Are All Melanomas the Same?

Spitzoid Melanoma Is a Distinct Subtype of Melanoma

David A. Lee, M.D.[1]
Jason A. Cohen, M.D.[2]
William S. Twaddell, M.D.[2]
Gustavo Palacios, M.D.[3]
Melissa Gill, M.D.[2]
Eyal Levit, M.D.[1]
Alan J. Halperin, M.D.[4]
Joan Mones, M.D.[5]
Klaus J. Busam, M.D.[6]
David N. Silvers, M.D.[1,2]
Julide Tok Celebi, M.D.[1]

[1] Department of Dermatology, Columbia University Medical Center, New York, New York.

[2] Department of Pathology, Columbia University Medical Center, New York, New York.

[3] Mailman School of Public Health, Columbia University Medical Center, New York, New York.

[4] Pathology Associates, P.C., New Rochelle, New York.

[5] Ackerman Academy of Dermatopathology, New York, New York.

[6] Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York.

Supported by the National Institutes of Health (NIAMS K08 AR050273 to J.T.C.), the Dermatology Foundation (to J.T.C.), the Doris Duke Charitable Foundation Clinical Research Fellowship (to D.A.L.), and the American Skin Association (to D.A.L.).

The authors are grateful to A. Bernard Ackerman, M.D. for his invaluable support of this project. The authors sincerely thank the following pathologists for contributing patients to this study: Nelson Amirkham, M.D.; Donald Bauermeister, M.D.; Katherine Chan, M.D.; James Durham, M.D.; Shokat Fatteh, M.D.; Fabio Gutierrez, M.D.; Jane Lindholm, M.D.; Homeira McDonald, M.D.; Cyrus Milani, M.D.; Linda Nims, M.D.; R. H. Patterson, M.D.; Husain Saleh, M.D.; Stan Shrago, M.D.; Hui C. Tsou, M.D.; Thomas Wade, M.D.; Noreen Walsh, M.D.; William Watkin, M.D.; and Ivo Sazunic Yanez, M.D. The authors also respectfully acknowledge the following physicians for providing survival data: Raymond Bernat, M.D.; Emil Bisaccia, M.D.; Jeffrey Bowden, M.D.; Michael Brown, M.D.; Kimberly Clark-Paul, M.D.; Jack Eisert, M.D.; Richard Fox, M.D.; Isabel Goldfaden, M.D.; Douglas Hawkins, M.D.; Jeffrey Kezis, M.D.; Joseph Masternick, M.D.; Galia J. Meiri, M.D.; Kalyanakrishnan Ramakrishnan, M.D.; Stephen Sener, M.D.; Gary Slater, M.D.; Barry Solomon, M.D.; and John Walczyk, M.D.

BACKGROUND. Although the majority of melanomas demonstrate high rates of mutations in *B-RAF* or *N-RAS* that result in constitutive activation of the mitogen-activated protein kinase-signaling pathway, emerging data suggest molecular differences among melanoma subtypes. In this study, the authors evaluated the contribution of *B-RAF* and *N-RAS* mutations to the pathogenesis of Spitzoid melanomas.

METHODS. In total, 33 Spitzoid melanomas were analyzed for clinical and pathologic characteristics as well as for hot-spot mutations in the *B-RAF* and *N-RAS* genes. In the majority of patients (28 of 33 melanomas), the tumors were confined to the skin with no evidence of metastasis (average follow-up, 32.5 mos). There were five metastasizing melanomas (5 of 33 tumors) with regional or systemic spread.

RESULTS. Of 33 Spitzoid melanomas, only 1 showed the V600E mutation in the *B-RAF* gene (1 of 33 tumors; 3%). It was noteworthy that none of the metastatic Spitzoid melanomas (0 of 5 tumors; 0%), of which 2 resulted in fatal outcomes, demonstrated mutations in *B-RAF* or *N-RAS*.

CONCLUSIONS. In contrast to the majority of cutaneous melanomas, activating hot-spot mutations in *B-RAF* or *N-RAS* were not involved in the pathogenesis of Spitzoid melanoma. These data suggested that Spitzoid melanoma is a distinct form of melanoma with unknown genes and/or signaling pathways involved in its development. ***Cancer* 2006;106:907–13.** © *2006 American Cancer Society.*

KEYWORDS: melanoma, mutation, *B-RAF*, spitz, nevus, oncogene.

