An Update on Molecular Genetic Aberrations in Spitz Melanocytic Proliferations: Correlation with Morphological Features and Biological Behavior

Daja Šekoranja, Jože Pižem, Boštjan Luzar
Institute of Pathology, Faculty of Medicine, University of Ljubljana, Korytkova 2, 1000 Ljubljana, Slovenia
Correspondence: bostjan.luzar@mf.uni-lj.si; Tel.: + 386 1 543 7130; Fax.: + 386 1 543 7101
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Abstract
The aim of the paper is to give an update on molecular genetic aberrations in Spitz melanocytic proliferations with special emphasis on their correlation with morphological features and biological behavior. The Spitz group of melanocytic proliferations is defined by a combination of distinctive morphological features and driver molecular genetic events. Morphologically, these neoplasms are characterized by large, oval, polygonal, or spindled melanocytes with abundant eosinophilic cytoplasm, vesicular nuclei with prominent nucleoli, often in association with epidermal hyperplasia. Molecular aberrations in Spitz melanocytic proliferations can be divided into two main groups, according to the driver genetic change: 1) 11p amplification/HRAS mutation, present in about 20% of cases, and 2) kinase fusions, present in about 50%, further subdivided into tyrosine kinase fusions (ALK, ROS1, NTRK1, NTRK3, MET, RET) or serine/threonine kinase fusions (MAP3K8, BRAF). Driver genetic aberrations can be detected along the whole biological spectrum of Spitz melanocytic proliferations, and are mutually exclusive. Although driver genetic aberrations enable proliferation of melanocytes, additional genetic events (often biallelic inactivation of CDKN2A and TERT promoter mutations) are necessary for the development of overt Spitz malignancy. Conclusions. Recent studies have demonstrated that certain driver genetic aberrations are more often associated with the benign spectrum of Spitz melanocytic proliferations and indolent biological behavior (11p amplification/HRAS mutation, tyrosine kinase fusions). In contrast, some driver aberrations are more frequent in the atypical/malignant spectrum of Spitz melanocytic proliferations with a potential for aggressive biological behavior (serine/threonine kinase fusions). In addition, certain driver aberrations are often associated with distinctive morphological features. However, none of the morphological features is entirely specific for any of these driver genetic aberrations. Immunohistochemistry for ALK, ROS1, and pan-TRK can be used for screening purposes to detect corresponding fusion proteins.

Key Words: Spitz Melanocytic Proliferations • Kinase Fusions • 11p Amplification/HRAS Mutation • Morphology • Clinical Behavior.

Introduction
According to the most recent 4th WHO Classification of Skin Tumours, the Spitz group of melanocytic proliferations is defined by a combination of morphological characteristics and driver molecular genetic abnormalities (1). Spitz melanocytic proliferations are characterized morphologically by a proliferation of large, oval, polygonal epithelioid or spindled melanocytes with abundant eosinophilic cytoplasm, vesicular nuclei with prominent nucleoli, often in association with epidermal hyperplasia (1). They encompass the whole biological spectrum of melanocytic proliferations, including Spitz nevi, atypical Spitz tumors, and Spitz melanomas, also referred to as malignant Spitz tumors (1). Based on the underlying genetic aberrations, Spitz melanocytic proliferations can be divided into four distinct groups: 1) 11p amplified/HRAS mutated proliferations, 2) proliferations with tyrosine kinase fusions (ALK, ROS1, NTRK1, NTRK3, MET, RET), 3) proliferations with serine/threonine kinase fusions (MAP3K8, BRAF) and 4) proliferations with Spitz morphology but lack-
ing an 11p/HRAS mutation, kinase fusions, BRAF, NRAS, GNAQ, GNA11 mutations and other driver genetic changes characteristic of other defined melanocytic subgroups (1). The last group is at present poorly defined and will not be discussed further in this review. Importantly, driver genetic events are mutually exclusive in a particular Spitz melanocytic proliferation and are by themselves insufficient for the development of overt malignancy, generally characterized by the development of distant metastases and aggressive biological behavior (2, 3).

As has been demonstrated by recent studies, such a combined morphological/genetic classification better correlates with the biological behavior of different groups of Spitz melanocytic proliferations. The vast majority of Spitz melanomas resulting in distant metastatic spread harbor serine/threonine kinase fusions (4-11). Furthermore, since several biological drugs are available to treat melanocytic proliferations with aggressive clinical behavior, the characterization of particular driver genetic events and additional genetic abnormalities is becoming increasingly important.

Herein, we review recent advances in the molecular genetics of Spitz melanocytic proliferations. Special emphasis is given to the correlation of molecular genetic aberrations with morphological features and with the biological behavior of Spitz melanocytic proliferations. Immunohistochemistry can be a reliable surrogate tool for certain molecular abnormalities to molecular genetic testing, as discussed further in this review (for practical purposes, the list of antibodies reflecting possible kinase fusions the authors are using routinely is summarized in the Table 1. Also, at the end of each section, a table is presented with short summary of key data for each particular Spitz group of melanocytic proliferations).

