Atypical Spitz Tumors

A Diagnostic Challenge

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• Spitzoid melanocytic lesions encompass a spectrum from benign Spitz nevi to malignant spitzoid melanomas. Spitzoid melanocytic neoplasms have significant morphologic and molecular differences from conventional melanocytic lesions, and prediction of biologic behavior and metastatic risk may be difficult. Most challenging is the atypical Spitz tumor, a borderline spitzoid melanocytic lesion of uncertain malignant potential that has overlapping histologic features with conventional Spitz nevus and spitzoid melanoma. Atypical Spitz tumors involve the sentinel lymph nodes at a greater frequency than conventional melanoma and frequently harbor chromosomal copy number changes, yet most cases follow an indolent course. Herein we review the clinical, microscopic, and molecular features of atypical Spitz tumors, including recent molecular advances, including the potential prognostic significance of chromosomal abnormalities, such as homozygous *CDKN2A* loss.

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Since they were first described by Sophie Spitz in 1948,[1] spitzoid melanocytic lesions have proven to be an enduring challenge for both clinicians and pathologists, given the difficulty in risk assessment and predicting which lesions have metastatic potential. Particularly challenging are the atypical Spitz tumors of uncertain biologic potential (ASTs), which share histologic features of both conventional Spitz nevi and spitzoid melanomas, but often follow a favorable clinical course. Herein, we summarize the current state of knowledge about ASTs, including recent large-scale studies and molecular discoveries that may provide a better understanding and more accurate diagnosis and prognostication of these rare and challenging lesions.

CLINICAL FEATURES AND COURSE

Spitzoid melanocytic lesions may be broadly categorized into Spitz nevi, ASTs, and spitzoid melanomas.[2–4] Classically, the Spitz nevus presents as a dome-shaped, pink to reddish-brown papule or nodule, usually less than 6 mm in diameter.[4] Spitz nevi commonly arise in childhood but may occur at any age. Lott et al[3] recently studied the clinical features of 484 Spitz nevi in comparison with 54 spitzoid melanomas from the Yale University Spitzoid Neoplasm Repository. They found that Spitz nevi were more common in females, the mean age at diagnosis was 22 years, and the most common location was on the lower extremity.[3]

Spitzoid melanomas present as a growing amelanotic or pigmented papule or nodule. In comparison with Spitz nevi, Lott et al[3] showed that spitzoid melanomas have a slight male predominance and tend to occur at a later age (mean, 55 years). In younger children (prepubertal or younger than 10 years), spitzoid melanoma is typically associated with a more favorable course, although mortalities may occur.[5,6] Some practitioners designate these tumors as "spitzoid melanomas of childhood" to distinguish them from tumors with a more aggressive course in adults.

The AST clinically presents as a raised or dome-shaped pink to red papule or nodule, often greater than 1 cm in diameter.[4] Ludgate et al[7] characterized the clinical features in 67 patients with ASTs at the University of Michigan. They found that ASTs were most commonly amelanotic (51%), the median age at diagnosis was 23.7 years, 61% of patients were female, 30% of tumors were located on the lower extremity, and 28% of tumors were on the head and neck.[7] The accurate classification of a lesion as AST is challenging and often requires assimilation of the clinical presentation, histology, and ancillary studies to differentiate AST from ordinary Spitz nevus and melanoma.

It is important to correctly differentiate among these spitzoid lesions because the clinical course and prognosis vary greatly. Spitz nevi are wholly benign, whereas spitzoid melanomas are malignant and have the potential to metastasize to the regional lymph node basin and distant sites. Multiple studies have clearly demonstrated that ASTs have a high rate of sentinel lymph node positivity, with an average rate of 38% to 39%.[8,9] However, despite relatively frequent micrometastatic spread to the regional basin, these patients have a favorable prognosis compared with patients with metastatic melanoma.[7,8,10] Documented fatal cases of

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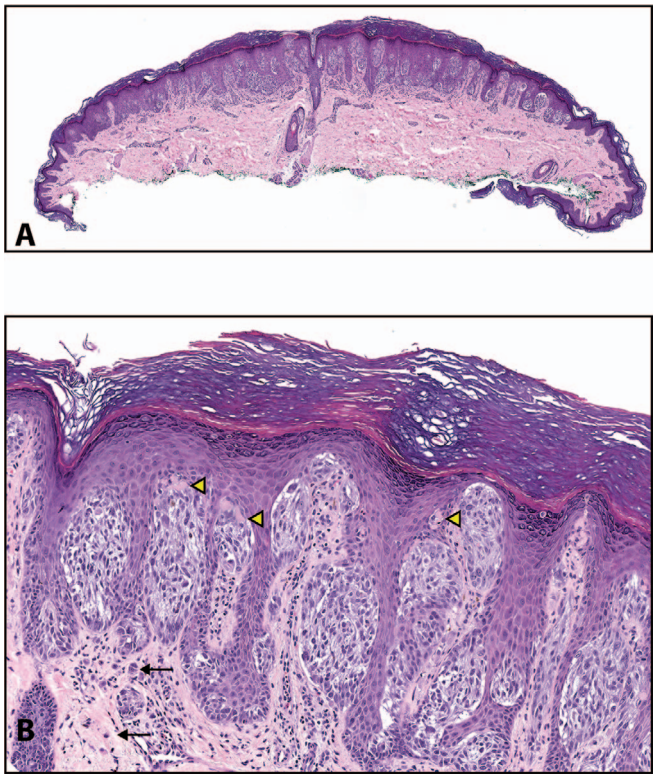

Figure 1. *Spitz nevus. A, Scanning magnification demonstrates a predominantly nested, symmetrical lesion with associated epidermal acanthosis. Nests are large, with clefting. B, Higher magnification demonstrates enlarged epithelioid melanocytes with prominent nucleoli arranged in nests. Kamino bodies (yellow arrowheads) may be rare or numerous. Dermal nevomelanocytes retain spitzoid morphology but show decreased size with descent into the dermis, indicated by arrows (hematoxylin-eosin, original magnifications ×15 [A] and ×100 [B]).*

AST are exceedingly rare.[7,8,10] The high rate of sentinel lymph node positivity in AST cases and low incidence of adverse outcome suggest that the biology of AST differs from that of conventional melanoma.[8,9] Thus, appropriate identification and classification of these challenging lesions are paramount in order to avoid overtreatment, as well as undertreatment.

