Tumorji s fuzijami NTRK

Jože Pižem

2019

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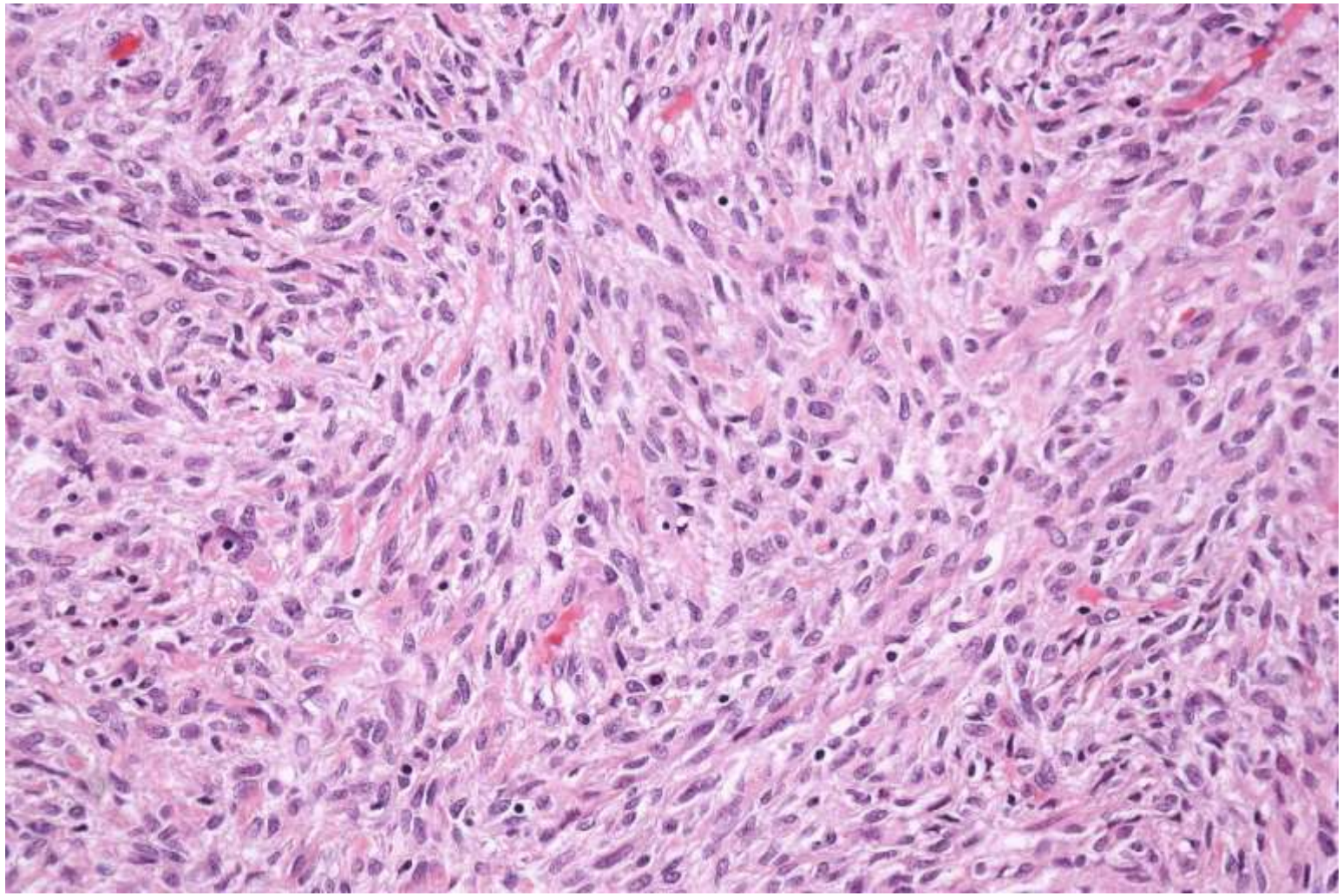

- Diagnoza?
- Lokacija?
- Prvotna diagnoza?

Uvod

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NTRK (=neurotrophic-tropomyosin tyrosine receptor kinase)

Gen	Beljakovina
NTRK1	TrkA
NTRK2	TrkB
NTRK3	TrkC

Fiziološka aktivacija:

- Centralni in periferni živčni sistem
- Gladke mišice
- Vezava nevrotropinov
- Aktivacija MAPK in PI3K/AKT poti

Fuzije NTRK

Posledici

- Konstitutivno izražanje Trk beljakovine
- Aktivacija poti brez vezave nevrotropinov

Gen	Znani partnerji
NTRK1	ARHGEF2, BCAN, CEL, CD74, CHTOP, CGN, CTRC, DDR2, EPHB2, EPS15, GATAD2B, GON4L, GRPAP1, IRF2BP2, LMNA, MDM4, MPRIP, MRPL24, NFASC, P2RY8, PEAR1, PLEKH6, PPL, RABGAP1L, RNF213, SCYL3, SQSTM1, SSBP2, TFG, TP53, TPM3, TPR, TRIM63, VANGL2 (n=33)
NTRK2	AFAP1, AGBL4, BCR, DAB2IP, NACC2, NAV1, PAN3, QK1, SLMAP, SQSTM1, STRN3, TLE4, TRAF2, TRIMM24, VCL, WNK2 (n=16)
NTRK3	AFAP1, AKAP13, BTBD1, COX5A, EML4, ETV6, FAT1, LYN, MYH9, MYO5A, RBPMS, TPM4, ZNF710 (n=13)

JCO Precis Oncol. 2018;2018. doi: 10.1200/PO.18.00183. Epub 2018 Nov 15.

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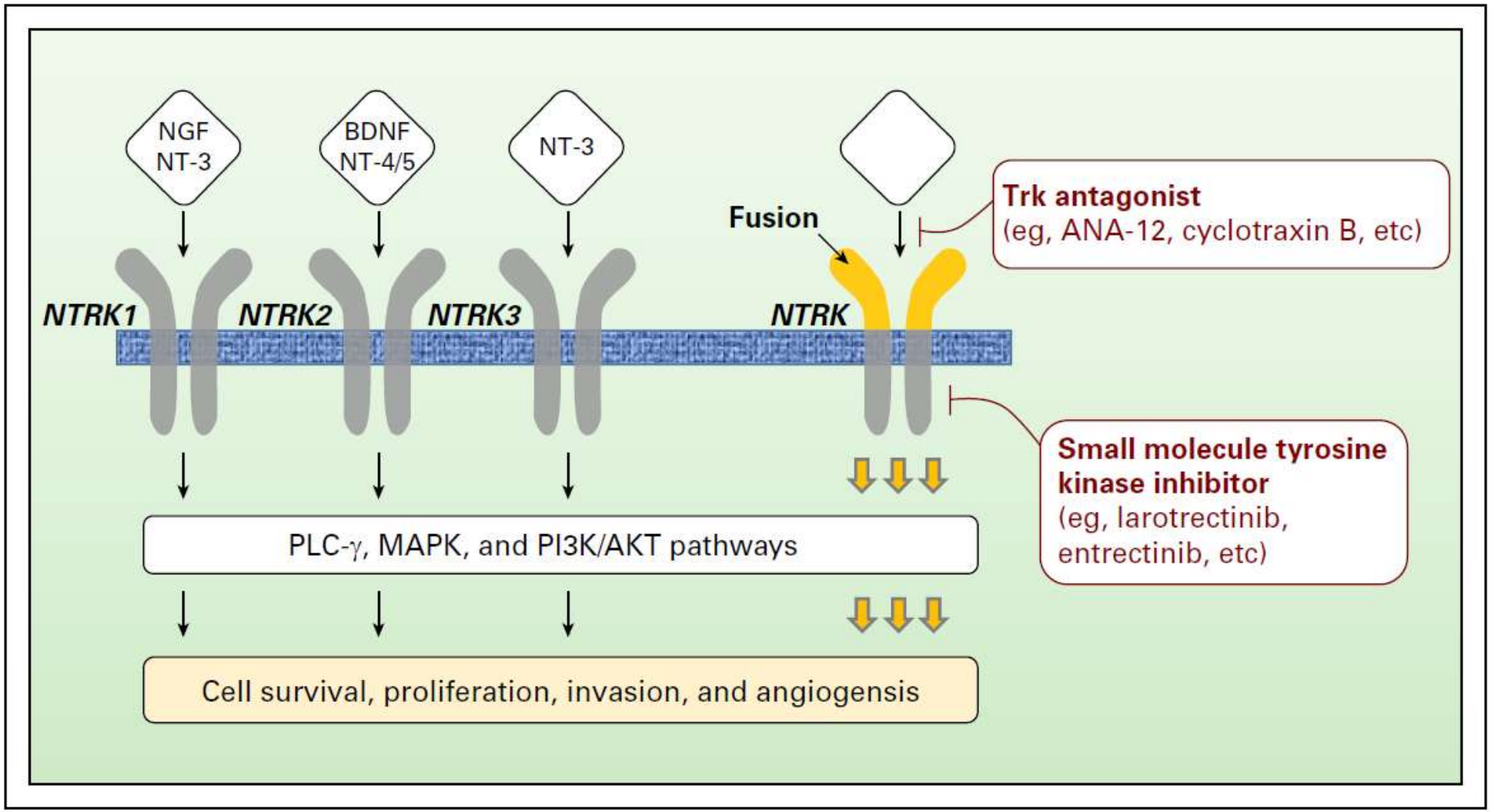

Pomen fuzij NTRK

- Tarčno zdravljenje s Trk zaviralci
  - Entrectinib (FDA, 2017)
  - Larotrectinib (FDA 2018, EMA 2019)

- Boljše poznavanje/klasifikacija tumorjev s fuzijami NTRK
  - Prepoznavanje tumorjev, ki bili prej opredeljeni kot MPNST,…

Dostopnost NGS

Trk zaviralci

- 75% odgovor na zdravljenje
- Neodvisno od
  - Starosti pacienta
  - Vrste tumorja
  - Gena NTRK
  - Fuzijskega partnerja

Fuzija NTRK sama po sebi ne pomeni, da je tumor maligen

Tumorji s fuzijami NTRK

Tumor	%
Sekretorni karcinom (dojka, slinavke, koža)	>95%
Infantilni fibrosarkom	>95%
Kongenitalni mezoblastni nefrom	>80%
Papilarni karcinom ščitnice	15-25%
Spitz tumorji	15%

Tumor	%
Adenokarcinom karcinom pljuč	0,2%
Kolorektalni karcinom	0,3%
Melanom	0,4%
Karcinom dojke	0,1%
Karcinom trebušne slinavke	0,3

33.997 tumorjev
87 tumorjev s fuzijami NTRK1-3
=0,25%

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USCAP

ARTICLE

NTRK fusion detection across multiple assays and 33,997 cases: diagnostic implications and pitfalls

James P. Solomon[1] · Irina Linkov[1] · Andrea Rosado[1] · Kerry Mullaney[1] · Ezra Y. Rosen[2] · Denise Frosina[1] · Achim A. Jungbluth[1] · Ahmet Zehir[1] · Ryma Benayed[1] · Alexander Drilon[2,3] · David M. Hyman[2,3] · Marc Ladanyi[1] · Anthony N. Sireci[4] · Jaclyn F. Hechtman[1]

Table 1 Prevalence of *NTRK* fusions in various tumor types

	Number of cases with *NTRK* fusions	Total patients for which molecular testing was performed	Percentage
Salivary gland carcinoma	13	256	5.08%
Thyroid carcinoma	13	571	2.28%
Sarcoma	13	1915	0.68%
Lung adenocarcinoma	9	3993	0.23%
Colorectal carcinoma	9	2929	0.31%
Glioma/neuroepithelial tumor	8	1465	0.55%
Breast carcinoma	6	4458	0.13%
Pancreatic adenocarcinoma	5	1492	0.34%
Melanoma	4	1125	0.36%
Inflammatory myofibroblastic tumor	3	17	17.7%
Cholangiocarcinoma	2	787	0.25%
Appendiceal adenocarcinoma	1	208	0.48%
Neuroendocrine tumor	1	322	0.31%

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Table 1. Frequency of NTRK Receptor Transcript Fusions in TCGA (n = 9,966 adult tumor samples) and St Jude Pediatric Cancer Database (n = 3,501 pediatric tumor samples), and Specific Tumors With High Incidence of *NTRK* Fusions in the Literature

		No. of Tumors (%)			
Tumor Sample	**No. of Samples**	**Any *NTRK* Fusion**	***NTRK1* Fusion**	***NTRK2* Fusion**	***NTRK3* Fusion**
Adult tumors (TCGA)*					
Total	9,966	31 (0.31)	9 (0.09)	6 (0.06)	16 (0.16)
Thyroid cancer	513	12 (2.34)	5 (0.97)	—	7 (1.36)
Colon adenocarcinoma	310	3 (0.97)	—	—	3 (0.97)
Low-grade glioma	534	5 (0.94)	1 (0.19)	3 (0.56)	1 (0.19)
Sarcoma	263	2 (0.76)	2 (0.76)	—	—
Glioblastoma multiforme	180	1 (0.56)	1 (0.56)	—	—
Pancreatic adenocarcinoma	179	1 (0.56)	—	—	1 (0.56)
Head and neck SCC	522	2 (0.38)	—	1 (0.19)	1 (0.19)
Cervical cancer	306	1 (0.33)	—	—	1 (0.33)
Melanoma	476	1 (0.21)	—	—	1 (0.21)
Breast cancer	1119	2 (0.18)	—	1 (0.09)	1 (0.09)
Lung adenocarcinoma	541	1 (0.18)	—	1 (0.18)	—
Pediatric tumors (St Jude PeCan)†					
Total	3,501	12 (0.34)	5 (0.14)	4 (0.11)	3 (0.09)
Melanoma	9	1 (11.11)	1 (11.11)	—	—
High-grade glioma	132	7 (5.3)	4 (3.03)	2 (1.52)	1 (0.76)
Low-grade glioma	120	3 (2.5)	—	2 (1.67)	1 (0.83)
B-cell ALL	716	1 (0.14)	—	—	1 (0.14)

