Inštitut za patologijo Medicinske fakultete Univerze v Ljubljani
Onkološki inštitut Ljubljana
Združenje za patologijo in sodno medicino pri Slovenskem zdravniškem društvu

MELANOCITNE PROLIFERACIJE S FUZIJAMI *NTRK*

Ljubljana, 14. november 2019

Boštjan Luzar
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2018 WHO KLASIFIKACIJA MELANOCITNIH PROLIFERACIJ

NOVOSTI

2018 WHO KLASIFIKACIJA KOŽNIH TUMORJEV
- *KLASIFIKACIJA MELANOCITNIH PROLIFERACIJ* -

2018 WHO KLASIFIKACIJA MELANOMA

Table 2.01 Classification of melanoma

Melanomas arising in sun-exposed skin	**Pathway I:**	Low-CSD melanoma/superficial spreading melanoma
	Pathway II:	High-CSD melanoma/lentigo maligna melanoma
	Pathway III:	Desmoplastic melanoma
Melanomas arising at sun-shielded sites or without known etiological associations with UV radiation exposure	**Pathway IV:**	Malignant Spitz tumour (Spitz melanoma)
	Pathway V:	Acral melanoma
	Pathway VI:	Mucosal melanoma
	Pathway VII:	Melanoma arising in congenital naevus
	Pathway VIII:	Melanoma arising in blue naevus
	Pathway IX:	Uveal melanoma
Low/high-CSD melanoma, melanoma in skin with a low/high degree of cumulative sun damage. Various: Nodular, naevoid, and metastatic melanomas.		

GENETSKO OZADJE MELANOCITNIH PROLIFERACIJ

Table 2.06 Classification of melanomas and precursor lesions on the basis of epidemiological, clinical, pathological, and genomic attributes

	Low UV radiation exposure / CSD				High UV radiation exposure / CSD	
Pathway	I				II	III
Endpoint of pathway	Low-CSD melanoma / SSM				High-CSD melanoma / LMM	Desmoplastic melanoma
Benign neoplasms (naevi)	Naevus				? IMP	? IMP
Intermediate / low-grade dysplasias and melanocytomas	Low-grade dysplasia	BIN	DPN		? IAMP/dysplasia	? IAMP/dysplasia
Intermediate / high-grade dysplasias and melanocytomas	High-grade dysplasia / MIS	*BAP1*-inactivated melanocytoma / MELTUMP	Deep penetrating melanocytoma / MELTUMP	PEM / MELTUMP	Lentigo maligna (MIS)	MIS
Malignant neoplasms	Low-CSD melanoma / SSM (VGP)	Melanoma in BIN (rare)	Melanoma in DPN (rare)	Melanoma in PEM (rare)	LMM (VGP)	Desmoplastic melanoma
Common mutations[a,b]	***BRAF*** p.V600E or ***NRAS***	***BRAF*** or ***NRAS*** + ***BAP1***	***BRAF***, ***MAP2K1***, or ***NRAS*** + ***CTNNB1*** or ***APC***	***BRAF*** + ***PRKAR1A*** or ***PRKCA***	***NRAS***; ***BRAF*** (non-p.V600E); ***KIT***; or ***NF1***	***NF1***; *ERBB2*; *MAP2K1*; *MAP3K1*; *BRAF*; *EGFR*; *MET*
	TERT; *CDKN2A*; *TP53*; *PTEN*				*TERT*; *CDKN2A*; *TP53*; *PTEN*; *RAC1*	*TERT*; *NFKBIE*; *NRAS*; *PIK3CA*; *PTPN11*

BIN, *BAP1*-inactivated naevus; **BN**, blue naevus; **CBN**, cellular blue naevus; **CN**, congenital naevus; **CSD**, cumulative sun damage; **DPN**, deep penetrating naevus; **IAMP**, intraepidermal atypical melanocytic proliferation; **IAMPUS**, intraepidermal atypical melanocytic proliferation of uncertain significance; **IMP**, intraepidermal melanocytic proliferation without atypia; **LMM**, lentigo maligna melanoma; low / high-CSD melaroma, melanoma in skin with a low / high degree of cumulative sun damage; **MELTUMP**, melanocytic tumour of uncertain malignant potential; **MIS**, melanoma in situ; **PEM**, pigmented epithelioid melanocytoma; **SSM**, superficial spreading melanoma; **STUMP**, spitzoid tumour of uncertain malignant potential; **UV**, ultraviolet; **VGP**, vertical growth phase (tumorigenic and/or mitogenic melanoma).

GENETSKO OZADJE MELANOCITNIH PROLIFERACIJ

Low to no (or variable/incidental) UV radiation exposure / CSD					
IV	**V**	**VI**	**VII**	**VIII**	**IX**
Malignant Spitz tumour / Spitz melanoma	Acral melanoma	Mucosal melanoma	Melanoma in CN	Melanoma in BN	Uveal melanoma
Spitz naevus	? Acral naevus	? Melanosis	CN	Blue naevus	? Naevus
Atypical Spitz tumour (melanocytoma)	IAMP / dysplasia	Atypical melanosis / dysplasia / IAMPUS	Nodule in CN (melanocytoma)	(Atypical) CBN (melanocytoma)	?
STUMP / MELTUMP	Acral MIS	Mucosal MIS	MIS in CN	Atypical CBN	?
Malignant Spitz tumour/ Spitz melanoma (tumorigenic)	Acral melanoma (VGP)	Mucosal lentiginous melanoma (VGP)	Melanoma in CN (tumorigenic)	Melanoma in blue naevus (tumorigenic)	Uveal melanoma
HRAS; ***ALK***; ***ROS1***; ***RET***; ***NTRK1***; ***NTRK3***; ***BRAF***; or ***MET***	***KIT***; ***NRAS***; ***BRAF***; ***HRAS***; ***KRAS***; ***NTRK3***; ***ALK***; or ***NF1***	***KIT***, ***NRAS***, ***KRAS***, or ***BRAF***	***NRAS***; **BRAF p.V600E** (small lesions); or ***BRAF***	***GNAQ***; ***GNA11***; or ***CYSLTR2***	***GNAQ***, ***GNA11***, ***CYSLTR2***, or ***PLCB4***
CDKN2A	*CDKN2A*; *TERT*; *CCND1*; *GAB2*	***NF1***; *CDKN2A*; *SF3B1*; *CCND1*; *CDK4*; *MDM2*		*BAP1*; *EIF1AX*; *SF3B1*	*BAP1*; *SF3B1*; *EIF1AX*

Definitions: *Melanocytoma* is a tumorigenic neoplasm of melanocytes that generally has increased cellularity and/or atypia (compared with a common naevus) and an increased (although generally still low) probability of neoplastic progression; *tumorigenic* means forming a mass of neoplastic cells.

[a] Common mutations in each pathway are listed; mutations already identified in benign or borderline low lesions are shown in bold.
[b] Blue, loss-of-function mutation; red, gain-of-function mutation; green, change-of-function mutation; orange, amplification; purple, rearrangement; grey, promoter mutation.

GENETSKO OZADJE MELANOCITNIH PROLIFERACIJ

Low to no (or variable/incidental) UV radiation exposure / CSD					
IV	**V**	**VI**	**VII**	**VIII**	**IX**
Malignant Spitz tumour / Spitz melanoma	Acral melanoma	Mucosal melanoma	Melanoma in CN	Melanoma in BN	Uveal melanoma
Spitz naevus	Acral naevus	? Melanosis	CN	Blue naevus	? Naevus
Atypical Spitz tumour (melanocytoma)	IAMP / dysplasia	Atypical melanosis / dysplasia / IAMPUS	Nodule in CN (melanocytoma)	(Atypical) CBN (melanocytoma)	?
STUMP / MELTUMP	Acral MIS	Mucosal MIS	MIS in CN	Atypical CBN	?
Malignant Spitz tumour/ Spitz melanoma (tumorigenic)	Acral melanoma (VGP)	Mucosal lentiginous melanoma (VGP)	Melanoma in CN (tumorigenic)	Melanoma in blue naevus (tumorigenic)	Uveal melanoma
HRAS; ***ALK***; ***ROS1***; ***RET***; ***NTRK1***; ***NTRK3***; ***BRAF***; or ***MET***	***KIT***; ***NRAS***; ***BRAF***; ***HRAS***; ***KRAS***; ***NTRK3***; ***ALK***; or ***NF1***	***KIT***, ***NRAS***, ***KRAS***, or ***BRAF***	***NRAS***; **BRAF p.V600E** (small lesions); or ***BRAF***	***GNAQ***; ***GNA11***; or ***CYSLTR2***	***GNAQ***, ***GNA11***, ***CYSLTR2***, or ***PLCB4***
CDKN2A	*CDKN2A*; *TERT*; *CCND1*; *GAB2*	***NF1***; *CDKN2A*; *SF3B1*; *CCND1*; *CDK4*; *MDM2*		*BAP1*; *EIF1AX*; *SF3B1*	*BAP1*; *SF3B1*; *EIF1AX*

Definitions: *Melanocytoma* is a tumorigenic neoplasm of melanocytes that generally has increased cellularity and/or atypia (compared with a common naevus) and an increased (although generally still low) probability of neoplastic progression; *tumorigenic* means forming a mass of neoplastic cells.

[a] Common mutations in each pathway are listed; mutations already identified in benign or borderline low lesions are shown in bold.

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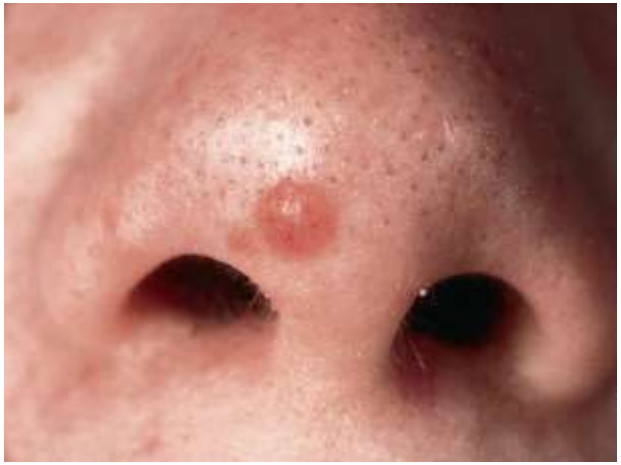
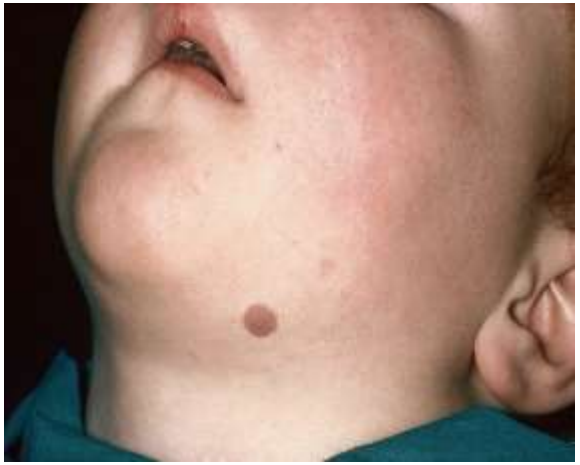
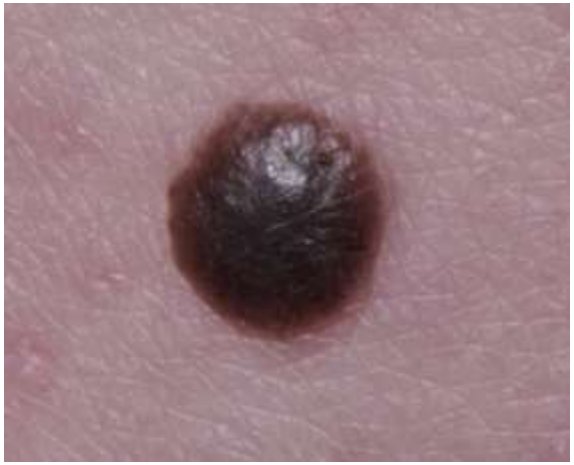
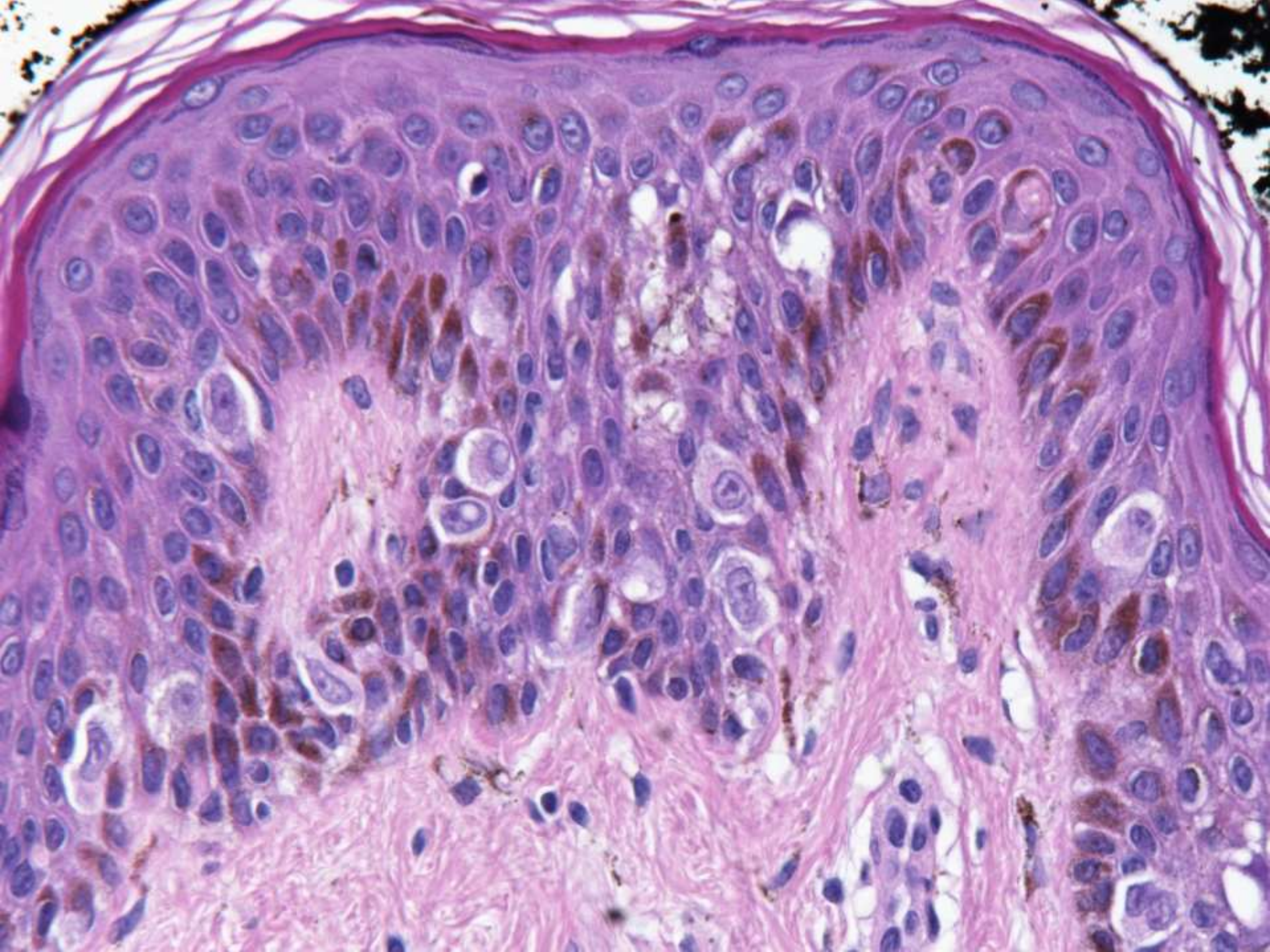
SPEKTER SPITZ MELANOCITNIH PROLIFERACIJ