HISTOPATHOLOGY

Benign Spitz nevi are well-circumscribed and symmetric melanocytic lesions comprising epithelioid and/or spindled nevomelanocytes with prominent nucleoli. Spitz nevi may be junctional, compound, or wholly dermal. There is generally epidermal hyperplasia and often artifactual clefting around junctional nests (Figure 1, A).[2,4,11] In addition, junctional nests often display a vertical orientation of cells within nests, which gives the appearance of "raining down" (Figure 1, B). Kamino bodies, eosinophilic globules comprising basement membrane material, are often present within the epidermis (Figure 1, B).[2,11] There may be pagetoid scatter of single melanocytes within the upper layers of the epidermis, most commonly in the center of a lesion, but well-formed nests predominate.[2,11] In the dermal component, there is evidence of architectural and cytologic maturation with decreased nest and cell size, respectively, with descent into the dermis. The dermal component displays symmetry from side to side at all levels of the lesion.[11] In contrast to the small, inconspicuous deep nevomelanocytes of conventional nevi, deeper dermal nevomelanocytes in Spitz nevi often retain epithelioid morphology and visible nucleoli (Figure 1, B), and are relatively larger than the nevomelanocytes in conventional nevi. Low-grade nuclear pleomorphism is typical; however, high-grade cytologic atypia is absent. Junctional mitoses are not infrequent; however, dermal mitoses should be inconspicuous. Pigmentation is usually minimal. When significant pigment is present in a spindled nevus, the diagnosis of pigmented spindle cell nevus of Reed is often appropriate (likely representing a Spitz variant).[2] On occasion, Spitz nevi may demonstrate greater degrees of atypia, characterized by higher-grade cytologic atypia, increased pagetoid scatter, and/or architectural overlap with dysplastic nevus, such that the terms *dysplastic Spitz nevus* or *atypical Spitz nevus* are used.

Spitzoid melanomas demonstrate some morphologic features of Spitz nevi, such as large epithelioid or spindled melanocytes with prominent nucleoli, associated epidermal hyperplasia, and vertical orientation of melanocytes in some junctional nests (Figure 2, A).[2] However, worrisome features of melanoma are also present, which may include asymmetry, poor circumscription, extensive pagetoid scatter of the junctional component (Figure 2, B), epidermal consumption, aberrant dermal growth, incomplete or absent dermal maturation (Figure 2, C), high-grade nuclear atypia, and/or increased dermal mitoses that may be deep, marginal, and/or atypical.[11] Conventional melanoma may also display a spitzoid cytomorphology; thus, there is a degree of subjectivity and interobserver variability in classifying a melanoma as spitzoid.[2]

Atypical Spitz tumors were historically described as large tumors comprising densely cellular fascicles of spindled spitzoid melanocytes displaying expansile growth associated with compression of the surrounding stroma (Figure 3, A and B).[2,12] In practice, ASTs demonstrate some features reminiscent of ordinary Spitz nevus, such as a spitzoid cytomorphology, epidermal hyperplasia, and/or a "raining down" vertical orientation of the junctional nests. There are also worrisome histologic features that overlap with melanoma, making a diagnosis of ordinary Spitz nevus untenable. These worrisome histopathologic features include large size (often >10 mm in diameter); asymmetry; poor circumscription; ulceration; higher-grade nuclear atypia; deep dermal extension, occasionally with involvement of the subcutis; aberrant dermal growth that demonstrates an increase in cellularity and is often expansile or sheetlike; incomplete or absent maturation; and increased dermal mitoses that may be deep or marginal (Figure 3, B).[4] An AST may not demonstrate all of these atypical histologic features, yet it must show enough of them that the lesion is regarded to not be an ordinary Spitz nevus and to not be unequivocally melanoma. Because the constellation of atypical histologic features falls into a borderline category, ASTs have historically been regarded as having an uncertain biologic potential. When present, sentinel lymph node deposits are typically small, subcapsular in location, and phenotypically resemble the primary lesion (Figure 3, C).[10] Frankly malignant features in a lymph node metastasis, such as dense sheets of pleomorphic tumor cells with necrosis, support classification of the lesion as malignant rather than borderline.[13]

Because ASTs are by definition lesions with borderline histopathologic features, diagnostic consensus is difficult to achieve. Recently, Gerami et al[14] studied the agreement of