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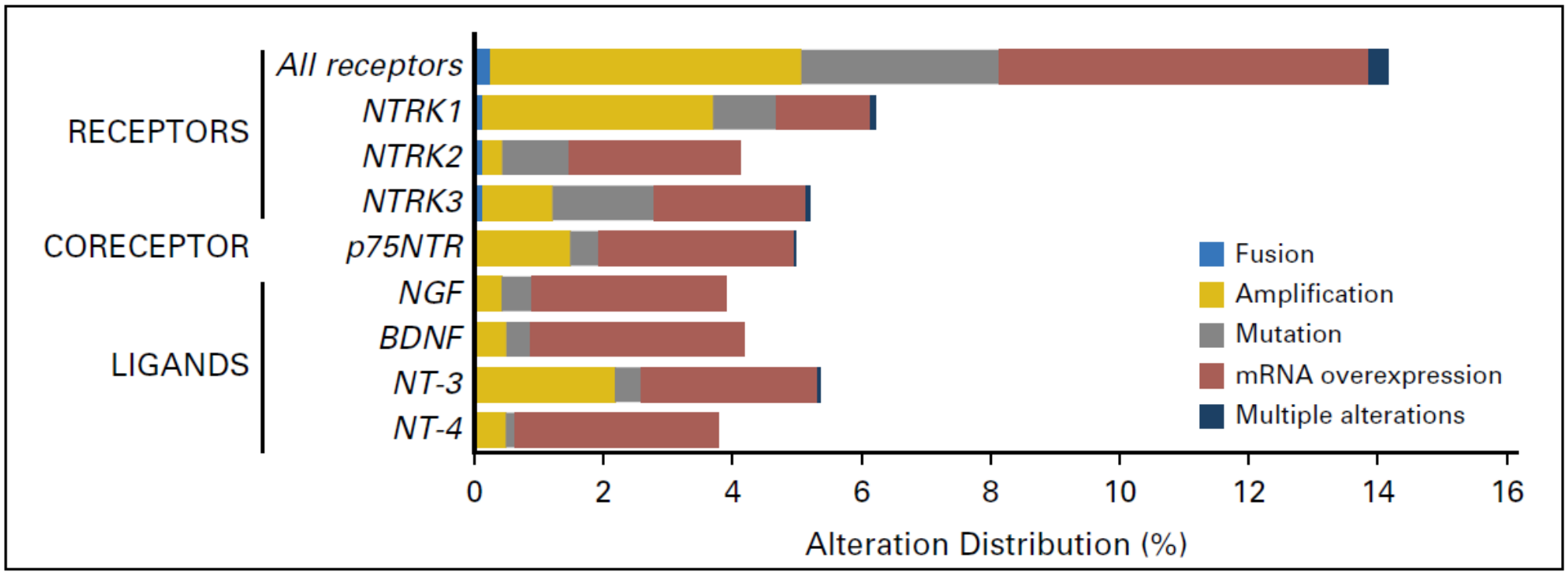
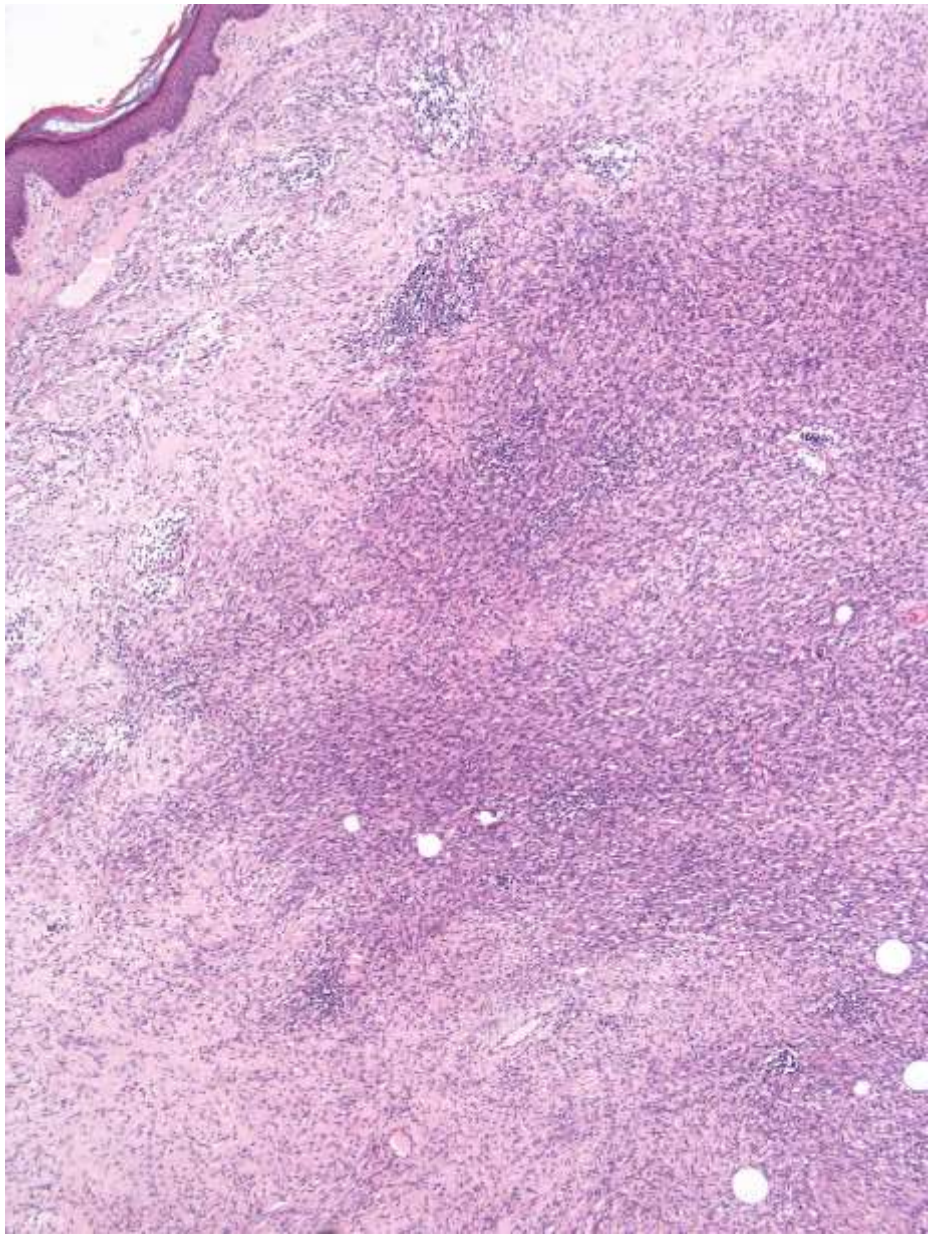
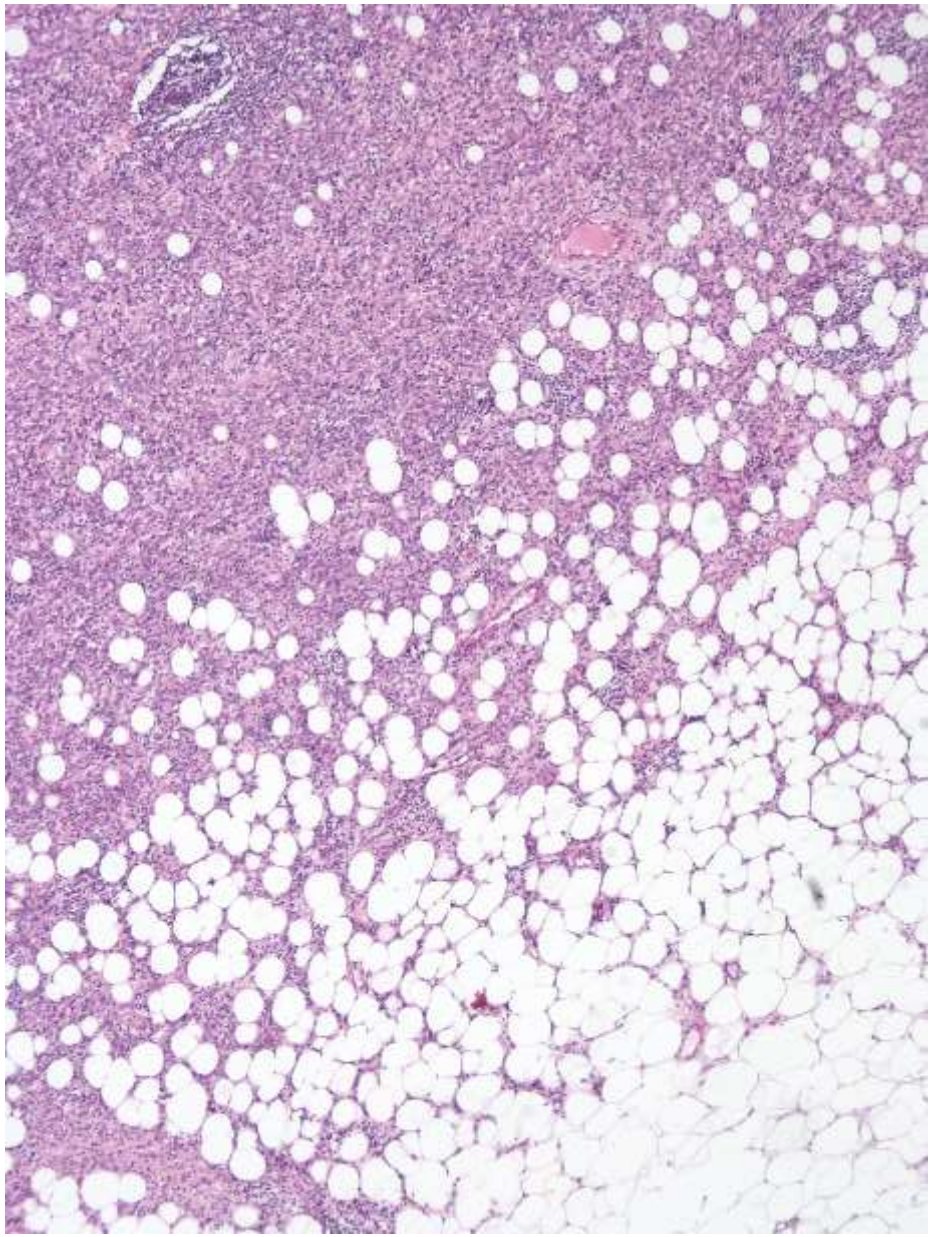
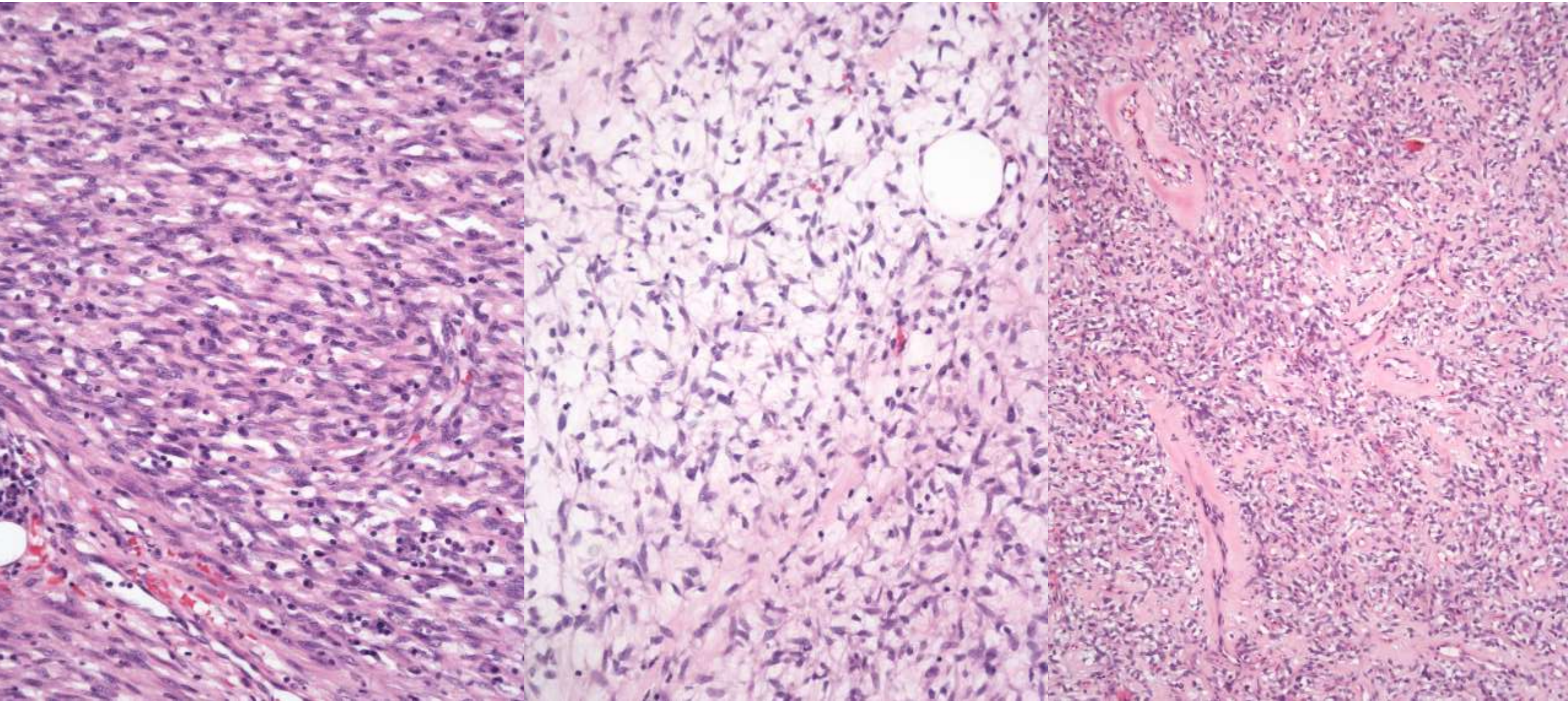
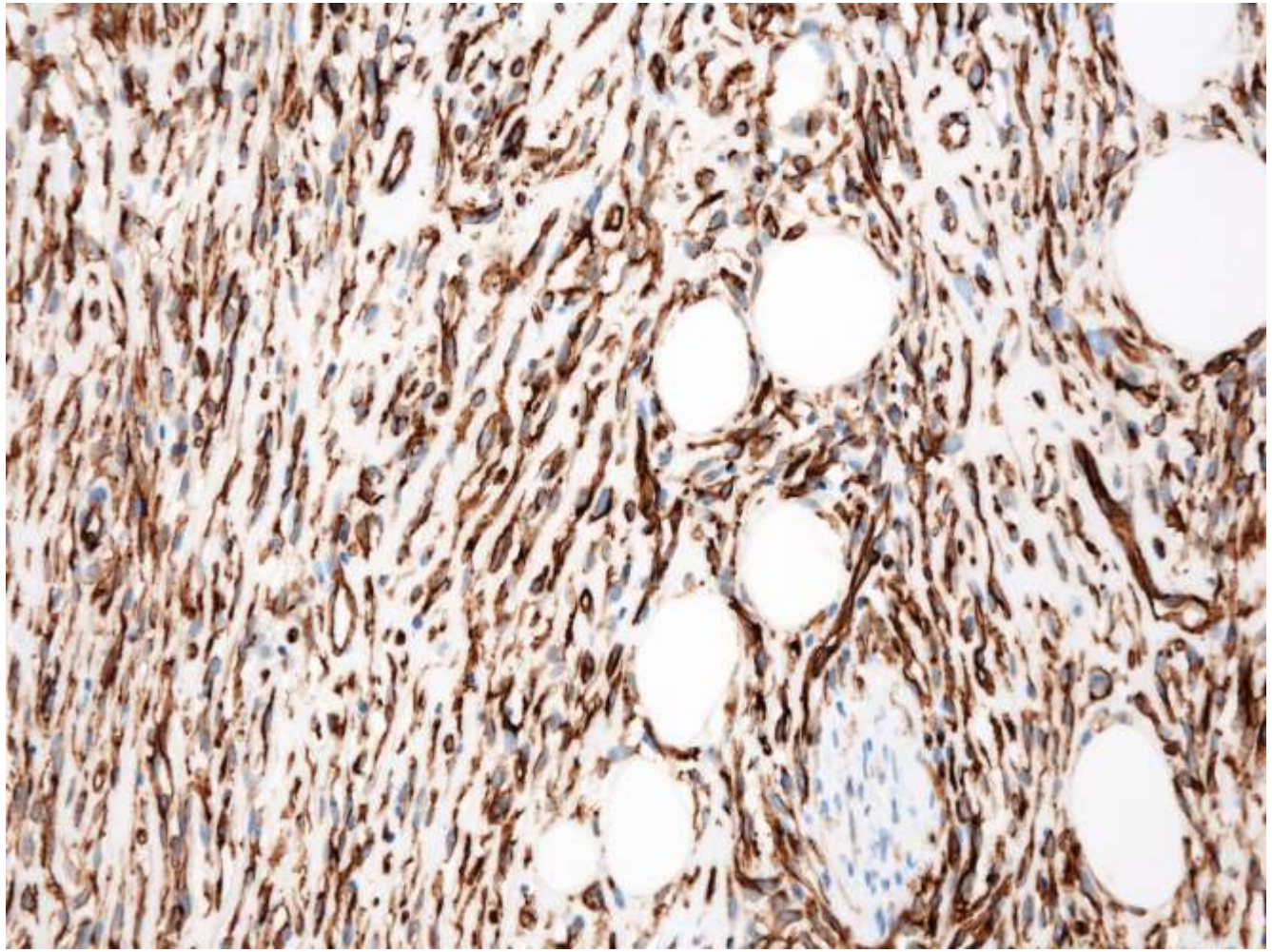




