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ESP, EORTC, and EURACAN Expert Opinion: practical recommendations for the pathological diagnosis and clinical management of intermediate melanocytic tumors and rare related melanoma variants

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Abstract

The recent WHO classification of skin tumors has underscored the importance of acknowledging intermediate grade melanocytic proliferations. A multistep acquisition of oncogenic events drives the progressive transformation of nevi into melanomas. The various pathways described are modulated by the initial oncogenic drivers that define the common, blue, and Spitz nevi groups. Intermediate lesions are most often the result of a clonal evolution within such nevi. Based on this established classification, we have suggested for each pathway a practical diagnostic approach, benefiting from the recently developed molecular tools, both in the setting of general pathology labs and expert centers. Moreover, recommendations regarding the re-excision and clinical follow-up are given to support decision-making in multidisciplinary tumor boards.

Keywords Intermediate melanocytic tumors · MELTUMP · Melanocytoma · Spitz tumors · Deep penetrating nevus · BAP-1 inactivated nevus · Pigmented epithelioid melanocytoma · Melanoma variants

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- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Introduction

The 4th edition of the WHO classification was published in 2018 [1, 2]. Since the previous edition published in 2006, a large amount of scientific data has been accrued and not only have many concepts evolved but a number of old views have changed [3–5]. The expansion of the group of intermediate melanocytic grade tumors is one of the many important developments in the recent classification. While most countries have specific national guidelines for the management of conventional melanomas [6–19], there are currently no established practical recommendations for the management of intermediate melanocytic tumors and rare variants of melanoma [20], whose optimal treatment and prognosis still remains to be more clearly elucidated and defined. A common practice, when confronted with cases belonging to this difficult group, pathologists and dermatopathologists often, seek a second opinion in a referral center, and this usually allows consensus with regard to the appropriate diagnosis [21].

Evolution-based classification of lesions according to the WHO classification

The new multidimensional classification of melanocytic tumors divides them into 9 pathways (“periodical table of elements-style”) [1, 2]. In some of these pathways, a progressive multistep evolution from a benign nevus to a melanoma is proposed and this evolution is associated with acquisition of increasing genomic abnormalities. Specific driver genes are listed for each of these pathways. Techniques enabling the identification of these driver genes help to determine the category that the lesion belongs to but do not allow assessment as to whether the tumor has progressed to one of the more advanced stages. For example, the presence of a canonical GNAQ mutation confirms a given skin lesion as part of the “blue group of melanocytic neoplasms” but does not help distinguishing a cellular blue nevus from an atypical cellular blue nevus or a melanoma ex-blue nevus; it however rules out a deep penetrating nevus or a pigmented epithelioid melanocytoma-related neoplasm, which are often confused with the former. Some gaps remain to be filled as the list of involved driver genes for each pathway is not definitive and some cases remain difficult to categorize with certitude. Also, some driver gene mutations (such as *BRAF*, *NRAS* or *GNAQ*) overlap in several pathways, when other contextual features, such as the presence of a congenital nevus or a specific location, are involved in their definition.

The known frequent secondary genomic events associated with progression are detailed for each category. Prognosis of advanced tumors is variable in each of the 9 pathways, and these are considered as different diseases, i.e., with potentially different treatments, although all of them are related in a given category.

Intermediate lesions

In pathways 2 (chronic sun damage), 3 (desmoplastic), 5 (acral), and 6 (mucosal), melanomas arise from intraepithelial melanocytes without any associated nevi. In the remaining pathways involving the skin, the intermediate melanocytic lesions arise from a pre-existing nevus (although such nevus is only identified histopathologically in ~ 30% of cases). They do not however fulfill the diagnostic criteria for malignancy. The WHO classification identifies 2 different grades of intermediate lesions, both covered under the term “melanocytomas.” There are distinctive low-grade and high-grade lesions. A list of the cyto-architectural features defined in these intermediate tumors is given in Table 1. As a rule of thumb, the criteria defining these grades are the same usual morphological