11p Amplified and/or HRAS Mutated Spitz Melanocytic Proliferations

The first recurrent genetic alterations discovered in Spitz melanocytic proliferations were 11p amplification and HRAS mutation. They were also the first genetic alterations associated with a specific morphologic phenotype in this group of melanocytic neoplasms (12, 13). Both 11p amplification and HRAS mutation can appear exclusively or simultaneously in a single lesion and are most commonly associated with a desmoplastic Spitz nevus morphology (13, 14). HRAS (Harvey rat sarcoma viral oncogene homolog) resides on 11p chromosome arm (11p15.5) and belongs to the Ras family of oncogenes, encoding a GTPase that is involved in cellular signaling (the MAP kinase-signaling pathway) (15, 16). Mutations in HRAS lead to constitutive activation of an altered protein that impacts the expression of various transcription factors involved in cell cycle progression, thus stimulating cell growth and differentiation (16). HRAS mutations have also been detected in urothelial and squamous cell carcinomas, adenocarcinomas of various origins, leukemias, and myelodysplastic syndromes (17-19). HRAS mutations in Spitz melanocytic proliferations are most commonly missense mutations involving codons 61 and 13, with the three most commonly reported mutations being Q61L (2, 6, 14, 20), Q61R (14, 20-23), and G13R (6, 21, 24-28).

The prototypic 11p amplified/HRAS mutated Spitz nevus is a symmetrical, predominantly dermal, relatively hypocellular proliferation composed of large, epithelioid and spindled melanocytes with desmoplastic stromal reaction (i.e., thickened collagen fibers between single neoplastic cells) and an infiltrative base (Figure 1) (2, 13, 14). Melanocytes have abundant eosinophilic or amphophilic cytoplasm, vesicular nuclei, and mild to moderate nuclear pleomorphism (2, 13, 14). The proliferation rate is usually low, although

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone</th>
<th>Manufacturer</th>
<th>Dilution</th>
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</thead>
<tbody>
<tr>
<td>ALK</td>
<td>S44</td>
<td>Leica Biosystems, Wetzlar, Germany</td>
<td>1:10</td>
</tr>
<tr>
<td>ROS1</td>
<td>SP384</td>
<td>Ventana, Roche, Tucson, USA</td>
<td>RTU (Ready To Use)</td>
</tr>
<tr>
<td>panTRK</td>
<td>EPR17341</td>
<td>Abcam, Cambridge, UK</td>
<td>1:50</td>
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</table>
isolated deep mitoses and, in rare instances, multiple mitoses may be present and are not associated with malignancy (2, 13, 14, 29). Atypical mitoses are generally absent.

In addition to solitary lesions with typical desmoplastic Spitz nevus morphology, other melanocytic proliferations with HRAS mutations and/or 11p gain have also been described, including agminated Spitz nevi with or without associated nevus spilus (25-28), recurrent Spitz nevi (30), melanocytic nevi with deep penetrating nevus-like morphology (21), pseudogranulomatous Spitz nevi (31) and a combination of syringocystadenoma papilliferum, tubular adenoma and a Spitz nevus (24). Moreover, the desmoplastic Spitz nevus phenotype is not restricted to HRAS mutated lesions since rare Spitz nevi harboring ROS1, ALK or even a BRAF fusion exhibiting a desmoplastic Spitz nevus morphology have been described (2, 32).

Even though the vast majority of Spitz proliferations with HRAS mutations are essentially associated with benign clinical behavior (14, 29, 33), there are occasional reports of HRAS mutated Spitz melanomas (6, 23). Recently, Raghavan et al. published a series of Spitz melanomas, two of which harbored HRAS hotspot mutations (23). Both lesions were associated with additional genetic aberrations, namely a loss of chromosome 9 (accompanying by negative p16 immunohistochemical reaction) in a case of a 50-year-old female, and a three-codon deletion in MAP2K1 (p.102_104del), a hemizygous mutation in ARID1A, homozygous deletion of CDKN2A and NOTCH2 amplification in a case of a 75-year-old female (23). Unfortunately, clinical follow-up was not available in either of the two cases (23). Lazova et al. also reported two melanomas with HRAS mutations in their series (6). One case was of a 73-year-old male diagnosed with Spitz melanoma that harbored an HRAS Q61L mutation, which developed metastases two years later, but he was still alive at a 4-year follow-up (6). The second case was a 60-year-old male diagnosed with conventional melanoma that
harbored an HRAS G13R mutation, who developed metastases two years later, and died at the age of 63 (6).

**Spitz Melanocytic Proliferations with Tyrosine Kinase Fusions**

**ALK Fusions**

The anaplastic lymphoma kinase (ALK) gene resides on chromosome 2p23 and encodes a tyrosine kinase receptor, a transmembrane protein that belongs to the insulin receptor family (34). Genetic alterations of the ALK gene include point mutations, gene fusions, ALK locus amplification, alternative transcription, and small deletions (35) and influence cell proliferation and survival via constitutive activation of the RAS-ERK, JAK3-STAT3, and PI3K-AKT-mTOR pathways (36-38). Fusions involving the ALK gene have been discovered in diverse cutaneous neoplasms, including primary cutaneous anaplastic large cell lymphoma (39), epithelioid fibrous histiocytoma (40), acral melanomas (41, 42), and Spitz melanocytic proliferations (2, 43-50).