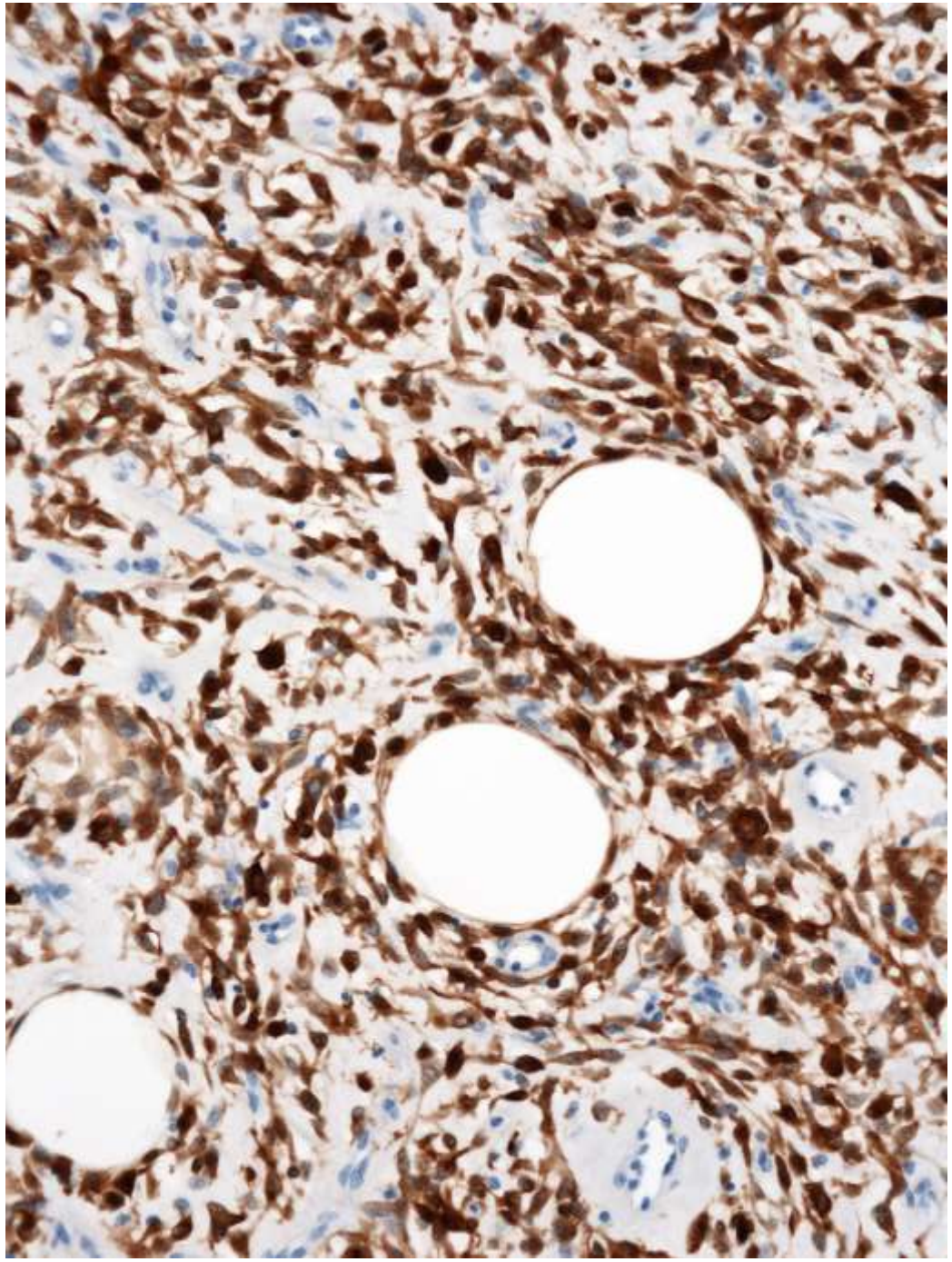


Leta 2007:
Ž, 25 let, 5 cm velik tumor na hrbtu





CD34



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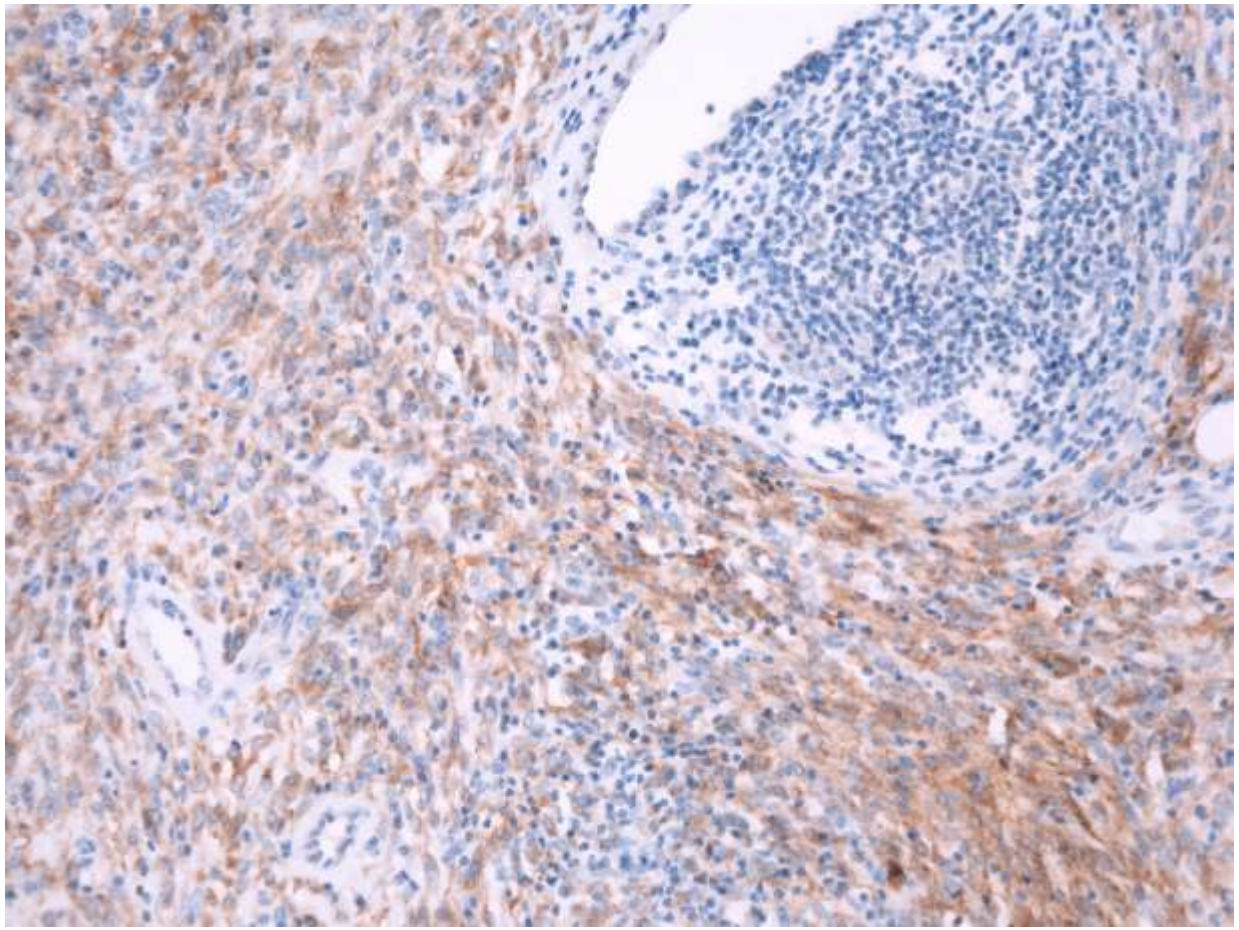

panTRK

NGS: TPR-NTRK1

Metode za določanje fuzij NTRK

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3 geni NTRK, številni fuzijski partnerji

Različni prelomi

V mnogih tumorjih fuzije NTRK prisotne v <1%

FISH

+	-
Razmeroma hitro (3-5 dni)	Potrebno izvesti 3 preiskave
Uporabno za določanje ETV6-NTRK3 (ETV6 ali NTRK3 break apart sonde)	Nepoznan fuzijski partner
Potrebnega malo tkiva	Preureditev na nivoju DNA ne pomeni, da ta funkcionalna
	Lahko lažno negativna

IHK

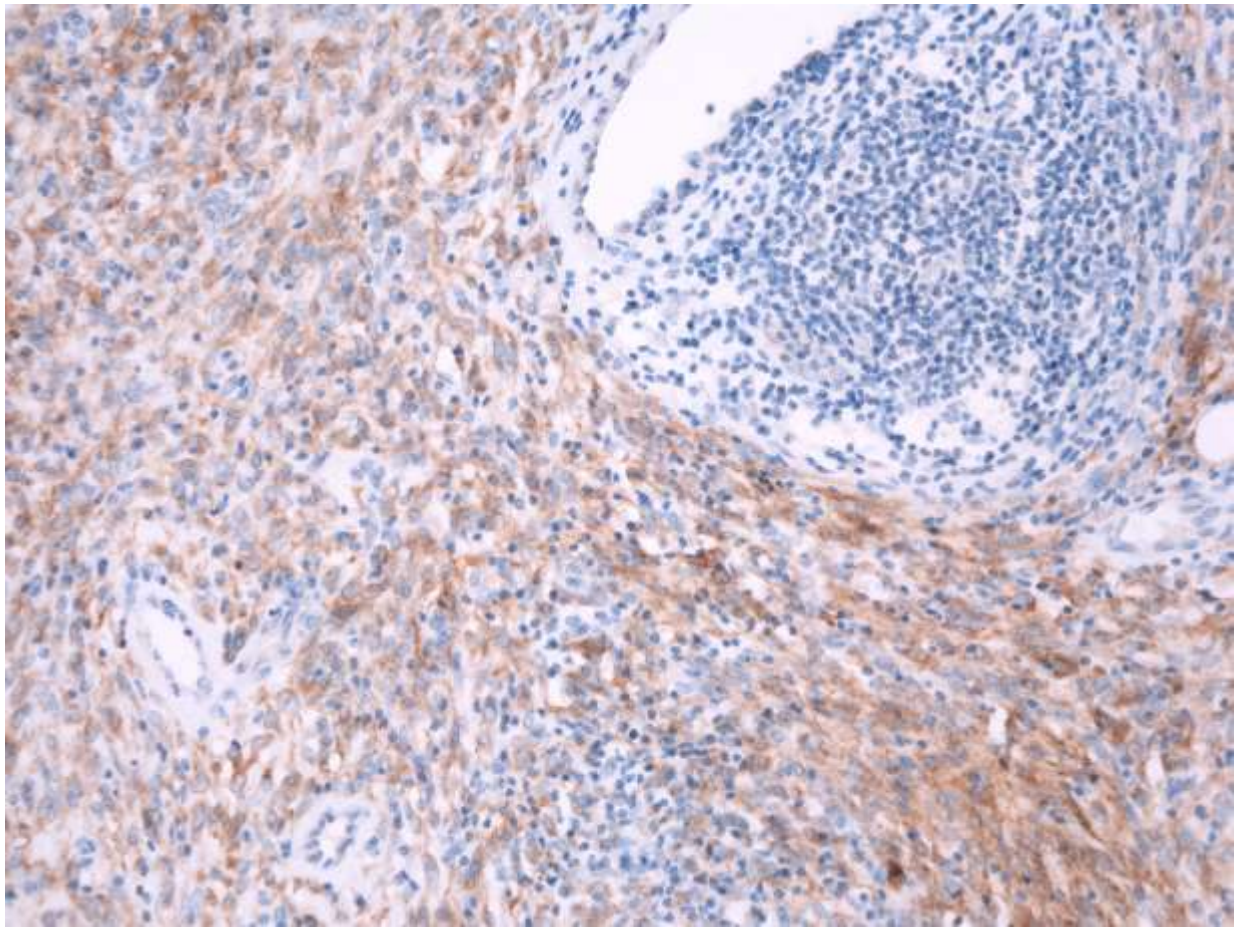
panTRK: EPR17341

- Abcam, Roche/Ventana
- Prepozna C-konec TrkA, TrkB in TrkC
- Občutljivost: 75-100%
- Specifičnost: 92-100%
- Občutljivost za NTRK3 fuzije nižja: 50-80% (ni izražanja)!
  - Pogosto le šibka citoplazemska reakcija
  - Pogosto jedrna reakcija
  - Lahko le fokalna reakcija
- Pozitivna notranja kontrola: živci
- Negativna notranja kontrola: vnetnice, žile,..