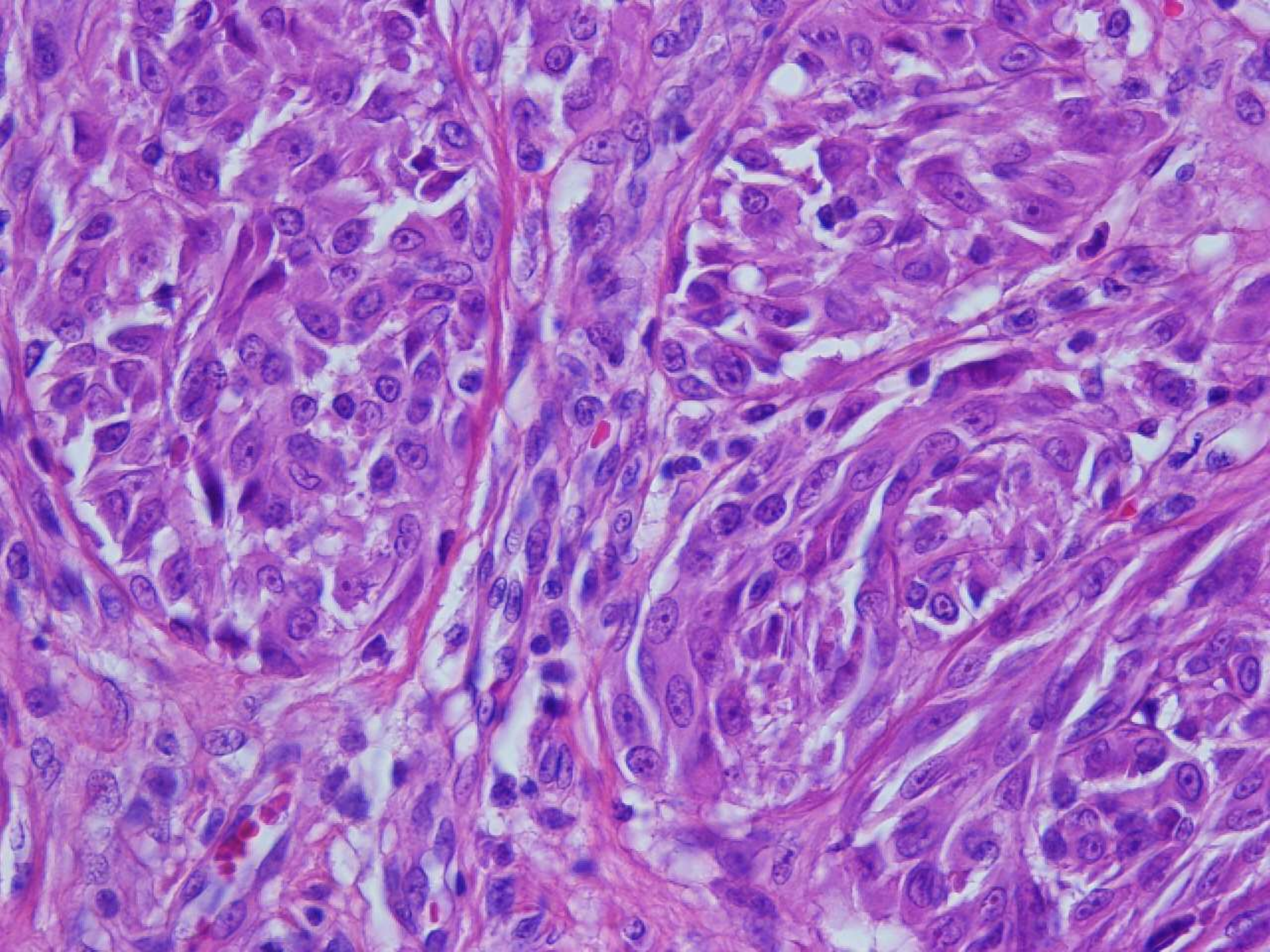
SPITZ NEVUS → **ATIPIČNI SPITZ TUMOR** → SPITZ MELANOM











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ARTICLE

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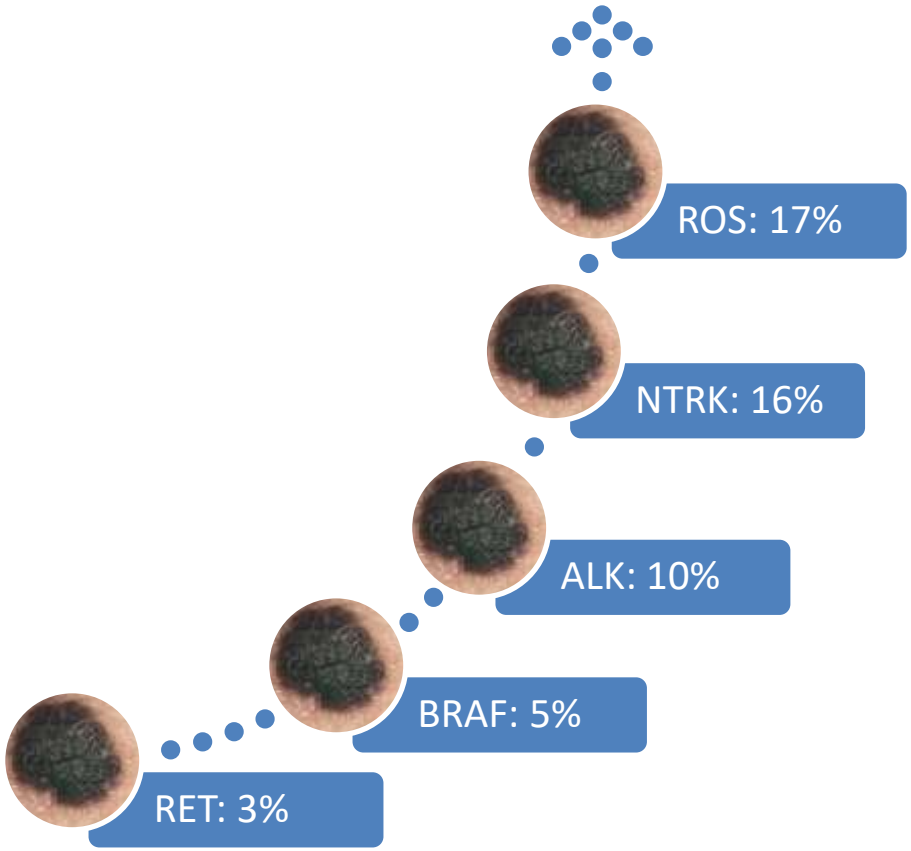
Kinase fusions are frequent in Spitz tumours and spitzoid melanomas

Thomas Wiesner[1,2,*], Jie He[3,*], Roman Yelensky[3,*], Rosaura Esteve-Puig[4], Thomas Botton[4], Iwei Yeh[4], Doron Lipson[3], Geoff Otto[3], Kristina Brennan[3], Rajmohan Murali[5,6], Maria Garrido[4], Vincent A. Miller[3], Jeffrey S. Ross[3], Michael F. Berger[1], Alyssa Sparatta[4], Gabriele Palmedo[7], Lorenzo Cerroni[2], Klaus J. Busam[5], Heinz Kutzner[7], Maureen T. Cronin[3], Philip J. Stephens[3] & Boris C. Bastian[1,4,5]

SPITZ MELANOCITNE PROLIFERACIJE IN KINAZNE FUZIJE

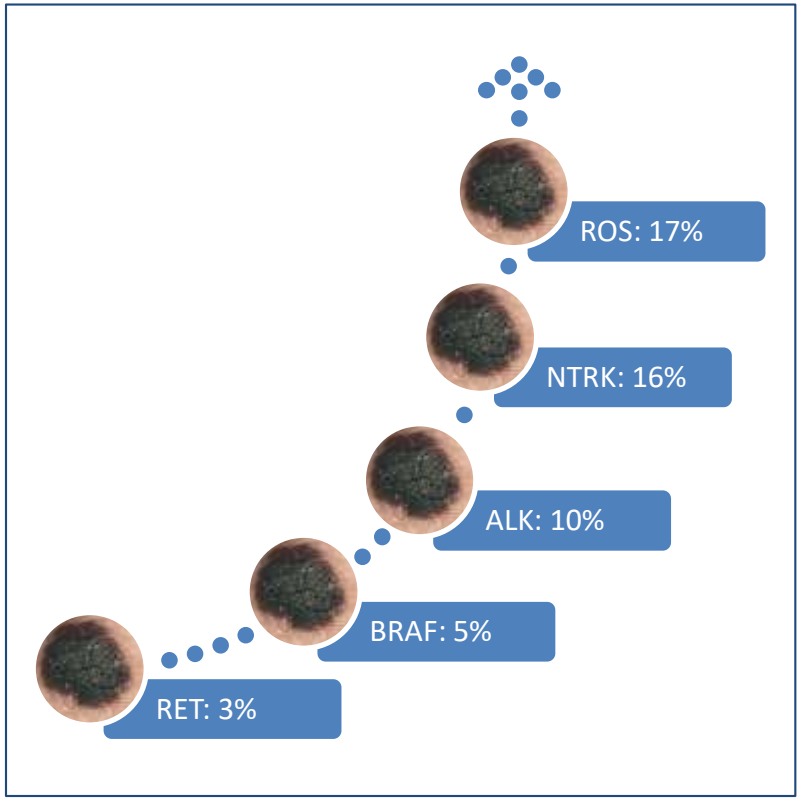
- *INCIDENCA*-



Wiesner T et al. Nat Commun 2014; 5: 3116.

SPITZ MELANOCITNE PROLIFERACIJE IN KINAZNE FUZIJE

- *INCIDENCA*-

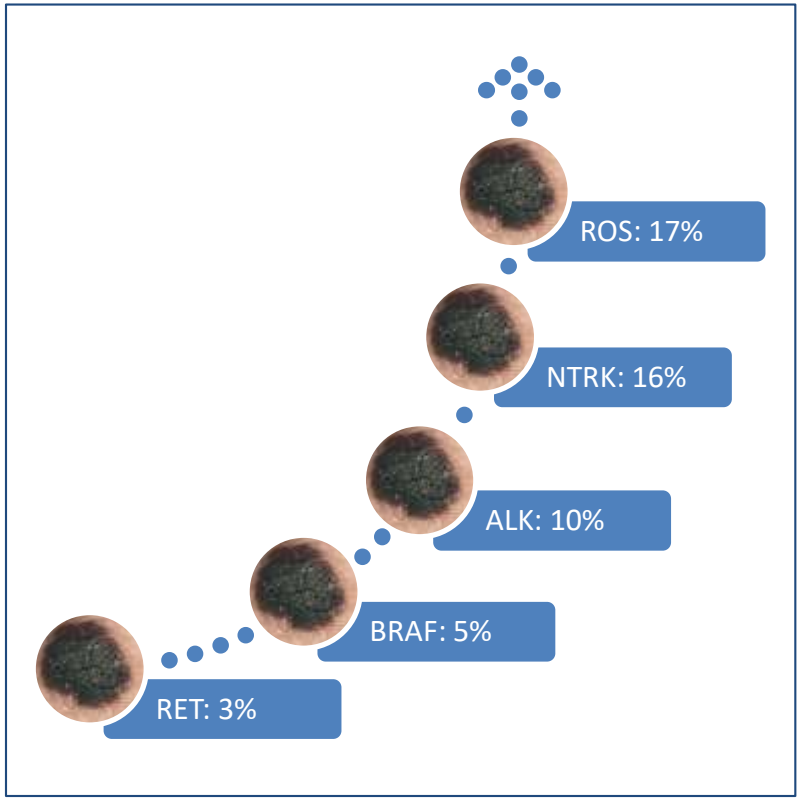


TIP TUMORJA (n=140)	(%)
SPITZ NEVUS (n=75)	55
ATIPIČNI SPITZ TUMOR (n=32)	56
SPITZ MELANOM (n=33)	39

Wiesner T et al. Nat Commun 2014; 5: 3116.

SPITZ MELANOCITNE PROLIFERACIJE IN KINAZNE FUZIJE

- *INCIDENCA*-



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SPITZ MELANOM (n=33)	39

FUZIJE SE MED SEBOJ IZKLJUČUJEJO

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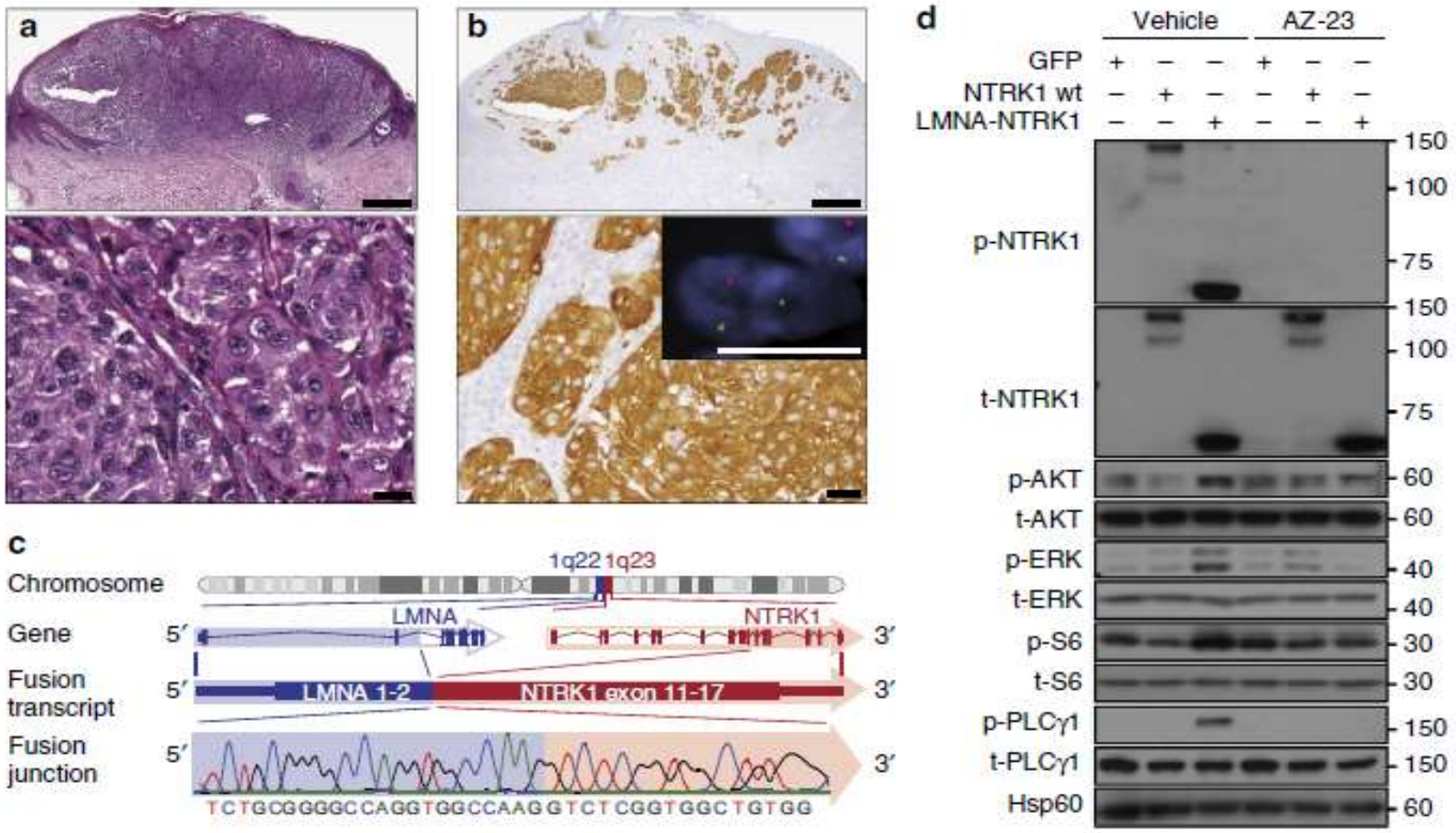
Wiesner T et al. Nat Commun 2014; 5: 3116.

Kinase fusions are frequent in Spitz tumours and spitzoid melanomas



Wiesner T et al. Nat Commun 2014; 5: 3116.

ARTICLE

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Kinase fusions are frequent in Spitz tumours and spitzoid melanomas

FUZIJE *NTRK* SO PRISOTNE V MANJ KOT 20% SPITZ PROLIFERACIJ

- BENIGNE
- INTERMEDIARNE
- MELIGNE

RAZLIČNE FUZIJE SE MED SEBOJ IZKLJUČUJEJO

Wiesner T et al. Nat Commun 2014; 5: 3116.