In Spitz melanocytic proliferations, various different fusion partners have been identified, including TPM3 (2, 11, 45, 51-54), DCTN1 (2, 23, 45, 50, 54), MLPH (9, 44, 45, 55), KANK1 (9, 45), CLIP1 (50), DDX3Y (9), EEF2 (45), GTF3C2 (50), MYOSA (45), NPM1 (47), PPFIBP1 (9), SPTAN1 (9) and TPR (50), in descending order of frequency. Lesions from the whole biological spectrum ranging from Spitz nevi, atypical Spitz tumors to Spitz melanomas have been distributed fairly equally among different fusion partners (2, 9, 11, 23, 44, 45, 47, 50-52, 54-56). Although different fusions in Spitz melanocytic proliferations are generally believed to be mutually exclusive with BRAF mutations, a few examples (two Spitz melanomas, one atypical Spitz tumor, and an acral melanoma) with concurrent ALK fusion and a BRAF mutation have been reported in the literature (41, 54, 57).

Such a combination of ALK fusion and BRAF mutation is, nevertheless, exceptionally rare.

Spitz melanocytic proliferations with ALK fusions have the largest average diameter among all Spitz melanocytic proliferations (43). The vast majority of Spitz proliferations with ALK fusion are polypoid/dome and/or wedge-shaped solitary lesions with a bulbous and/or infiltrative base. They are composed of plexiform and intersecting fascicles of fairly large, fusiform/spindle cell or mixed spindle and epithelioid cell melanocytes with amphiphilic cytoplasm and vesicular nuclei with prominent nucleoli (Figure 2) (2, 23, 43-46, 49, 50, 54, 55, 58). Nuclear pleomorphism is usually mild and occasionally moderate. Melanocytes may appear discohesive with clefts or small vesicle-like spaces in between (23, 50, 55, 59). Ulceration may be present, as may be dermal (even deep) mitoses and perineural invasion, but Kamino bodies are rare (23, 43, 45, 47, 50, 58). Melanin pigment is typically lacking or presents in limited amounts in the cytoplasm of melanocytes. Focal mucin deposits have been described (45). Proliferations with abundant myxoid areas with ALK+/SOX10+/MelanA− spindle cells underneath the superficial nevoid or Spitzoid component have been termed melanocytic myxoid spindle cell tumors with ALK rearrangement (MMySTAR) (56). They have been shown to harbor ALK fusions with different fusion partners, namely FBXO28, NPAS2, PPFIBP1, and TPM3 (56). Interestingly, a single example of a desmoplastic Spitz nevus harboring a TPM3-ALK fusion has also been reported (2).

ALK immunohistochemistry is a reliable surrogate marker for molecular genetic techniques in cases with diffuse and strong immunopositivity in most melanocytes (Figure 2c) (2, 43-46, 50). In contrast, weak and focal or heterogeneous ALK staining has been demonstrated in non-Spitz melanocytic proliferations with ALK overexpression due to other molecular mechanisms (e.g., alternative transcription initiation that leads to the expression of a novel ALK isoform ALKATI (60, 61) or in cases with chromosome 2p23 gain (62), in rare cases of cellular blue nevi and in a single case of deep penetrating nevus (63).

Exceptionally rare examples of Spitz melanocytic proliferations with ALK fusion have har-
bored additional molecular changes, i.e., homozygous 9p21 (CDKN2A) deletion in combination with 6p25 (RREB1) gain in two cases (49) and a hotspot TERT-promoter mutation (C228T) in one case (45), which had no effect on the biological behavior of the proliferation. Only two cases of Spitz melanocytic proliferations with ALK fusion and with deposits in lymph nodes have been described, one of which had an additional homozygous 9p21 deletion (49, 55). Importantly, however, none of the cases of Spitz melanocytic proliferations with ALK fusion and with available follow-up data were found to be associated with systemic metastases or death from the disease.

**ROS1 Fusions**

ROS1 protooncogene resides on chromosome 6q22.1 and, like ALK, encodes a protein receptor tyrosine kinase that is part of the intracellular signaling pathways Ras-Raf-MEK-ERK, JAK3-STAT3, and PI3K-AKT-mTOR. (64) ROS1 fusions have been found in a variety of tumors, including non-small cell lung carcinomas, glioblastomas, pediatric gliomas, cholangiocarcinomas, inflamma-

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**Figure 2.** Spitz nevus with ALK fusion. A. A large diameter of the proliferation is a characteristic feature of ALK-fused melanocytic proliferations. B. A plexiform growth pattern with deep extension of melanocytes is also frequently observed. C. ALK immunohistochemistry.