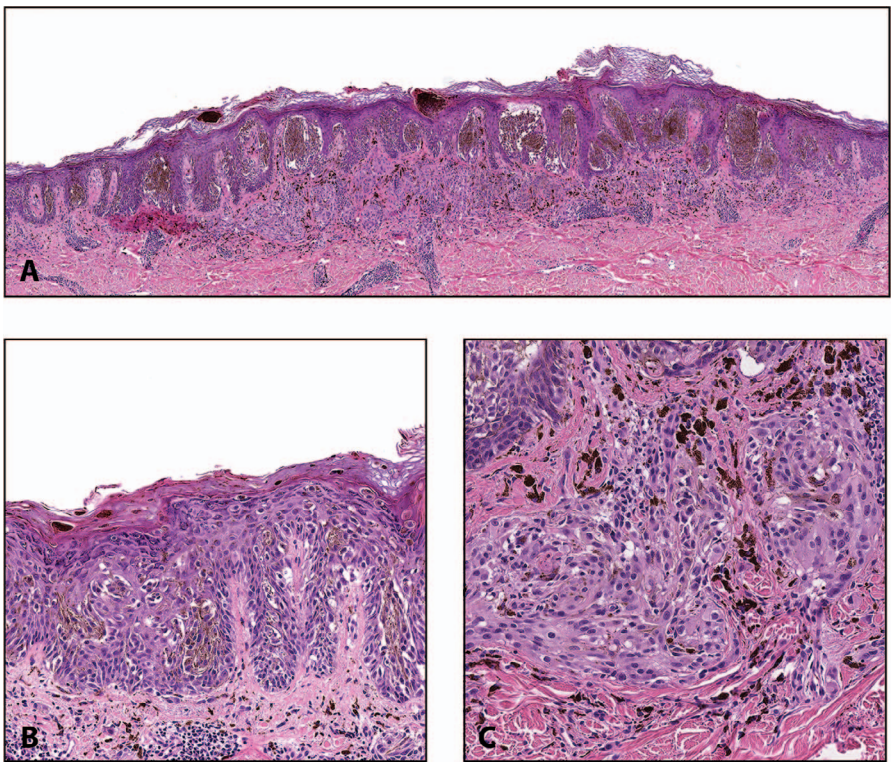

Figure 2. *Spitzoid melanoma. A, Scanning magnification reveals an asymmetric lesion with large nests and associated epidermal acanthosis. B, Higher magnification reveals poorly nested areas with pagetoid scatter, consistent with melanoma in situ. C, Dermal component displays atypical spitzoid cells without evidence of maturation (hematoxylin-eosin, original magnifications ×20 [A] and ×200 [B and C]).*

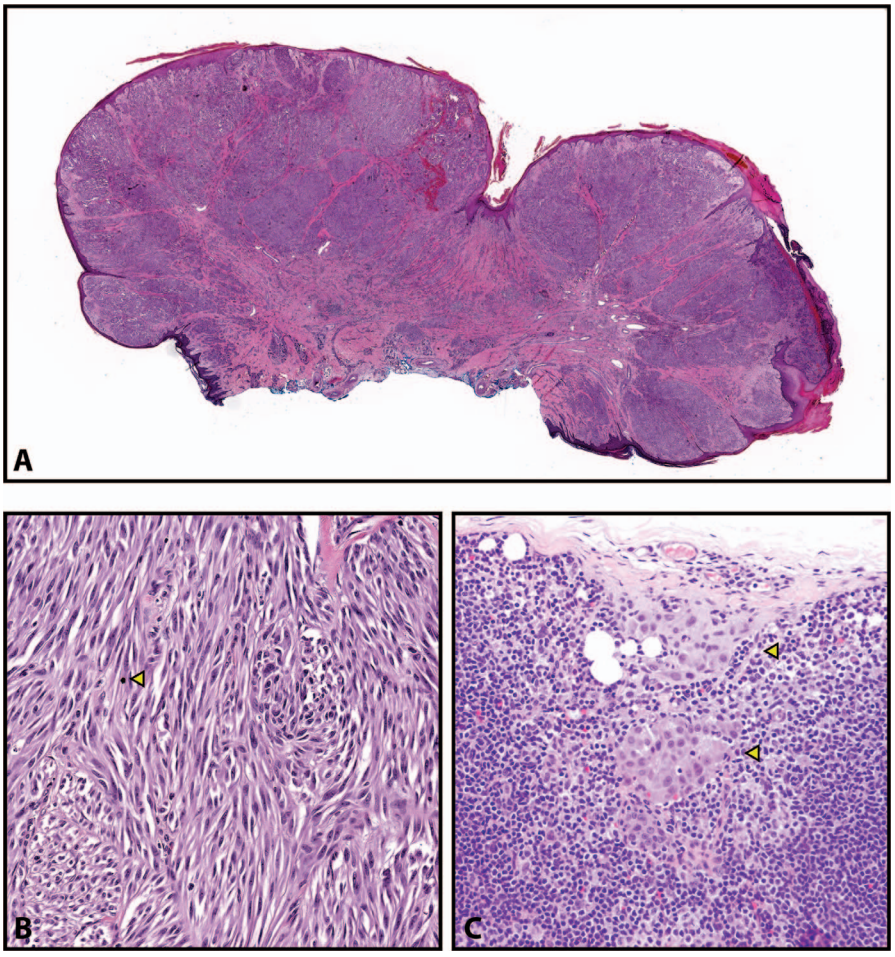

Figure 3. *Atypical Spitz tumor of uncertain biologic potential. A, Large, polypoid tumor with ulceration. B, Cellular fascicles of spindled spitzoid cells with minimal pleomorphism and occasional mitotic figures (yellow arrowhead). C, Subcapsular deposit of spitzoid cells (yellow arrowheads) in sentinel lymph node for atypical Spitz tumor (hematoxylin-eosin, original magnifications ×5 [A], ×400 [B], and ×200 [C]).*

13 expert dermatopathologists for 75 atypical Spitz tumors. They found low interobserver agreement. However, within this cohort, frequent mitoses, deep mitoses, asymmetry, high-grade cytologic atypia, and ulceration correlated with disease progression.[14] Given the diagnostic and prognostic challenges posed by ASTs, ancillary studies, including immunohistochemical staining, comparative genomic hybridization, and fluorescence in situ hybridization (FISH), have become potentially useful adjunctive tools to aid in diagnosis, appropriate classification, and potential risk stratification of ASTs.