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ORIGINAL PAPER

NTRK3 kinase fusions in Spitz tumours

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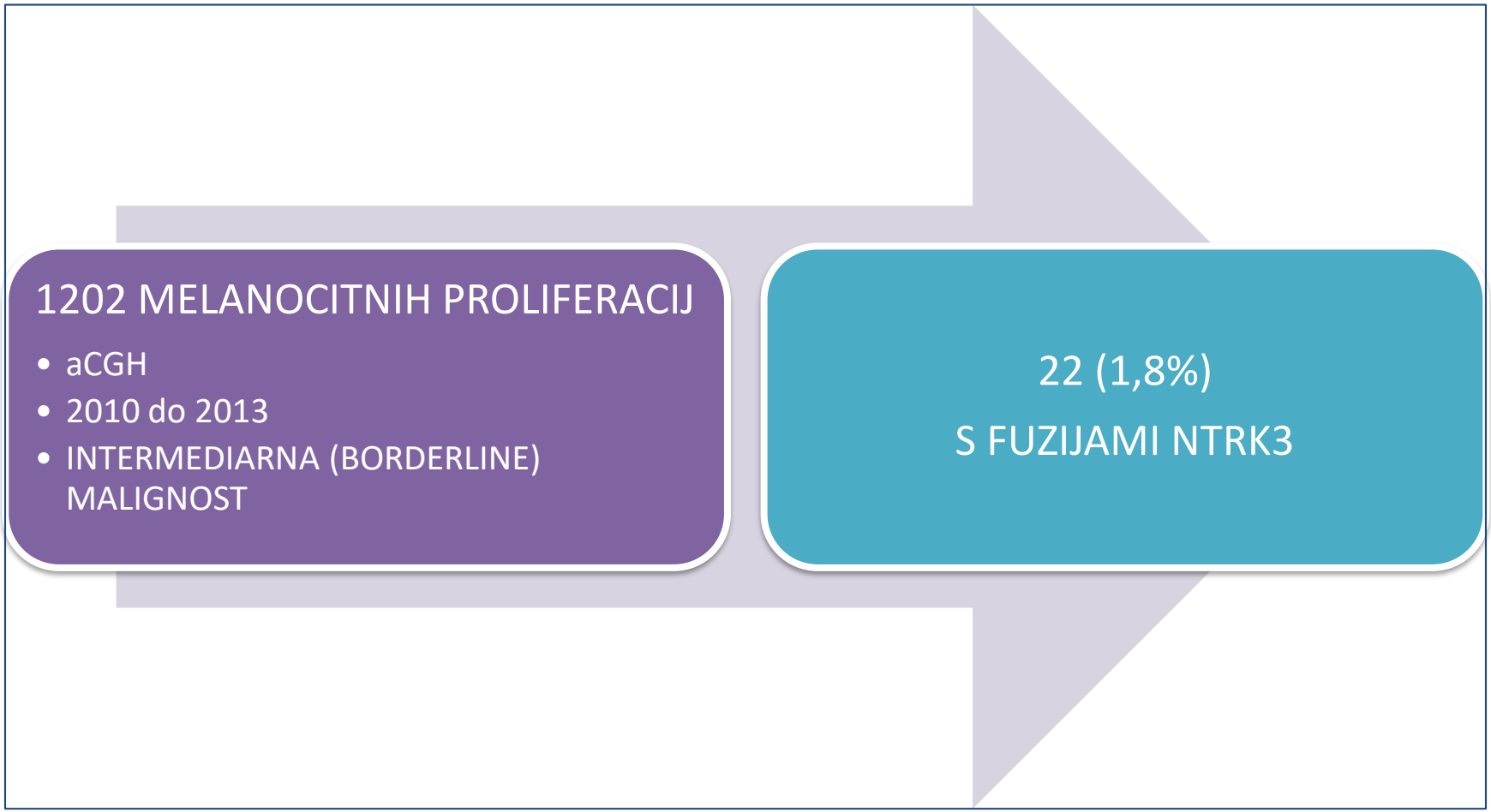
Abstract

Oncogenic fusions in *TRK* family receptor tyrosine kinases have been identified in several cancers and can serve as therapeutic targets. We identified ETV6–NTRK3, MYO5A–NTRK3 and MYH9–NTRK3 fusions in Spitz tumours, and demonstrated that NTRK3 fusions constitutively activate the mitogen-activated protein kinase, phosphoinositide 3-kinase and phospholipase Cγ1 pathways in melanocytes. This signalling was inhibited by DS-6051a, a small-molecule inhibitor of NTRK1/2/3 and ROS1. NTRK3 fusions expand the range of oncogenic kinase fusions in melanocytic neoplasms and offer targets for a small subset of melanomas for which no targeted options currently exist.

SPITZ MELANOCITNE PROLIFERACIJE IN FUZIJE *NTRK3*



YEH I ET AL. J PATHOL 2016; 240: 282-290.

SPITZ TUMORJI – PARTNERSKI GENI PRI *NTRK3* FUZIJAH

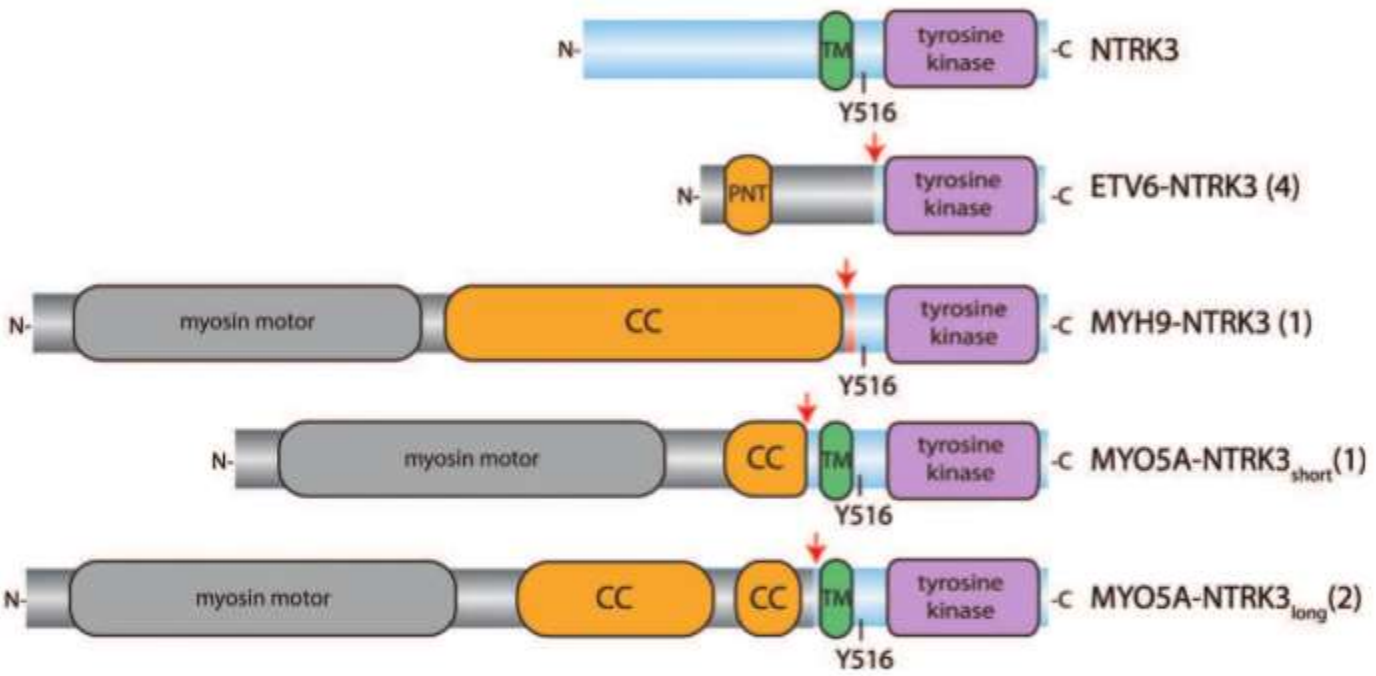


Figure 1. Structural details of NTRK3 fusions. The protein domain structure of NTRK3 is shown at the top with the N-terminal portion to the left and the transmembrane (TM, green) and tyrosine kinase (purple) domains highlighted. Below, the protein domain structures of the NTRK3 fusions identified are shown. Red arrows denote the fusion junctions. The blue backbone denotes the contribution from NTRK3, and the grey backbone denotes the contribution from the N-terminal partner. The domains contributed by the N-terminal partners that mediate dimerization of the native proteins [coiled-coil (CC) and sterile alpha motif) are coloured orange. The red backbone in MYH9–NTRK3 represents the novel sequence contributed by *NTRK3* intron 13.

YEH I ET AL. J PATHOL 2016; 240: 282-290.

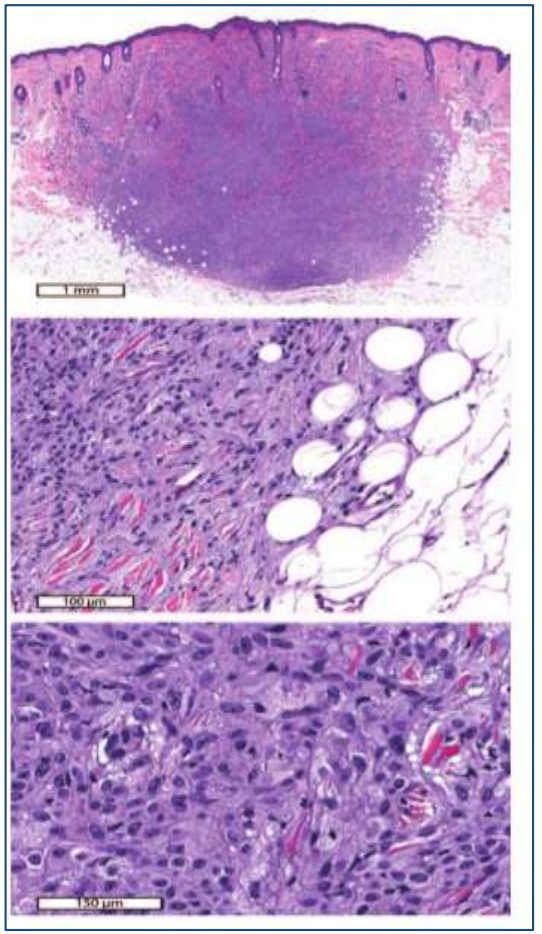
MELANOCITNE PROLIFERACIJA S FUZIJAMI *NTRK3*
- KLINIČNO PATOLOŠKE ZNAČILNOSTI -

MLAJŠI BOLNIKI
(MEDIANA 10 LET)

- 75% MLAJŠI OD 18 LET

SPITZ MORFOLOGIJA

- SPITZ NEVUS (25%)
- ATIPIČNI SPITZ TUMOR (75%)



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2016, Vol. 24(3) 200–206

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Spitz Tumors: Comparison of Histological Features in Relationship to Immunohistochemical Staining for ALK and NTRK1

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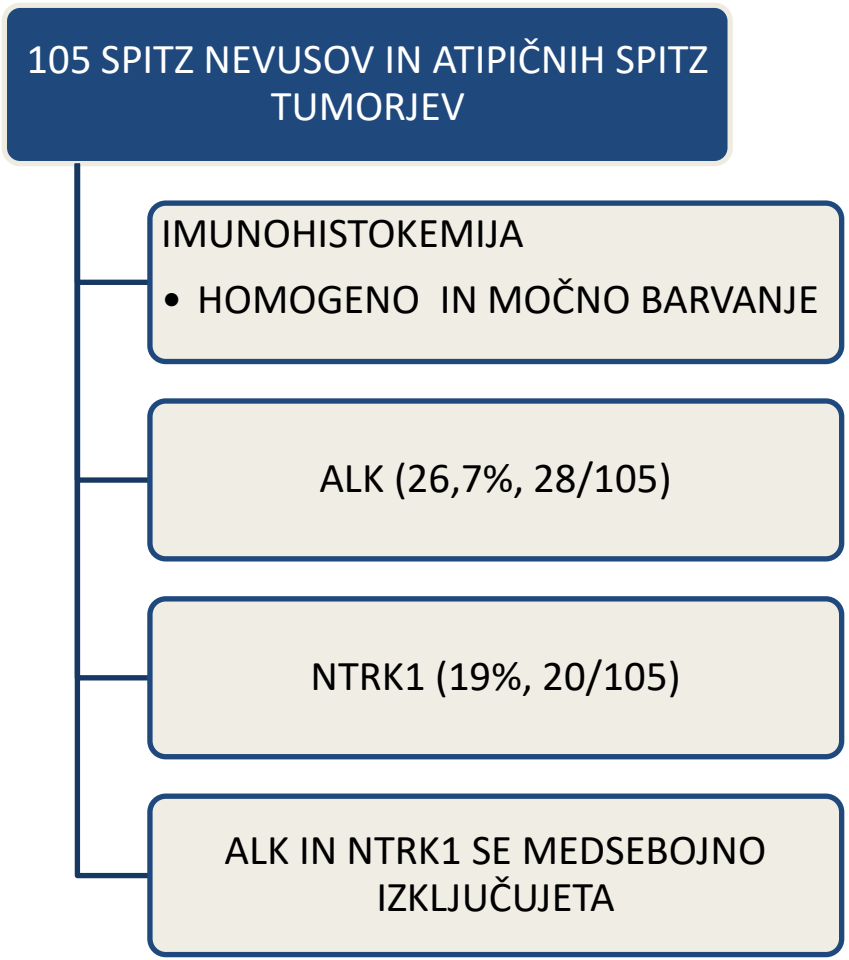
Abstract

Spitz tumors are a group of melanocytic neoplasms with distinct morphological features that tend to affect young individuals. Distinguishing benign from malignant Spitz tumors can be challenging, but cytogenetic and molecular tests have contributed to improvements in diagnostic accuracy. Spitz tumors harbor diverse genetic alterations, including mutations in *HRAS*, loss of *BAP1*, or kinase fusions in *ROS1*, *NTRK1*, *ALK*, *BRAF*, and *RET* genes. Limited data exist on the correlation between histopathological features and kinase fusions. Here, we describe the histopathological features of 105 Spitz tumors (Spitz nevi and atypical Spitz tumors), comparing lesions according to their immunoreactivity for ALK or NTRK1. Intersecting fascicular growth of fusiform melanocytes was seen in all but one ALK-positive tumor (27 of 28 or 96.4%), whereas it was infrequent in NTRK1-positive tumors (5 of 20 or 25.0%) and tumors negative for both ALK and NTRK1 (96.4% vs 25.0% vs 8.7%, $P < .0027$). There was a trend toward ALK-positive tumors being amelanotic compared with NTRK1-positive tumors and combined ALK-/NTRK1-negative tumors (89.3% vs 45% vs 47.4%, respectively, $P = .1023$) and lacking epithelioid cell morphology (0% vs 45.0% vs 41%, respectively, $P = .6985$). In conclusion, this study confirms that although not specific, the growth pattern of intersecting fascicles of amelanotic fusiform melanocytes is strongly associated with ALK expression.

SPITZ MELANOCITNE PROLIFERACIJE IN FUZIJE *NTRK1*



KIURU M ET AL. INT J SURG PATHOL 2016; 24: 200-206.

MELANOCITNE PROLIFERACIJA S FUZIJAMI *NTRK3*
- KLINIČNO PATOLOŠKE ZNAČILNOSTI -

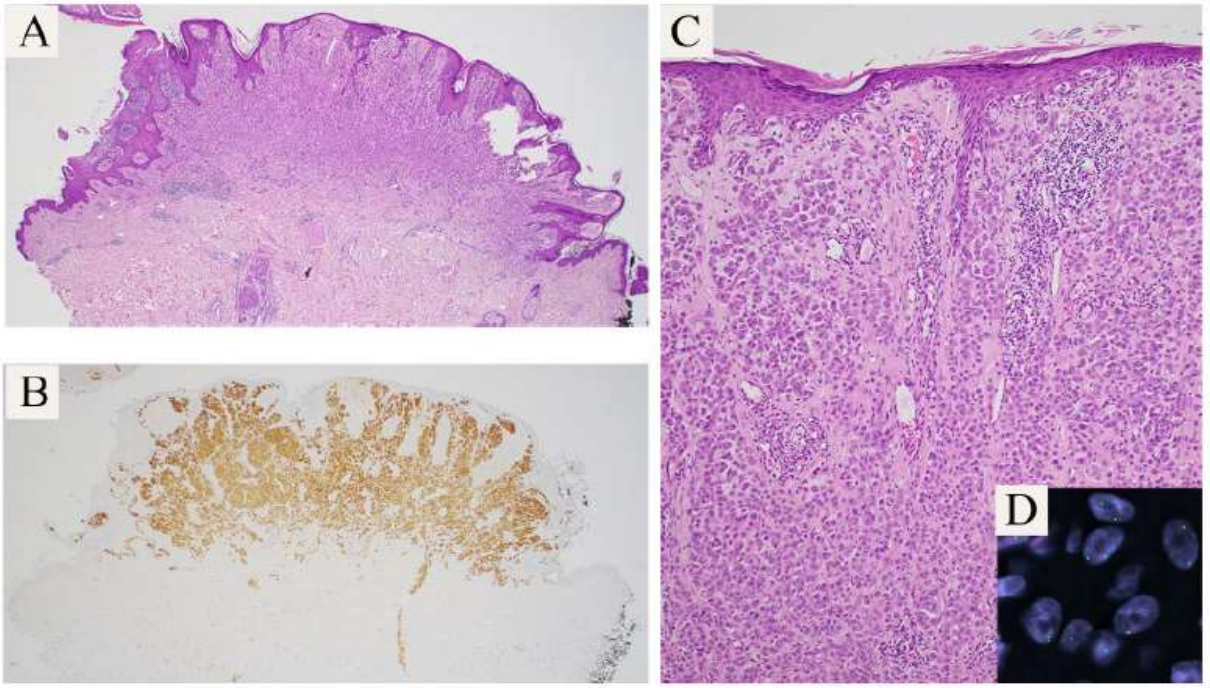


Figure 2. NTRK1-positive Spitz nevus: A. Silhouette of a raised papule of a compound epithelioid melanocytic proliferation (hematoxylin and eosin). B. Immunohistochemistry for NTRK1 highlights a plaque-like growth of dense sheet-like aggregates of melanocytes in the superficial dermis. C. The lesion is predominantly composed of large amelanotic epithelioid melanocytes. Scattered lymphocytic aggregates are present. D. Fluorescence in situ hybridization for NTRK1 documents rearrangement (break-apart probe).