**Table 3. Spitz Melanocytic Proliferations with ALK Fusions**

<table>
<thead>
<tr>
<th>Morphological features</th>
<th>Biological behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symmetrical, polypoid/dome and/or wedge-shaped</td>
<td>Generally favorable (benign)</td>
</tr>
<tr>
<td>Large diameter</td>
<td>Regional lymph node deposits</td>
</tr>
<tr>
<td>Plexiform growth pattern</td>
<td>uncommon</td>
</tr>
<tr>
<td>Epithelioid and spindled melanocytes</td>
<td>No distant metastases or death from the disease</td>
</tr>
<tr>
<td>– Mild to moderate pleomorphism</td>
<td></td>
</tr>
<tr>
<td>– Mitoses rare</td>
<td></td>
</tr>
<tr>
<td>– Pigmentation absent or scant</td>
<td></td>
</tr>
<tr>
<td>– Low proliferation rate</td>
<td></td>
</tr>
<tr>
<td>– Ulceration rare</td>
<td></td>
</tr>
<tr>
<td>– Kamino bodies usually absent</td>
<td></td>
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</tbody>
</table>

**Confirmatory test**

- Immunohistochemistry
- Next generation sequencing
- Fluorescence in situ hybridization

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tory myofibroblastic tumors, etc (64). \textit{ROS1} fusions are present in up to 17\% of Spitz melanocytic proliferations (2). Thirteen different fusion partners have been reported: \textit{PWWP2A}, \textit{TPM3}, \textit{PPFIBP1}, \textit{CAPRIN1}, \textit{MYO5A}, \textit{PPFIBP1}, \textit{CLIP1}, \textit{ERC1}, \textit{FIP1L1}, \textit{HLA-A}, \textit{KIAA1598}, \textit{MYH9}, and \textit{ZCCHC8}, in descending order of frequency (2, 23, 32, 65).

Although \textit{ROS1}-fused Spitz proliferations lack unique identifying morphological features, most \textit{ROS1}-fused melanocytic proliferations are compound, composed either of spindle cells or of a combination of spindle and epithelioid cells with mild to moderate cytological atypia and with limited pigmentation of melanocytes (2, 32, 65). The junctional melanocytic component is frequently prominent, with floating nests or trans-epidermal elimination of melanocytic nests, often colonizing the epithelium of skin adnexa (Figure 3) (32, 65). Kamino bodies seem to be more frequently present in \textit{ROS1} fused Spitz melanocytic proliferations, along with signs of maturation, lack of high-grade cytological atypia, lack of large cells, and fewer mitoses - all statistically significant features differing between \textit{ROS1} and non-\textit{ROS1} Spitz neoplasms in a study by Gerami et al. (32). Nonetheless, large cells were described in 9 of 11 \textit{ROS1} fused Spitz neoplasms in a study by Wiesner et al. (2), and up to 8 mitoses per square millimeter were reported in a study of such proliferations by Donati et al. (65).

Immunohistochemistry for \textit{ROS1} protein is a reliable surrogate for molecular testing (Figure 3c). Studies have confirmed that the vast majority (97.4\%) of Spitz melanocytic proliferations harboring \textit{ROS1} fusions display \textit{ROS1} cytoplasmic positivity on immunohistochemistry (2, 32). It is important to note, though, that immunohistochemical staining is often weak yet diffuse.

The vast majority of hitherto reported Spitz neoplasms with \textit{ROS1} fusion in the literature were classified as either Spitz nevi or atypical Spitz tumors (2, 23, 32, 47, 65-67). Three lesions were of desmoplastic Spitz nevus phenotype (2, 32), four
were pigmented spindle cell nevi or Reed nevi (2, 67), and one was an eruptive Spitz nevus (48). The last was reported in a 49-year old female, who developed over 100 similar lesions over four years. Further molecular characterization of the proliferation revealed identical \( \text{TPM3-ROS1} \) fusions in the three analyzed lesions (48). Only five Spitz melanomas with \( \text{ROS1} \) fusion have been described, and none of them resulted in distant metastases or death from the disease (2, 47).

**NTRK Fusions**

Neurotrophic tyrosine kinase receptor genes \( \text{NTRK1}, \text{NTRK2} \) and \( \text{NTRK3} \), are oncogenes encoding the Trk family of tyrosine kinase receptors (TrkA, TrkB, and TrkC, respectively) (68). These tyrosine kinase receptors are all single-pass transmembrane enzymes that stimulate different pathways once activated, namely the MAPK/ERK, PI3K-AKT-mTOR, and phospholipase C-\( \gamma \) pathways (3, 68). In most \( \text{NTRK} \) fusions identified, the 3' portion encoding the kinase domain is retained, and the 5' portion encoding dimerization domains is provided by the fusion partner. The resultant chimeric Trk protein is an oncogenic, constitutively active tyrosine kinase (69).

In Spitz melanocytic proliferations, \( \text{NTRK1} \) fusions predominate over \( \text{NTRK3} \) fusions, the latter being more common in the pigmented spindle cell nevus of Reed, a special subtype of Spitz nevus (2, 9, 11, 23, 43, 46, 47, 67, 69-74). Only a single case of superficial spreading melanoma with an \( \text{TRAF2-NTRK2} \) fusion has been reported so far (7).