ANCILLARY STUDIES

Immunohistochemical analysis of markers for melanocyte lineage (HMB-45), cell cycle regulators (p16), and proliferation (Ki-67) are often useful in the evaluation of melanocytic lesions.[15] These markers should not be used in isolation, but they may aid in the distinction between benign nevi and melanoma, including the distinction between Spitz nevi and spitzoid melanomas. For example, in many cases (albeit not all of them), benign Spitz nevi demonstrate maturation with loss of HMB-45 staining at the base of the lesion, whereas spitzoid melanomas often retain HMB-45 staining throughout the lesion.[11,16,17] Although reports are mixed, most studies find that Spitz nevi commonly retain expression of p16, a cyclin-dependent kinase inhibitor, whereas spitzoid melanomas may show a loss of p16 in a minority of cases.[18–21] Additionally, as determined by immunostaining for Ki-67, Spitz nevi often show a low proliferation index, whereas spitzoid melanomas show a higher proliferation index.[11,18,22,23] There is significant variability among studies regarding the percentages of Ki-67 labeling index in benign and malignant lesions, although one study recommended a value of less than 2% Ki-67 labeling index as favoring benign, greater than 10% as favoring malignant, and 2% to 10% as indeterminate.[23] The accuracy of Ki-67 proliferative index staining may be improved by double staining for a melanocytic marker, such as Melan-A, to assist in excluding stromal and inflammatory cells from analysis. In the context of ASTs, immunohistochemistry has not been extensively explored, and thus provides less definitive information. Ki-67 staining patterns in ASTs are thought to be intermediate between Spitz nevi and spitzoid melanoma.[22,24] In addition, loss of p16 expression may occur in a minority of ASTs.[25]

Although spitzoid lesions may be generally similar to other melanocytic lesions with respect to immunohistochemical staining patterns in benign and malignant lesions, extensive evidence indicates that spitzoid neoplasms are distinct from conventional melanocytic lesions with regard to chromosomal copy number aberrations and oncogenic driver mutations. Therefore, numerous studies have focused on molecular evidence that may be useful for risk stratification in spitzoid lesions.

Conventional melanomas are characterized by high genomic instability, with copy number gains and losses (often multiple) in 96% of cases.[26] In contrast, Spitz nevi generally lack chromosomal aberrations, with the exception of gains of 11p (20%), gains of 7q (rare), or tetraploidy (5%–10%).[27–29] Heterozygous loss of 9p21 has been reported in 1 Spitz nevus.[30] The finding of more than a single chromosomal aberration in Spitz nevi is exceedingly rare. Comparative genomic hybridization, a molecular technique that analyzes copy number variations, takes advantage of this observation to characterize the genetic instability of a melanocytic lesion. In 2011, a study by Raskin et al[31] showed that ASTs may harbor chromosomal aberrations that are distinct from melanoma and ordinary Spitz nevus. This study, the largest to date, analyzed comparative genomic hybridization on 16 cases of AST with clinical follow-up. A total of 8 cases had a positive sentinel lymph node biopsy, and 1 case had a fatal outcome. Comparative genomic hybridization demonstrated chromosomal copy number changes in 7 of the 16 cases. Importantly, the alterations were not those commonly identified in conventional melanoma, and the presence of aberrations did not correlate with sentinel lymph node positivity. In addition, the aberrations identified in the fatal case may be seen in melanoma, such as the loss of 8p and 9 and gain of 8q; however, the most common melanoma aberrations involving 6p, 6q, and 11q were not detected. Thus, this study suggests that ASTs represent a distinct morphologic entity or category of borderline melanocytic tumors with its own heterogeneous molecular signature.

Given the technical challenges of comparative genomic hybridization, there is an interest in developing alternative assays for evaluation of copy number variation in melanocytic lesions. Fluorescence in situ hybridization is a molecular technique that evaluates genetic alterations at specific loci. When using a 4-probe FISH panel evaluating loci commonly altered in melanoma—*MYB* (6q23), *RREB1* (6p25), *CCND1* (11q13), and centromere 6 (Cep6)—studies have found high sensitivity and specificity when distinguishing unequivocal nevi from melanoma.[32] Because ASTs have been proven to be distinct morphologically, with a heterogeneous molecular profile, compared with conventional melanocytic neoplasms, it is not surprising that this original 4-probe FISH panel was negative in all of the 16 ASTs evaluated by Raskin et al.[31] Additionally, Gaiser and colleagues[33] studied 12 ambiguous melanocytic lesions with clinical follow-up and found that the 4-probe FISH panel did not have clinically useful sensitivity or specificity for these ambiguous melanocytic lesions.

More recently, an alternative FISH panel has been tested that replaces *MYB* and centromere 6 with *CDKN2A* and *MYC*, thus evaluating genetic alterations at *RREB1* (6p25), *MYC* (8q24), *CDKN2A* (9p21), and *CCND1* (11q13). This alternative panel increases the sensitivity for detecting spitzoid melanoma.[34] Given the need for ancillary studies to aid in the identification of AST, Gerami et al[35] studied the utility of the conventional 4-probe FISH and the alternative FISH panel in childhood ASTs, childhood spitzoid melanomas with known copy number changes, and conventional childhood melanomas with clinical follow-up. In the outcome analysis, this study combined the ASTs and the spitzoid melanomas into a group termed *spitzoid neoplasms*. In the spitzoid neoplasms, homozygous deletion of *CDKN2A* (9p21) and sentinel lymph node status correlated with the development of tumor extension beyond the sentinel lymph node. Interestingly, none of the 14 spitzoid neoplasms with isolated deletion of *MYB* (6q23), a target in the original 4-probe FISH panel, developed tumor spread beyond the sentinel lymph node. The FISH results for cases of conventional childhood melanoma demonstrated more molecular heterogeneity compared with the spitzoid neoplasms. A potential limitation of this study was that ASTs and spitzoid melanomas were combined into a single category. Later studies that specifically examined ASTs found that homozygous 9p21 loss was associated histolog-

ically with severe cytologic atypia, epithelioid cytomorphology, and increased dermal mitotic activity, and prognostically with poor outcome.[25,36] In contrast, ASTs with heterozygous 9p21 deletion were not associated with distant metastasis.[25] However, a later study reported a fatal outcome in a pediatric AST with heterozygous 9p21 loss.[37] In summary, current evidence suggests that the presence of chromosomal numeric abnormalities alone may not be predictive of clinical course in ASTs, but specific abnormalities may be associated with low risk (no abnormality, 6q23 deletion), intermediate risk (6p25 gain, 11q13 gain), or high risk (homozygous 9p21 deletion).[25,35,36,38]