KIURU M ET AL. INT J SURG PATHOL 2016; 24: 200-206.

SPITZ PROLIFERACIJE Z *NTRK1* FUZIJO
- *HISTOLOŠKE ZNAČILNOSTI* -

A Comparison of Morphologic and Molecular Features of *BRAF*, *ALK*, and *NTRK1* Fusion Spitzoid Neoplasms

Sapna M. Amin, MD, Alexandra M. Haugh, BA,* Christina Y. Lee, BA,* Bin Zhang, MS,* Jeffrey A. Bubley, BA,* Emily A. Merkel, BA,* Anna Elisa Verzi, MD,* and Pedram Gerami, MD*†*

Abstract: Recent studies have identified translocations involving the kinase domains of *ALK*, *NTRK1*, *BRAF*, *RET*, and *ROS* in spitzoid neoplasms. Subsequent studies have also characterized morphologic features corresponding to *ALK* and *NTRK1* translocations. In this study, we sought to further compare morphologic features across a range of 49 genetically defined spitzoid neoplasms with *ALK*, *NTRK1*, *BRAF*, or *RET* fusions to determine discriminating features. We also compared them with a group of 22 spitzoid neoplasms, which were confirmed to be negative for fusions in *ALK*, *NTRK1*, *BRAF*, and *RET*. Features with the highest discriminatory value included diameter of the lesion, dermal architecture, and certain cytomorphologic features. Specifically, cases with a large diameter (≥9 mm) and wedge-shaped, plexiform dermal architecture of nests of large, spindle-shaped cells were most likely to have an *ALK* fusion. *NTRK1*-fused cases were most likely of the fusions to have Kamino bodies and were typically arranged in smaller nests with smaller predominantly spindle-shaped cells, occasionally forming rosettes. *BRAF* fusion cases were the only fusion subtype to have a predominance of epithelioid cells, were less organized in nests, and commonly had a sheet-like growth pattern or dysplastic Spitz architecture. *BRAF* fusion cases were most likely to have high-grade nuclear atypia, to be diagnosed as spitzoid melanoma, to have a positive result by melanoma fluorescence in situ hybridization assay, and to develop copy number gains in the kinase domain of the fusion protein. On the basis of experience from this cohort, *BRAF*-fused cases appear most likely to progress to melanoma.

Key Words: *ALK*, *NTRK1*, *BRAF*, *RET*, *ROS*, Spitz tumors, melanoma, spitzoid melanoma, Spitz nevi, FISH

(*Am J Surg Pathol* 2017;41:491–498)

The initiating genomic event for a significant proportion of spitzoid neoplasms has now been described. This includes gene mutations and/or copy number gains involving *HRAS*,[1] and, more recently, a number of translocations involving either *BRAF*, *RET*, *ROS*, *ALK*, or *NTRK1* have been described. The translocations in these cases result in loss of the normal regulatory domain of these proteins and ultimately activation of the kinase domain.[2] There have been a number of studies describing the morphologic features of spitzoid neoplasms with *HRAS* mutations, *ALK* translocations, and *NTRK1* translocations.[1–5] However, the overall data are relatively limited, particularly with regard to studying the morphologic features of the *BRAF*-translocated, *RET*-translocated, and *ROS*-translocated spitzoid neoplasms.

The above translocations have been described in Spitz nevi, Spitz tumors, and spitzoid melanoma. Yet there is relatively sparse information regarding whether any of these translocation subgroups of spitzoid neoplasms is at a higher risk of transforming to melanoma and what results to expect when assessing these cases by fluorescence in situ hybridization (FISH) or comparative genomic hybridization (CGH). For example, using the *ALK* break-apart probe, our group recently noted copy number gains involving the *ALK* kinase domain of *ALK*-translocated spitzoid tumors that lack sufficient morphologic and molecular criteria of a diagnosis of melanoma.[6] In the current study, we describe a series of 17 *ALK*-translocated spitzoid neoplasms, 17 *NTRK1*-translocated spitzoid neoplasms, 14 *BRAF*-translocated neoplasms—13 of which were spitzoid and 1 of which had balloon cell morphology—1 *RET*-translocated spitzoid

SPITZ PROLIFERACIJE Z *NTRK1* FUZIJO
- *HISTOLOŠKE ZNAČILNOSTI* -

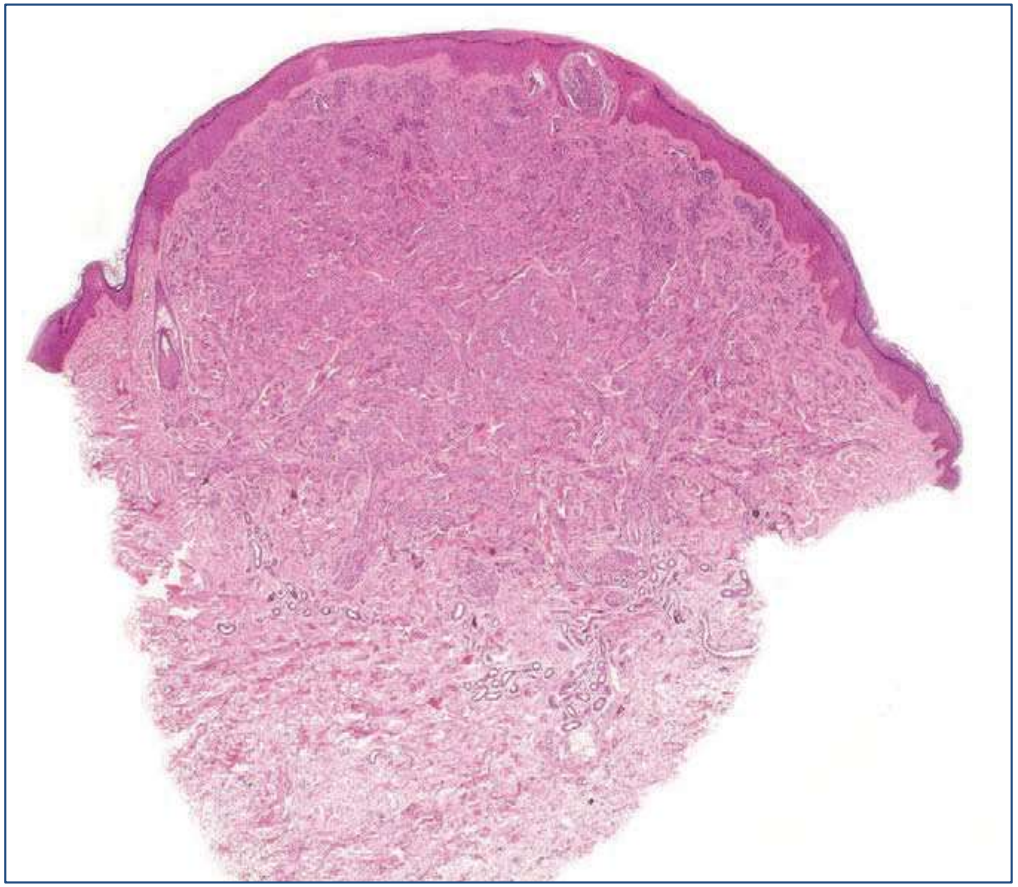
- KLINASTA SILHUETA
- KAMINO-JEVA TELESCA
- MANJŠA MELANOCITNA GNEZDA
- VRETENASTI MELANOCITI
- MELANOCITNE ROZETE

AMIN SM ET AL. AM J SURG PATHOL 2017; 41: 491-498.

SPITZ PROLIFERACIJE Z *NTRK1* FUZIJO

HISTOLOŠKE ZNAČILNOSTI

- KLINASTA SILHUETA
- KAMINO-JEVA TELESCA
- MANJŠA MELANOCITNA GNEZDA
- VRETENASTI MELANOCITI
- MELANOCITNE ROZETE



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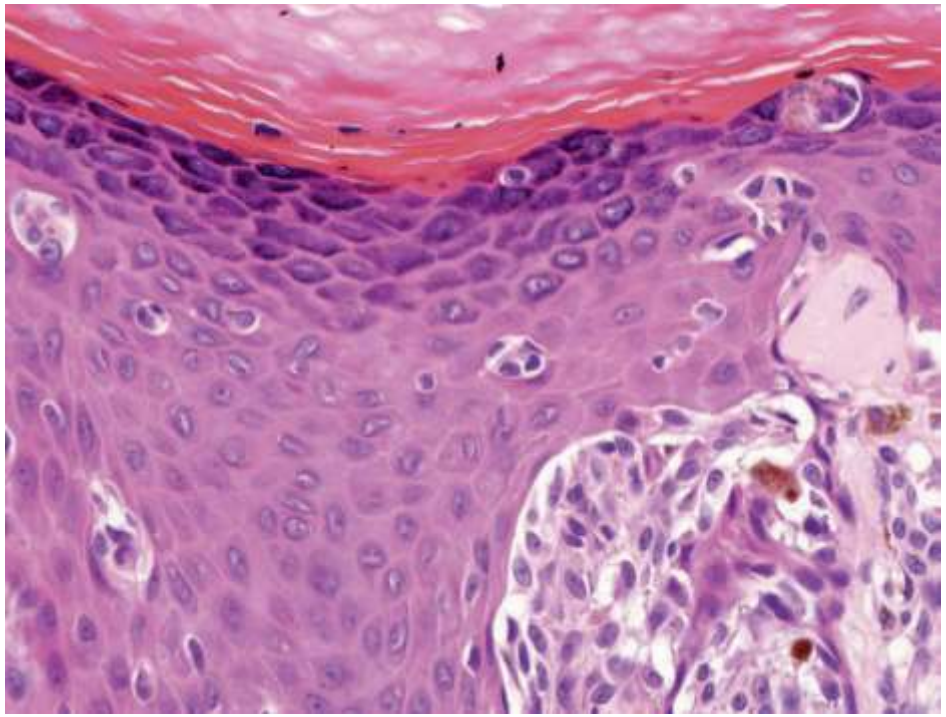

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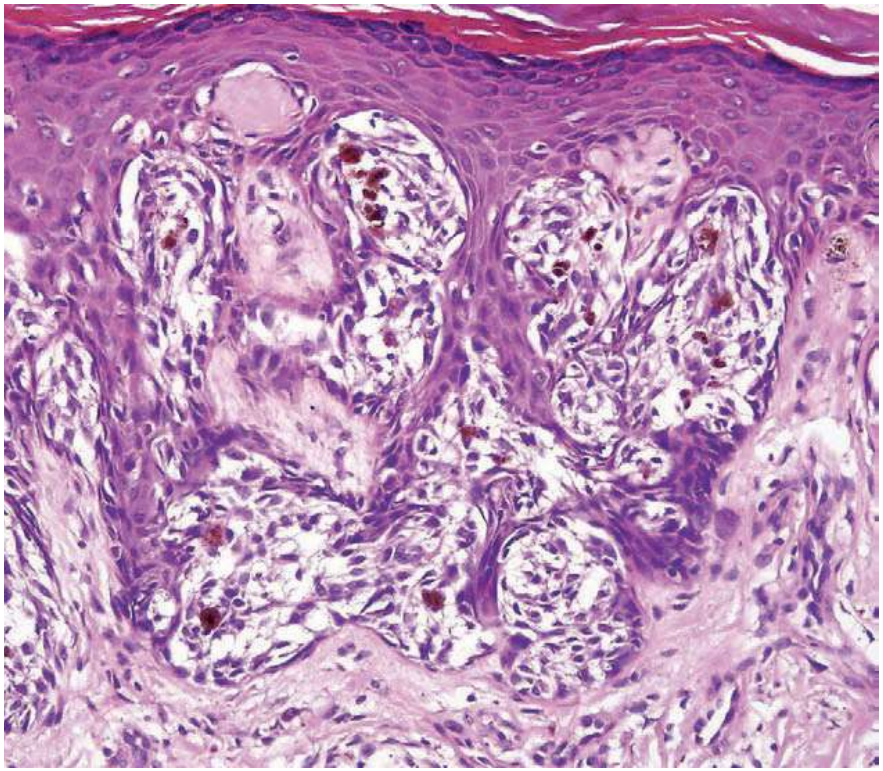

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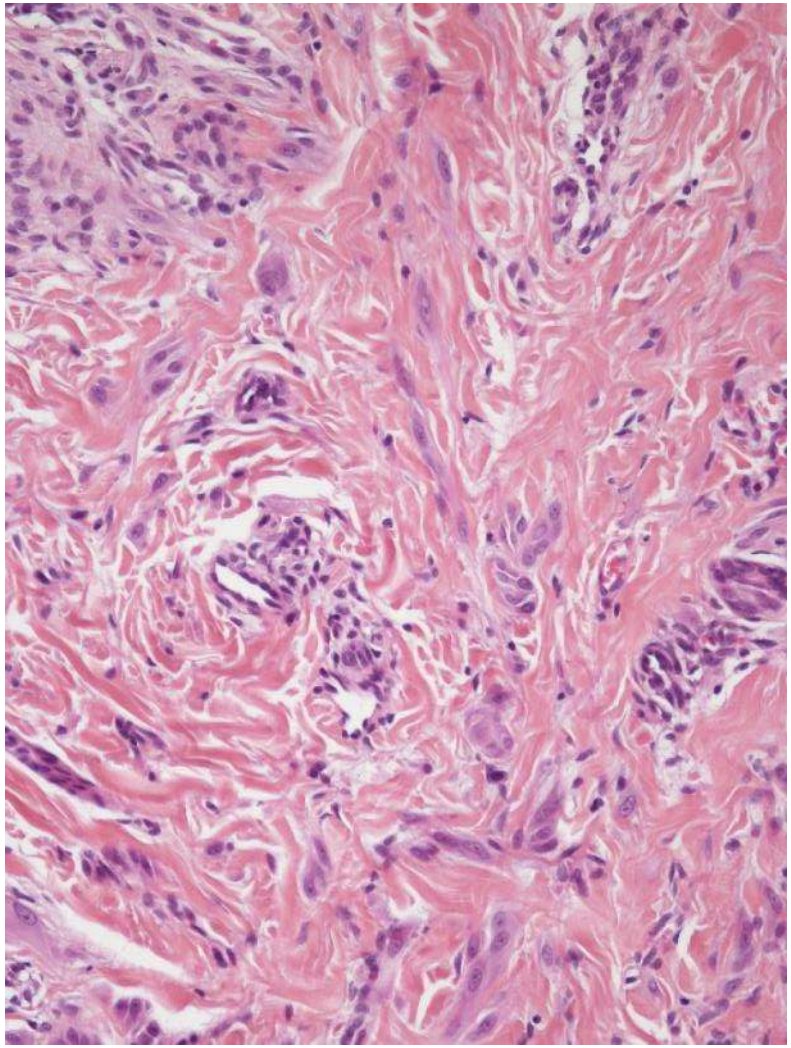

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HISTOLOŠKE ZNAČILNOSTI

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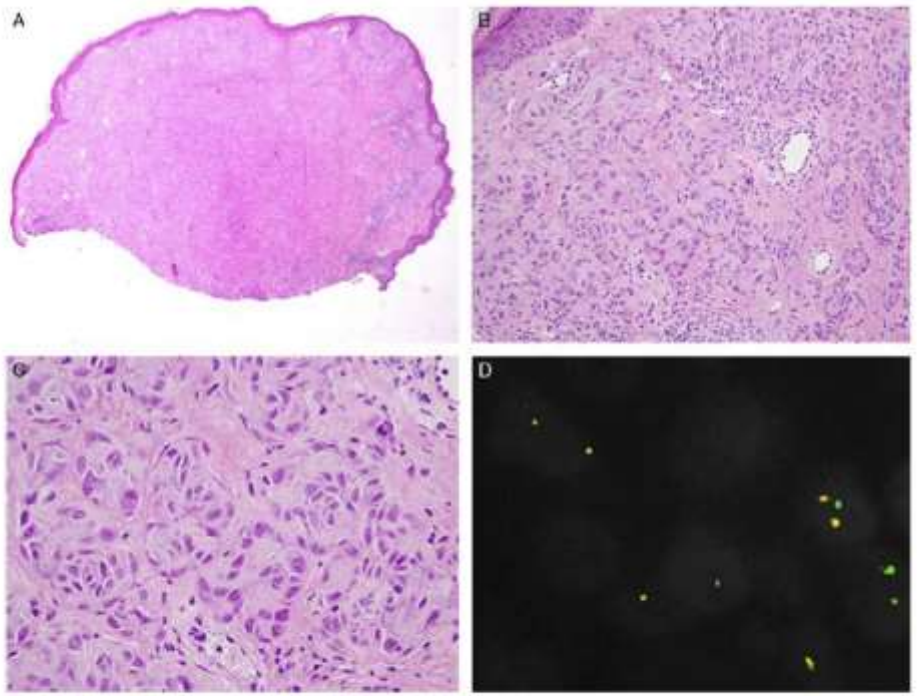


FIGURE 2. A, *NTRK1* fusion Spitz tumor organized in relatively smaller sized nests (H&E). B, Within the nests, there are smaller-sized spitzoid melanocytes with a predominance of spindle-shaped cytomorphology (H&E). C, A rosette-like pattern of the nuclei can occasionally be seen (H&E). D, The last panel shows FISH with a break-apart probe targeting the kinase domain of *NTRK1* with the yellow fluorochrome and the regulatory domain with the green fluorochrome (FISH). There are cells with separation of the yellow and green signals as well as cells with the yellow signal with no accompanying green signal, suggestive of an *NTRK1* fusion. H&E indicates hematoxylin and eosin.