The \( \text{NTRK1} \) fusion partners in Spitz melanocytic proliferations include \( \text{LMNA} (2, 9, 72), \text{TPM3} (11, 23, 47, 72), \text{TP53} (2, 72), \) and \( \text{KHDRBS1} (9, 72) \), in descending order of frequency. Even though the number of cases for each particular known \( \text{NTRK1} \) fusion partner is relatively low (\( \text{LMNA} \) was identified in 16 cases, while \( \text{TP53} \) and \( \text{KHDRBS1} \) in two cases each), 4 of 5 Spitz melanocytic proliferations with \( \text{TPM3-NTRK1} \) fusion were diagnosed as Spitz melanomas, all with several additional chromosomal aberrations, including (most commonly) homozygous deletions of \( \text{CDKN2A} (11, 23, 47, 72) \). Furthermore, Spitz melanoma was also diagnosed in one of the two reported cases with an \( \text{KHDRBS1-NTRK1} \) fusion (9, 72).

Four different fusion partners have been identified so far for \( \text{NTRK3} \) fusions, namely \( \text{MYO5A} (67, 69, 70, 73), \text{ETV6} (67, 70, 73), \) \( \text{MYH9} (70, 73), \) and \( \text{SQSTM1} (9) \), in descending order of frequency. While no \( \text{NTRK1} \) fusions have been identified in Reed nevi, they harbor \( \text{NTRK3} \) fusions (with \( \text{MYO5A} \) and rarely \( \text{ETV6} \) fusion partners) in up to 57% of cases (67). In addition to pigmented spindle cell nevi of Reed, Spitz proliferations with \( \text{NTRK3} \) fusions are usually diagnosed as Spitz nevi or atypical Spitz tumors and much less frequently as Spitz melanomas (9, 67, 69, 70, 73).

\( \text{NTRK1} \) and \( \text{NTRK3} \) fusions are also occasionally detected in non-Spitz melanocytic proliferations, e.g., pigmented epithelioid melanocytomas (75, 76), acral melanomas (42), and in a wide variety of non-melanocytic tumors, e.g., infantile fibrosarcoma, secretory carcinoma of the breast, secretory carcinoma of the salivary gland, congenital mesoblastic nephroma, lung carcinoma, thyroid papillary carcinoma and high grade gliomas (77).

Histologically, \( \text{NTRK1} \)-fused Spitz melanocytic proliferations are characterized by filigree-like rete ridges (elongated, thin rete ridges), lobulated dermal melanocytic nests (composed of smaller nests inside the larger ones), and by the formation of...
Pseudorosettes (23, 43, 72). Exaggerated maturation of spindled and/or epithelioid melanocytes displaying mild to moderate nuclear pleomorphism is also a characteristic finding (23, 43, 72). Mild to moderate and sometimes even marked lymphocytic infiltrate is often present (2, 46, 72). While the pagetoid spread of melanocytes has been observed in up to 25% of cases, Kamino bodies are variably present (Figure 4) (2, 43, 46, 72).

*NTRK1* fused Spitz melanocytic proliferations can occasionally resemble those with *ALK* fusions, exhibiting an intersecting fascicular growth pattern in the dermis (46).

*NTRK3* fusions are the most common driver genetic aberrations in this subgroup of Spitz nevi, *NTRK1* fusions are generally absent (67). It has been recently demonstrated that various *NTRK3* fusion partners have different intracellular localizations, ultimately determining the morphological characteristics of the Spitz melanocytes (62). For example, *MYO5A-NTRK3* chimeric protein is localized to cell dendritic processes and is associated with a fusiform/spindled morphology of melanocytes with a fascicular or sometimes plexiform or syncytial growth pattern (70). Besides, the formation of pseudo-Verocay bodies or pseudorosettes is associated with a more neuroid appearance of these proliferations (70). In contrast, the *ETV6-NTRK3* chimeric protein is localized to both the nucleus and cytoplasm of melanocytes and is linked to an epithelioid morphology with well-defined cell borders. Melanocytes have abundant, glassy cytoplasm and somewhat large, pleomorphic nuclei. They are arranged in large coalescing and also lobulated nests. Signs of maturation are fairly discrete (70). Finally, Spitz melanocytic proliferations with *MYH9-NTRK3* fusion are distinguished by fibrotic stroma and peripheral collagen trapping (70).

Immunohistochemistry with pan-TRK antibody can be used to detect the fusion protein (Figure 4c). Both available clones, clone A7H6R (Cell Signaling Technology) and EPR17341 (Abcam/Ventana) are highly sensitive and specific,
EPR17341 being slightly superior in terms of specificity (78). The staining pattern can hint at the presence of the NTRK fusion subtype. However, most pan-TRK immunohistochemistry studies in different NTRK fusion subtypes were performed on mostly non-Spitz NTRK-fused tumors (79, 80). Pan-TRK immunohistochemical staining is more intense and cytoplasmic in NTRK1-fused tumors, with additional nuclear accentuation in cases with an LMNA-NTRK1 fusion (79, 80). On the other hand, up to 50% of tumors with an NTRK3 fusion exhibit a cytoplasmic and nuclear pan-TRK immunohistochemical reaction (79, 80). However, a study by de la Fouchardière et al., which included only NTRK3-fused Spitz melanocytic proliferations, demonstrated that more intense nuclear and less intense cytoplasmic immunoreactivity is indicative of an ETV6-NTRK3 fusion. At the same time, linear staining along dendritic processes can point to the presence of an MYO5A-NTRK3 fusion (70).