Other markers and genetic alterations are under investigation to assess whether they have a role in identifying or further defining ASTs. Based on current evidence, spitzoid neoplasms may be divided into 3 molecular categories, specifically those with (1) *HRAS* activation and/or amplification, (2) *BAP1* inactivation (frequently with *BRAF* activation), and (3) kinase fusions.[39] Additional as-yet undescribed drivers may be present in a small minority of spitzoid neoplasms. *HRAS* mutation occurs in a minority of Spitz nevi and ASTs, and it may be associated with a wedge-shaped dermal profile and desmoplastic morphology.[40–42] Importantly, van Engen-van Grunsven et al[43] found that in their cohort of 24 AST cases harboring *HRAS* mutation, none developed recurrence or metastasis. However, *HRAS* mutations have been described in a small minority of melanomas, including one case of spitzoid melanoma with metastasis and multiple recurrences.[39,44] Publicly available sequencing data from 278 cutaneous melanomas in The Cancer Genome Atlas indicates the incidence of *HRAS*-activating mutations across all cutaneous melanomas to be less than 1%.[45] In summary, these findings suggest that *HRAS*-activating mutations are much more likely to occur in spitzoid neoplasms with a favorable course. Reports are mixed regarding mutations of RAS-RAF pathway components other than *HRAS* in spitzoid lesions, but most evidence suggests *BRAF* and *NRAS* mutations are infrequent events, with the exception of tumors with concomitant BAP1 loss (discussed below).[41,46–49]

BAP1 inactivation by mutation and/or copy number loss was recently identified by next-generation sequencing in a subset of Spitzoid lesions.[46,47] Nomenclature for these tumors is not yet settled; proposed names include Wiesner nevus, melanocytic *BAP1*-mutated atypical intradermal tumors (MBAITs), and nevoid melanomalike melanocytic proliferations (NEMMPs).[50] The ASTs with *BAP1* inactivation display a distinctive morphology.[46,47,51] Tumors are predominantly composed of epithelioid cells with two populations (smaller epithelioid cells more similar to conventional nevus, and larger, more atypical epithelioid cells) that blend together. More atypical cells of the lesion do not show expected maturation with depth. Mitotic activity is typically lacking. Some inflammatory response may be present. These tumors lack BAP1 expression by immunohistochemistry. This is useful because morphologic findings may not allow for distinction from other ASTs.[50] In addition to BAP1 loss, *BRAF* V600E mutations are often detected.[46,47] *BAP1*-inactivating mutations may be either sporadic or germ line, with an associated heritable cancer syndrome and multiple ASTs.[46,47] Therefore, the finding of multiple ASTs with epithelioid morphology in a patient should prompt consideration for BAP1 immunohistochemical staining and possibly genetic screening. It is notable that *BAP1* mutations occur in melanomas at an estimated frequency of 3% to 5%, and loss of BAP1 protein expression is seen in a minority of melanomas.[45,52,53] Hence, the finding of BAP1 loss should be correlated with morphologic and clinical findings.

Recently, Wiesner et al[54] studied spitzoid lesions, including Spitz nevi, ASTs, and spitzoid melanoma, with targeted DNA and transcriptome sequencing, and they identified that approximately 50% of cases harbor kinase fusions involving the kinases *ROS1*, *ALK*, *NTRK1*, *BRAF*, or *RET*.[54] Further studies have demonstrated a distinctive plexiform morphology in spitzoid neoplasms with *ALK* fusions.[55,56] Although the discovery of kinase fusions in spitzoid neoplasms has clear implications for improved biologic understanding and targeted therapy, kinase fusions occur in both benign and malignant spitzoid neoplasms. Therefore, at present, their identification has limited, if any, utility in risk stratification of these borderline melanocytic tumors.

DIFFERENTIAL DIAGNOSIS

In most cases, the differential diagnosis for AST rests between a benign Spitz nevus and a malignant spitzoid melanoma. This distinction may be exceedingly difficult, if not impossible, with light microscopy alone. Thus, using supplementary ancillary studies, such as immunohistochemistry and molecular studies, as described above and summarized in the Table, may help to refine the diagnosis. In addition, it is essential to incorporate clinical data, such as the clinical course of the lesion and the age of the patient, when arriving at a diagnosis. For instance, a spitzoid neoplasm that is of new onset and rapidly growing in an older patient is worrisome for a more aggressive clinical course based on the clinical information alone.

To expand the differential diagnosis, some spitzoid lesions may need to be distinguished from cellular neurothekeomas or histiocytic/fibrohistiocytic lesions. This can usually be accomplished by immunohistochemistry for markers of melanocytic differentiation, such as S100 and/or Melan-A, which would be positive in melanocytic lesions and negative in cellular neurothekeoma and fibrohistiocytic lesions. In addition, spindled or epithelioid melanocytic tumors that do not display definitive spitzoid features might better be considered unclassified borderline melanocytic tumors, because literature on molecular and prognostic findings in ASTs may not be applicable to all borderline melanocytic tumors.

CURRENT TREATMENT

Treatment recommendations for Spitz nevi, atypical Spitz nevi, and spitzoid melanoma are relatively straightforward. Most sources recommend conservative excision of Spitz nevi to ensure complete removal if atypical features are present, or if the patient is an adult.[2,9] Recommendations are more variable regarding whether conventional Spitz nevi in children should be completely removed.[2,9,57] For spitzoid melanoma, treatment recommendations follow the National Comprehensive Cancer Network guidelines for melanoma and include wide excision with treatment margins based on the Breslow depth of the lesion, with or without additional staging of the regional nodal basin with sentinel lymph node biopsy also determined by the Breslow depth of the lesion.[58] Further treatment, in concordance with NCCN guidelines, depends on the sentinel lymph node status.