Spitzoid melanoma is a subtype of melanoma that clinically and histopathologically resembles a benign melanocytic neoplasm called Spitz nevus.[1] Although Spitz nevi are considered benign neoplasms, Spitzoid melanomas are malignant. Like other melanocytic neoplasms, the gold standard for diagnosis is histopathologic examination. The diagnosis and classification of these tumors can be achieved in most patients by histopathology; however, there are tumors in which it is difficult or impossible to differentiate between these groups.[2] Consequently, there are Spitzoid melanomas that originally were diagnosed as Spitz nevi that metastasized and led to fatal outcomes.[3–6]

Address for reprints: Julide Tok Celebi, M.D., Department of Dermatology, Columbia University, 630 West 168th Street, VC-15-202, New York, NY 10032; Fax: (212) 305-7391; E-mail: jt165@columbia.edu

Received July 22, 2005; revision received September 9, 2005; accepted September 16, 2005.

DOI 10.1002/cncr.21686
Published online 18 January 2006 in Wiley InterScience (www.interscience.wiley.com).

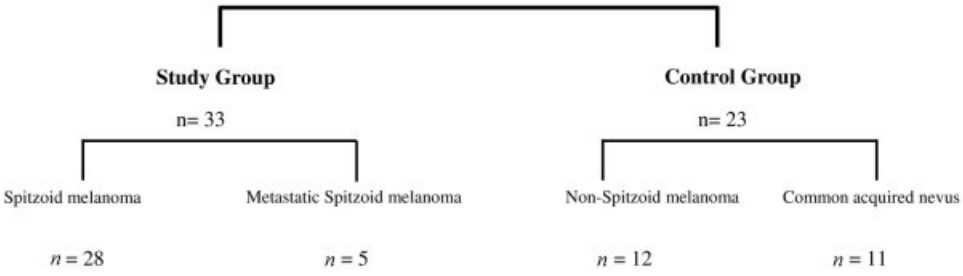

FIGURE 1. This chart illustrates the study design and tumor classification. Tumors in the Spitzoid melanoma group were confined to the skin, and patients with those tumors had no evidence of metastasis at an average follow-up of 32.5 months.

Mutations in the *B-RAF* gene (≈ 50%) and the *N-RAS* gene (≈ 10%) that lead to activation of the mitogen-activated protein kinase (MAPK) pathway play a role in melanoma.[7–9] Similarly, a high rate of activating mutations in *B-RAF* are found in benign melanocytic nevi,[10] suggesting a role for *B-RAF* in both benign and malignant melanocytic tumors. However, the molecular basis of Spitzoid melanoma and Spitz nevi remains uncharacterized. Mutations in *B-RAF* or *N-RAS* are not found in Spitz nevi,[11–14] although one study showed constitutive activation of the MAPK pathway in Spitz nevi.[11] We recently reported the absence of *B-RAF* and *N-RAS* mutations in a unique but small cohort of Spitzoid melanomas in children.[13] In the current study, we evaluated the contribution of *B-RAF* and *N-RAS* to the pathogenesis of Spitzoid melanomas from patients in varying age groups.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Review Boards of Columbia University and Memorial Sloan-Kettering Cancer Center.

Study Design and Tumor Classification

Tumor specimens were collected for two groups: the study group and the control group (for a summary of the study design, see Fig, 1). Tumor samples were retrieved from the archives of the Dermatopathology Laboratory at Columbia University; Pathology Associates, P.C.; Ackerman Academy of Dermatopathology; and Memorial Sloan-Kettering Cancer Center.

The study group consisted of tumor samples that were diagnosed as Spitzoid melanoma (melanoma with Spitz nevus-like features) by the dermatopathologists who participated in this study (D.N.S., A.J.H., J.M., K.J.B., and their associates). The histopathologic features of these tumors have been described previously.[2,15] Once the samples were retrieved, the histopathology of each tumor was reviewed again independently by two dermatopathologists (D.N.S. and K.J.B.) to confirm the diagnosis. Although the majority of the tumor specimens were considered Spitzoid melanomas, other dermatopathologists may classify some of these tumors as *atypical Spitz tumors* (a group of tumors that shares some histopathologic features of both Spitz nevus and melanoma and does not fit neatly into either category). In the majority of samples, there was concordance among the reviewing dermatopathologists in classifying these tumors as Spitzoid melanoma; however, the few tumor samples for which there was discordance were removed from the study group. In this study, we did not select any tumors that were diagnosed as *classic* Spitz nevus, because we studied that group of tumors previously.[11] The lack of concordance among dermatopathologists in diagnosing these tumors is well established, and these diagnoses do not necessarily correlate with biologic behavior.[2] To classify these tumors based on biologic behavior and to select the *unequivocal* Spitzoid melanomas (proven by metastasis), we then obtained survival information on each patient. Patients' physicians were contacted, and information from the last clinical examination was obtained. Our control group was composed of non-Spitzoid melanomas and common acquired nevi.