AMIN SM ET AL. AM J SURG PATHOL 2017; 41: 491-498.

Filigree-like Rete Ridges, Lobulated Nests, Rosette-like Structures, and Exaggerated Maturation Characterize Spitz Tumors With *NTRK1* Fusion

Iwei Yeh, MD, PhD, Klaus J. Busam, MD,† Timothy H. McCalmont, MD,* Philip E. LeBoit, MD,* Daniel Pissaloux, PhD,‡ Laurent Alberti, PhD,‡ Arnaud de la Fouchardière, MD, PhD,‡§ and Boris C. Bastian, MD, PhD**

Abstract: Activating *NTRK1* fusions have been described as oncogenic events across the spectrum of Spitz tumors. Herein we report a series of 38 Spitz tumors with *NTRK1* fusion. These Spitz tumors have distinctive histopathologic features characterized by filigree-like rete ridges which are elongated, thin and branched, dermal melanocytes arranged in a rosette-like configuration, and marked diminishment of melanocyte size with descent into the dermis. These features are distinct from those of other genetically defined subtypes of Spitz tumors and can aid in microscopic diagnosis and help prioritize in case selection for molecular testing in the rare patients that need targeted therapy.

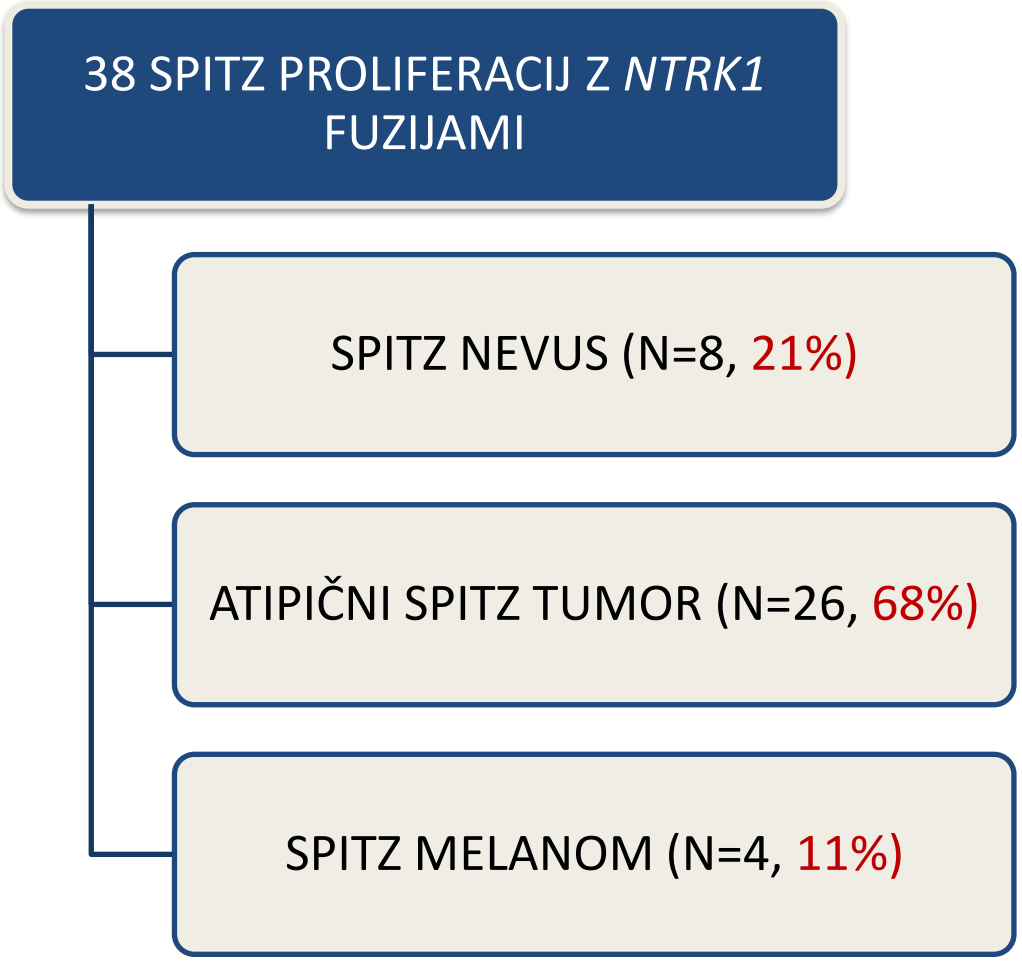
Key Words: Spitz nevus, Spitz tumor, Spitz melanoma, NTRK1, kinase fusion

(*Am J Surg Pathol* 2019;43:737–746)

pending on the presence or absence of features worrisome for melanoma, tumors are classified as benign (Spitz nevus), intermediate (atypical Spitz tumor or melanocytic tumor of unknown potential [MELTUMP]), or malignant (malignant Spitz tumor or spitzoid melanoma depending on their genetic characteristics).[2] There is considerable uncertainty and interobserver variation in categorizing Spitz tumors into these 3 subcategories.[3–5] Although some atypical Spitz tumors metastasize to regional lymph nodes they rarely cause distant metastases,[6,7] indicating that bona fide Spitz melanomas are rare. Most Spitz nevi occur during childhood, adolescence, or early adult life[8] and their incidence decreases with age, a pattern opposite to that of cutaneous melanoma.

Spitz nevi lack *BRAF* or *NRAS* mutations which are found in a mutually exclusive pattern in the majority of common and congenital nevi and cutaneous melanomas.[9(p2),10–14]

SPITZ PROLIFERACIJE IN *NTRK1* FUZIJA
- HISTOLOŠKE ZNAČILNOSTI -



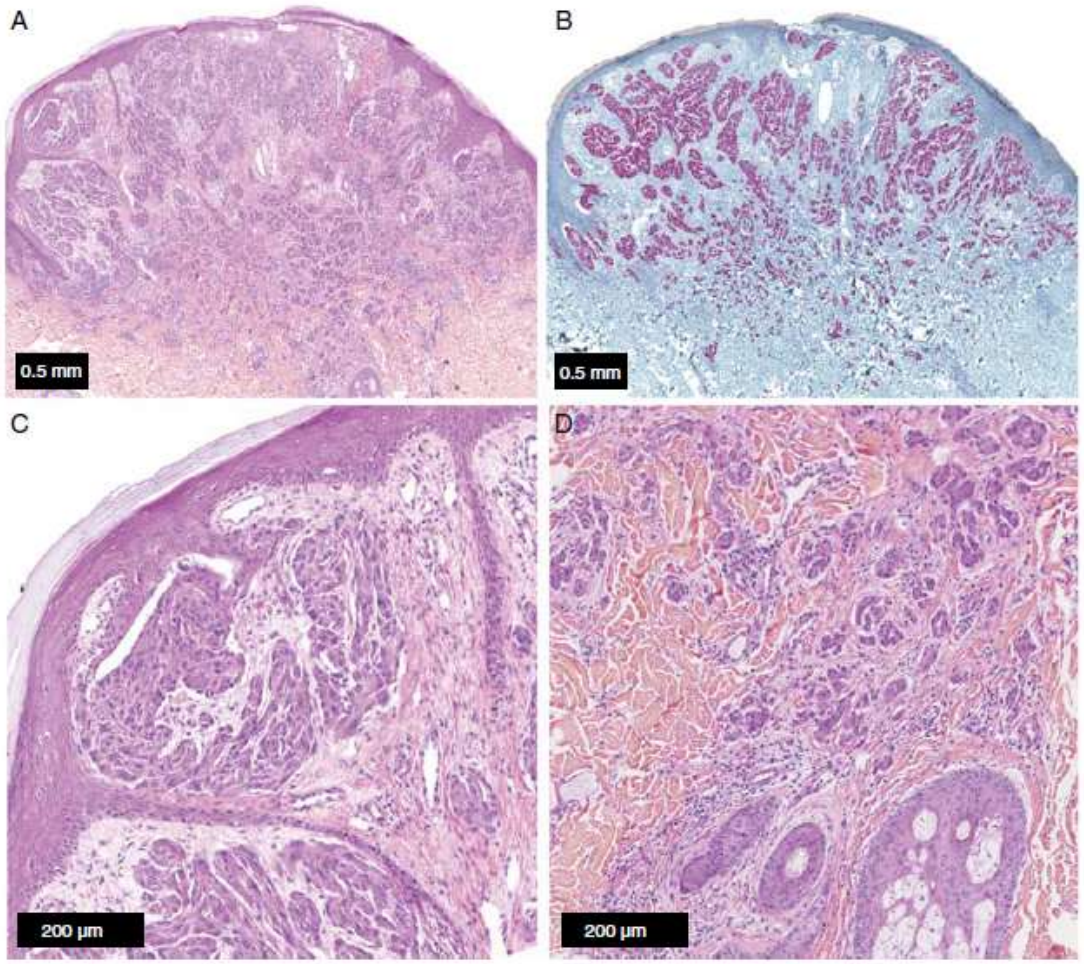
YEH I. AM J SURG PATHOL 2019; 43: 737-746.

SPITZ TUMORJI IN *NTRK1* FUZIJE
- HISTOLOŠKE ZNAČILNOSTI -

YEH I. AM J SURG PATHOL 2019; 43: 737-746.

SPITZ TUMORJI IN *NTRK1* FUZIJE
- HISTOLOŠKE ZNAČILNOSTI -

- EPIDERMALNI POGANJKI
  - TANKI
  - RAZVEJANI
- LOBULIRANA GNEZDA MELANOCITOV
- ROZETAM PODOBNE STRUKTURE
- IZRAZITA MATURACIJA MELANOCITOV



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SPITZ TUMORJI IN *NTRK1* FUZIJE
- HISTOLOŠKE ZNAČILNOSTI -

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  - TANKI
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- ROZETAM PODOBNE STRUKTURE
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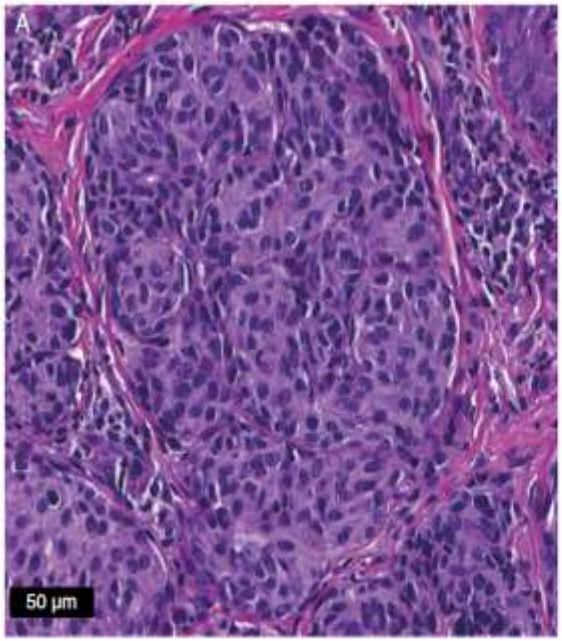


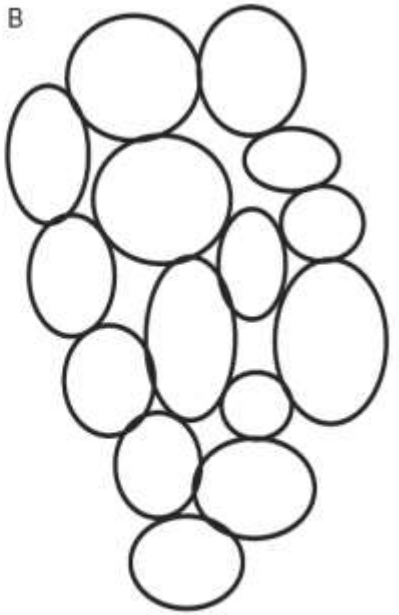


FIGURE 4. Lobulated nests in Spitz tumors with *NTRK1* fusion. A large lobulated nest from case 7 (A), accompanied by a diagram highlighting the internal small nests within (B).

YEH I. AM J SURG PATHOL 2019; 43: 737-746.

SPITZ TUMORJI IN *NTRK1* FUZIJE
- HISTOLOŠKE ZNAČILNOSTI -

- EPIDERMALNI POGANJKI
  - TANKI
  - RAZVEJANI
- LOBULIRANA GNEZDA MELANOCITOV
- ROZETAM PODOBNE STRUKTURE
- IZRAZITA MATURACIJA MELANOCITOV