Treatment recommendation for ASTs is challenging and controversial because there is considerable interobserver

Features of Spitz Nevi, Spitzoid Melanoma, and Atypical Spitz Tumor			
	Spitz Nevus	**Atypical Spitz Tumor**	**Spitzoid Melanoma**
Clinical presentation	Typically younger patients (subset occur in adults) Small papule or nodule	Typically younger patients Papule or nodule	Typically older patients Large, changing lesion
Scanning magnification	Symmetrical Well circumscribed Epidermal acanthosis Well-formed nests, limited pagetoid scatter	Large, deep tumor May be asymmetrical	Asymmetrical Poorly nested Some architectural features of Spitz nevus (large nests with clefting, epidermal acanthosis)
High magnification	*Cytologic atypia:* Limited pleomorphism Lack of high-grade cytologic atypia *Junctional component:* Well nested, may be central pagetoid scatter *Dermal component:* Dermal maturation Few/no mitoses	*Cytologic atypia:* Most cases lack extreme pleomorphism, hyperchromasia, atypical mitoses *Junctional component:* Typically lacks findings of melanoma in situ May be ulceration *Dermal component:* Cellular, with spindled or epithelioid cells, often in fascicles Multiple dermal mitoses Some investigators allow for focal necrosis	*Cytologic atypia:* High-grade cytologic atypia *Junctional component:* Poor nesting, pagetoid scatter Consumption May be ulceration *Dermal component:* Lack of maturation Mitoses may be numerous, deep/marginal, or atypical May have tumor necrosis
Immunohistochemistry	p16 typically retained HMB-45 lost in deeper dermal component Low Ki-67 index	p16 loss in minority Intermediate Ki-67 index	p16 loss in minority Elevated Ki-67 proliferative index Deep HMB-45 expression in minority
Molecular	FISH: no aberration (or rare heterozygous loss of *CDKN2A*) CGH: isolated gains of 7p, 11q, tetraploidy Other: *HRAS*-activating mutations Tyrosine kinase fusions	FISH: aberrations overlap with spitzoid melanoma; homozygous 9p21 loss may herald aggressive course CGH: may have one or multiple chromosomal abnormalities Loss of 3 (associated with BAP1 inactivation) Other: Tyrosine kinase fusions BAP1 mutation	FISH: aberrations of 9p21, 6p25, 11q13, and 8q24 CGH: multiple chromosomal abnormalities Other: Tyrosine kinase fusions *BRAF*, *NRAS* mutations *HRAS* mutations rare
Prognosis	Benign	Typically indolent, but there are rare cases with widespread metastases, death	Malignant (may be slightly better outcome relative to conventional melanoma)

Abbreviations: CGH, comparative genomic hybridization; FISH, fluorescence in situ hybridization.

variability in the classification of AST, even among experts, and there is no consensus on treatment regimens.[14] Given the more favorable prognosis of AST with reasonable long-term follow-up, some centers recommend excision only, with careful clinical observation for recurrence.[59] The role, if any, of sentinel lymph biopsy in the staging and/or management of these patients is evolving. Clearly, the goal of patient management is to not undertreat or overtreat. Careful assessment of risk is important in our therapeutic algorithm at the University of Michigan. If the light microscopic features are disturbing such that melanoma remains in the differential diagnosis, then molecular studies are often employed in an effort to better define the lesion and assess risk. If there are no or rare chromosomal aberrations not typically seen in conventional melanoma, the lesion is regarded as a low-risk AST, and re-excision alone is recommended. If there are multiple aberrations, especially those overlapping with those reported in conventional melanoma, including homozygous loss of 9p21, the lesion is regarded as more genomically unstable, with a higher risk potential. In such cases, in the context of an atypical borderline melanocytic tumor, we counsel patients and their families on the risks and benefits of additional staging with sentinel lymph node biopsy.[7] If the sentinel lymph node harbors larger tumor aggregates and/or there is effacement of lymph node architecture or necrosis, the lesion is considered more aggressive, and further treatment is based on melanoma guidelines. At a minimum, every patient should receive long-term follow-up and be counseled that our understanding of these lesions is evolving.

CONCLUSIONS

In summary, distinguishing ASTs from Spitz nevi and spitzoid melanoma is often challenging. Assimilation of the clinical presentation, histology, and immunohistochemical and molecular studies may help to clarify the diagnosis. When these lesions are misdiagnosed as benign Spitz nevi, there is a risk for undertreatment. Conversely, if diagnosed as spitzoid melanoma, there is a risk of overtreatment. Importantly, many studies agree that patients with ASTs have a much better prognosis compared with those with

melanoma. It has been controversial whether ASTs should be considered spitzoid neoplasms sui generis or a provisional category of diagnostically challenging lesions. Both may be true. Atypical Spitz tumors have distinct, yet somewhat diverse, molecular profiles harboring multiple genomic aberrations, yet they usually follow an indolent course, a combination not seen in nevi or melanomas.[7,26,31] Further studies are needed to expand molecular diagnostics, as well as to confirm whether molecular findings, such as homozygous loss of *CDKN2A*, are sufficient to guide diagnosis and management of these challenging lesions.