Tissue Sample Preparation and DNA Extraction

Paraffin embedded tumor specimens were cut into 5-μm-thick sections and stained with eosin. Tumor cells were isolated (≈ > 90% of tumor cells) by using a PixCell II Laser Capture microdissection system (Arcturus, Mountain View, CA). Total cellular DNA was isolated by using the Qiagen DNA extraction kit according to the manufacturer's recommendations.

Mutation Screening of the *B-RAF* and *N-RAS* Genes

Exon 15 of *B-RAF* and exons 2 and 3 of *N-RAS* were amplified by using polymerase chain reaction (PCR) with specific primers that were described previously.[7] These exons contain the hot-spot regions in these genes (amino acid 600 for *B-RAF* and amino acids 12, 13, 61, and 62 for *N-RAS*) in which the majority of mutations cluster. For amplification, 10–25 ng of DNA, 1.25 mMol of each primer, 10 × PCR buffer, 2.5 mMol $MgCl_2$, 0.2 mMol of each dinucleotide triphosphate, and Bio-X-Act Taq polymerase (Bioline, Ran-

TABLE 1
Summary of Clinical Data of Patients with Spitzoid Melanoma

Characteristic	No. of patients (%)
No. of patients	33
Age	6–71
< 21 yrs	6 (18)
≥ 21 yrs	27 (82)
Gender	
Male	16 (48)
Female	17 (52)
Primary tumor site	
Head and neck	4 (13)
Trunk	11 (34)
Extremity	17 (53)
Tumor depth (Breslow thickness)	
Average depth	2.58 mm
< 1.0 mm	2 (6.4)
1.01–2.0 mm	15 (48)
2.01–4.0 mm	8 (26)
> 4.01 mm	6 (19)
Follow-up range (mean)	2–98 mos (32.5 mos)

dolph, MA) were used in the following PCR protocol: 94 °C for 2 minutes; 42 cycles at 94 °C for 45 seconds, 58 °C for 45 seconds, and 72 °C for 45 seconds; and a final extension at 72 °C for 10 minutes. Purified PCR products were then sequenced by using a Big Dye Terminator cycle-sequencing kit and an ABI Prism 310 automated sequencer system (Applied Biosystems, Foster City, CA). For sequencing reactions, 2–5 μL of the purified PCR product, 5 × Big Dye sequencing buffer, and 0.02 mMol of each primer were used in the following cycle conditions: 96 °C for 10 seconds, 25 cycles at 50 °C for 5 seconds, 60 °C for 4 minutes.

RESULTS

Clinicopathologic Data and Survival

In total, 56 tumor specimens were retrieved for this study. The study group consisted of 33 tumors that were classified as Spitzoid melanoma. The control group included 12 non-Spitzoid melanomas and 11 nevus samples (Fig. 1). Clinical characteristics of the patients with Spitzoid melanoma were evaluated (Table 1). Ages ranged from 6 years to 71 years, and the majority of tumors were from adults (age 21 yrs and older; 27 of 33 patients; 82%). There were approximately equal numbers of males (48%) and females (52%). The extremities (53%) followed by the trunk (34%) were the most common primary tumor sites, whereas the head and neck was the least common tumor site (13%). Clinical photographs, which were available for some of the tumors, showed that individual lesions were erythematous amelanotic papules or nodules (Fig. 2A). Primary skin tumors from all 33 patients were examined histopathologically and confirmed as Spitzoid melanoma. The average tumor thickness was 2.58 mm. Survival data were obtained from 29 of 33 patients, and follow-up ranged from 2 months to 98 months (mean, 32.5 mos).

Five of 33 patients had regional or systemic metastasis. Among the 5 patients with metastasis, 3 patients had regional metastasis to lymph nodes, and 2 patients had both regional and widespread visceral metastases that resulted in fatal outcomes (tumors ST53 and ST1LN). Histopathologic evaluation of the lymph node metastases showed sheets of tumor cells invading the parenchyma, consistent with metastatic melanoma (Fig. 2B).