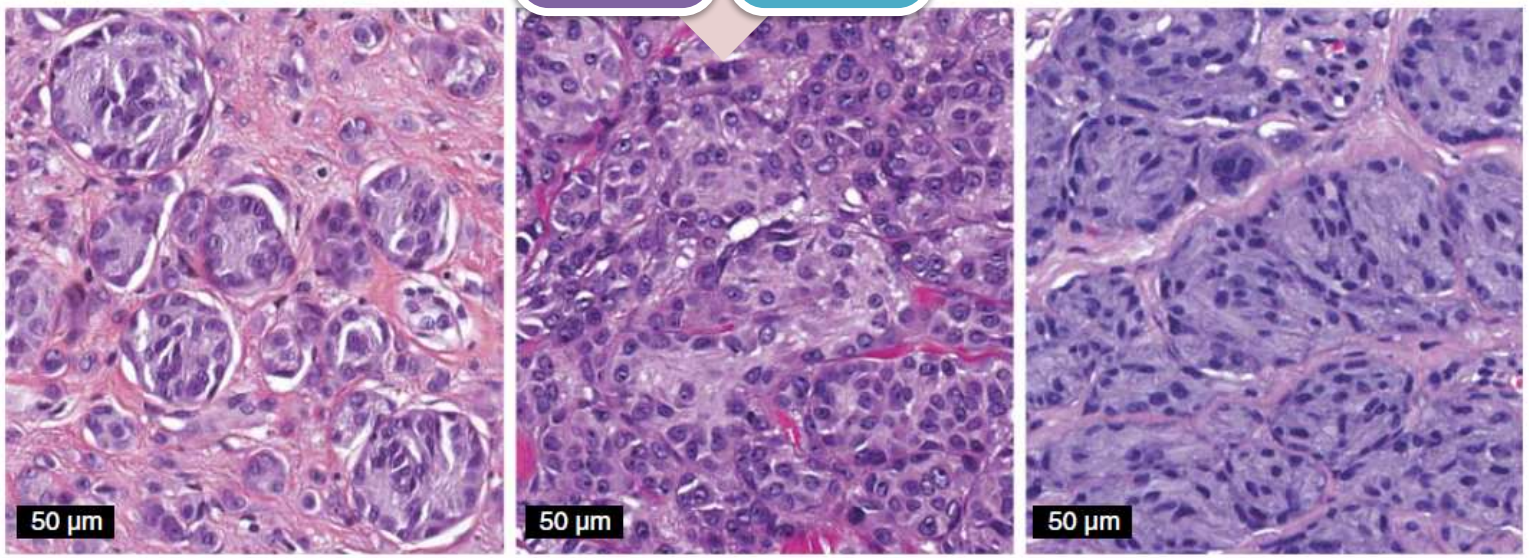
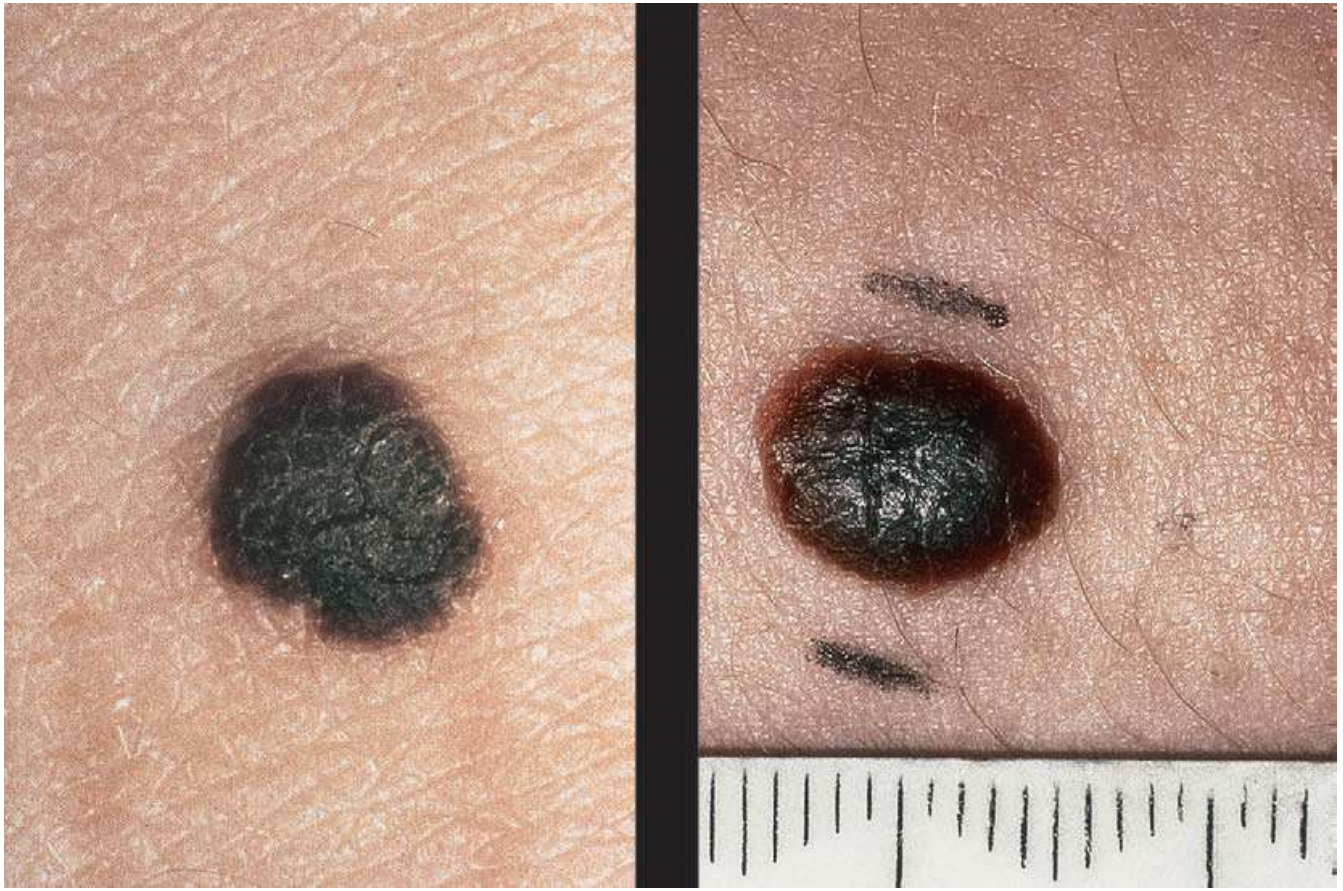


FIGURE 5. Rosette-like structures in Spitz tumors with *NTRK1* fusion. From left to right: case 30, case 37, case 14.

YEH I. AM J SURG PATHOL 2019; 43: 737-746.

REEDOV NEVUS IN *NTRK* FUZIJE



Genomic Fusions in Pigmented Spindle Cell Nevus of Reed

Timothy VandenBoom, MD, Victor L. Quan, BA,* Bin Zhang, MS,* Erin M. Garfield, BS,* Betty Y. Kong, MD, PhD,* Maria C. Isales, MD, MPH,* Elnaz Panah, MD,* Catherine Igartua, PhD,† Timothy Taxter, MD,† Nike Beaubier, MD,† Kevin White, PhD,† and Pedram Gerami, MD**

Abstract: Recent molecular studies of spitzoid neoplasms have identified mutually exclusive kinase fusions involving ROS1, ALK, RET, BRAF, NTRK1, MET, and NTRK3 as early initiating genomic events. Pigmented spindle cell nevus (PSCN) of Reed is a morphologic variant of Spitz and may be very diagnostically challenging, having histologic features concerning for melanoma. Their occurrence in younger patients, lack of association to sun exposure, and rapid early growth phase similar to Spitz nevi suggest fusions may also play a significant role in these lesions. However, to date, there is little data in the literature focused on the molecular characterization of PSCN of Reed with next-generation sequencing. We analyzed a total of 129 melanocytic neoplasms with RNA sequencing including 67 spitzoid neoplasms (10 Spitz nevi, 44 atypical Spitz tumors, 13 spitzoid melanomas) and 23 PSCN of Reed. Although only 2 of 67 (3.0%) of spitzoid lesions had NTRK3 fusions, 13 of 23 (57%) of PSCN of Reed harbored NTRK3 fusions with 5′ partners ETV6 (12p13) in 2 cases and MYO5A (15q21) in 11 cases. NTRK3 fusions were confirmed with a fluorescent in situ hybridization break-apart probe. The presence of a NTRK3 fusion correlated with younger age ($P=0.021$) and adnexal extension ($P=0.001$). Other minor fusions identified in PSCN of Reed included MYO5A-MERTK (2), MYO5A-ROS1, MYO5A-RET, and ETV6-PITX3 leading to a total of 78% with fusions. Our study suggests that the majority of PSCN of Reed are the result of genomic fusions, and the most frequent and characteristic genomic aberration is an NTRK3 fusion.

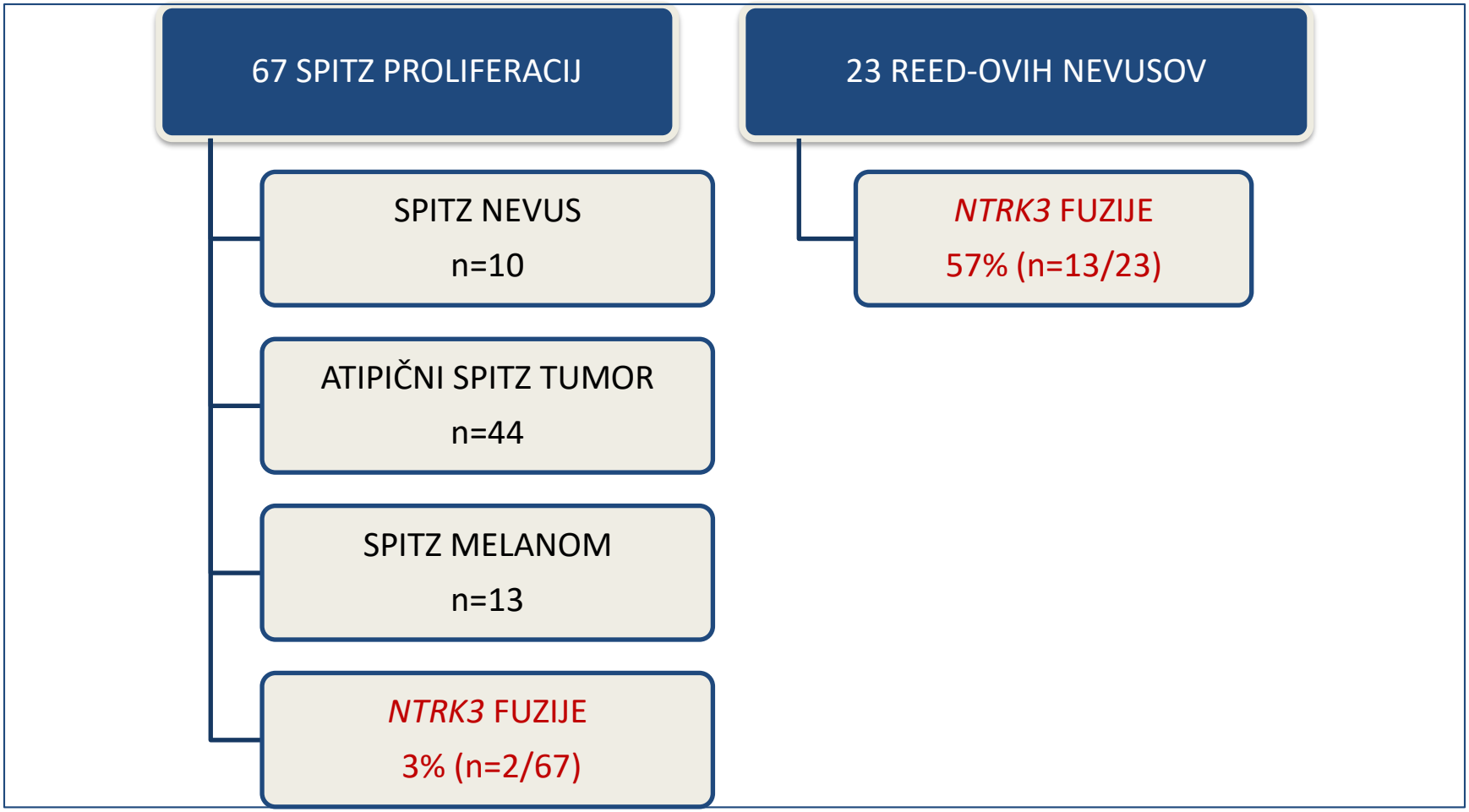
Key Words: pigmented spindle cell nevus, Reed nevus, NTRK3, kinase fusions, melanocytic nevus, melanoma, spitz nevus, spitz tumor

(*Am J Surg Pathol* 2018;42:1042–1051)

The diagnosis of Spitz and Reed nevi can be highly challenging and these neoplasms can be easily misdiagnosed as melanoma.[1–4] Integration of genomic findings with morphology may assist in more precise classification of these lesions with better ability to predict clinical behavior. Recent studies have described specific, mutually exclusive kinase fusions that occur in roughly 50% of spitzoid neoplasms including ROS1, ALK, RET, BRAF, NTRK1, MET, and more recently NTRK3. Some of these fusions yield characteristic morphologic features. Rearrangement to a variable partner results in constitutive activation of the kinase and subsequent activation of downstream signaling pathways with effects on factors such as cell size, proliferation, and survival.[5–8] Kinase fusions are present across the spectrum of benign to intermediate grade to malignant spitzoid lesions and are therefore likely early initiating genomic events.

Pigmented spindle cell nevus (PSCN) of Reed was first described in 1975 by Reed et al[9] as a distinct entity of melanocytic nevus but is now regarded as a heavily pigmented and spindle cell variant of Spitz nevus. Like Spitz nevi, Reed nevi may morphologically show confluent and expansile junctional nesting, pagetoid dispersion, and cytologic atypia raising concern for melanoma. As in many Spitz nevi, PSCN of Reed often occurs in younger patients, occurs in a distribution independent of sun exposure, and may have a rapid early growth phase followed by senescence suggesting a high likelihood that fusions play a role in their development. While there have been numerous recent studies focused on the genetic characterization of the different morphologic variants of Spitz nevus,[10–13] only minimal data is available in the literature regarding the genetic characterization of PSCN of Reed

SPITZ PROLIFERACIJE IN *NTRK* FUZIJE



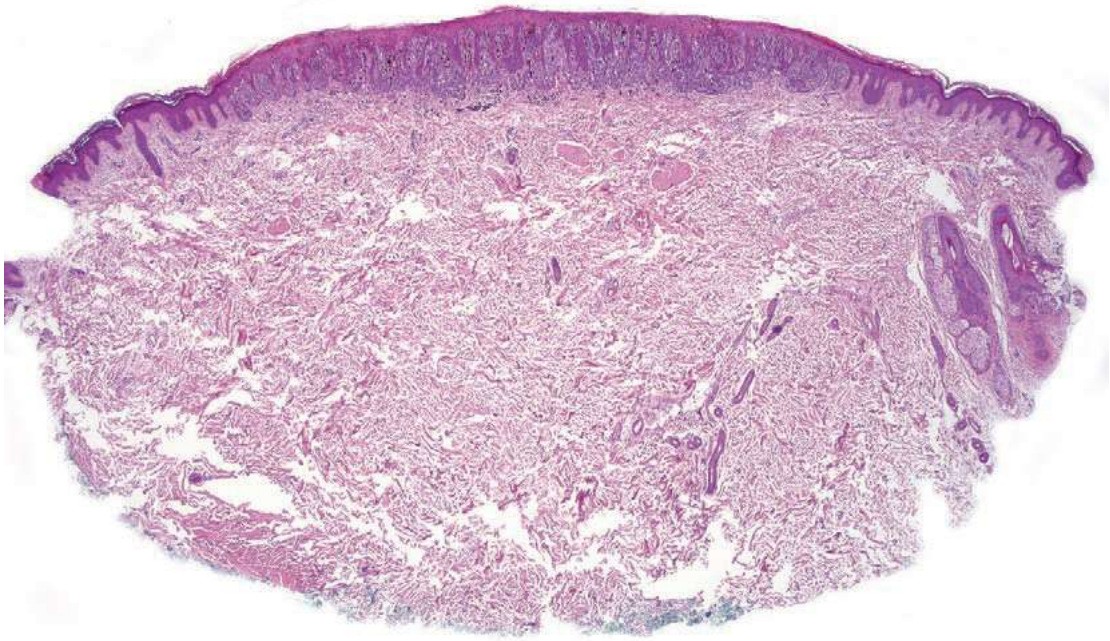
VAN DEN BOOM T ET AL. AM J SURG PATHOL 2018; 42: 1042-1051.

