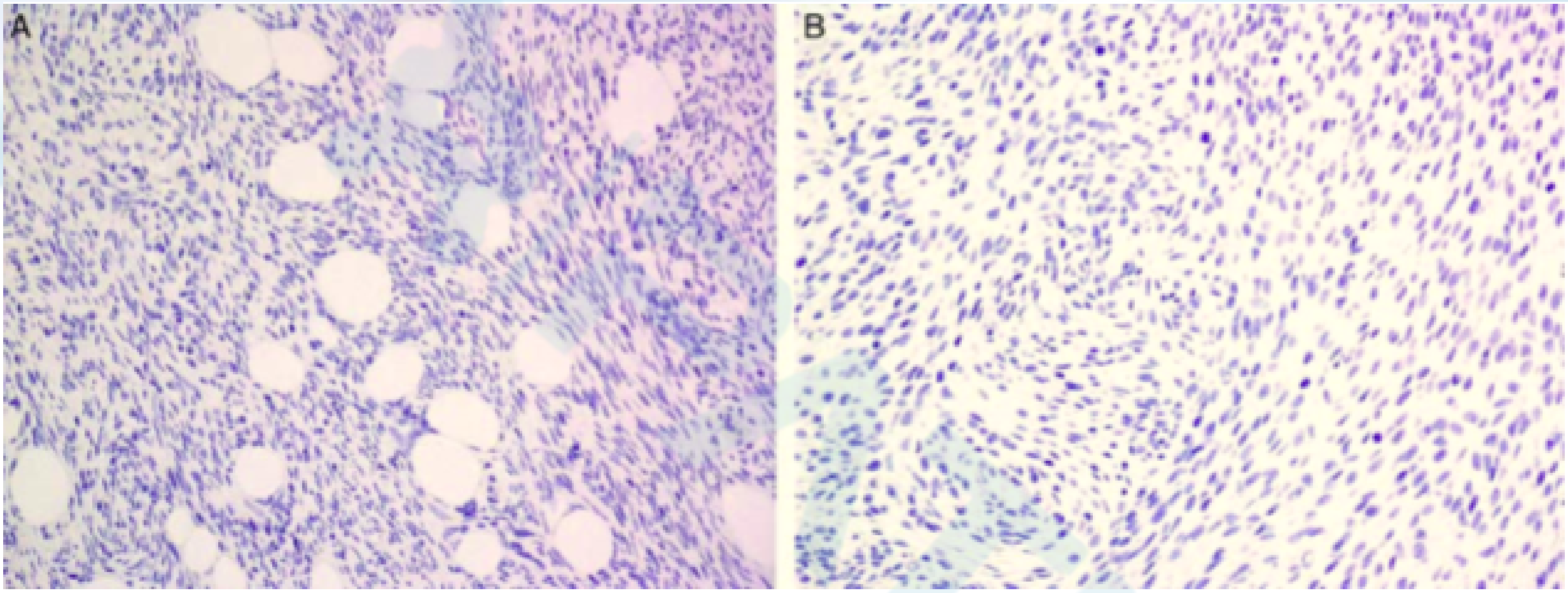


FIGURE 5. Pan-Trk IHC was negative in non–NTRK-rearranged cases; (A) dermatofibrosarcoma protuberans (case 1: pan-Trk) and (B) malignant peripheral nerve sheath tumor (case 20: pan-Trk).

讨论

1：通过免疫组化的方法证实儿童间叶源性肿瘤是否存在NTRK基因融合，Pan-Trk抗体更为可靠；

2：存在NTRK3融合的病例免疫组化染色比NTRK1/NTRK2染色更弱；

3：与传统检测方法（NGS）相比，Pan-Trk在检测儿童间叶源性肿瘤检测中具有省时、经济适用、易于推广、需较少组织样本等优点。故推荐使用Pan-Trk作为独立或辅助检测NTRK基因融合，指导临床用药。

ORIGINAL ARTICLE

Pan-Trk Immunohistochemistry Is an Efficient and Reliable Screen for the Detection of *NTRK* Fusions

Jaclyn F. Hechtman, MD, Ryma Benayed, PhD,* David M. Hyman, MD,† Alexander Drilon, MD,† Ahmet Zehir, PhD,* Denise Frosina, BS,* Maria E. Arcila, MD,* Snjezana Dogan, MD,* David S. Klimstra, MD,* Marc Ladanyi, MD,* and Achim A. Jungbluth, MD**

结直肠癌、膀胱癌、胶质瘤、肺癌、肉瘤、黑色素瘤、乳腺分泌性癌、胰腺分泌性癌共21例，均检测到NTRK基因融合。20/21Pan-Trk免疫组化染色阳性。

阴性对照组共20例，20/20Pan-Trk免疫组化染色阴性。

and 20 cases negative for *NTRK* fusions on Archer. Of 23 cases with *NTRK* rearrangements, 15 had known activating fusions. Archer detected fusion transcripts in 6 of 8 novel *NTRK* rearrangements of uncertain functional significance. Pan-Trk IHC was positive in 20 of 21 cases with *NTRK* fusion transcripts confirmed by Archer. The discordant negative case was a mismatch repair- deficient colorectal carcinoma with an *ETV6-NTRK3* fusion. All 20 additional Archer-negative cases had concordant pan-TRK IHC results. Pan-Trk IHC sensitivity and specificity for transcribed *NTRK* fusions was 95.2% and 100%, respectively. All positive IHC cases had cytoplasmic staining while the following fusion partner-specific patterns were discovered: all 5 *LMNA-NTRK1* fusions displayed nuclear mem-

sponse rate for patients with *NTRK* fusions.[4–6]

Screening *NTRK* fusions is usually done on a molecular level and can be achieved with next-generation sequencing (NGS) of DNA, or targeted RNA testing. However, molecular analyses are still expensive, comparable time-consuming and sampling error or nucleic acid degradation can pose a technical risk. Immunohistochemistry (IHC) is a well established method, usually less expensive and fast compared with current molecular tests. Here, we investigate pan-Trk IHC as a faster and more tissue-efficient method to identify *NTRK* fusions.

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