Exceptional cases of non-Spitz (‘spitzoid’) melanomas with NTRK fusions have been reported resulting in widespread hematogenous metastases (7). In contrast, Spitz melanomas with NTRK fusions do not carry a dismal prognosis since only rare metastases to lymph nodes, but not beyond, have been described (47, 71). At present, no examples of Spitz melanocytic proliferations with NTRK fusions and with distant metastases or death from the disease have been reported. In the unlikely event of an NTRK-fused metastatic Spitz melanoma, specific therapy with TRK inhibitors is available (81, 82).

**RET Fusions**

The RET protooncogene resides on chromosome 10q11.21 and encodes a protein receptor tyrosine kinase involved in the MAPK/ERK, PI3K/AKT/mTOR and phospholipase C-γ1 intracellular signaling pathways (2). Only a handful of Spitz melanocytic proliferations with RET fusions have been reported (2, 9, 43, 67, 83). Four different fusion partners have been identified: CCDC6 (9), GOLGA5 (2), KIF5B (2), and MYO5A (67). Similar fusions have also been detected in thyroid cancer (84) and lung adenocarcinomas (85).

RET fusions have been reported in the whole biological spectrum of Spitz melanocytic proliferations, including ordinary Spitz nevi and pigmented spindle cell nevus of Reed, atypical Spitz tumors, and Spitz melanomas (2, 9, 43, 67, 83). Although the morphologic features of RET fused Spitz melanocytic proliferations lack specificity, such proliferations are often well-circumscribed, symmetrical, compound melanocytic proliferations with a plaque-like silhouette, a nested growth pattern of small to intermediate-sized epithelioid and spindled melanocytes with only mild cytological atypia (2, 43, 67).

RET-fused Spitz melanocytic proliferations generally follow an indolent clinical course (2, 9, 43, 67, 83). At present, no Spitz melanomas with RET fusion and a dismal outcome have been reported in the literature (2, 9, 43, 67, 83). Nevertheless, in the unlikely event of aggressive clinical behavior, potential therapy with RET inhibitors is available (2).

**MET Fusions**

The MET protooncogene is localized on chromosome 7q31.2 and encodes a tyrosine kinase receptor with high affinity for hepatocyte growth factor.

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**Table 5. Spitz Melanocytic Proliferations with NTRK1 Fusions**

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<thead>
<tr>
<th>Morphological features</th>
<th>Biological behavior</th>
<th>Confirmatory test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filigree-like rete ridges</td>
<td>Generally favorable</td>
<td>Immunohistochemistry with pan-TRK antibody</td>
</tr>
<tr>
<td>Lobulated melanocytic nests</td>
<td>Regional lymph node deposits uncommon</td>
<td>Next generation sequencing (preferred)</td>
</tr>
<tr>
<td>Rosette-like structures</td>
<td>No distant metastases or death from the disease</td>
<td>Fluorescence in situ hybridization</td>
</tr>
<tr>
<td>Epithelioid and spindled melanocytes</td>
<td>in proliferations classified as Spitz melanomas</td>
<td></td>
</tr>
<tr>
<td>– Mild to moderate pleomorphism</td>
<td></td>
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<tr>
<td>– Mitoses rare</td>
<td></td>
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<tr>
<td>– Kamino bodies frequent</td>
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(86). Only eight Spitz melanocytic proliferations, including a Spitz nevus, five atypical Spitz tumors, and two Spitz melanomas with MET fusions, have been reported (74, 83, 86). The largest series of six Spitz neoplasms with MET fusions demonstrated a breakpoint in intron 14 in all of the cases (86). The breakpoint event is localized upstream of the kinase domain-encoding exons 15 to 21, which are fully retained. In contrast, the auto-inhibitory domain encoded in exon 14 is absent in the chimeric protein (86). The N-terminal fusion partners identified in their series were ZKSCAN1, PPFIBP1, TRIM4, LRRFIP1, EPS15, and DCTN1 (86).

MET fusions result in constitutive activation of tyrosine kinase with subsequent activation of the MAPK/ERK, PI3K/AKT/mTOR, and phospholipase C-γ1 pathways, which can be inhibited by cabozantinib (inhibitor of c-MET and VEGFR2) or PF-04217903 (c-MET inhibitor) (86). The number of reported Spitz melanocytic proliferations with MET fusions is too small to conclude specific morphologic features and prognosis. However, none of the cases with available follow-up resulted in aggressive clinical behavior (86).

**Spitz Melanocytic Proliferations with Serine/Threonine Kinase Fusions**

The largest proportion of Spitz melanocytic proliferations with serine/threonine kinase fusions involves the MAP3K8 or BRAF genes. Nevertheless, other serine/threonine kinase fusions involving the RAF1, PRKCA/B, and ARAF genes have exceptionally been reported in a few examples of atypical Spitz tumors and Spitz melanomas (9, 83). Notably, the vast majority of Spitz melanocytic proliferations with serine/threonine kinase fusions are classified as atypical Spitz tumors or Spitz melanomas and are infrequently detected in Spitz nevi.