REED NEVUS

- *HISTOLOŠKE ZNAČILNOSTI* -

REEDOV NEVUS

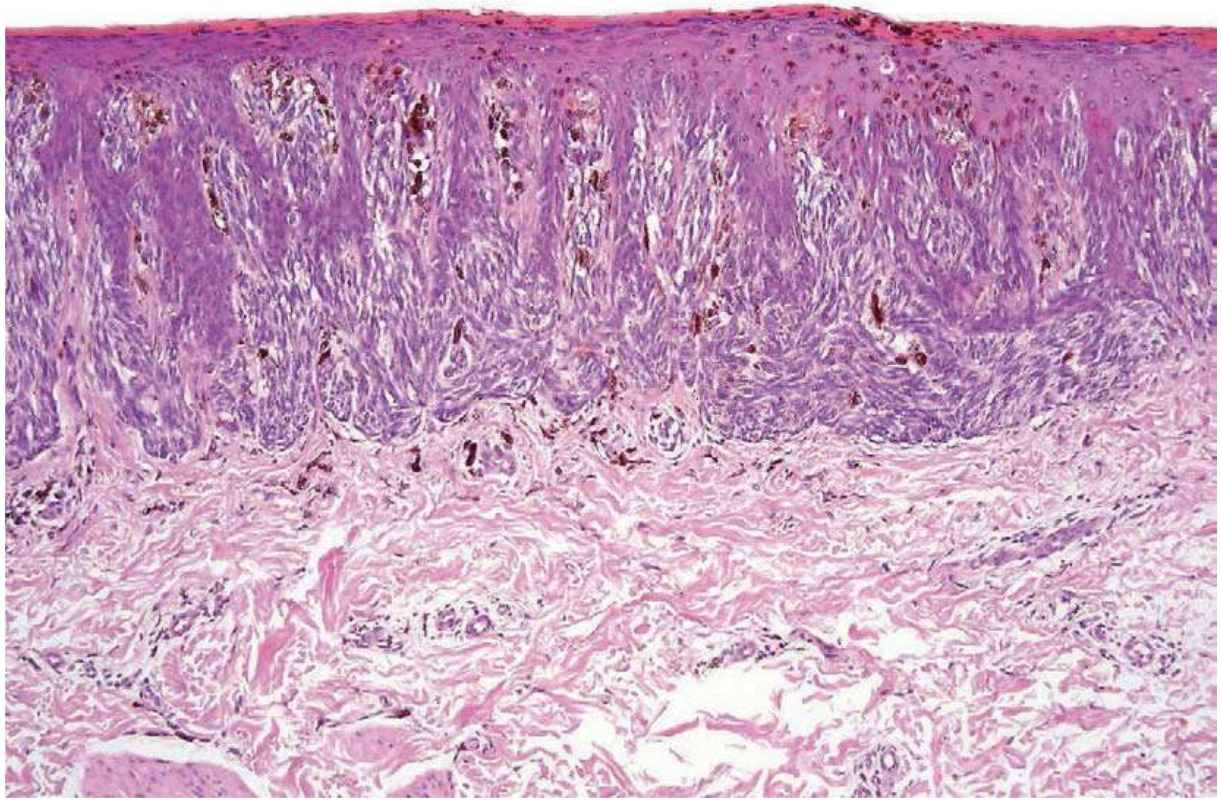
- SIMETRIJA
- OSTRA RAZMEJENOST
- DNO PARALELNO Z EPIDERMISOM
- HIPERPLAZIJA EPIDERMISA



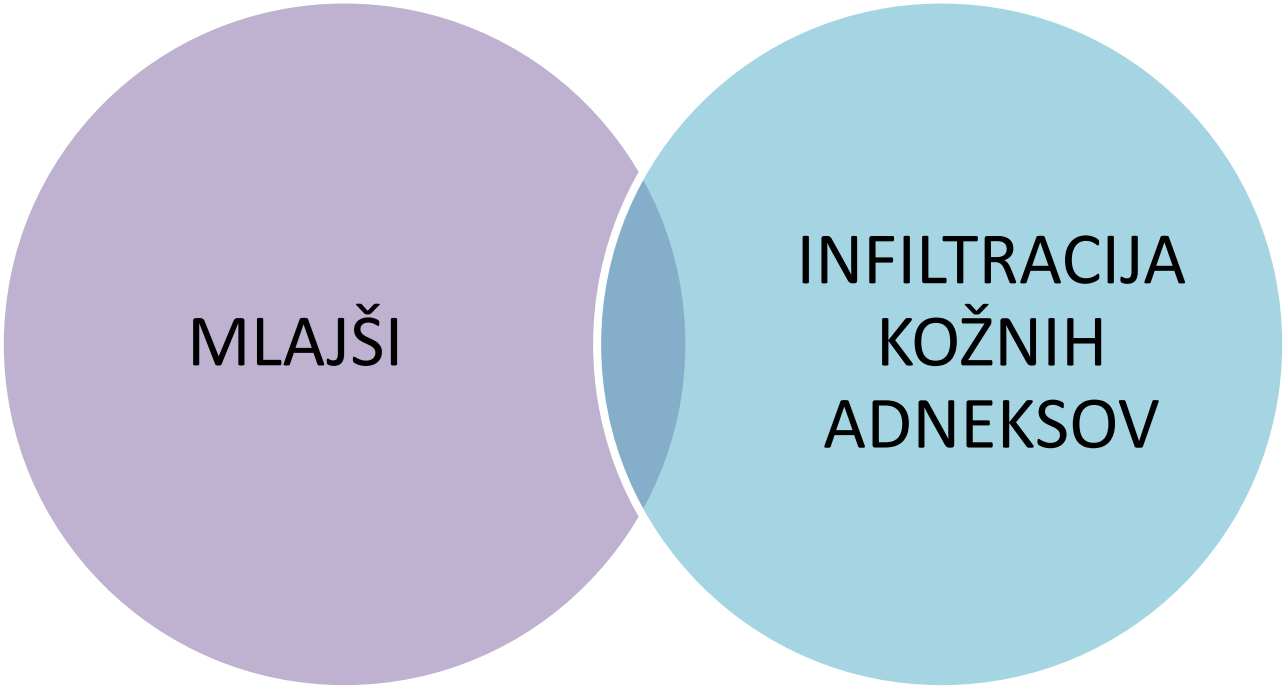
REED NAEVUS

- *HISTOLOGICAL FEATURES* -

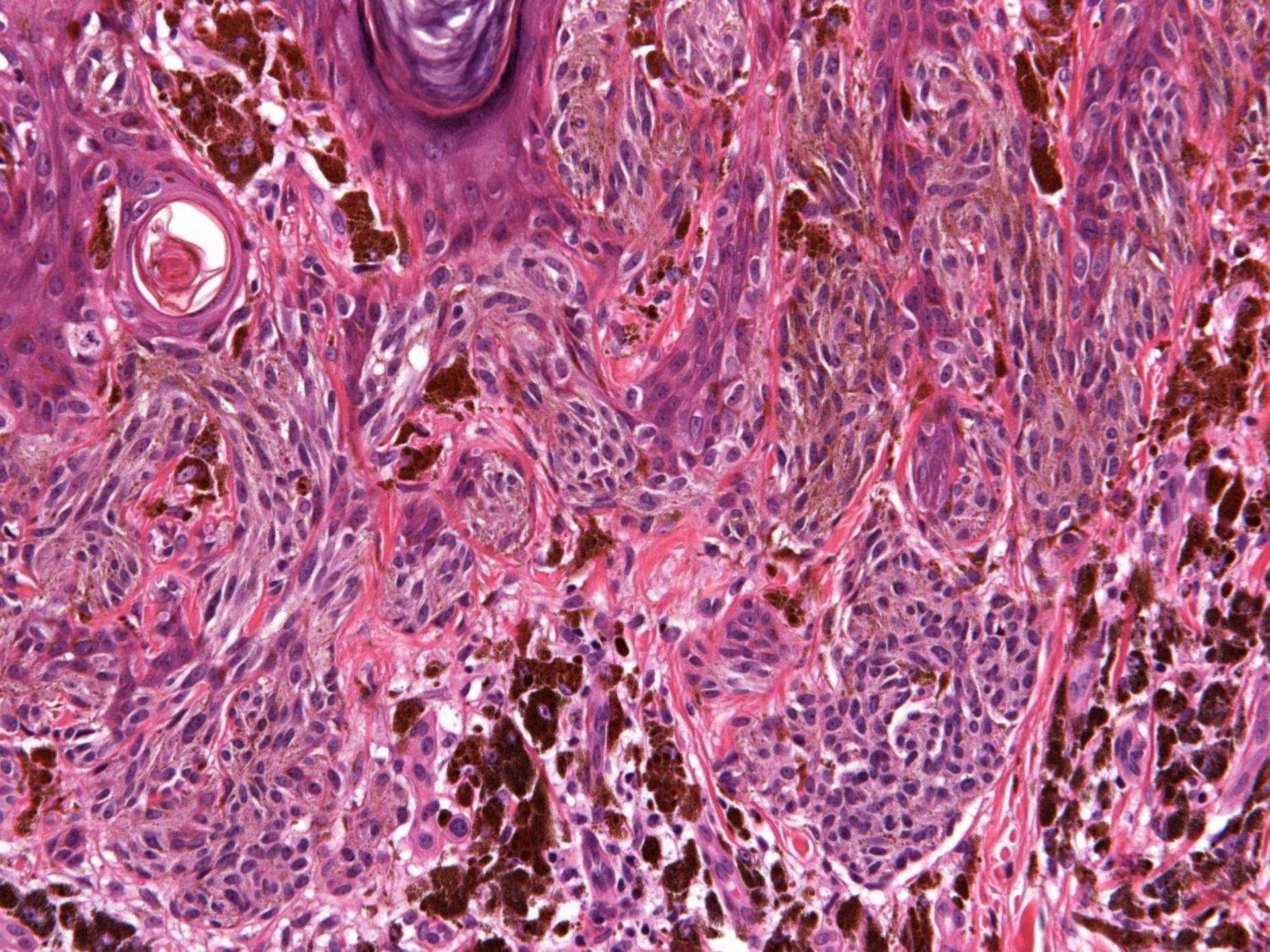
- JUNKCIJSKA GNEZDA
  - HRUŠKASTE OBLIKE GNEZD
  - VRETENASTE CELICE
  - PODOLGOVATA JEDRA
  - PIGMENTIRANOST



NTRK3 FUZIJE IN REEDOV NEVUS
- KLINIČNO-PATOLOŠKE ZNAČILNOSTI -



VAN DEN BOOM T ET AL. AM J SURG PATHOL 2018; 42: 1042-1051.



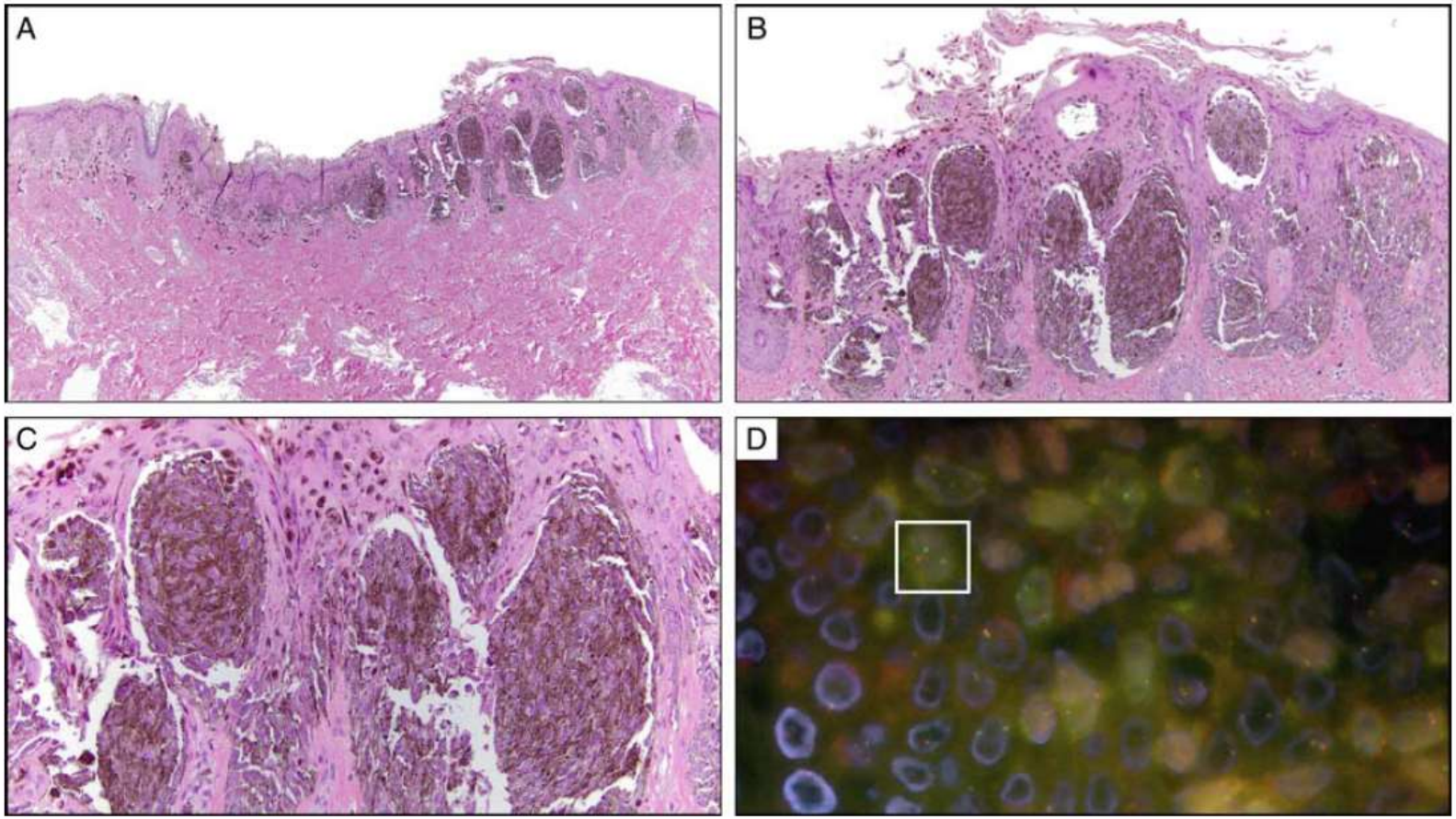


FIGURE 3. NTRK3 fusion PSCN of Reed (hematoxylin and eosin). A, Low-power view showing a well-circumscribed melanocytic lesion encased by epidermal hyperplasia. B, Expansile, heavily pigmented nests with prominent clefting from the adjacent epidermis. C, Highly spindled, heavily pigmented melanocytes without significant nuclear atypia. D, NTRK3 FISH break-apart probe showing separation of green and red signals, confirming NTRK3 fusion status. The white box highlights a representative cell showing separation of 5' and 3' ends of NTRK3.

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REEDOV NEVUS IN FUZIJE

TABLE 1. Clinical and Histopathologic Characteristics of PSCN of Reed and NTRK3-positive Melanocytic Neoplasms

Case	Diagnosis	Age (y)	Sex	Location	Fusion	Silhouette	Cell Size	Cytologic Atypia
1	Tumor of Reed	2	M	Canthus, left lateral	MYO5A-NTRK3	Dome-shaped	Large; epithelioid component	Moderate
2	PSCN of Reed	30	F	Arm, right upper	MYO5A-NTRK3	Plaque-like	Small	Moderate
3	PSCN of Reed	35	M	Thigh, left posterior	MYO5A-NTRK3	Plaque-like	Small	Mild
4	PSCN of Reed	8	M	Leg, right lower	MYO5A-NTRK3	Plaque-like	Large	Moderate
5	PSCN of Reed	27	F	Thigh, right	MYO5A-NTRK3	Plaque-like	Large	Moderate
6	PSCN of Reed	39	F	Thigh, right	MYO5A-NTRK3	Plaque-like	Large	Moderate
7	PSCN of Reed	9	F	Knee, left	MYO5A-NTRK3	Plaque-like	Large	Moderate
8	PSCN of Reed	6	F	Thigh, left	MYO5A-NTRK3	Plaque-like	Large	Moderate
9	PSCN of Reed	17	F	Trunk, left	MYO5A-NTRK3	Plaque-like	Small	Moderate
10	PSCN of Reed	26	M	Shoulder, right anterior	MYO5A-NTRK3	Plaque-like	Large	Moderate
11	PSCN of Reed	2	M	Helix, right	MYO5A-NTRK3	Plaque-like	Large	Moderate
12	PSCN of Reed	13	F	Neck, right	ETV6-NTRK3	Plaque-like	Large	Moderate
13	PSCN of Reed	28	M	Back, mid	ETV6-NTRK3	Plaque-like	Small	Mild
14	PSCN of Reed	34	M	Back, left lower	MYO5A-MERTK	Plaque-like	Small	Moderate
15	PSCN of Reed	29	F	Arm, right upper	MYO5A-MERTK	Plaque-like	Large	Moderate
16	PSCN of Reed	30	F	Arm, left	MYO5A-ROS1	Plaque-like	Large	Moderate
17	PSCN of Reed	62	F	Thigh, right lateral	MYO5A-RET	Plaque-like	Small	Mild
18	PSCN of Reed	27	F	Elbow, left	ETV6-PITX3	Plaque-like	Large	Moderate
19	PSCN of Reed	39	F	Arm, left upper lateral	—	Plaque-like	Large	Moderate
20	PSCN of Reed	33	F	Arm, left upper	—	Plaque-like	Large	Moderate
21	PSCN of Reed	23	F	Wrist, right	—	Plaque-like	Large	Moderate
22	PSCN of Reed	33	F	Forearm, left	—	Plaque-like	Large	Severe
23	PSCN of Reed	26	F	Hip, right	—	Plaque-like	Large	Moderate
24	Spitz tumor	18	M	Mandible, right	MYO5A-NTRK3	Plaque-like	Small	Moderate
25	Spitz nevus	41	F	Thigh, left	MYO5A-NTRK3	Dome-shaped	Small	Mild

F indicates female; M, male.

VAN DEN BOOM T ET AL. AM J SURG PATHOL 2018; 42: 1042-1051.