Mutation Analysis

Mutation data are presented in Table 2. In all but 2 samples (ST1LN and ST20LN), the primary skin tumors were analyzed for mutations. For samples ST1LN and ST20LN, lymph node metastases were studied. Mutations in the hot-spot regions for *N-RAS* were not identified in any of the 33 tumors studied, and *B-RAF* mutation frequency was low (1 of 33 tumors; 3%). A V600E mutation in *B-RAF* was found only in 1 tumor (ST24) (Fig. 2C). It is noteworthy that no *B-RAF* or *N-RAS* mutations were found in the Spitzoid melanomas that metastasized. In contrast, high rates of *B-RAF* mutations were identified in the control group, and this finding was consistent with the literature, validating the mutation detection strategy that we used. Of the 12 non-Spitzoid melanomas, 7 tumors had the V600E mutation identified, and 1 tumor had a V600K mutation identified (total, 8 of 12 tumors; 67%). Common acquired nevi also showed a high *B-RAF* mutation frequency, with 8 of 11 tumors (73%) demonstrating the V600E mutation in *B-RAF*. Mutation results from the study group and the control group were confirmed further by using the same techniques in a different laboratory at Columbia University (G.P.).

DISCUSSION

Clinically, Spitzoid melanomas resemble Spitz nevus and typically present as an amelanotic (nonpigmented) papule or nodule on the skin, often mimicking pyogenic granulomas or insect bites.[3] However, some Spitzoid melanomas may be pigmented and resemble common melanocytic nevi. It is noteworthy that the majority of these tumors do not conform to the *ABCD rule* for melanoma (asymmetry, border irregularity, color variation, and > 6 mm in greatest dimension) and, thus, are difficult to recognize as suspicious lesions even by expert clinicians.[3,16–18]

In addition to these clinical diagnostic difficulties, these tumors are among the most challenging neo-

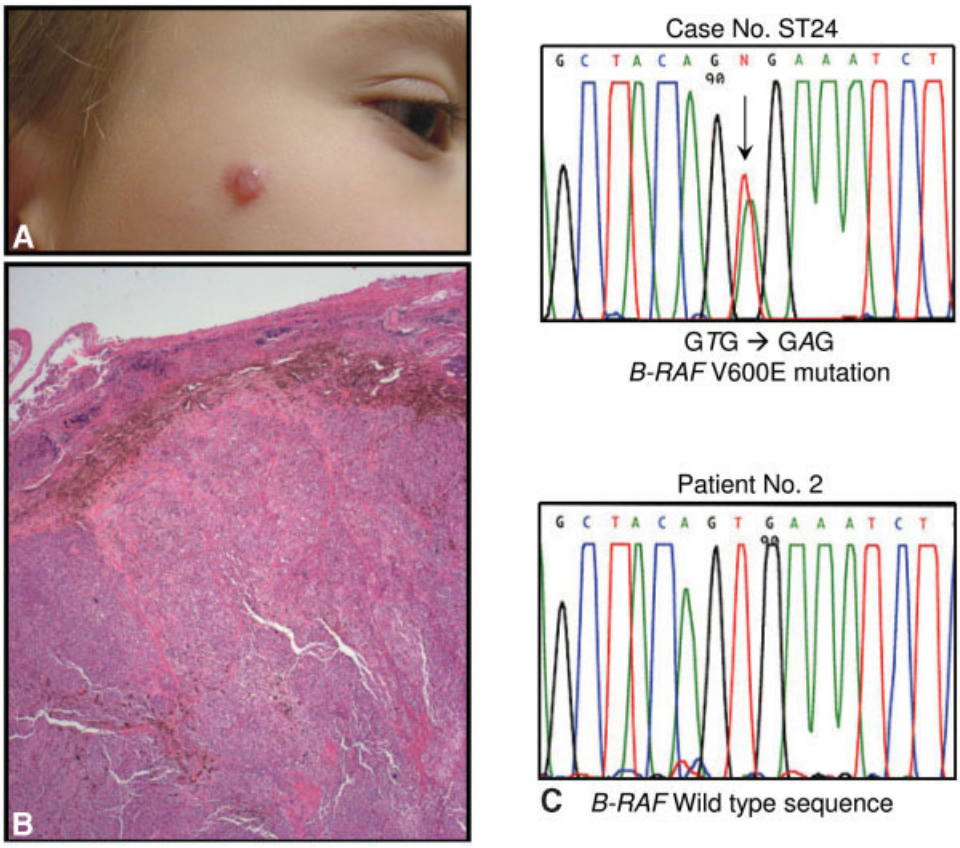

FIGURE 2. Clinical and histopathologic data and mutation analysis are illustrated. (A) Clinical features of a Spitzoid melanoma are observed in a child from the current cohort who presented with an erythematous papule on the cheek. (B) Histopathologic findings of lymph node metastasis are illustrated in this photomicrograph of a Spitzoid melanoma (ST1LN) that shows sheets of tumor cells invading the parenchyma. (C) The sequence of *B-RAF* exon 15 shows a V600E mutation in tumor ST24 (top) and a wild-type sequence of the corresponding region (bottom).