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References

1. Spitz S. Melanomas of childhood. *Am J Pathol*. 1948;24(3):591–609.
2. Zedek DC, McCalmont TH. Spitz nevi, atypical spitzoid neoplasms, and spitzoid melanoma. *Clin Lab Med*. 2011;31(2):311–320.
3. Lott JP, Wititsuwannakul J, Lee JJ, et al. Clinical characteristics associated with Spitz nevi and Spitzoid malignant melanomas: the Yale University Spitzoid Neoplasm Repository experience, 1991 to 2008. *J Am Acad Dermatol*. 2014; 71(6):1077–1082.
4. McCormack CJ, Conyers RK, Scolyer RA, et al. Atypical Spitzoid neoplasms: a review of potential markers of biological behavior including sentinel node biopsy. *Melanoma Res*. 2014;24(5):437–447.
5. Pol-Rodriquez M, Lee S, Silvers DN, Celebi JT. Influence of age on survival in childhood spitzoid melanomas. *Cancer*. 2007;109(8):1579–1583.
6. Paradela S, Fonseca E, Pita-Fernandez S, Prieto VG. Spitzoid and non-spitzoid melanoma in children: a prognostic comparative study. *J Eur Acad Dermatol Venereol*. 2013;27(10):1214–1221.
7. Ludgate MW, Fullen DR, Lee J, et al. The atypical Spitz tumor of uncertain biologic potential: a series of 67 patients from a single institution. *Cancer*. 2009; 115(3):631–641.
8. Lallas A, Kyrgidis A, Ferrara G, et al. Atypical Spitz tumours and sentinel lymph node biopsy: a systematic review. *Lancet Oncol*. 2014;15(4):e178–e183.
9. Luo S, Sepehr A, Tsao H. Spitz nevi and other Spitzoid lesions part II: natural history and management. *J Am Acad Dermatol*. 2011;65(6):1087–1092.
10. Busam KJ, Murali R, Pulitzer M, et al. Atypical spitzoid melanocytic tumors with positive sentinel lymph nodes in children and teenagers, and comparison with histologically unambiguous and lethal melanomas. *Am J Surg Pathol*. 2009; 33(9):1386–1395.
11. Crotty KA, Scolyer RA, Li L, et al. Spitz naevus versus Spitzoid melanoma: when and how can they be distinguished? *Pathology*. 2002;34(1):6–12.
12. Reed RJ, Ichinose H, Clark WH Jr, Mihm MC Jr. Common and uncommon melanocytic nevi and borderline melanomas. *Semin Oncol*. 1975;2(2):119–147.
13. Busam KJ, Pulitzer M. Sentinel lymph node biopsy for patients with diagnostically controversial Spitzoid melanocytic tumors? *Adv Anat Pathol*. 2008; 15(5):253–262.
14. Gerami P, Busam K, Cochran A, et al. Histomorphologic assessment and interobserver diagnostic reproducibility of atypical spitzoid melanocytic neoplasms with long-term follow-up. *Am J Surg Pathol*. 2014;38(7):934–940.
15. Ordonez NG. Value of melanocytic-associated immunohistochemical markers in the diagnosis of malignant melanoma: a review and update. *Hum Pathol*. 2014;45(2):191–205.
16. Puri PK, Ferringer TC, Tyler WB, et al. Statistical analysis of the concordance of immunohistochemical stains with the final diagnosis in spitzoid neoplasms. *Am J Dermatopathol*. 2011;33(1):72–77.
17. Paradela S, Fonseca E, Pita S, et al. Spitzoid melanoma in children: clinicopathological study and application of immunohistochemistry as an adjunct diagnostic tool. *J Cutan Pathol*. 2009;36(7):740–752.
18. Al Dhaybi R, Agoumi M, Gagne I, et al. p16 expression: a marker of differentiation between childhood malignant melanomas and Spitz nevi. *J Am Acad Dermatol*. 2011;65(2):357–363.
19. George E, Polissar NL, Wick M. Immunohistochemical evaluation of p16INK4A, E-cadherin, and cyclin D1 expression in melanoma and Spitz tumors. *Am J Clin Pathol*. 2010;133(3):370–379.
20. Hilliard NJ, Krahl D, Sellheyer K. p16 expression differentiates between desmoplastic Spitz nevus and desmoplastic melanoma. *J Cutan Pathol*. 2009; 36(7):753–759.
21. Mason A, Wititsuwannakul J, Klump VR, Lott J, Lazova R. Expression of p16 alone does not differentiate between Spitz nevi and Spitzoid melanoma. *J Cutan Pathol*. 2012;39(12):1062–1074.
22. Kapur P, Selim MA, Roy LC, et al. Spitz nevi and atypical Spitz nevi/tumors: a histologic and immunohistochemical analysis. *Mod Pathol*. 2005;18(2):197–204.
23. Vollmer RT. Use of Bayes rule and MIB-1 proliferation index to discriminate Spitz nevus from malignant melanoma. *Am J Clin Pathol*. 2004; 122(4):499–505.
24. Barnhill RL. The Spitzoid lesion: rethinking Spitz tumors, atypical variants, 'Spitzoid melanoma' and risk assessment. *Mod Pathol*. 2006;19(suppl 2):S21–S33.
25. Yazdan P, Cooper C, Sholl LM, et al. Comparative analysis of atypical spitz tumors with heterozygous versus homozygous 9p21 deletions for clinical outcomes, histomorphology, BRAF mutation, and p16 expression. *Am J Surg Pathol*. 2014;38(5):638–645.
26. Bastian BC, Olshen AB, LeBoit PE, Pinkel D. Classifying melanocytic tumors based on DNA copy number changes. *Am J Pathol*. 2003;163(5):1765–1770.
27. Bastian BC, Wesselmann U, Pinkel D, Leboit PE. Molecular cytogenetic analysis of Spitz nevi shows clear differences to melanoma. *J Invest Dermatol*. 1999;113(6):1065–1069.
28. Isaac AK, Lertsburapa T, Pathria Mundi J, et al. Polyploidy in spitz nevi: a not uncommon karyotypic abnormality identifiable by fluorescence in situ hybridization. *Am J Dermatopathol*. 2010;32(2):144–148.
29. Harvell JD, Kohler S, Zhu S, et al. High-resolution array-based comparative genomic hybridization for distinguishing paraffin-embedded Spitz nevi and melanomas. *Diagn Mol Pathol*. 2004;13(1):22–25.
30. Horst BA, Terrano D, Fang Y, Silvers DN, Busam KJ. 9p21 gene locus in Spitz nevi of older individuals: absence of cytogenetic and immunohistochemical findings associated with malignancy. *Hum Pathol*. 2013;44(12):2822–2828.
31. Raskin L, Ludgate M, Iyer RK, et al. Copy number variations and clinical outcome in atypical spitz tumors. *Am J Surg Pathol*. 2011;35(2):243–252.
32. Gerami P, Jewell SS, Morrison LE, et al. Fluorescence in situ hybridization (FISH) as an ancillary diagnostic tool in the diagnosis of melanoma. *Am J Surg Pathol*. 2009;33(8):1146–1156.
33. Gaiser T, Kutzner H, Palmedo G, et al. Classifying ambiguous melanocytic lesions with FISH and correlation with clinical long-term follow up. *Mod Pathol*. 2010;23(3):413–419.
34. Gerami P, Li G, Pouryazdanparast P, et al. A highly specific and discriminatory FISH assay for distinguishing between benign and malignant melanocytic neoplasms. *Am J Surg Pathol*. 2012;36(6):808–817.
35. Gerami P, Cooper C, Bajaj S, et al. Outcomes of atypical spitz tumors with chromosomal copy number aberrations and conventional melanomas in children. *Am J Surg Pathol*. 2013;37(9):1387–1394.
36. Gerami P, Scolyer RA, Xu X, et al. Risk assessment for atypical spitzoid melanocytic neoplasms using FISH to identify chromosomal copy number aberrations. *Am J Surg Pathol*. 2013;37(5):676–684.
37. Massi D, Tomasini C, Senetta R, et al. Atypical Spitz tumors in patients younger than 18 years. *J Am Acad Dermatol*. 2015;72(1):37–46.
38. Shen L, Cooper C, Bajaj S, et al. Atypical spitz tumors with 6q23 deletions: a clinical, histological, and molecular study. *Am J Dermatopathol*. 2013;35(8): 804–812.
39. van Engen-van Grunsven AC, Kusters-Vandevelde H, Groenen PJ, Blokx WA. Update on molecular pathology of cutaneous melanocytic lesions: what is new in diagnosis and molecular testing for treatment? *Front Med (Lausanne)*. 2014;1:39.
40. van Dijk MC, Bernsen MR, Ruiter DJ. Analysis of mutations in B-RAF, N-RAS, and *HRAS* genes in the differential diagnosis of Spitz nevus and spitzoid melanoma. *Am J Surg Pathol*. 2005;29(9):1145–1151.
41. Da Forno PD, Pringle JH, Fletcher A, et al. BRAF, NRAS and *HRAS* mutations in spitzoid tumours and their possible pathogenetic significance. *Br J Dermatol*. 2009;161(2):364–372.
42. Bastian BC, LeBoit PE, Pinkel D. Mutations and copy number increase of *HRAS* in Spitz nevi with distinctive histopathological features. *Am J Pathol*. 2000; 157(3):967–972.
43. van Engen-van Grunsven AC, van Dijk MC, Ruiter DJ, et al. *HRAS*-mutated Spitz tumors: a subtype of Spitz tumors with distinct features. *Am J Surg Pathol*. 2010;34(10):1436–1441.
44. Roychowdhury S, Iyer MK, Robinson DR, et al. Personalized oncology through integrative high-throughput sequencing: a pilot study. *Sci Transl Med*. 2011;3(111):111ra121.
45. The Cancer Genome Atlas, cutaneous melanoma (TCGA provisional). cBioPortal Web site. http://cbioportal.org. Accessed May 15, 2015.
46. Wiesner T, Murali R, Fried I, et al. A distinct subset of atypical Spitz tumors is characterized by BRAF mutation and loss of BAP1 expression. *Am J Surg Pathol*. 2012;36(6):818–830.
47. Wiesner T, Obenauf AC, Murali R, et al. Germline mutations in BAP1 predispose to melanocytic tumors. *Nat Genet*. 2011;43(10):1018–1021.
48. Dubruc E, Balme B, Dijoud F, et al. Mutated and amplified NRAS in a subset of cutaneous melanocytic lesions with dermal spitzoid morphology: report of two pediatric cases located on the ear. *J Cutan Pathol*. 2014;41(11):866–872.