Table 1 General morphological criteria for atypia

<u>Architectural criteria</u> (low-power view analysis)
Diameter > 6 mm
Asymmetry
Epidermal effacement
Ulceration
High dermal cellularity
Tumor clones
Loss of grenz zone
Absence of vertical “maturation” (progressive reduction of size of nests, cells and pigmentation load)
Expansile nodule formation
Destructive growth pattern (tumor destroys entrapped adnexal structures)
Deep subcutaneous extension
Ascending cells/pagetoid spread within the epidermis
<u>Cytological criteria</u> (high-power view analysis)
Cellular pleomorphism (important variation in adjacent cells of nuclear and cytoplasmic size)
Macro-eosinophilic nucleoli
Variable density of nuclear chromatin
Irregular nuclear membrane (cleavage or folding)
Dermal mitotic activity (> 1/mm ² , atypical and deep/marginal mitosis)
Overlapping nuclei
Tumor necrosis

malignancy criteria used generally in pathology. Basically, in a nevus, almost none of these criteria will be found; in a low-grade melanocytoma, few of them would be identified; in a high-grade melanocytoma, nearly half of them would be present; and in a melanoma, the majority would be seen.

We herein adopted the terminology “high-grade melanocytoma” rather than MelTUMP (melanocytic tumor of unknown malignant potential) as some may consider that lesions diagnosed as MELTUMP should be treated as melanoma according to national guidelines (i.e., because melanoma cannot be ruled out) [22].

For practical purposes, we will break down each specific pathway category of tumors, with a subheading on each, listing specific molecular findings. Whenever possible, a three-tier system (benign, intermediate and malignant) will be used. The practical management (molecular diagnosis, surgery and follow-up) of the intermediate tumors within each subgroup is the main objective of these recommendations and focuses on intradermal proliferations.

Molecular tests are helpful in the diagnosis of lesions associated with several of the predefined criteria of atypia. Table 2 provides a list of ancillary tests that are usually performed in most pathology laboratories allowing the screening of complex cases and, also, the more complex molecular techniques available only in referral centers. This list will likely be modified and expanded in coming years as our experience and knowledge in this field advances. Specific tests will be described in each of the subsections where appropriate.

Table 2 Molecular testing of ambiguous lesions

Methods	Proteins and genes studied	Average turnaround time for results
(A) Tests that could be routinely performed in all pathology labs with a high volume of melanocytic tumors:		
IHC	PMEL (HMB-45); SOX10; MiTF, tyrosinase, MART-1; P16; Ki-67/MIB1; BRAF V600E; ALK1; ROS1; BAP1; beta-catenin; PRAME.	24–48 h
Molecular biology methods (Sanger sequencing, real-time PCR, PCR, NGS)	<i>BRAF</i> codon 600 mutations: c.1799T>A and c.1799_1800TG>AA p.(V600E); c.1798_1799GT>AA p.(V600K); V600D); 1798_1799GT>AG p.(V600R). <i>NRAS</i> , exon 2 codon 12,13; exon 3 codons 49,61; exon 4 codons 117,146	7 days 7 days
(B) Tests that are currently performed mainly in specialized referral centers		
IHC	PRKAR1A; NRAS Q61R; NTRK1; Pan-TRK; HRAS WT; MET; P21 (spitzoid lesions).	48 h
Mutation detection by sequencing or others molecular biology methods (NGS; Sanger sequencing, real-time PCR, pyrosequencing)	<i>BRAF</i> exon 11, 15; <i>N/H/K-RAS</i> exon 2, 3; <i>KIT</i> exon 11, 13, 17 and 18; <i>NFI</i> ; <i>GNAQ</i> exon 4 and 5; <i>GNAI1</i> exon 4 and 5; <i>CYSLTR2</i> p.(L129Q); <i>PLCB4</i> ; <i>BAP1</i> ; <i>SF3B1</i> ; <i>EIF1AX</i> ; <i>MAP2K1</i> ; <i>TERT</i> promoter mutations; <i>CTNNB1</i> .	7 days
Gene amplifications or deletions by FISH	<i>CDKN2A</i> ; <i>TERT</i> ; <i>CCND1</i> ; <i>MDM2</i> ; <i>RREB1</i> ; <i>MYB</i> ; <i>CCND1</i> ; <i>CEP6</i> .	72 h
Fusion detections (FISH, RT-PCR, NGS targeted RNAseq, nanostring)	<i>ALK</i> ; <i>ROS1</i> ; <i>NTRK1</i> ; <i>NTRK3</i> ; <i>MAP3K8</i> ; <i>BRAF</i> ; <i>MET</i> ; <i>RET</i> ; <i>PRKCA</i> .	3–21 days
CNV analysis (CGH array, NGS, nanostring; shallow NGS, MLPA)	3p21 loss (<i>BAP1</i>); 9p21 loss (<i>CDKN2A</i>); gain 11p (<i>HRAS</i>), specific breakpoints areas in fusion genes, assessment of number, and type of CNVs.	14–21 days

Initial excision of the lesion

The initial excision is usually performed outside of specialized centers, and the melanocytic nature of lesions is not always obvious before the procedure.