plasms for pathologists. Despite detailed characterization of the histopathologic features of these tumors over the past few decades, there is an absence of objective criteria for differentiating Spitz nevus from Spitzoid melanoma.[2] An elegant study by Barnhill et al. illustrated the lack of consensus among pathologists in diagnosing Spitz tumors and the difficulties in predicting clinical outcome based on histopathologic examination.[2] In this study, 13 of 19 Spitzoid melanomas, which were confirmed as melanoma by regional or systemic metastases, were designated as Spitz nevus or atypical Spitz tumor by some of the pathologists. Furthermore, in a review of the literature, we found several patients who had Spitzoid melanoma that initially was misdiagnosed as Spitz nevus, leading to fatal outcomes.[3–6] Due to these diagnostic difficulties of Spitzoid melanoma clinically and histopathologically, misdiagnoses or delays in diagnosis occur. This diagnostic dilemma is much more obvious in children, because melanomas of all types are extremely rare in this age group, whereas Spitz nevi are common. Taken together, these data demonstrate the need to identify molecular signatures to differentiate between these tumors and predict clinical outcomes.

It has been shown that the MAPK pathway plays an important role in melanocytic neoplasia.[19] Constitutive activation of this pathway in melanoma occurs through mutations in *B-RAF* or *N-RAS*. A high percentage of melanomas have mutations in *B-RAF* ($\approx$ 50%), the majority of which show the V600E missense mutation ($>$ 90%).[7,8,20,21] A high rate of activating mutations in *B-RAF* also are found in benign melanocytic nevi,[10,12,14] similar to what has been observed in melanoma, suggesting a role for *B-RAF* in both benign and malignant melanocytic tumors. There are several subtypes of melanoma and melanocytic nevi that are classified based on clinical and histopathologic characteristics. It is noteworthy that the frequency of *B-RAF* mutations shows differences among melanoma and melanocytic nevi subtypes. In contrast to cutaneous melanoma, *B-RAF* mutations are rare in uveal melanomas[22] and mucosal melanomas.[23] A recent study on uveal melanoma demonstrated constitutive activation of the MAPK pathway in the absence of *B-RAF* or *N-RAS* mutations, suggesting that activation of this pathway in these tumors occurs through a different mechanism than that observed in cutaneous melanoma.[24] Among the cutaneous melanomas, acral melanoma, a subtype of melanoma seen on the palms and soles, shows a low frequency of *B-RAF* mutations.[25] No *B-RAF* mutations are reported in cutaneous melanoma subtypes, such as Spitzoid melanoma[13] and

TABLE 2
Clinical and Histopathologic Data and Mutation Analysis of *B-RAF* and *N-RAS* in Spitzoid Melanoma