Pozitivni tudi

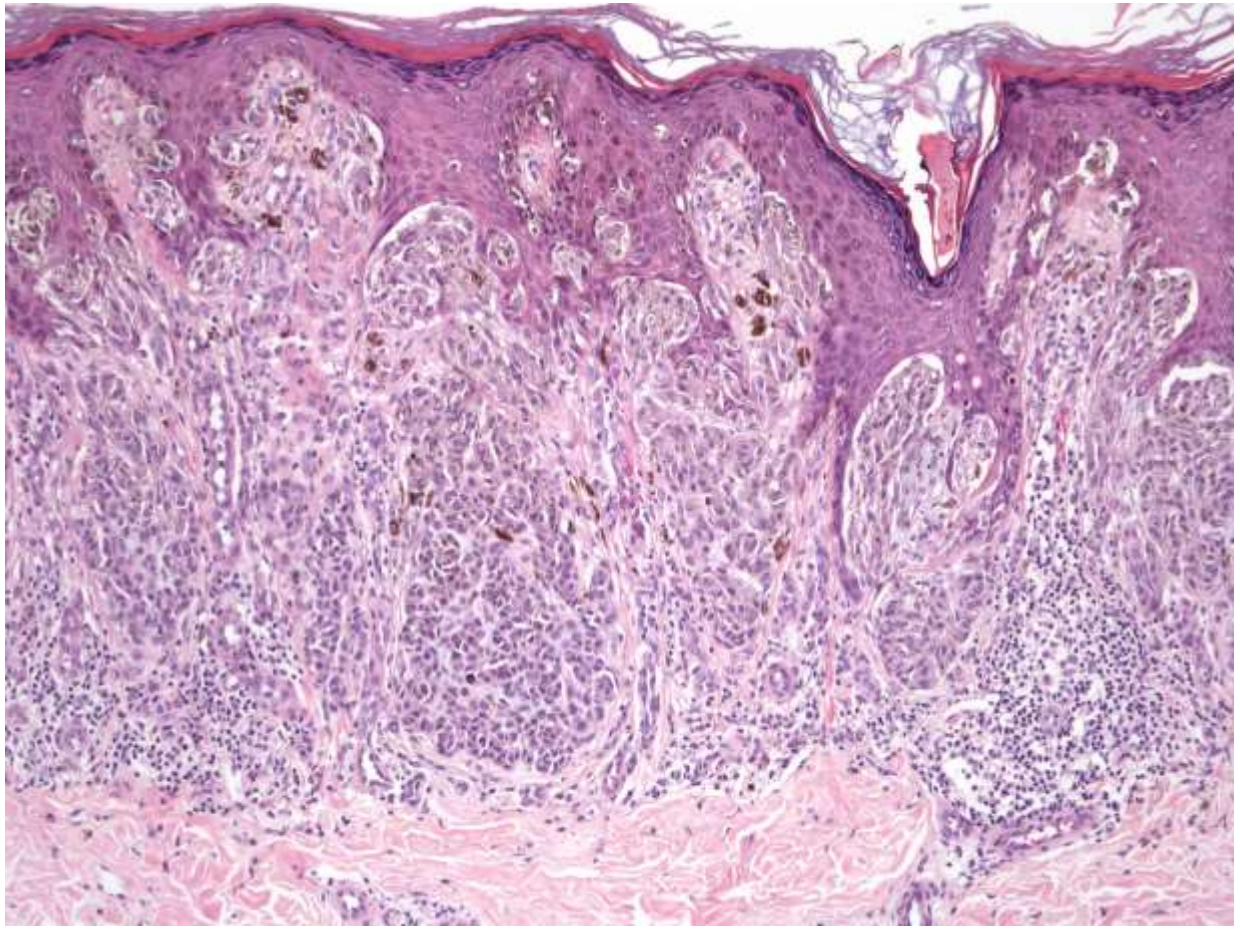
- Nevronalna tkiva
- Gladke mišice
- Testis
- Tumorji brez fuzije NTRK
  - Leiomiosarkom
  - GIST
  - Glioblastom
  - Nevroblastom
  - Fibrozni hamartom otroške dobe
  - Primitivni miksoidni mezenhimski tumor otroške dobe