As a reminder, as cited in several existing melanoma guidelines, in order for an accurate histological diagnosis of a melanocytic lesion to be made, every effort should be made to provide the following clinical information:

- Time of presentation (since birth, childhood, adulthood)
- Topography
- Clinical size
- Modifications leading to consultation/removal (change in size, color, thickness, presence or absence of ulceration)
- Dermoscopic and/or clinical pictures
- Suggested clinical diagnosis

Complete excision with a 2-mm minimal clinical margin is preferable (i.e., no incisional biopsies) if a lesion is clearly identified as melanocytic. In rare instances, when due to the location or size of the lesion, more extensive or complex surgery is required, an incisional biopsy can be performed. For optimal interpretation, an annotated picture or schematic drawing of the lesion should be provided.

General surgical and clinical recommendations for all subgroups

Table 3 summarizes the resection margins suggested for each subtype of intermediate melanocytic lesions included in these recommendations. All margins discussed herein refer to clinical margins as skin samples suffer from shrinkage related both to devascularization and fixation techniques. Figure 1 displays a schematic view of the workflow.

The management of these lesions should be discussed in a multidisciplinary tumor board (MTB) with results of appropriate ancillary techniques when needed.

In most low-grade intermediate lesions, a 2-mm margin is recommended. This clinical margin is to ensure a complete removal of the histologically verified lesion. As we have underscored in the “Initial excision paragraph” above, recommendations suggest pigmented lesions should be excised ideally with 2-mm margins. Therefore, lesions that already have clear, albeit narrow, margins from an excisional biopsy do not require additional excision to some arbitrary limit, although some room for exceptions based on personal preferences could be permitted following MTB discussion.

In most high-grade intermediate lesions, a 5–10-mm re-excision is recommended. Clinicians should evaluate the patient individually based on several factors affecting the risk of recurrence. The recommended follow-up physical examinations and loco-regional lymph node sonography is every 6 months for at least 5 years. Longer follow-up care may change in consideration of additional individual risk factors. If a diagnosis of melanoma cannot be ruled out following molecular pathology studies, we recommend the case should be treated as a melanoma of similar Breslow thickness.

Table 3 Suggested resection margins for intermediate melanocytic tumors

	Pathway 1-BAP1	Pathway 1-DPN	Pathway 1-PEM	Pathway 4 Spitz	Pathway 8 Ex-BN	Unclassified atypical dermal lesions
Low grade	2 mm	2 mm	NA	2 mm	2 mm	2–5 mm
High grade	5–10 mm	5–10 mm	5–10 mm	5–10 mm	5–10 mm	5–10 mm

DPN deep penetrating nevus, PEM pigmented epithelioid melanocytoma, Ex-BN ex blue nevus, NA not applicable

Specific subgroup recommendations

Pathway 1 (low chronic sun damage (CSD) melanoma/SSM endpoint pathway)

Genetic background: *BRAF* (exon 15) or *NRAS* (exon 2 and 3) hotspot mutations predominate but are not exclusive.

The WHO breaks down this group into subcategories that will be described separately with regard to available ancillary tests and recommendations.

Lesions with dysplastic features and leading to in situ SSM-type melanomas have been excluded from this contribution, as they do not fit the general recommendations for the mainly dermal lesions described herein. We advocate for specific separate recommendations for this subgroup as they can also occasionally be explored by molecular techniques.

Pathway 1 (BAP1-inactivated melanocytic tumors, Example 1)

BAP1-inactivated nevus (BIN) (low-grade dysplasia/melanocytomas):

BIN is usually a combined nevus. Cellularity, atypia, and mitotic activity are usually low in the clone. The diagnosis is best confirmed by BAP1 IHC with loss of nuclear expression in the altered melanocytes. Optionally, a *BRAF* V600E mutation can easily be confirmed by immunohistochemistry (IHC) and can be helpful, when present, to rule out a lesion of the Spitz group. Proliferation index is low (< 5%) in the melanocytes.