**MAP3K8 Fusions**

Mitogen-activated protein kinase kinase kinase 8 (MAP3K8), also known as Tpl-2 and COT, is an enzyme belonging to the group of serine/threonine protein kinases (87, 88) and is encoded by the MAP3K8 gene that resides on chromosome 10p11. The enzyme consists of a kinase domain encoded by exons 1-8 of the MAP3K8 gene and an inhibitory C-terminal domain encoded by exon 9 of the MAP3K8 gene. The inhibitory C-terminal domain covers the kinase domain in its inactive state, preventing it from phosphorylating MEK proteins. The inhibitory C-terminal domain is also essential for targeting the MAP3K8 enzyme for proteolytic degradation. Fusions involving the MAP3K8 gene and truncation of the MAP3K8 gene follow the same basic mechanism, resulting in a fusion/truncated transcript including the intact kinase domain while lacking the inhibitory C-terminal domain. Consequently, kinase activity is unopposed by the C-terminal inhibitory action and, at the same time, the MAP3K8 is not targeted for proteolytic degradation, resulting in significant MAP3K8 overexpression and increased phosphorylation of MEK proteins, which in turn phosphorylate and activate ERK1/2 proteins that influence cell proliferation, division and differentiation (88-90).

Similarly, one of the MEK proteins (MAP2K1, also called MEK1) has an autoinhibitory domain in amino acids 98 to 104, and deletions in this region (e.g., p.I103_K104del of MAP2K1) also result in constitutive activation of downstream ERK1/2 proteins (91).

A number of different MAP3K8 fusion partners have been identified, including SVIL (9, 23, 92, 93), DIP2C (53, 83, 91, 92), UBL3 (9, 83, 92), SPECC1 (9, 92), STX7 (9, 92), ATP2A2 (91), CCNY (92), CDC42EP3 (92), CUBN (92), GNG2 (9), LINC00703 (92), MIR3681HG (92), PCDH7 (91), PIP4K2A (92), PRKACB (9), SFMBT2 (92), SLC4A4 (92), SUBN (9) and ZFP36L1 (23).

MAP3K8 fusions and truncations have also been identified in ovarian, lung, and breast carcinomas, mesotheliomas, cutaneous myxoinflammatory fibroblastic sarcoma, squamous cell carcinomas, and melanocytic tumors – in rare acral melanomas and Spitz neoplasms (9, 23, 53, 83, 92-97).

Morphologically, Spitz proliferations with a MAP3K8 fusion are often ulcerated tumors (more than 50%) with predominantly epithelioid mor-
Additional characteristic features include focal hyperpigmented dermal clones and giant multinucleated melanocytes. Deep mitotic activity is not uncommon (9, 23, 53, 91, 92). Furthermore, desmoplastic stromal reaction and focal pagetoid scatter can be seen in 73% and 45% of cases, respectively (92).

A literature review revealed that most Spitz proliferations with MAP3K8 fusion or truncations, a MAP3K3 fusion, and a MAP2K1 p.I103_K104del were classified either as atypical Spitz tumors or Spitz melanomas (40% and 52%, respectively). In comparison, Spitz nevi represented only a small portion (8%) of cases in this Spitz subgroup (23, 53, 91, 92, 98). Houlier et al. reported the largest series of 33 cases of Spitz melanocytic proliferations with MAP3K8 fusions, of which 13 (40%) were classified as atypical Spitz tumors and 15 (45%) as Spitz melanomas (92). Moreover, 77% of these atypical Spitz tumors and Spitz melanomas harbored CDKN2A (92) inactivation, which was also reported as one of the most common secondary genetic events in some other series (23, 83, 91).

The biological behavior of Spitz melanocytic proliferations with a MAP3K8 fusion is variable; it appears that the prognosis depends on the presence of these additional genetic aberrations. Biallelic inactivation of CDKN2A, demonstrated either by p16 immunohistochemistry (with focal or diffuse complete loss of p16 expression) or molecular ge-
BRAF Fusions

The **BRAF** gene encoding a serine-threonine protein kinase is composed of three highly conserved regions (CRs) (99, 100). CR1 contains N-terminal RAS-binding and cysteine-rich domains, while CR2 contains serine-threonine-rich domains, and both CR1 and CR2 act as auto inhibitors of CR3, the kinase domain (99, 100). In **BRAF** fusions, the resulting chimeric protein retains only the intact kinase domain (CR3) of the **BRAF** gene. The loss of autoinhibitory domains results in increased kinase activity, evidenced by increased phosphorylation and activation of downstream MEK1/2 and ERK1/2 proteins (4, 5).