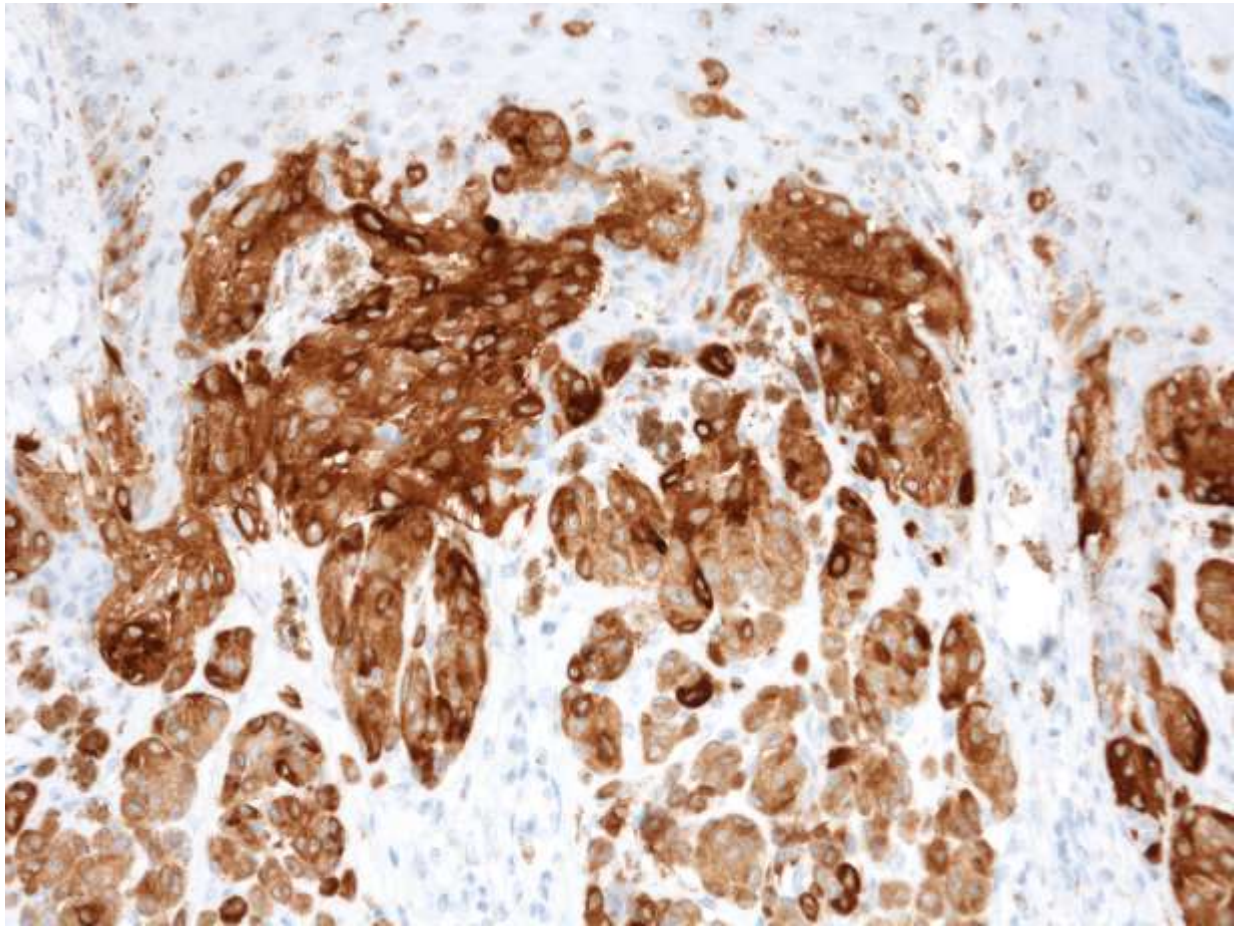
TRK-IHK – vzorci pozitivnosti

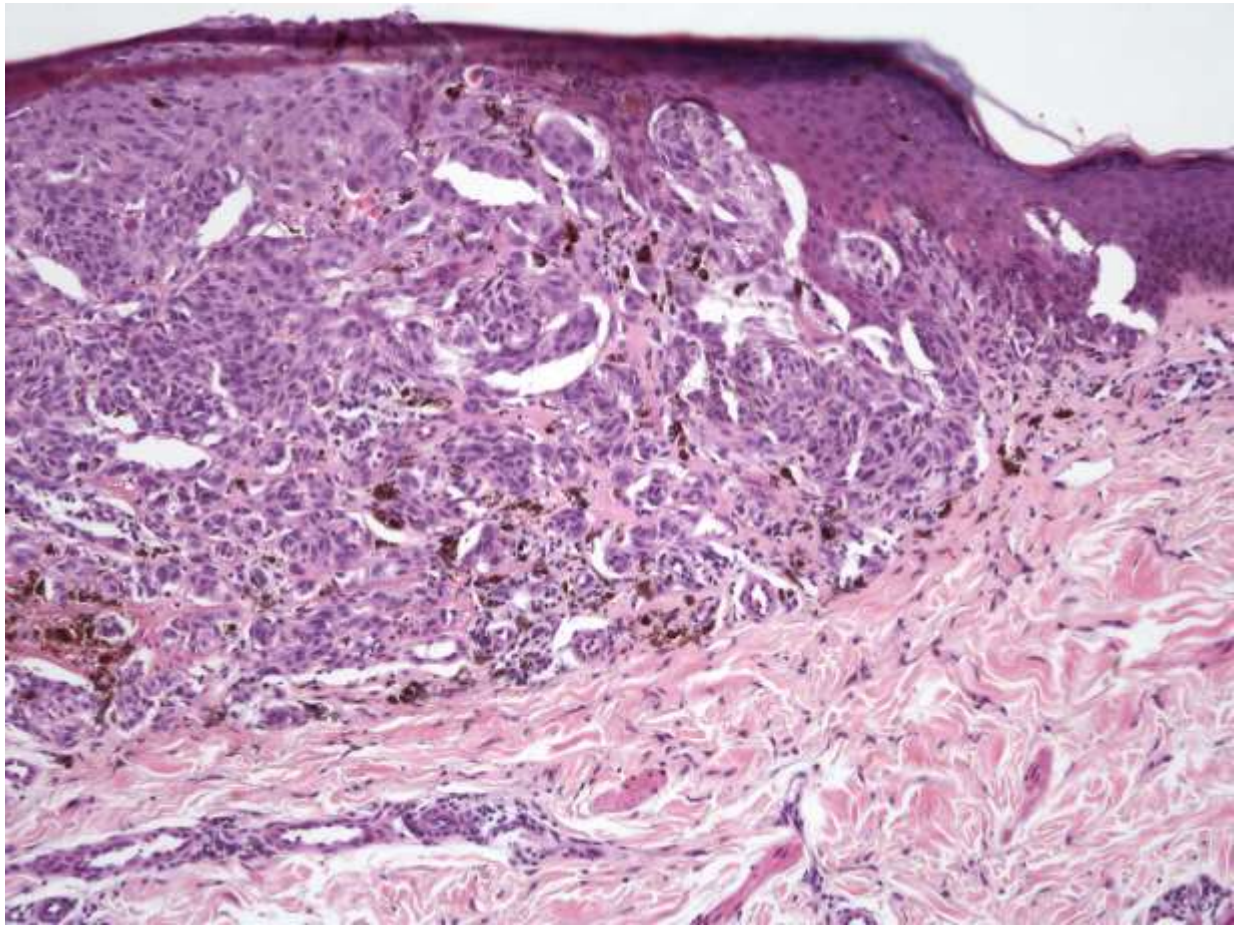


V osnovi citoplazemska reakcija

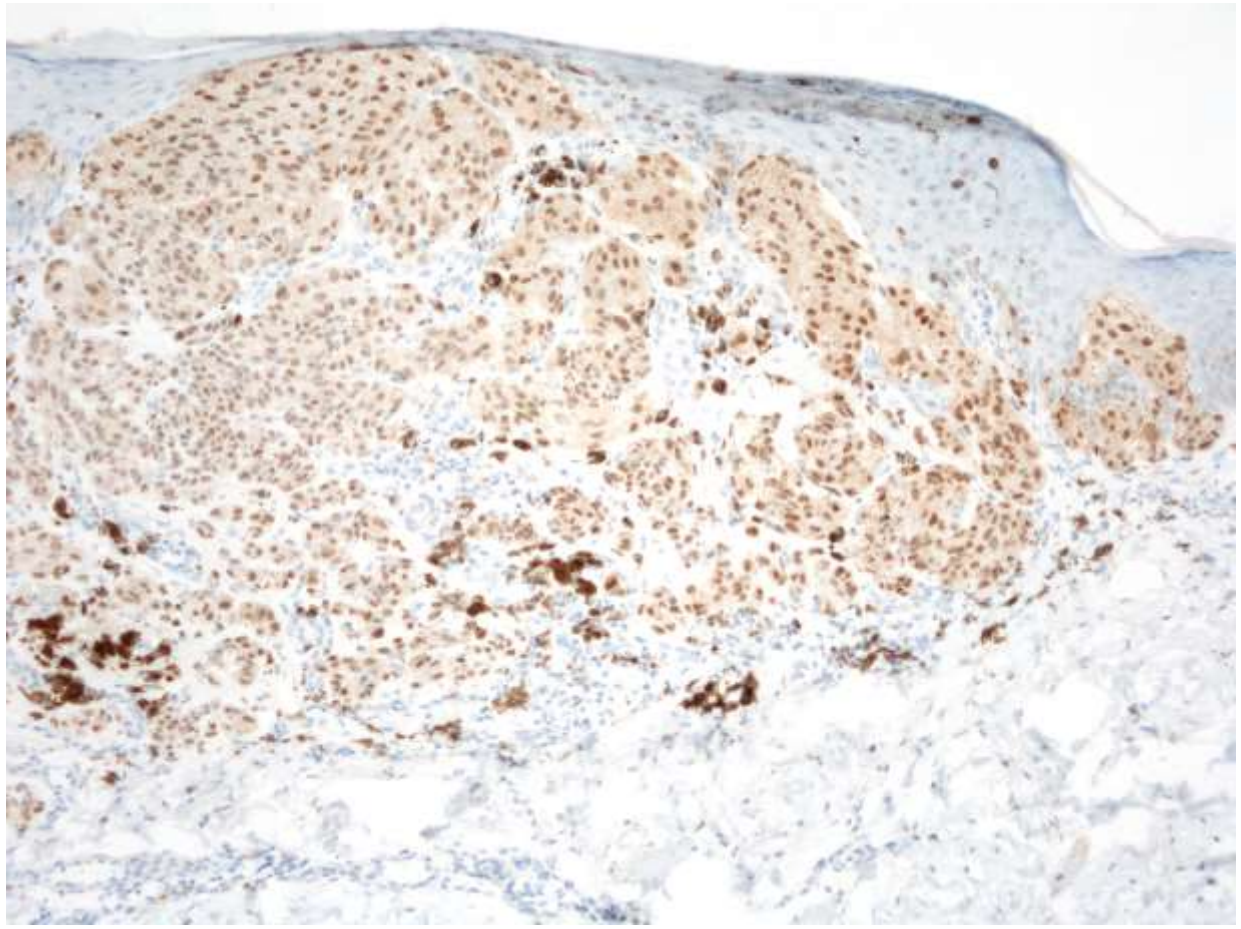
Lahko le fokalna (>1% celic)



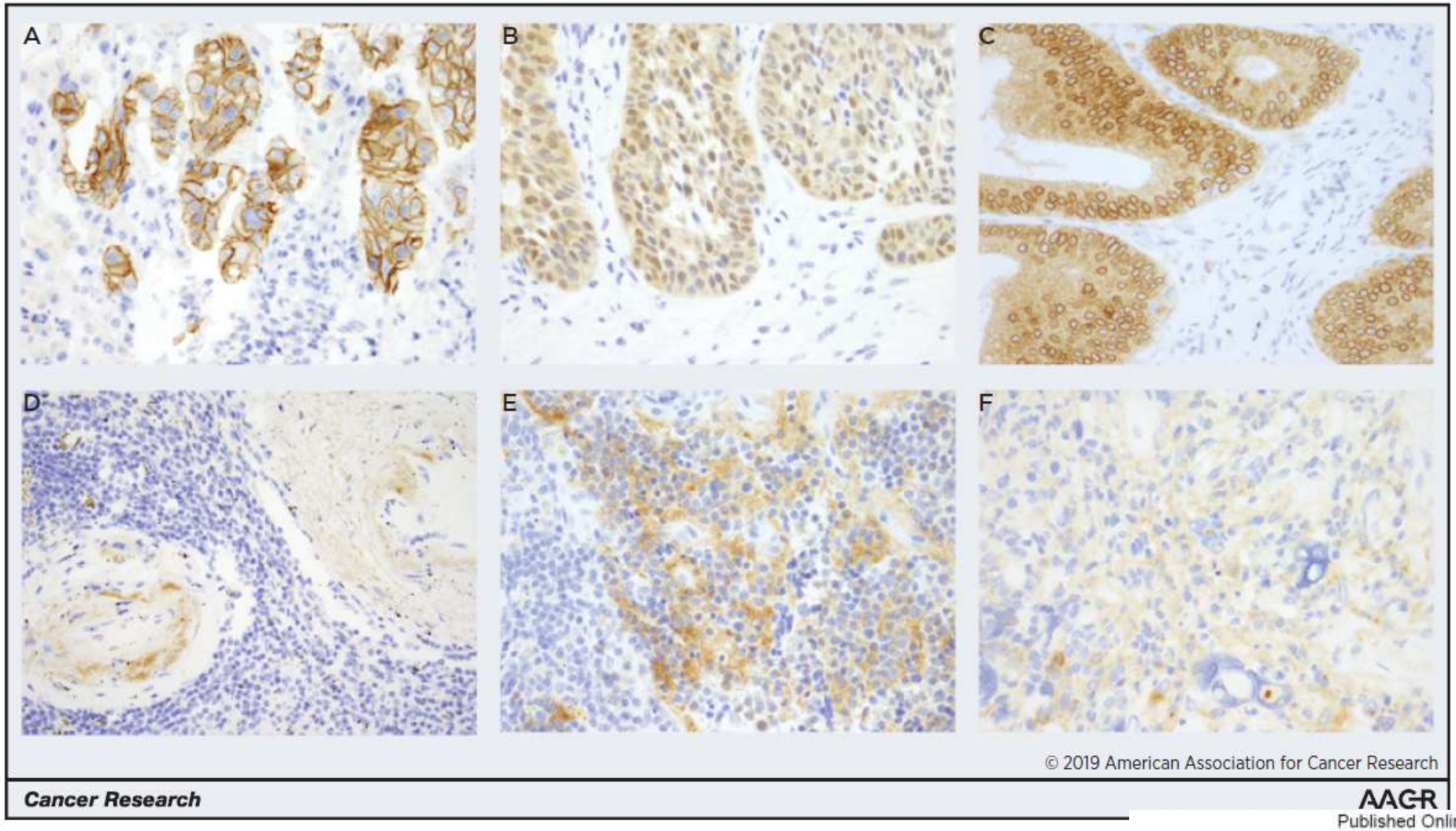




Jedrna reakcija vedno kaže na fuzijo!



ETV6-NTRK3: pogosto (ne vedno) jedrna reakcija



TPM-NTRK
PLEKH6-NTRK
membranska reakcija

ETV6-NTRK3:
pogosto (ne vedno) jedrna reakcija

LMNA-NTRK1
jedrna membrana

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Figure 1.

IHC staining with pan-Trk antibody (clone EPR 17341, Abcam) demonstrates a variety of staining patterns in malignancies with / patterns correlate with the fusion partner. **A,** A membranous staining pattern is seen in this case of intrahepatic cholangiocarcir fusion. **B,** A nuclear and cytoplasmic staining pattern is seen in this case of secretory carcinoma of the salivary gland with the ca **C,** Colonic adenocarcinoma with an *LMNA-NTRK1* fusions exhibits a cytoplasmic and perinuclear staining pattern. **D,** Physiologic muscle, as seen in arterial walls. **E** and **F,** Physiologic staining can also be seen in tumors of neural differentiation, such as neurol (**F**), making interpretation difficult.

Review

Detection of *NTRK* Fusions: Merits and Limitations of Current Diagnostic Platforms

James P. Solomon and Jaclyn F. Hechtman

Table 3 Sensitivity and specificity of pan-Trk immunohistochemistry for detecting *NTRK* fusions

	Sensitivity
NTRK1	96.2% (26/27)
NTRK2	100% (5/5)
NTRK3	79.4% (27/34)

	Sensitivity	Specificity
Total	87.9% (58/66)	81.1% (257/317)
Colon	87.5% (7/8)	100% (25/25)
Lung	87.5% (7/8)	100% (24/24)
Thyroid	81.8% (9/11)	100% (27/27)
Salivary	88.9% (8/9)	52% (13/25)
Breast	80% (4/5)	82.1% (23/28)
Inflammatory myofibroblastic tumor	100% (3/3)	100% (5/5)
Sarcoma	80% (8/10)	74.4% (29/39)
Pancreas	(0/0)[a]	100% (20/20)
Appendix	100% (1/1)	100% (1/1)
Cholangio	100% (2/2)	100% (19/19)
Glioma	100% (6/6)	20.8% (5/24)
Melanoma	100% (3/3)	100% (17/17)
Neuroendocrine	(0/0)[a]	88.9% (8/9)
Small round cell tumor[b]	(0/0)	45.8% (11/24)
Other[c]	(0/0)	100% (30/30)

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Received: 31 May 2019 / Revised: 14 June 2019 / Accepted: 15 June 2019

N=66
Referenčna metoda: RNA Archer

IHK

+	-
Hitro (1 dan)	Lažno + v tumorjih z nevronalno ali gladkomišično diferenciacijo
Potrebnega malo tkiva	Nizka občutljivost za fuzije NTRK3
Razmeroma visoka občutljivost, 95-100% (razen za NTRK3)	Ne loči med različnimi fuzijami
Nizki stroški	Potrebna dokončna potrditev fuzije z molekularnimi metodami
Primerna za presejanje	Nizka specifičnost (gliomi, drobnocelični okroglocecelični sarkomi, nevroblastom, sarkomi, nevroendokrini tumorji)

DNA-NGS (veliki paneli: MSK-IMPACT, FoundationOne)

+	-
Mutacije, delecije, amplifikacije, fuzije, mikrosatelitna nestabilnost, mutacijsko breme tumorja	Visoki stroški
	Izvedba v 2-4 tednih
	Lahko lažno -, ni zanesljivo za določanje fuzij NTRK3 (prelomi znotraj intronov, ki v testu niso pokriti)
	Lažno – ob premajhnem deležu tumorskih celic (<20%)
	Potrebnega razmeroma veliko tkiva (20 rezin po 5 mikrometrov)
	Prisotnost spremembe še ne pomeni, da je ta tudi funkcionalna

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Table 2 Sensitivity and specificity of DNA-based cancer gene panel next generation sequencing (MSK-IMPACT) for detecting *NTRK* fusions

	Sensitivity	Specificity
NTRK1	96.8% (30/31)	
NTRK2	0% (0/4)	
NTRK3	76.9% (30/39)	
Total	81.1% (60/74)	99.86% (33877/33923)

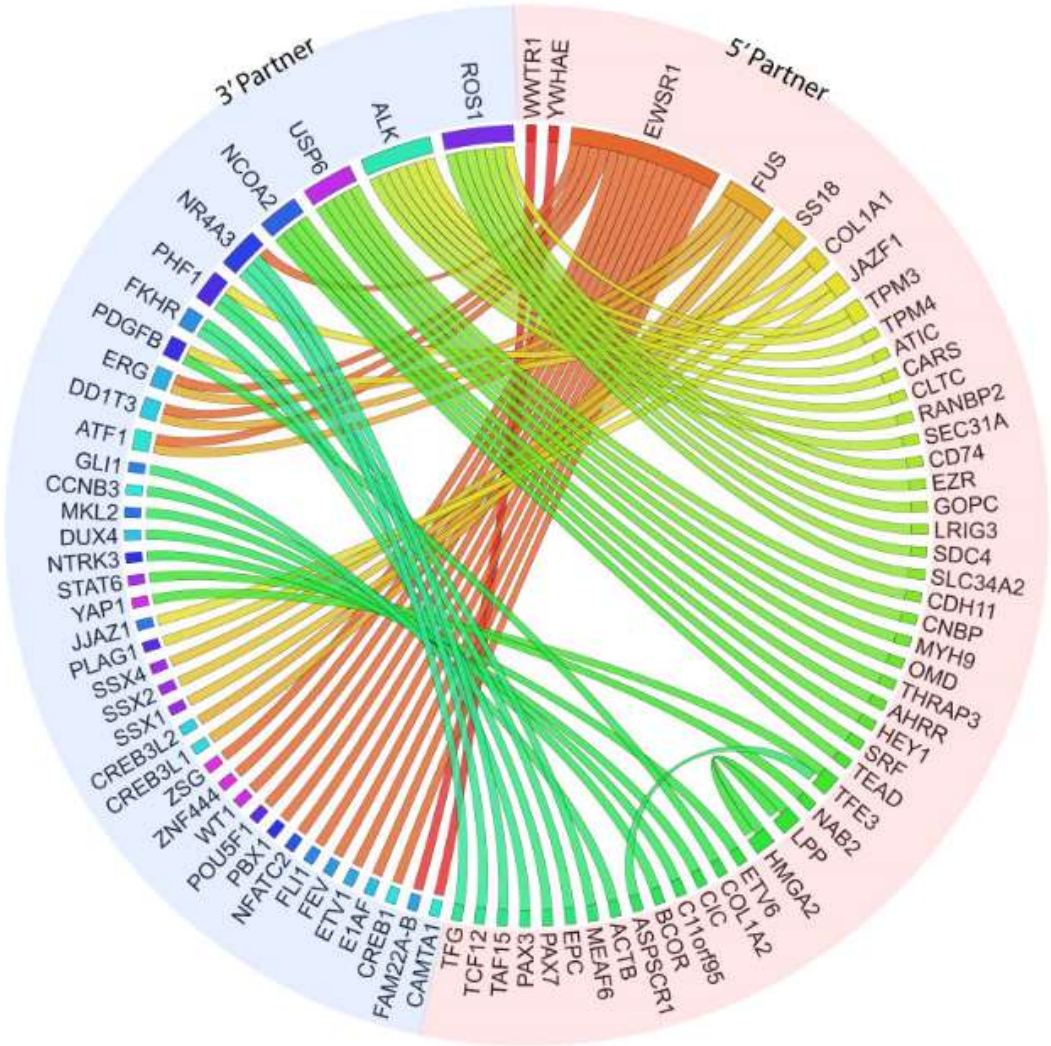
Strukturne različice NTRK: n=107
Ni fuzije z RNA testiranjem: n=47
Na DNA nivoju nezaznane fuzije: n=14