The clonal area of a BIN should be completely removed with ideally 2-mm margins and genetic counseling should be given according to personal and familial history.

BAP1-inactivated melanocytoma (BIM) (high-grade dysplasia/melanocytoma):

In rare occurrences, a cellular, large (> 6mm) dermal clone within a compound nevus with important proliferation (> 10%), mitotic activity and loss of nuclear BAP1 expression

suggest a diagnosis of BIM. We recommend such cases be sent to a referral center for confirmation. This requires ruling out a melanoma ex-BAP1-inactivated nevus.

Pathway 1—deep penetrating nevus (DPN) type lesions (Example 2)

Deep penetrating nevus (low-grade dysplasia/melanocytomas):

DPN is a morphological diagnosis and no ancillary techniques are needed in typical lesions. In ambiguous cases, DPN can be confirmed by beta-catenin IHC with cytoplasmic and nuclear staining of deep-seated melanocytes; however, variants located in the superficial dermis are also recognized, usually but not exclusively, as a part of a combined melanocytic proliferation. Mutation studies can identify hotspot mutations in beta catenin exon 3 or APC as well as activating mutations of MAP Kinase pathway (*BRAF*, *NRAS*, *HRAS*, and *MAP2K1* mutations). These lesions should be completely removed, especially with regard to their deep expansion, ideally with a 2-mm margin.

Atypical DPN—high-grade dysplasia/melanocytoma:

These rare lesions should be sent to a referral center for confirmation and to rule out a plexiform melanoma that often mimics these neoplasms. Tumors should be removed with a 5–10-mm margins.

Pathway 1—pigmented epithelioid melanocytoma (PEM, Example 3)

This group of tumors described in 2004 has originally been considered as “low-grade melanomas” [23]. In the current WHO 2018 classification, all cases are classified under the intermediate/high-grade category.

PEMs are rare, and their morphological diagnosis is difficult. We recommend that all suspected cases are sent to a referral center for confirmation of the diagnosis.

The diagnosis of PEM can be confirmed by IHC with loss of cytoplasmic PRKARIA expression or identification of a *PRKCA* fusion either by FISH break-apart techniques, real-time PCR, or NGS [24–26]. The later define two different subtypes of PEM, and only those with *PRKARIA* alterations are potentially linked to Carney complex. Combined PEM is observed in the context of a pre-existing *BRAF* p.(V600E)-mutated background nevus. Confirmed cases should be removed with a 5–10-mm margin, and genetic counseling should be given when needed.

Pathway 2 (high CSD melanoma/LMM endpoint)

These melanomas should be completely removed with margins as recommended in other national/international guidelines.

Pathway 3 (desmoplastic melanoma)

These melanomas should be completely removed with margins as recommended in other national/international guidelines.

Pathway 4 (Spitz tumors, Example 4)

Spitz and Reed nevi represent a morphological diagnosis and no ancillary techniques are needed in typical lesions. The initial excision should be complete, with narrow margins of 2 mm.

The Spitz group of tumors has overlapping morphologic features characterized by a variety of molecular anomalies including *HRAS* hotspot mutations, tyrosine kinase fusions (*ALK*, *ROS1*, *NTRK1/3*, *RET*, and *MET*), and serine-threonine kinase fusions (*BRAF*, *MAP3K8*). Truncating mutations of *MAP3K8* are also present. The presence of molecular features related to another pathway of melanocytic proliferation (a *BRAF* p.(V600E) mutation for example) de facto excludes a case from the Spitz group even if it has “spitzoid” morphology [27]. When genetic testing or IHC Spitz markers are not available, positive *BRAF* IHC can be used as a screening tool to exclude a lesion from the Spitz group. However, if the staining is negative, a non-p.(V600E) *BRAF* mutation cannot be excluded, respectively.