49. Fullen DR, Poynter JN, Lowe L, et al. BRAF and NRAS mutations in spitzoid melanocytic lesions. *Mod Pathol*. 2006;19(10):1324–1332.

50. Murali R, Wiesner T, Scolyer RA. Tumours associated with BAP1 mutations. *Pathology*. 2013;45(2):116–126.

51. Llamas-Velasco M, Perez-Gonzalez YC, Requena L, Kutzner H. Histopathologic clues for the diagnosis of Wiesner nevus. *J Am Acad Dermatol*. 2014; 70(3):549–554.

52. Piris A, Mihm MC Jr, Hoang MP. BAP1 and BRAFV600E expression in benign and malignant melanocytic proliferations. *Hum Pathol*. 2015;46(2):239–245.

53. Murali R, Wilmott JS, Jakrot V, et al. BAP1 expression in cutaneous melanoma: a pilot study. *Pathology*. 2013;45(6):606–609.

54. Wiesner T, He J, Yelensky R, et al. Kinase fusions are frequent in Spitz tumours and spitzoid melanomas. *Nat Commun*. 2014;5:3116.

55. Busam KJ, Kutzner H, Cerroni L, Wiesner T. Clinical and pathologic findings of Spitz nevi and atypical Spitz tumors with ALK fusions. *Am J Surg Pathol*. 2014;38(7):925–933.

56. Yeh I, de la Fouchardiere A, Pissaloux D, et al. Clinical, histopathologic, and genomic features of Spitz tumors with ALK fusions. *Am J Surg Pathol*. 2015; 39(5):581–591.

57. Gelbard SN, Tripp JM, Marghoob AA, et al. Management of Spitz nevi: a survey of dermatologists in the United States. *J Am Acad Dermatol*. 2002;47(2): 224–230.

58. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: melanoma. http://www.nccn.org/professionals/physician_gls/f_guidelines.asp. Accessed May 17, 2015.

59. Cerrato F, Wallins JS, Webb ML, et al. Outcomes in pediatric atypical spitz tumors treated without sentinel lymph node biopsy. *Pediatr Dermatol*. 2012; 29(4):448–453.