RNA-NGS (Archer FusionPlex)

+
Možno določiti fuzije s katerimkoli fuzijskim partnerjem
Zelo visoka senzitivnost
Zelo visoka specifičnost

-
Visoki stroški
Pogoj ustrezna kakovost izolirane RNA

Sarcoma Gene Fusion Map



Assay Targets

Includes the following genes and their fusion partners:

ALK	FUS	NTRK3	TCF12
CAMTA1	GLI1	PDGFB	TFE3
CCNB3	HMGA2	PLAG1	TFG
CIC	JAZF1	ROS1	USP6
EPC1	MEAF6	SS18	YWHAE
EWSR1	MKL2	STAT6	
FOXO1	NCOA2	TAF15	

Kombinirani DNA/RNA NGS paneli

Thermo Fisher/Ion Torrent

- OFA: 53 genov
- OCA: 161 genov
- Amplicon-based
- Za detekcijo morata biti znana oba fuzijskega partnerja

Illumina

- TruSight Tumor: 170 genov
- Hybridization-capture

Oncomine Focus Assay

- geni z visoko frekvenco klinično pomembnih somatskih mutacij (»hotspot«, skupaj 35 genov): AKT1, ALK, AR, BRAF, CDK4, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FGFR2, FGFR3, GNA11, GNAQ, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, PDGFRA, PIK3CA, RAF1, RET, ROS1 in SMO;

- fuzije (skupaj 23 genov): ALK, RET, ROS1, NTRK1, NTRK2, NTRK3, FGFR1, FGFR2, FGFR3, MET, BRAF, RAF1, ERG, ETV1, ETV4, ETV5, ABL1, AKT3, AXL, EGFR, ERBB2, PDGFRA in PPARG;

- spremembe števila kopij (skupaj 19 genov): ALK, AR, BRAF, CCND1, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, FGFR4, KIT, KRAS, MET, MYC, MYCN, PDGFRA in PIK3CA;

Oncomine Comprehensive Assay

Hotspot genes			
AKT1	ESR1	KIT	PDGFRB
AKT2	EZH2	KNSTRN	PIK3CB
AKT3	FGFR1	KRAS	PIK3CA
ALK	FGFR2	MAGOH	PPP2R1A
AR	FGFR3	MAP2K1	PTPN11
ARAF	FGFR4	MAP2K2	RAC1
AXL	FLT3	MAP2K4	RAF1
BRAF	FOXL2	MAPK1	RET
BTK	GATA2	MAX	RHEB
CBL	GNA11	MDM4	RHOA
CCND1	GNAQ	MED12	ROS1
CDK4	GNAS	MET	SF3B1
CDK6	H3F3A	MTOR	SMAD4
CHEK2	HIST1H3B	MYC	SMO
CSF1R	HNF1A	MYCN	SPOP
CTNNB1	HRAS	MYD88	SRC
DDR2	IDH1	NFE2L2	STAT3
EGFR	IDH2	NRAS	TERT
ERBB2	JAK1	NTRK1	TOP1
ERBB3	JAK2	NTRK2	U2AF1
ERBB4	JAK3	NTRK3	XPO1
ERCC2	KDR	PDGFRA	

Full-length genes		
ARID1A	FBXW7	PTEN
ATM	MLH1	RAD50
ATR	MRE11	RAD51
ATRX	MSH6	RAD51B
BAP1	MSH2	RAD51C
BRCA1	NBN	RAD51D
BRCA2	NF1	RNF43
CDK12	NF2	RB1
CDKN1B	NOTCH1	SETD2
CDKN2A	NOTCH2	SLX4
CDKN2B	NOTCH3	SMARCA4
CHEK1	PALB2	SMARCB1
CREBBP	PIK3R1	STK11
FANCA	PMS2	TP53
FANCD2	POLE	TSC1
FANCI	PTCH1	TSC2

Copy number genes	
AKT1	FGFR4
AKT2	FLT3
AKT3	IGF1R
ALK	KIT
AXL	KRAS
AR	MDM2
BRAF	MDM4
CCND1	MET
CCND2	MYC
CCND3	MYCL
CCNE1	MYCN
CDK2	NTRK1
CDK4	NTRK2
CDK6	NTRK3
EGFR	PDGFRA
ERBB2	PDGFRB
ESR1	PIK3CB
FGF19	PIK3CA
FGF3	PPARG
FGFR1	RICTOR
FGFR2	TERT
FGFR3	

Gene fusions (inter- and intragenic)		
AKT2	FGFR2	NUTM1
ALK	FGFR3	PDGFRA
AR	FGR	PDGFRB
AXL	FLT3	PIK3CA
BRCA1	JAK2	PRKACA
BRCA2	KRAS	PRKACB
BRAF	MDM4	PTEN
CDKN2A	MET	PPARG
EGFR	MYB	RAD51B
ERBB2	MYBL1	RAF1
ERBB4	NF1	RB1
ERG	NOTCH1	RELA
ESR1	NOTCH4	RET
ETV1	NRG1	ROS1
ETV4	NTRK1	RSPO2
ETV5	NTRK2	RSPO3
FGFR1	NTRK3	TERT

Illumina TruSight Tumor 170

SNVs and Indels (from DNA)									
AKT1	*BRIP1*	*CREBBP*	*FANCI*	*FGFR2*	*JAK3*	*MSH3*	*PALB2*	*RAD51D*	*TSC1*
AKT2	*BTK*	*CSF1R*	*FANCL*	*FGFR3*	*KDR*	*MSH6*	*PDGFRA*	*RAD54L*	*TSC2*
AKT3	*CARD11*	*CTNNB1*	*FBXW7*	*FGFR4*	*KIT*	*MTOR*	*PDGFRB*	*RB1*	*VHL*
ALK	*CCND1*	*DDR2*	*FGF1*	*FLT1*	*KMT2A (MLL)*	*MUTYH*	*PIK3CA*	*RET*	*XRCC2*
APC	*CCND2*	*DNMT3A*	*FGF2*	*FLT3*	*KRAS*	*MYC*	*PIK3CB*	*RICTOR*	
AR	*CCNE1*	*EGFR*	*FGF3*	*FOXL2*	*MAP2K1*	*MYCL1*	*PIK3CD*	*ROS1*	
ARID1A	*CD79A*	*EP300*	*FGF4*	*GEN1*	*MAP2K2*	*MYCN*	*PIK3CG*	*RPS6KB1*	
ATM	*CD79B*	*ERBB2*	*FGF5*	*GNA11*	*MCL1*	*MYD88*	*PIK3R1*	*SLX4*	
ATR	*CDH1*	*ERBB3*	*FGF6*	*GNAQ*	*MDM2*	*NBN*	*PMS2*	*SMAD4*	
BAP1	*CDK12*	*ERBB4*	*FGF7*	*GNAS*	*MDM4*	*NF1*	*PPP2R2A*	*SMARCB1*	
BARD1	*CDK4*	*ERCC1*	*FGF8*	*HNF1A*	*MET*	*NOTCH1*	*PTCH1*	*SMO*	
BCL2	*CDK6*	*ERCC2*	*FGF9*	*HRAS*	*MLH1*	*NOTCH2*	*PTEN*	*SRC*	
BCL6	*CDKN2A*	*ERG*	*FGF10*	*IDH1*	*MLLT3*	*NOTCH3*	*PTPN11*	*STK11*	
BRAF	*CEBPA*	*ESR1*	*FGF14*	*IDH2*	*MPL*	*NPM1*	*RAD51*	*TERT*	
BRCA1	*CHEK1*	*EZH2*	*FGF23*	*INPP4B*	*MRE11A*	*NRAS*	*RAD51B*	*TET2*	
BRCA2	*CHEK2*	*FAM175A*	*FGFR1*	*JAK2*	*MSH2*	*NRG1*	*RAD51C*	*TP53*	
Amplifications (from DNA)									
AKT2	*BRCA2*	*CHEK1*	*ERCC2*	*FGF5*	*FGF14*	*FGFR4*	*MDM4*	*NRG1*	*RAF1*
ALK	*CCND1*	*CHEK2*	*ESR1*	*FGF6*	*FGF19*	*JAK2*	*MET*	*PDGFRA*	*RET*
AR	*CCND3*	*EGFR*	*FGF1*	*FGF7*	*FGF23*	*KIT*	*MYC*	*PDGFRB*	*RICTOR*
ATM	*CCNE1*	*ERBB2*	*FGF2*	*FGF8*	*FGFR1*	*KRAS*	*MYCL1*	*PIK3CA*	*RPS6KB1*
BRAF	*CDK4*	*ERBB3*	*FGF3*	*FGF9*	*FGFR2*	*LAMP1*	*MYCN*	*PIK3CB*	*TFRC*
BRCA1	*CDK6*	*ERCC1*	*FGF4*	*FGF10*	*FGFR3*	*MDM2*	*NRAS*	*PTEN*	
Fusions and Splice Variants (from RNA)									
ABL1	*BRAF*	*EML4*	*ETV4*	*FGFR4*	*KIF5B*	*MYC*	*NTRK2*	*PIK3CA*	*TMPRSS2*
AKT3	*BRCA1*	*ERBB2*	*ETV5*	*FLI1*	*KIT*	*NOTCH1*	*NTRK3*	*PPARG*	
ALK	*BRCA2*	*ERG*	*EWSR1*	*FLT1*	*KMT2A (MLL)*	*NOTCH2*	*PAX3*	*RAF1*	
AR	*CDK4*	*ESR1*	*FGFR1*	*FLT3*	*MET*	*NOTCH3*	*PAX7*	*RET*	
AXL	*CSF1R*	*ETS1*	*FGFR2*	*JAK2*	*MLLT3*	*NRG1*	*PDGFRA*	*ROS1*	
BCL2	*EGFR*	*ETV1*	*FGFR3*	*KDR*	*MSH2*	*NTRK1*	*PDGFRB*	*RPS6KB1*	

Fuzije NTRK

OFA OCA

Gen	Znani partnerji
NTRK1	ARHGEF2, BCAN, CEL, CD74, CHTOP, CGN, CTRC, DDR2, EPHB2, EPS15, GATAD2B, GON4L, GRPAP1, IRF2BP2, LMNA, MDM4, MPRIP, MRPL24, NFASC, P2RY8, PEAR1, PPL, RABGAP1L, RNF213, SCYL3, SQSTM1, SSBP2, TFG, TP53, TPM3, TPR, TRIM63, VANGL2 (n=32)
NTRK2	AFAP1, AGBL4, BCR, DAB2IP, NACC2, NAV1, PAN3, QK1, SLMAP, SQSTM1, STRN3, TLE4, TRAF2, TRIMM24, VCL, WNK2 (n=16)
NTRK3	AFAP1, AKAP13, BTBD1, COX5A, EML4, ETV6, FAT1, LYN, MYH9, MYO5, RBPMS, TPM4, ZNF710 (n=13)

RESEARCH ARTICLE | Full Access

Testing *NTRK* testing: wet-lab and *in silico* comparison of RNA-based targeted sequencing assays

Nicole Pfarr, Martina Kirchner, Ulrich Lehmann, Jonas Leichsenring ... See all authors