Numerous fusion partners have been identified: **AKAP9** (9, 10, 101), **AGK** (10, 102), **CLIP2** (102, 103), **BAIAP2L1** (11, 47), **CEP89** (2), **CUX1** (10), **DYNC1L2** (10), **EML4** (11, 47), **LSM14A** (2), **MAD1L1** (9), **MLANA** (102), **MYO5A** (102), **MZT1** (10), **NRFI** (23), **SKAP2** (102), **SLC12A7** (10), **SOX6** (23), **TRIM24** (10) and **ZKSCAN1** (10). In non-Spitz melanoma subtypes, additional fusion partners have been identified, such as **KIAA1549** in a case of an acral melanoma (8), **ZNF767** in two cases of mucosal melanomas (5, 10), **PPFIBP2** in a case of superficial spreading melanoma (8), **GTF2I** in a metastatic melanoma of unknown primary origin (10), **AGAP3**, **CCDC91**, **CDC27**, **PAPSS1**, **RAD18** and **TAX1BP1** in melanomas either classified as non-Spitz (spitzoid) or unclassified (4, 10).

Similar to the **MAP3K8** fused Spitz subgroup of melanocytic proliferations, the vast majority of **BRAF** melanocytic proliferations belong to atypical Spitz tumor, 41% to Spitz melanoma and only 14% to Spitz nevi (2, 9-11, 23, 43, 47, 71, 74, 83, 101-103). **BRAF** fusions are present in various tumors, including gliomas, thyroid, and pancreatic carcinomas, non-small cell lung adenocarcinomas, and colorectal carcinomas (10).

Secondary genetic alterations in **BRAF** fused Spitz melanocytic proliferations were similar to those in other Spitz subgroups. The most common secondary changes were the homozygous deletion of 9p21, **TERT** promoter mutations, 6p25 gains, and, in single cases, **MDM2** amplification and **ARID2** p.Q720 mutation (11, 43, 47, 101-103).

Histologically, **BRAF** fusions are present in various tumors, including gliomas, thyroid, and pancreatic carcinomas, non-small cell lung adenocarcinomas, and colorectal carcinomas (10).

### **Table 6. Spitz Melanocytic Proliferations with **MAP3K8** Fusions**

<table>
<thead>
<tr>
<th>Morphological features</th>
<th>Ulceration common</th>
<th>Epithelioid morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Moderate to high grade cytological atypia</td>
<td>Lack of maturation</td>
</tr>
<tr>
<td>-</td>
<td>Giant multinucleated melanocytes</td>
<td>Focal hyperpigmented dermal clones</td>
</tr>
<tr>
<td>Biological behavior</td>
<td>Mostly in atypical Spitz tumors and Spitz melanomas</td>
<td></td>
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<tr>
<td>Confirmatory test</td>
<td>Next generation sequencing</td>
<td>Fluorescence in situ hybridization</td>
</tr>
</tbody>
</table>

of genetic techniques, has been detected in about 35% of all reported cases of this **MAP3K8** fused Spitz subgroup of melanocytic proliferation (23, 83, 91, 92), followed by **TERT** promoter mutations and a complex **TERT** structural rearrangement, albeit less frequently (9, 83).

A single case has been reported that resulted in the death of an 11-year old boy, who was diagnosed as having a Spitz melanoma with **MAP3K8-GNG2** fusion, additional complex structural rearrangement in the **TERT** gene, and a homozygous **CDKN2A/B** deletion (9). Three other Spitz melanomas with **MAP3K8** fusions, all three with additional biallelic **CDKN2A** inactivation, demonstrated tumor cells’ deposits in at least one lymph node. However, none of these cases resulted in widespread metastatic disease during the 6 to 18 months follow up period (91, 92). Two atypical Spitz tumors with **MAP3K8** fusions locally recurred, with otherwise no signs of distant metastases (83, 91).
cytoplasm (11, 23, 43, 101-103). Interestingly, some authors have described a common, distinct growth pattern comprising of densely cellular sheet-like proliferation in the superficial part of the lesion, overlying a less cellular, desmoplastic base with prominent dermal sclerosis (Figure 6) (2, 43, 101, 102).

Widespread metastatic disease (i.e., metastases beyond the sentinel lymph node) has been described in 19 patients with melanomas with \textit{BRAF} fusion (4, 5, 8, 10, 11, 47), ten of which were called Spitz melanomas (10, 11, 47). One case of non-Spitz metastasizing melanoma in a 54-year-old male harbored a \textit{BRAF V600E} mutation and an \textit{AGAP3-BRAF} fusion (10). Another case reported as a Spitz melanoma harbored concurrent \textit{BRAF} fusion, \textit{NRAS} mutation, and a \textit{TERT} promoter mutation (83).

### Conclusion

Spitz melanocytic proliferations are defined by distinctive morphological and molecular genetic features. They encompass the whole biological spectrum of proliferations ranging from Spitz nevi, atypical Spitz tumors to Spitz melanomas. While most Spitz nevi can be reliably diagnosed on morphological grounds alone, additional molecular genetic testing is generally necessary to classify atypical Spitz tumors and Spitz melanomas and, significantly, to predict their biological behavior. The proposed algorithm of how to approach Spitz melanocytic proliferations is summarized in Figure 7. Molecular testing includes the detection of different driver fusions and additional genetic events associated with biologic behavior. In addition, since several biological drugs are available to treat melanocytic proliferations with aggressive clinical behavior, characterization of particular driver genetic events and additional genetic abnormalities is becoming increasingly important.
Conflict of Interest: The authors declare that they have no conflict of interest.

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