MELANOM IN *NTRK* FUZIJE

Primary and Metastatic Melanoma With NTRK Fusions

Cecilia Lezcano, MD, Alexander N. Shoushtari, MD,† Charlotte Ariyan, MD,‡ Travis J. Hollmann, MD, PhD,* and Klaus J. Busam, MD**

Abstract: A number of oncogenic driver mutations have been identified in melanocytic nevi and melanoma, but translocations also play a role in tumorigenesis and provide potential therapeutic targets for malignant lesions. Various translocations, such as those involving the anaplastic lymphoma kinase (*ALK*), neurotrophic tropomyosin receptor kinase 1 (*NTRK1*), and *NTRK3* have been reported in spitzoid melanocytic neoplasms leading to kinase-fusion proteins that result in immunohistochemically detectable ALK or NTRK expression. We have previously reported that ALK expression can be found in nonspitzoid primary and metastatic cutaneous melanomas. In this study we report that nonspitzoid metastasizing melanomas of adults may also harbor *NTRK* fusions and that NTRK expression can be immunohistochemically detected in these tumors. Of 751 melanomas analyzed by next-generation sequencing, 4 metastatic melanomas were identified with *NTRK* fusions, 3 involving *NTRK1*, 1 involving *NTRK2*. They occurred in 3 women and 1 man. Two of the corresponding primary tumors were from the trunk, 1 from an extremity and 1 tumor arose in anal skin. One primary tumor displayed features of superficial spreading melanoma and 3 were nodular melanomas. All tumors were cytologically characterized by the presence of large epithelioid melanocytes. All tumors were immunoreactive with anti-Trk antibody. Next-generation sequencing documented that the *NTRK1* fusion partners included *TRIM63*, *DDR2*, and *GON4L*. One tumor harbored an *NTRK2-TRAF2* fusion. Thus, our findings document that *NTRK* kinase fusions can occur in nonspitzoid metastasizing melanomas of adults. The presence of an *NTRK* family fusion in these tumors may provide a therapeutic opportunity in a small subset of patients with metastatic melanoma.

Key Words: NTRK, immunohistochemistry, melanoma, pathology

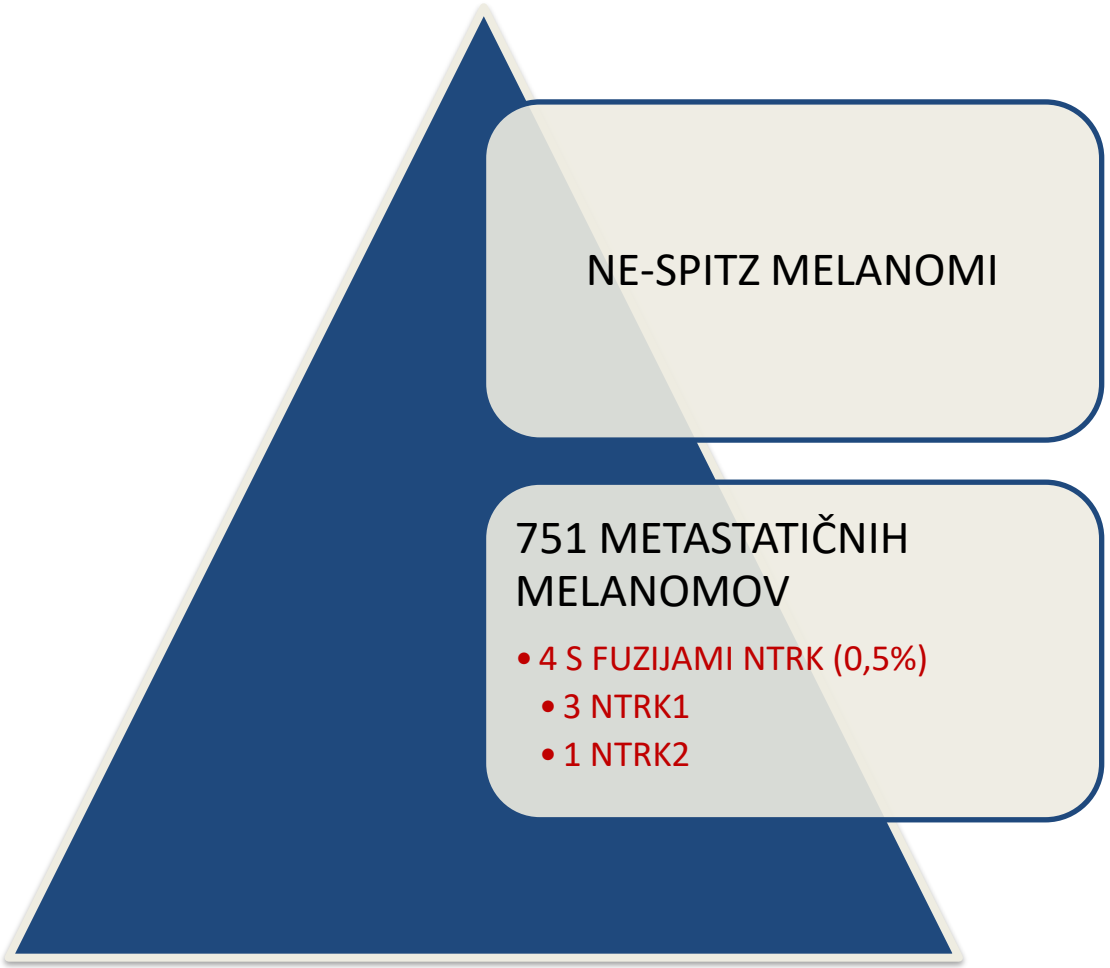
(*Am J Surg Pathol* 2018;42:1052–1058)

for cutaneous melanoma: mutant *BRAF*, *RAS*, *NF1*, and triple wild-type tumors.[1] The latter group encompasses melanomas with molecular aberrations that are infrequent, such as mutations of *KIT*,[2,3] *GNA11*,[4] and *GNAQ*,[4] or genomic rearrangements of *BRAF*[5,6] and *RAF1*.[7] Aside from mutations, the pathogenesis of melanoma is also influenced by other events, including, but not limited to DNA copy number changes,[8–11] translocations,[12–15] DNA methylation,[16] and alternative transcriptional initiation (ATI).[17]

With regard to genomic rearrangements in melanocytic tumors, much has been learned from the study of spitzoid neoplasms.[10,18] The most common type of aberrations in this subclass of melanocytic tumors involve various receptor-tyrosine kinases, including anaplastic lymphoma kinase (*ALK*), *ROS1*, neurotrophic tropomyosin receptor kinase 1 (*NTRK1*), *NTRK3*, *RET*, or *MET*, and the serine-threonine kinases *BRAF*.[12,19,20] The rearrangements link the kinase domain of these signaling proteins to a wide range of fusion partners, leading to highly expressed and kinase-active proteins. The resulting chimeric proteins stimulate multiple oncogenic signaling pathways and promote tumor initiation and progression.[12]

Recent searches for possible similar genomic rearrangements in melanomas led to the discovery of a novel *ALK* isoform.[17] This transcript is expressed from a de novo ATI site in *ALK* intron 19, and was accordingly named *ALK*^{ATI}. Melanomas with ALK^{ATI} expression show positive staining by ALK immunohistochemistry (IHC).[17] In a large set of primary and metastatic melanomas ALK immunoreactivity was found only rarely, and if present, was not associated with *ALK* translocations, but with the truncated ALK^{ATI} isoform.[21] Nonetheless, for the few patients whose tumors express ALK, it may provide a unique therapeutic opportunity.

MELANOM IN *NTRK* FUZIJE



LEZCANO C ET AL. AM J SURG PATHOL 2018; 42: 1052-1058.

MELANOM IN *NTRK* FUZIJE

TABLE 1. Clinical, Pathologic, and Molecular Findings of Melanomas With NTRK Fusions

	Case 1	Case 2	Case 3	Case 4
Age (y)	63	47	55	36
Sex	Male	Female	Female	Female
Site of primary	Shin	Perianal	Umbilical	Back
Breslow (mm)	2.3	2.8	4.5	6.2
Ulceration	No	No	No	Present
Tumor mitotic rate (/mm^2)	11	8	4	27
Type	Nodular	Superficial spreading pattern	Nodular	Nodular
Cytology	Large epithelioid	Large epithelioid	Large epithelioid	Large epithelioid
Melanin	Absent	Present	Absent	Absent
Age at metastasis (y)	65	47	58	39
Site of metastasis	Skin, lymph node	Lymph node	Colon	Duodenum
NTRK1 fusion	NTRK1-TRIM63	NTRK2-TRAF2	NTRK1-DDR2	NTRK1-GON4L
Other driver mutations	None	None	NF1 truncation; RAC1 p295	NRAS Q61L

LEZCANO C ET AL. AM J SURG PATHOL 2018; 42: 1052-1058.

MELANOM IN *NTRK* FUZIJE

EPITELIOIDNA MORFOLOGIJA

ODSOTNOST PIGMENTA

LEZCANO C ET AL. AM J SURG PATHOL 2018; 42: 1052-1058.

MELANOM IN *NTRK* FUZIJE

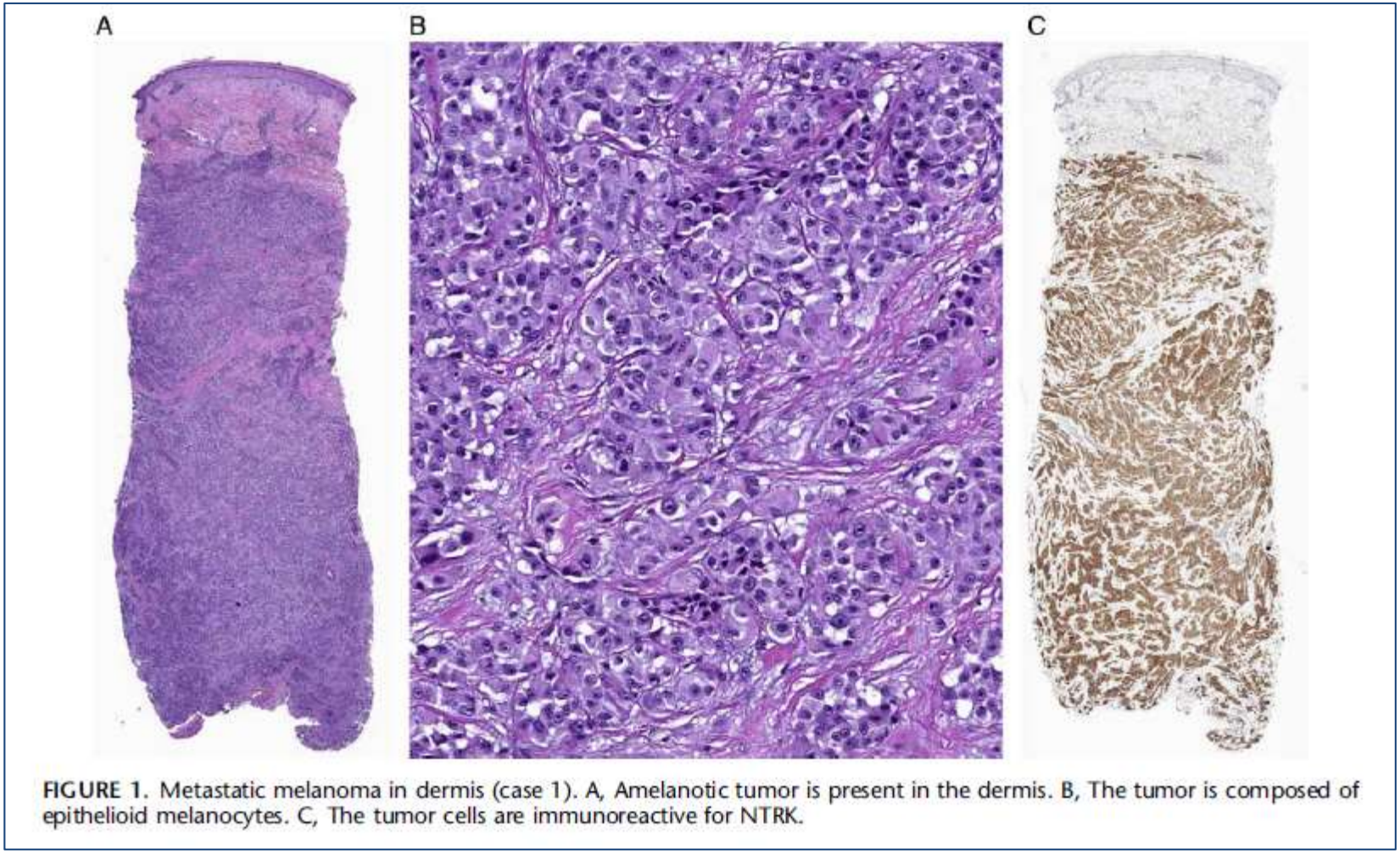


FIGURE 1. Metastatic melanoma in dermis (case 1). A, Amelanotic tumor is present in the dermis. B, The tumor is composed of epithelioid melanocytes. C, The tumor cells are immunoreactive for NTRK.

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MELANOM IN *NTRK* FUZIJE

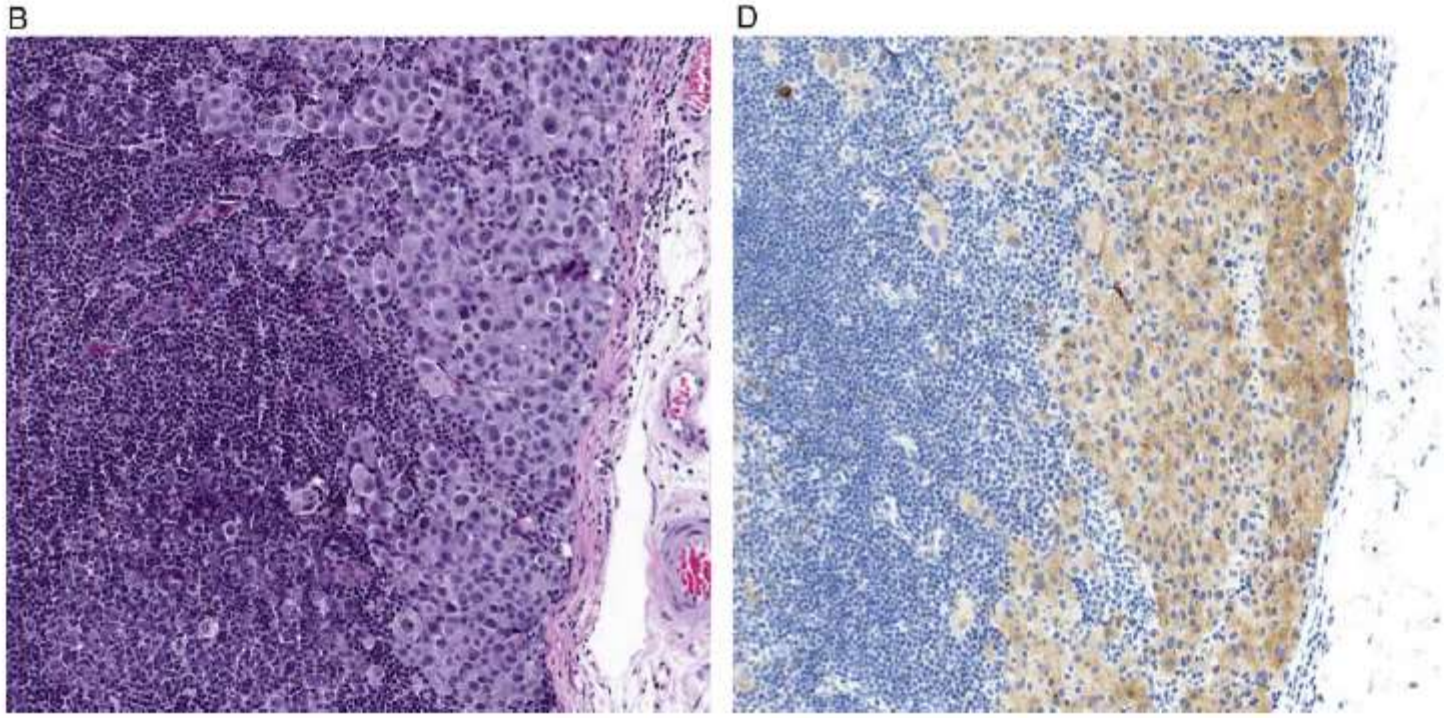

FIGURE 2. Metastatic melanoma in lymph node (case 2). A, Amelanotic melanoma is located predominantly at the periphery of the node. B, The tumor cells display epithelioid cell features. C, The tumor cells are immunoreactive for NTRK. D, The epithelioid melanoma cells are immunoreactive for NTRK.

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MELANOCITNE PROLIFERACIJE S FUZIJAMI *NTRK* - SKLEPI I.-

FUZIJE *NTRK* REDKE

- SPITZ PROLIFERACIJE (**16%**)

CELOTEN SPEKTER MELANOCITNIH PROLIFERACIJ

- BENIGNE
- INTERMEDIARNE
- MALIGNE

POVEZAVA MED FUZIJAMI *NTRK* IN MORFOLOŠKIMI SPREMEMBAMI

MELANOCITNE PROLIFERACIJE S FUZIJAMI *NTRK* - SKLEPI II.-