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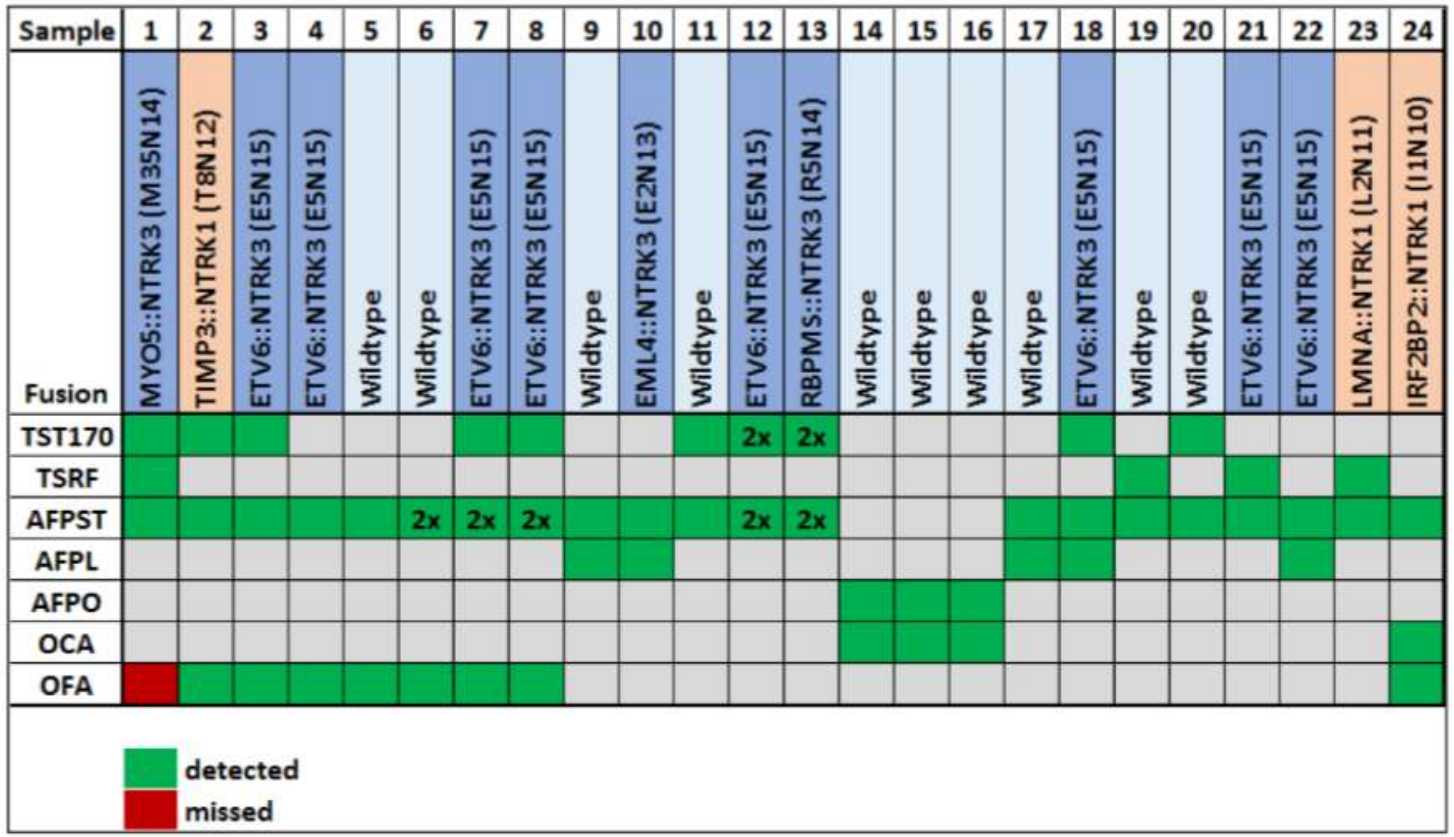
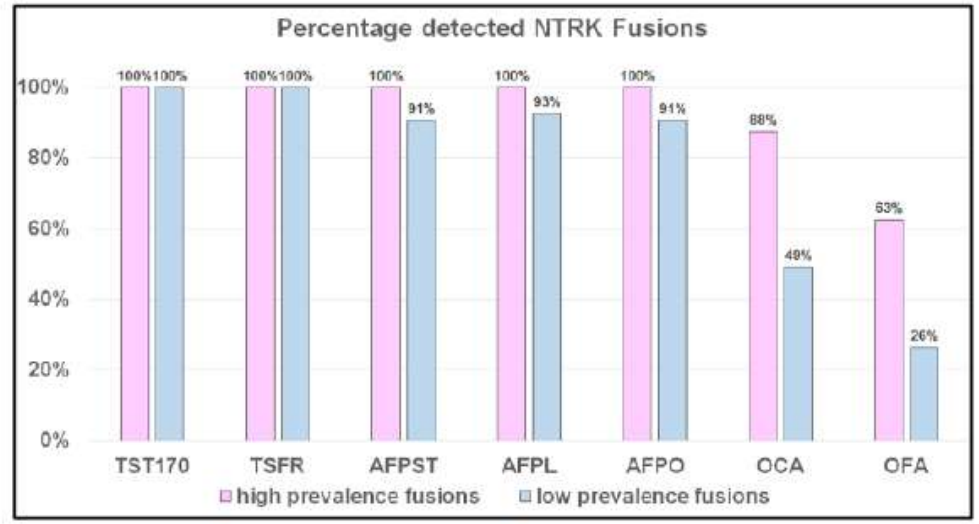


	Fusion partner	Exons	TST170	TSRF	AFPST	AFPL	AFPO	OCA	OFA
NTRK1	AFAP1::NTRK1	A4N9							
	ARHGEF2::NTRK1	A21N10							
	BCAN::NTRK1	B13N16							
	CD74::NTRK1	C8N12							
	CHTOP::NTRK1	C5N10							
	EPHB2::NTRK1	E3N8							
	IRF2BP2::NTRK1	I1N9							
		I1N2							
		I1N10							
	LMNA::NTRK1	L2N10							
		L2N11							
		L2N12							
		L4N12							
		L8N12							
		L12N12							
	LRRC71::NTRK1	L1N10							
	MPRIP::NTRK1	M21N14							
	NFASC::NTRK1	N20N10							
	NTRK1::RPL8	N16R6							
	P2RY8::NTRK1	P2N5							
	PHF20::NTRK1	P2N5							
	PLEKHA6::NTRK1	P21N9							
	PPL::NTRK1	P21N10							
	SCYL3::NTRK1	S11N12							
	TFG::NTRK1	T5N9							
		T6N10							
	TMB3::NTRK1	T7N10							
	TPM3::NTRK1	T10N9							
		T8N10							
	TP53::NTRK1	T11N9							
	TPR::NTRK1	T10N9							
	TRIM63::NTRK1	T8N10							
	SQSTM1::NTRK1	S5N10							
	SSBP2::NTRK1	S12N12							

	Fusion partner	Exons	TST170	TSRF	AFPST	AFPL	AFPO	OCA	OFA
NTRK2	AFAP1::NTRK2	A13N12							
	BCR::NTRK2	B1N17							
	NACC2::NTRK2	N4N13							
	PAN3::NTRK2	P13N9							
	QKI::NTRK2	Q6N16							
	SFAP1::NTRK2	F4N9							
	STRN3::NTRK2	S7N16							
	SQSTM1::NTRK2	S5N17							
	TRAF2::NTRK2	T9N15							
	TRIM24::NTRK2	T12N16							
	WNK2::NTRK2	W24N16							

	Fusion partner	Exons	TST170	TSRF	AFPST	AFPL	AFPO	OCA	OFA
NTRK3	AKAP13::NTRK3	A14N14							
	EML4::NTRK3	E2N14							
		E6N14							
	ETV6::NTRK3	E4N14							
		E4N15							
		E5N14							
		E5N15							
		E6N15							
		E12N15							
	FAT1::NTRK3	F4N15							
	LYN::NTRK3	L8N14							
	RBPMS::NTRK3	R5N14							
	SQSTM1::NTRK3	S8N14							
	TPM4::NTRK3	T6:N11							
	VPS18::NTRK3	V4N18							
	ZNF710::NTRK3	Z1N14							

- "non-targeted fusion"
- previous exon covered, possibly detected
- not detected
- detected
- primer in different pools > undetected
- high prevalence fusion
- low prevalence fusion



Kombinirani DNA/RNA NGS paneli

+	-
Hkratno določanje vseh relevantnih sprememb/tarč (mutacije, CNV, fuzije) z enim samim testom	Razmeroma visoki stroški
Možna izvedba v 5 delovnih dneh ob začetka izolacije RNA/DNA, ob pogoju, da vzorcev dovolj	10-40 ng RNA, >20% tumorskih celic
Senzitivnost 98-100%	Za racionalno delo potrebnih dovolj vzorcev za hkratno analizo (8-16)
Specifičnost 96-100%	Večji paneli, večji stroški, daljši čas analize, več informacij

Praktični pristop – Memorial Slone Kattering Cancer Center

- **FISH**: če je pričakovana fuzija ETV6-NTRK3
- **DNA NGS**: rutinsko vsi maligni tumorji, če je dovolj tumorskega tkiva
- **RNA NGS (Archer):** če so v DNA NGS strukturne variante nejasnega pomena ali RAS/BRAF divji tip pri tumorjih, kjer pogosto mutacije (karcinom kolona, pljučni adenokarcinom)
- **IHK:** kadar fuzije nejasnega pomena ali premalo tkiva za NGS

Fuzije NTRK se izključujejo z:

- Mutacije KRAS, NRAS, BRAF, EGFR
- Fuzije ALK, ROS1, RET

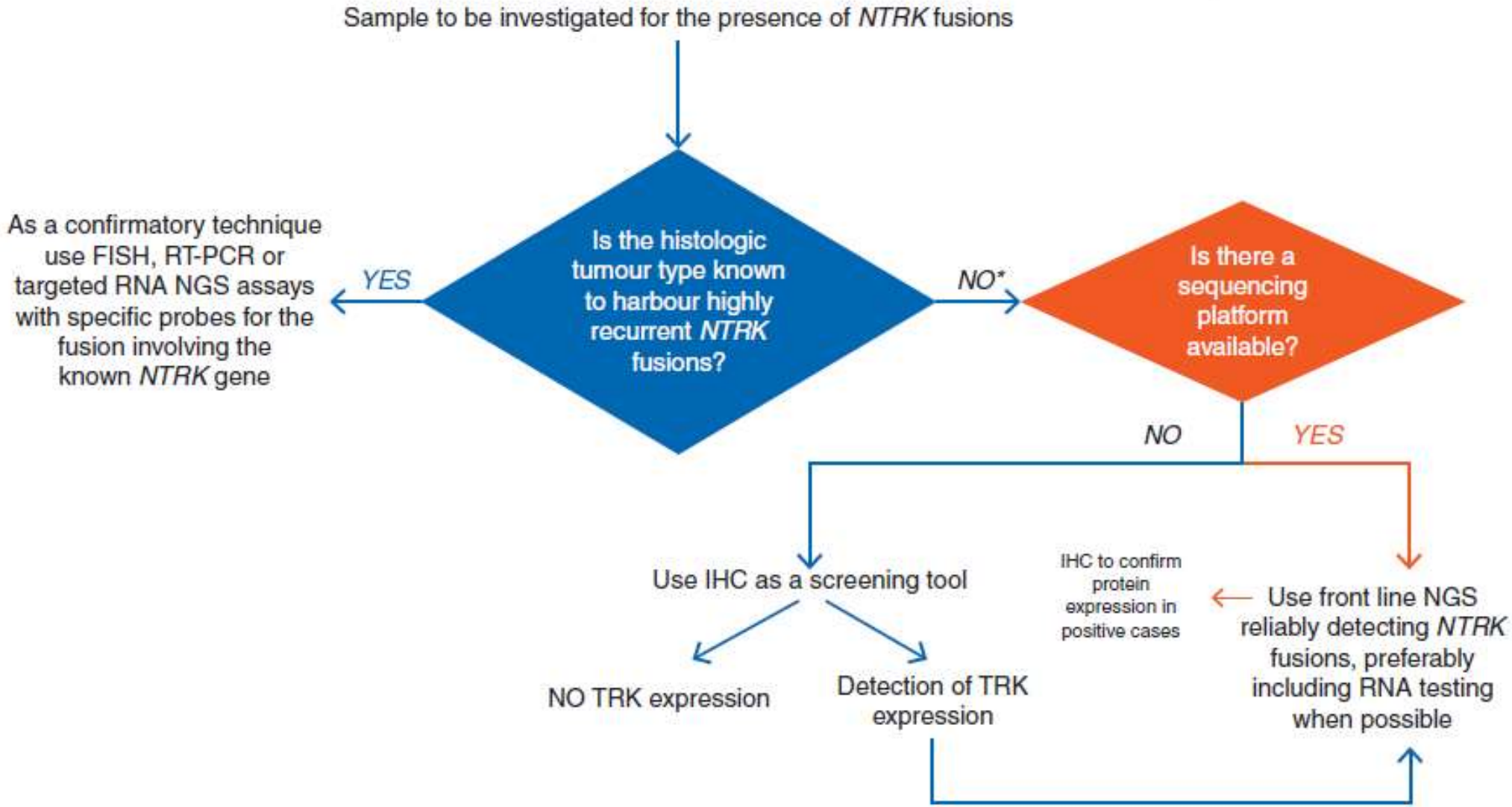


Figure 2. Summary of the ESMO Translational Research and Precision Medicine Working Group recommendations. Following the review of

Fuzije NTRK – testiranje v Sloveniji

IHK (panNTRK, EPR17341)

NGS (DNA/RNA, RNA)

Fuzije NTRK – indikacije za testiranje

Potrditev določene vrste tumorja/opredelitev vrste tumorja

Določanje prediktivnih dejavnikov

Fuzije NTRK – indikacije za testiranje

Potrditev določene vrste tumorja/opredelitev vrste tumorja

- Potrditev tumorja z veliko verjetnostjo fuzije NTRK (sekretorni karcinom, infantilni fibrosarkom,…)
- Prepoznava tumorjev s fuzijo NTRK („fibrosarkom“, „MPNST“, „DFSP“, “IMT“, nejasni mezenhimski tumorji z monomorfno sliko,..

Določanje prediktivnih dejavnikov

- Refleksno testiranje prediktivnih dejavnikov
- Testiranje malignih tumorjev v napredovalem stadiju na zahtevo

Fuzije NTRK – indikacije za testiranje

Potrditev določene vrste tumorja/opredelitev vrste tumorja	
• Potrditev tumorja z veliko verjetnostjo fuzije NTRK (sekretorni karcinom, infantilni fibrosarkom,…)	NGS→IHK
• Prepoznava tumorjev s fuzijo NTRK („fibrosarkom“, „MPNST“, „DFSP“, nejasni mezenhimski tumorji z monomorfno sliko,..	NGS→IHK ali IHK→NGS
Določanje prediktivnih dejavnikov	
• Refleksno testiranje prediktivnih dejavnikov	NGS→IHK
• Testiranje malignih tumorjev v napredovalem stadiju na zahtevo	NGS→IHK ali IHK→NGS

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WILEY

RESEARCH ARTICLE

A novel group of spindle cell tumors defined by S100 and CD34 co-expression shows recurrent fusions involving RAF1, BRAF, and NTRK1/2 genes