IHC screening can be performed with antibodies against *ALK*, *ROS1*, *MET*, *HRAS*, *NTRK1*, and pan-TRK (*NTRK1-3*) to identify high cytoplasmic level of protein expression. If the results of this screening implies additional surgical procedures, immuno-positive cases should be confirmed for the respective genomic aberration by molecular methods. This can be quickly done by FISH-specific break-apart probes. However, only real-time PCR or NGS allow the

identification of specific 3' or 5' fusion partners. No specific IHC is available for *BRAF*, *RET*, *NTRK3*, and *MAP3K8* fusion products. It is important to note that it is likely that not all genetic anomalies related to this group have yet been identified, and the list could be expanded in the coming years as more cases are analyzed.

Loss of p16 IHC expression, especially if only present in a clone, is an important feature leading to regard a Spitz proliferation as not a standard Spitz nevus. Preservation of p16 expression is reassuring in this morphological and genetic setting, but loss of p16 is not necessarily associated with bad outcome. *CDKN2A* FISH techniques or array techniques can be used to evaluate if a heterozygous or homozygous deletion of 9p21 is present, as the latter could be used as a diagnostic element in favor of a malignant proliferation.

AST

Currently, according to the latest suggestions of the WHO Classification, most pathologists merge low- and high-grade atypical Spitz tumors into a single atypical Spitz tumor group [1, 2]. Well-defined criteria do not exist to separate between the two groups.

This group of uncommon lesions displays many atypical clinicopathological features (size > 10 mm, high dermal density and/or high mitotic activity) and, in addition, can have immunophenotypic aberrations such as heterogeneous dermal expression of HMB45, a high proliferation rate (> 10%) or clonal p16 loss. The latter can be explored by *CDKN2A* FISH techniques for homo/heterozygous deletion [28, 29]. In addition, array-based techniques can be used for detection of copy number variations of *CDKN2A*. Atypical Spitz tumors and malignant Spitz tumors often have one or more chromosomal abnormalities. The actual number that discriminates AST and MST with certainty, however, needs to be determined (a cut-off of 3 or more alterations has been suggested by some authors for most melanomas) [30, 31]. Genetic alterations involving the promoter region of *TERT*, mainly but not exclusively mutations, can also be detected and favor malignancy. AST is often a diagnosis of exclusion, and we advise sending these lesions to a referral center for genetic subtyping and to rule out a malignant Spitz tumor (Spitz melanoma). The latter is not always possible due to overlapping histological and molecular alterations between the two groups. Sometimes only long-term follow-up with distant metastases will confirm the initial diagnosis of malignant Spitz tumor (Spitz melanoma).

Excision with a 5–10-mm margin is recommended in AST. It is desirable that excision margins are tailored to individual cases based on size and depth of involvement. A SLN biopsy procedure is not currently recommended in these tumors

because in principle, this procedure is not predictive of outcome even when positive [32, 33]. However, sentinel node staging may be considered in selected situations (e.g., when a malignant Spitz tumor cannot be ruled out) following MTB discussion.

Although the number of reported cases is low, we advise more caution in the management of *BRAF* or *MAP3K8*-fused cases that may follow a more aggressive course (10-mm margins recommended) compared with tyrosine kinase–fused cases, especially if *TERT* promoter mutations are also present [34–36].

Pathway 5 (acral melanoma)

Most of the melanomas arising in this group do not develop on pre-existing nevi. It has been suggested that a major chromosomal rearrangement, such as chromothripsis, in a normal melanocyte leads to in situ transformation from which an invasive melanoma can arise. These melanomas should be completely removed with margins as recommended in standard national/international guidelines.

Pathway 6 (mucosal melanoma)

Most of the melanomas arising in this group do not develop on pre-existing nevi. As with acral melanomas, it has been suggested that chromosomal rearrangements are the initial drivers of malignant transformation. These melanomas should be completely removed with margins as recommended in standard national/international guidelines.

Pathway 7 (melanoma in congenital nevus)

This group includes melanoma arising in both small and large pre-existing nevi and is associated with a canonical mutation in *NRAS* exon 2 or 3 or *BRAF* exon 15.

Excluded from this group are all the previously described lesions in pathway 1 (*BAP1*-inactivated, *PRKAR1A*-negative and beta catenin-activated DPN clones) and lesions in which the congenital status of the nevus is ambiguous (congenital-like architecture).