Patient no	Age (yrs)	Tumor thickness (mm)	Gender	Anatomic site	Clinical outcome (mos)	*B-RAF* exon 15	*N-RAS* exon 2	*N-RAS* exon 3
Spitzoid melanoma								
ST19	6	UN	Male	Scalp	NED (32)	WT	WT	WT
ST11	12	3.5	Female	Arm	NED (2)	WT	WT	WT
ST21	12	5.0	Female	Cheek	NED (24)	WT	WT	NA
ST37	17	2.3	Female	Knee	NED (36)	WT	WT	WT
ST5	23	2.0	Female	Buttock	NED (19)	WT	WT	WT
ST35	24	6.5	Female	Back	NED (32)	WT	WT	NA
ST4	25	1.2	Female	Hip	NED (8)	WT	WT	WT
ST47	28	4.4	Male	Thigh	UN	WT	WT	NA
ST9	30	7.5	Male	Thigh	NED (7)	WT	WT	WT
ST25	30	1.4	Male	Foot	NED (30)	WT	WT	WT
ST41	33	2.0	Male	Leg	NED (20)	WT	WT	WT
ST32	34	2.4	Male	Cheek	UN	WT	WT	WT
ST6	35	1.5	Female	Ankle	NED (8)	WT	WT	WT
ST30	35	2.1	Female	Arm	NED (40)	WT	WT	WT
ST36	36	4.7	Male	Thigh	NED (43)	WT	WT	WT
ST45	37	0.8	Female	Knee	NED (81)	WT	WT	WT
ST23	40	1.7	Male	Knee	UN	WT	WT	WT
ST51	42	1.1	Female	Foot	NED (52)	WT	WT	WT
ST13	42	3.1	Male	Arm	NED (2)	WT	WT	WT
ST46	43	1.2	Female	Arm	NED (98)	WT	WT	WT
ST8	46	1.6	Male	Back	NED (16)	WT	WT	WT
ST24	47	1.9	Male	Scapula	NED (19)	V600E	WT	WT
ST49	53	0.7	Female	Back	NED (73)	WT	WT	WT
ST15	54	2.3	Female	Back	NED (27)	WT	WT	WT
ST7	57	1.5	Female	Leg	UN	WT	WT	WT
ST27	58	5.0	Female	Back	NED (12)	WT	WT	WT
ST34	63	1.4	Male	Thigh	NED (39)	WT	WT	NA
ST18	71	3.5	Male	Breast	NED (2)	WT	WT	WT
Metastatic Spitzoid melanoma[a]								
ST40	23	1.7	Female	Leg	AW (26)[b]	WT	WT	WT
ST3	46	2.3	Male	Neck	AW (36)[c]	WT	WT	NA
ST53	28	1.9	Male	Chest	DOD (78)	WT	WT	WT
ST1LN	14	UN	Female	Back	DOD	WT	WT	NA
ST20LN	14	1.7	Male	UN	AW (48)[c]	WT	WT	WT

UN: unknown; NED: no evidence of disease; WT: wild type; NA: not available; AW: alive with disease; LN: lymph node; DOD: died of disease.

[a] Indicates that all melanomas in this group metastasized (Stage III or IV disease).

[b] Sentinel LN positive according to hematoxylin and eosin staining and immunohistochemistry.

[c] Palpable lymph node.

desmoplastic melanoma,[26] suggesting the involvement of different genes or signaling pathways in these tumors. Similar findings have been observed in melanocytic nevi. Although common melanocytic nevi have high rates of *B-RAF* mutations, alterations in this gene are infrequent in blue nevi or Spitz nevi.[11,12,14]

Desmoplastic melanoma is a form of cutaneous melanoma with distinct clinical and histopathologic features in addition to biologic behavior that differs from that of other cutaneous melanomas.[27] They typically occur on the head and neck as amelanotic (nonpigmented) nodules and show a predisposition for hematogeneous rather than lymphatic metastasis.[28] Although constitutive activation of the MAPK signaling pathway in these tumors has not been studied, they lack *B-RAF* mutations[26] and also can be distinguished from other cutaneous melanomas by gene expression profiling,[29] suggesting that desmoplastic melanoma represents a subtype with differences at the molecular level. Similarly, Spitzoid melanoma is a subset of cutaneous melanoma with clinical and histopathologic features different from other melanomas. We and others reported the absence of B-RAF mutations in Spitz nevi.[11,12,14] To date, our group has studied a total of 73 tumors, including Spitz nevi and

TABLE 3
Summary of B-RAF Mutation Frequency in Spitz Nevi and Spitzoid Melanomas

	B-RAF	
Tumor type	Current study (%)	To date by our group (%)
Spitz nevus	—	0/30 (0)
Spitzoid melanoma	1/28 (3.5)	1/28 (3.5)
Metastatic Spitzoid melanoma[a]	0/5 (0)	0/14 (0)
Control groups		
Non-Spitzoid melanoma	8/12 (67)	21/35 (60)
Common acquired nevus	8/11 (73)	8/11 (73)

[a] Indicates that all melanomas in this group metastasized (Stage III or IV disease).

Spitzoid melanoma, for mutations in *B-RAF* and *N-RAS* (summarized in Table 3).[11,13] Our data demonstrated infrequent mutations in *B-RAF* (1 of 73 tumors; $\approx$ 1%) and no mutations in *N-RAS*, supporting the notion that these are distinct melanocytic tumors with unknown genes and signaling pathways for their development. The classification of melanoma subtypes based on molecular signatures and identification of the genes and signaling pathways involved in their pathogenesis will be crucial in determining therapeutic regimens as molecular-targeted therapies are being developed for patients with melanoma.[30]

The controversies with respect to the prognosis for patients according to melanoma subtypes and the influence of age on outcomes also are noteworthy. Spitzoid melanoma can be seen in children and adults. Melanomas of all types in children are rare: Approximately 3–4% arise before age 20 years, and 0.4% arise before puberty.[31] Because of the extremely low rates of other cutaneous melanoma subtypes in children, such as superficial spreading melanoma, the incidence of Spitzoid melanoma appears disproportionately greater in this age group. In general, the prognosis for patients with childhood melanoma remains controversial because of the rarity of these tumors in this age group and the absence of studies with long-term follow-up.