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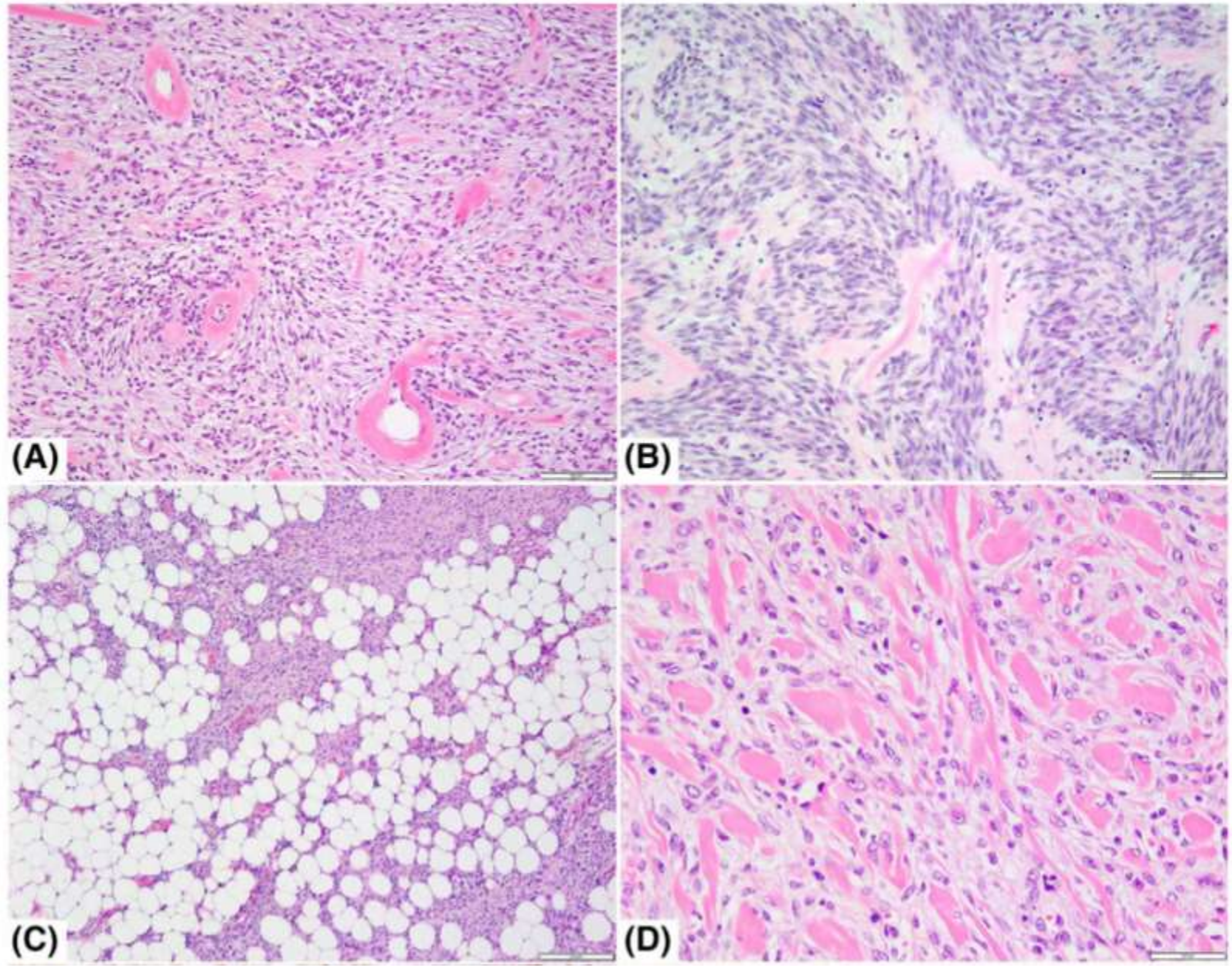

(A)
(B)
(C)
(D)

TABLE 1 RAF1/BRAF and NTRK1/2 rearranged tumors

	Age/sex	Location	Gene fusion/rearrangement	Cellularity	FU
Case 1	67/F	Abdomen	*PDZRN3–RAF1*[a]	IC	AWD (7 mo), lung, liver, and periton mets
Case 2	41/M	Thigh	*SLMAP–RAF1*[a]	LC	NA
Case 3	2/F	Rectum	*TMF1–RAF1*[b]	LC	NA
Case 4	45/F	Back	*RAF1*	LC	NED (36 mo)
Case 5	10/M	Thigh	*RAF1*	LC	NA
Case 6	38/F	Shoulder	*RAF1*	IC	NED (9 mo)
Case 7	27/F	Back	*RAF1*	LC	NED (26 mo)
Case 8	59/M	Chest wall	*RAF1*	IC	NED (25 mo), s/p RT
Case 9	48/F	Hand	*BRAF*	LC	AWD (22 mo) (persistent local disease)
Case 10	18/F	Thigh	*BRAF*	IC	NA
Case 11	13/M	Maxilla	*LMNA–NTRK1*	LC	NED (648 mo)
Case 12	4/M	Mandible	*LMNA–NTRK1*	LC	NED (144 mo, s/p LR, RT)
Case 13	16/F	Chest wall	*LMNA–NTRK1*	IC	NA
Case 14	61/F	Lower leg	*LMNA–NTRK1*	IC	DOO (375 mo), lung mets
Case 15	18/M	Stomach	*LMNA–NTRK1*	IC	DOD (25 mo)
Case 16	5/M	Leg	*LMNA–NTRK1*	LC	NED (3 mo)
Case 17	15/F	Thigh	*LMNA–NTRK1*	LC	NED (14 mo)
Case 18	15/F	Thigh	*LMNA–NTRK1*	LC	NA
Case 19	38/M	Wrist	*TPM3–NTRK1*[c]	LC	NED (16 mo)
Case 20	23/M	Arm	*TPM3–NTRK1*	IC	AWD (36 mo), lung and abd mets
Case 21	35/M	Chest wall	*TPM3–NTRK1*	LC	NA
Case 22	77/F	Thigh	*TPR–NTRK1*	LC	NA
Case 23	17/F	Chest wall	*NTRK1*	LC	NA
Case 24	3/M	Shoulder	*NTRK1*	LC	NA
Case 25	20/M	Forearm	*SPECC1L–NTRK2*[a]	LC	NA

Oncomine Focus Assay

- geni z visoko frekvenco klinično pomembnih somatskih mutacij (»hotspot«, skupaj 35 genov): AKT1, ALK, AR, BRAF, CDK4, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FGFR2, FGFR3, GNA11, GNAQ, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, PDGFRA, PIK3CA, RAF1, RET, ROS1 in SMO;

- fuzije (skupaj 23 genov): ALK, RET, ROS1, NTRK1, NTRK2, NTRK3, FGFR1, FGFR2, FGFR3, MET, BRAF, RAF1, ERG, ETV1, ETV4, ETV5, ABL1, AKT3, AXL, EGFR, ERBB2, PDGFRA in PPARG;

- spremembe števila kopij (skupaj 19 genov): ALK, AR, BRAF, CCND1, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, FGFR4, KIT, KRAS, MET, MYC, MYCN, PDGFRA in PIK3CA;

Oncomine Focus Assay – adenokarcinom pljuč

- geni z visoko frekvenco klinično pomembnih somatskih mutacij (»hotspot«, skupaj 35 genov): AKT1, ALK, AR, BRAF, CDK4, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FGFR2, FGFR3, GNA11, GNAQ, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, PDGFRA, PIK3CA, RAF1, RET, ROS1 in SMO;

- fuzije (skupaj 23 genov): ALK, RET, ROS1, NTRK1, NTRK2, NTRK3, FGFR1, FGFR2, FGFR3, MET, BRAF, RAF1, ERG, ETV1, ETV4, ETV5, ABL1, AKT3, AXL, EGFR, ERBB2, PDGFRA in PPARG;

- spremembe števila kopij (skupaj 19 genov): ALK, AR, BRAF, CCND1, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, FGFR4, KIT, KRAS, MET, MYC, MYCN, PDGFRA in PIK3CA;

Oncomine Focus Assay - melanom

- geni z visoko frekvenco klinično pomembnih somatskih mutacij (»hotspot«, skupaj 35 genov): AKT1, ALK, AR, BRAF, CDK4, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FGFR2, FGFR3, GNA11, GNAQ, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, PDGFRA, PIK3CA, RAF1, RET, ROS1 in SMO;

- fuzije (skupaj 23 genov): ALK, RET, ROS1, NTRK1, NTRK2, NTRK3, FGFR1, FGFR2, FGFR3, MET, BRAF, RAF1, ERG, ETV1, ETV4, ETV5, ABL1, AKT3, AXL, EGFR, ERBB2, PDGFRA in PPARG;

- spremembe števila kopij (skupaj 19 genov): ALK, AR, BRAF, CCND1, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, FGFR4, KIT, KRAS, MET, MYC, MYCN, PDGFRA in PIK3CA;

Rezistenca na inhibitorje NTRK – točkovne mutacije

NTRK1: p.G667C
NTRK3: p.G696A

Hotspot genes			
AKT1	ESR1	KIT	PDGFRB
AKT2	EZH2	KNSTRN	PIK3CB
AKT3	FGFR1	KRAS	PIK3CA
ALK	FGFR2	MAGOH	PPP2R1A
AR	FGFR3	MAP2K1	PTPN11
ARAF	FGFR4	MAP2K2	RAC1
AXL	FLT3	MAP2K4	RAF1
BRAF	FOXL2	MAPK1	RET
BTK	GATA2	MAX	RHEB
CBL	GNA11	MDM4	RHOA
CCND1	GNAQ	MED12	ROS1
CDK4	GNAS	MET	SF3B1
CDK6	H3F3A	MTOR	SMAD4
CHEK2	HIST1H3B	MYC	SMO
CSF1R	HNF1A	MYCN	SPOP
CTNNB1	HRAS	MYD88	SRC
DDR2	IDH1	NFE2L2	STAT3
EGFR	IDH2	NRAS	TERT
ERBB2	JAK1	NTRK1	TOP1
ERBB3	JAK2	NTRK2	U2AF1
ERBB4	JAK3	NTRK3	XPO1
ERCC2	KDR	PDGFRA	

Full-length genes		
ARID1A	FBXW7	PTEN
ATM	MLH1	RAD50
ATR	MRE11	RAD51
ATRX	MSH6	RAD51B
BAP1	MSH2	RAD51C
BRCA1	NBN	RAD51D
BRCA2	NF1	RNF43
CDK12	NF2	RB1
CDKN1B	NOTCH1	SETD2
CDKN2A	NOTCH2	SLX4
CDKN2B	NOTCH3	SMARCA4
CHEK1	PALB2	SMARCB1
CREBBP	PIK3R1	STK11
FANCA	PMS2	TP53
FANCD2	POLE	TSC1
FANCI	PTCH1	TSC2

Copy number genes	
AKT1	FGFR4
AKT2	FLT3
AKT3	IGF1R
ALK	KIT
AXL	KRAS
AR	MDM2
BRAF	MDM4
CCND1	MET
CCND2	MYC
CCND3	MYCL
CCNE1	MYCN
CDK2	NTRK1
CDK4	NTRK2
CDK6	NTRK3
EGFR	PDGFRA
ERBB2	PDGFRB
ESR1	PIK3CB
FGF19	PIK3CA
FGF3	PPARG
FGFR1	RICTOR
FGFR2	TERT
FGFR3	

Gene fusions (inter- and intragenic)		
AKT2	FGFR2	NUTM1
ALK	FGFR3	PDGFRA
AR	FGR	PDGFRB
AXL	FLT3	PIK3CA
BRCA1	JAK2	PRKACA
BRCA2	KRAS	PRKACB
BRAF	MDM4	PTEN
CDKN2A	MET	PPARG
EGFR	MYB	RAD51B
ERBB2	MYBL1	RAF1
ERBB4	NF1	RB1
ERG	NOTCH1	RELA
ESR1	NOTCH4	RET
ETV1	NRG1	ROS1
ETV4	NTRK1	RSPO2
ETV5	NTRK2	RSPO3
FGFR1	NTRK3	TERT

Zaključki

INŠTITUT ZA PATOLOGIJO
UNIVERZA V LJUBLJANI ◊ MEDICINSKA FAKULTETA

NTRK

RNA NGS→IHK

IHK→RNA NGS

Diagnostika fuzij NTRK in izbor primarne metode mora biti v korelaciji histopatološko sliko, vrsto tumorja, diferencialno diagnozo, pričakovano fuzijo, kliničnim kontekstom in vzorcem, ki je na voljo za testiranje

SLO NA LETO

Melanomi
2

Atipični Spitz tumorji
5-10

Mezenhimski tumorji
5-10

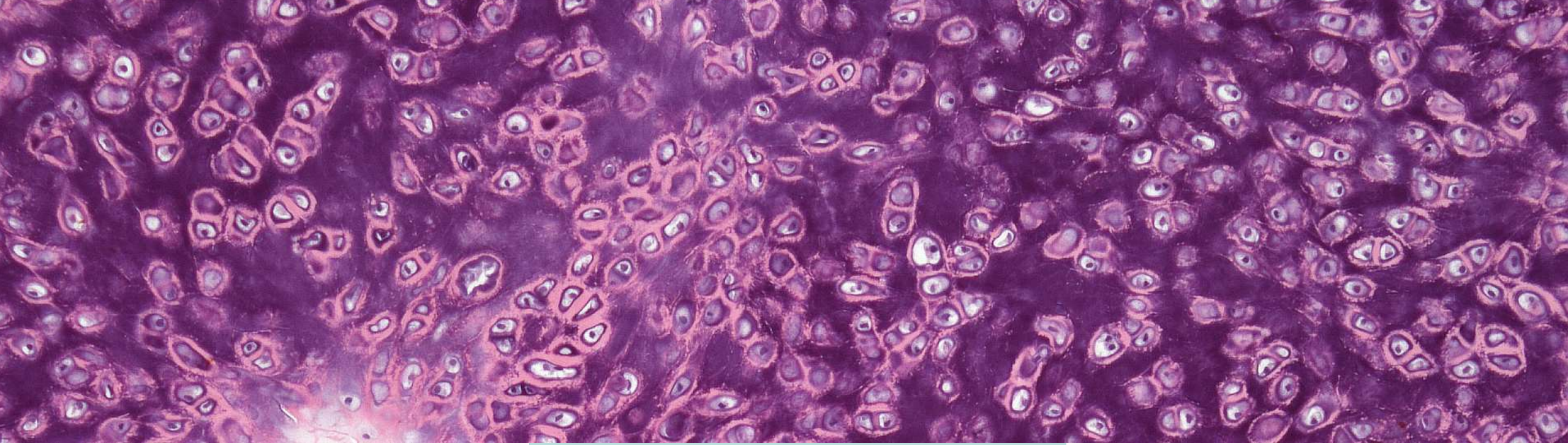
Karcinomi
20-30

Glialni tumorji
2

Pred zdravljenjem je potrebno fuzijo NTRK potrditi z molekularno metodo (RNA NGS) in IHK

DOLOČANJE FUZIJ NTRK MORA BITI DEL PATOLOŠKE DIAGNOSTIKE IN CELOVITEGA OPREDELJEVANJA PREDIKTIVNIH DEJAVNIKOV IN NE IZOLIRAN PROCES

IZBOR TESTIRANJA NTRK JE ODVISEN OD VEČ OKOLIŠČIN IN DOSTOPNIH METOD



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