The main diagnostic consideration in this category, especially in children, is the distinction between a proliferative nodule and a nevoid melanoma arising in a congenital nevus (ex-nevus). Array techniques are helpful in making the distinction between these two entities [37]. Proliferative nodules show gains and losses of complete chromosomes in contrast to gains and losses of individual chromosome arms in melanoma. A few alterations such as an amplification of canonical mutated exon 2, 3 *NRAS*, or in the context of Li-Fraumeni syndrome have been reported, but a wider range of anomalies is likely to be identified in the future. These lesions should be completely removed with a 2–5-mm margin. It should be

noted, however, that frequently these nodules occur in giant congenital nevi in which a complete resection cannot be performed. Thus, the resection will be performed with positive nevus margins and further clinical follow-up of the residual nevus is maintained.

Pathway 8 (melanoma in blue nevus)

Common blue nevi and morphologically typical cellular blue nevi (CBN) do not require further IHC, molecular testing, or referral to a specialized center for confirmation of the diagnosis. In CBN, deep involvement of the subcutis is often seen, and as a result of this, tumors are often incompletely excised in the deep margin requiring further excision. Unusual variants or those displaying morphological overlap with atypical DPN and PEM may require confirmation of canonical mutations characteristic of this group (exons 4 and 5 of *GNAQ* or *GNA11*, *CYSLTR2*, and *PLCB4*).

BAP1 IHC is recommended in all atypical and highly cellular blue nevi. Extensive loss of nuclear expression usually suggests malignant transformation.

Array-CGH is a useful tool as an aid in the diagnosis of malignant lesions [38]. Atypical blue nevi (see below) tend to show multiple whole chromosome gains. Malignant lesions display class I/II-type profiles similar to those found in uveal melanomas [39].

Molecular biology techniques may be necessary to assess and confirm the presence of secondary events associated with tumor progression (*BAP1*, *SF3B1*, and *EIF1AX* exon 1 or 2 mutations).

Atypical cellular blue nevi: high-grade dysplasia/melanocytoma

Atypical blue nevus is a diagnosis of exclusion that should only be considered once malignant transformation of a blue nevus has been ruled out. IHC is a useful screening tool. Loss of nuclear expression of *BAP1* and loss of p16 by IHC along with a proliferation rate of more than 20% (with MIB1 or Ki-67) favor a diagnosis of malignancy. Array-CGH is of great help in cases of atypical cellular blue nevus, as the molecular profile either is flat or displays multiple whole chromosome gains. By contrast, the profiles in malignant lesions show segmental gains; losses on chromosomes 1, 6, and 8; and a monosomy of chromosome 3 related to *BAP1* inactivation.

There are no available published studies with scientifically sound evidence based on follow-up providing guidance with regard to resection margins. Margins in the scope of 5–10 mm and follow-up are advised.

Pathway 9 (uveal melanoma)

This group is not skin-related and will not be covered in these recommendations.

Histologically and genetically unclassified borderline melanocytic proliferations

Despite significant progress in classification of melanocytic proliferations, a minority of cases will defy a clear-cut morphological diagnosis. We recommend that these cases be sent to a referral center. Those presenting morphological atypia as described in Table 1 should be evaluated with screening tools to attempt, when possible, to rule out malignancy. They should be treated as a subgroup of unclassified melanocytomas with 2–5-mm margins in low-grade lesions and 5–10-mm margins in high grade as well as follow-up.

Conclusion

The current 2018 WHO classification of skin tumors separates melanocytic proliferations according to 9 different genetic pathways, each with specific background driver anomalies as well as secondary molecular events. In the pathways involving a pre-existing benign nevus, the classification has underscored the presence of intermediate steps of transformation denominated as “low- and high-grade melanocytomas.” The majority of these lesions are initially assessed in general pathology laboratories. We have defined a number of morphological features and specific screening tools for pathologists to confidently identify most of the diagnostic categories and decide when referral/expert consultation is needed to perform more specialized molecular testing for the most uncommon/difficult types of intermediate tumors. We suggest whenever possible a multidisciplinary approach to advise surgical margins and clinical follow-up for each subgroup. Also, when appropriate, education on lifelong regular self-skin examinations should be promoted.

Further follow-up studies involving multiple referral centers worldwide are needed to obtain reliable feedback with regard to these practical recommendations as there is a risk of both over- and under-treatment.

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Authors' contributions AF and DM conceived the study. AF provided the cases for the examples and wrote the manuscript. WB, LCK, BL, SPN, SP, LA, EC, and DM edited and reviewed the manuscript.










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