Several studies have suggested a similar prognosis for patients with childhood melanoma compared with adults that is dependent on the initial stage of the tumor.[30,31] By contrast, there are data suggesting better survival rates in children with melanoma, especially before age 10 years.[6,32,33] A recent study of childhood melanoma emphasized the strong influence of age on outcomes and suggested a survival advantage in children younger than age 10 years compared with older children, whose outcomes appeared similar to the outcomes observed in adults.[32] It is worth mentioning that one of the melanomas in our series that had a fatal outcome occurred in a child age 14 years. However, because of the rarity of these tumors, most of those data were based on studies that evaluated childhood melanoma without examining different clinical and histopathologic subtypes.

Similarly, it has been suggested that the prognosis for children with the Spitzoid melanoma subtype is better compared with the prognosis for adults; however, this assertion remains controversial.[6,34] Although a number of patients who had Spitzoid melanomas with regional lymph node metastasis and had no further progression have been reported, such tumors leading to widespread metastasis and fatal outcomes also are well documented.[6,34] Mones and Ackerman described the histopathologic features of a series of Spitzoid melanomas that occurred in children before puberty (age 10 yrs or younger).[35] All melanomas in that unique group had metastasized, confirming the diagnosis of melanoma.

We previously analyzed nine patients from that cohort and reported the absence of mutations in *B-RAF* and *N-RAS*.[13] The patients we evaluated had regional lymph node metastasis (Stage III), and all were alive at the time of the study and had a median follow-up of 20 months. We recently updated the survival information on this cohort and were able to obtain outcome data on eight of the nine patients. All eight children were alive with no evidence of disease with a median follow-up of 64 months (5.3 yrs). Although it is important to emphasize that this group showed a better survival rate compared with patients who had adult cutaneous melanomas, it will be essential to identify a larger cohort that enables statistical analysis with longer follow-up. Moreover, it would be considerably significant to design prospective clinical trials that can evaluate whether chemotherapy, immunotherapy, and/or molecular-targeted therapy regimens improve the survival of patients with childhood Spitzoid melanomas.

REFERENCES

1. Weedon D, Little JH. Spindle and epithelioid cell nevi in children and adults. A review of 211 cases of the Spitz nevus. *Cancer.* 1977;40:217–225.
2. Barnhill RL, Argenyi ZB, From L, et al. Atypical Spitz nevi/tumors: lack of consensus for diagnosis, discrimination from melanoma, and prediction of outcome. *Hum Pathol.* 1999;30:513–520.
3. Mehregan AH, Mehregan DA. Malignant melanoma in childhood. *Cancer.* 1993;71:4096–4103.
4. Tate PS, Ronan SG, Feucht KA, et al. Melanoma in childhood and adolescence: clinical and pathological features of 48 cases. *J Pediatr Surg.* 1993;28:217–222.

5. Peters MS, Goellner JR. Spitz naevi and malignant melanomas of childhood and adolescence. *Histopathology*. 1986;10: 1289–1302.
6. Barnhill RL, Flotte TJ, Fleischli M, et al. Cutaneous melanoma and atypical Spitz tumors in childhood. *Cancer*. 1995; 76:1833–1845.
7. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature*. 2002;417:949–954.
8. Brose MS, Volpe P, Feldman M, et al. BRAF and RAS mutations in human lung cancer and melanoma. *Cancer Res*. 2002;62:6997–7000.
9. Gray-Schopfer VC, da Rocha Dias S, Marais R. The role of B-RAF in melanoma. *Cancer Metast Rev*. 2005;24:165–183.
10. Pollock PM, Harper UL, Hansen KS, et al. High frequency of BRAF mutations in nevi. *Nat Genet*. 2003;33:19–20.
11. Gill M, Renwick N, Silvers DN, et al. Lack of BRAF mutations in Spitz nevi. *J Invest Dermatol*. 2004;122:1325–1326.
12. Yazdi AS, Palmedo G, Flaig MJ, et al. Mutations of the BRAF gene in benign and malignant melanocytic lesions. *J Invest Dermatol*. 2003;121:1160–1162.
13. Gill M, Cohen J, Renwick N, et al. Genetic similarities between Spitz nevus and Spitzoid melanoma in children. *Cancer*. 2004;101:2636–26340.
14. Saldanha G, Purnell D, Fletcher A, et al. High BRAF mutation frequency does not characterize all melanocytic tumor types. *Int J Cancer*. 2004;111:705–710.
15. Paniago-Pereira C, Maize JC, Ackerman AB. Nevus of large spindle and/or epithelioid cells (Spitz's nevus). *Arch Dermatol*. 1978;114:1811–1823.
16. Ceballos PI, Ruiz-Maldonado R, Mihm MC Jr. Melanoma in children. *N Engl J Med*. 1995;332:656–662.
17. Sybert V. Six children with malignant melanoma. *J Am Acad Dermatol*. 1991;24:666–667.
18. Pratt CB, Palmer MK, Thatcher N, et al. Malignant melanoma in children and adolescents. *Cancer*. 1981;47:392–397.
19. Satyamoorthy K, Li G, Gerrero MR, et al. Constitutive mitogen-activated protein kinase activation in melanoma is mediated by both BRAF mutations and autocrine growth factor stimulation. *Cancer Res*. 2003;63:756–759.
20. Thomas NE, Alexander A, Edmiston SN, et al. Tandem BRAF mutations in primary invasive melanomas. *J Invest Dermatol*. 2004;122:1245–1250.
21. Kumar R, Angelini S, Hemminki K. Activating BRAF and N-Ras mutations in sporadic primary melanomas: an inverse association with allelic loss on chromosome 9. *Oncogene*. 2003;22:9217–9124.
22. Cruz F 3rd, Rubin BP, Wilson D, et al. Absence of BRAF and NRAS mutations in uveal melanoma. *Cancer Res*. 2003;63: 5761–5766.
23. Edwards RH, Ward MR, Wu H, et al. Absence of BRAF mutations in UV-protected mucosal melanomas. *J Med Genet*. 2004;41:270–272.
24. Zuidervaart W, van Nieuwpoort F, Stark M, et al. Activation of the MAPK pathway is a common event in uveal melanomas although it rarely occurs through mutation of BRAF or RAS. *Br J Cancer*. 2005;92:2032–2038.
25. Maldonado JL, Fridlyand J, Patel H, et al. Determinants of BRAF mutations in primary melanomas. *J Natl Cancer Inst*. 2003;95:1878–1890.
26. Davison JM, Rosenbaum E, Barrett TL, et al. Absence of V599E BRAF mutations in desmoplastic melanomas. *Cancer*. 2005;103:788–792.
27. Busam KJ. Cutaneous desmoplastic melanoma. *Adv Anat Pathol*. 2005;12:92–102.
28. Hawkins WG, Busam KJ, Ben-Porat L, et al. Desmoplastic melanoma: a pathologically and clinically distinct form of cutaneous melanoma. *Ann Surg Oncol*. 2005;12:207–213.
29. Busam KJ, Zhao H, Coit DG, et al. Distinction of desmoplastic melanoma from non-desmoplastic melanoma by gene expression profiling. *J Invest Dermatol*. 2005;124:412–418.
30. Gibbs P, Moore A, Robinson W, et al. Pediatric melanoma: are recent advances in the management of adult melanoma relevant to the pediatric population. *J Pediatr Hematol Oncol*. 2000;22:428–432.
31. Saenz NC, Saenz-Badillos J, Busam K, et al. Childhood melanoma survival. *Cancer*. 1999;85:750–754.
32. Ferrari A, Bono A, Baldi M, et al. Does melanoma behave differently in younger children than in adults? A retrospective study of 33 cases of childhood melanoma from a single institution. *Pediatrics*. 2005;115:649–654.
33. Schmid-Wendtner MH, Berking C, Baumert J, et al. Cutaneous melanoma in childhood and adolescence: an analysis of 36 patients. *J Am Acad Dermatol*. 2002;46:874–879.
34. Smith KJ, Barrett TL, Skelton HG 3rd, et al. Spindle cell and epithelioid cell nevi with atypia and metastasis (malignant Spitz nevus). *Am J Surg Pathol*. 1989;13:931–939.
35. Mones JM, Ackerman AB. Melanomas in prepubescent children: review comprehensively, critique historically, criteria diagnostically, and course biologically. *Am J Dermatopathol*. 2003;25:223–238.