MELANOMA CARE OPTIONS™

JANUARY 2009

FOSTERING AN INTERDISCIPLINARY APPROACH TO MELANOMA CARE

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Issue 1: Pathogenesis and Predictors of Prognosis in Melanoma

Editor's Note...

Dear Colleague,

This issue of *Melanoma Care Options* begins a new 3-part series designed to provide expert interpretation of recent and emerging information on melanoma research. Issue 1 examines various predictors of prognosis that may impact the management of patients with or at risk for primary cutaneous melanoma. Issue 2 will focus on advances in melanoma staging and surgical technique, while Issue 3 will deliberate on recent developments in melanoma treatment.

Dr. Sancy Leachman reviews the latest data on hereditary (familial) melanoma and issues related to genetic testing, including guidelines for determining who should be referred for genetic counseling and possible testing. She also presents results from a recent study examining the impact of genetic testing on photoprotective and screening behaviors in patients from high-risk melanoma families.

Dr. Mohammed Kashani-Sabet evaluates the potential of tumor vascularity and vascular involvement to act as as prognostic markers for overall survival and evaluates recent results pointing to interactions between these factors and ulceration, with transcription factor nuclear factor-κB acting as a potential intermediary. He also examines other molecules for their ability to serve as novel markers of disease-specific survival and predictors of sentinel lymph node (SLN) metastasis.

Dr. Vernon K. Sondak and Dr. Jane L. Messina discuss the rationale for SLN biopsy (SLNB) in the management of patients with cutaneous melanoma and review data supporting its use for staging, with particular focus on initial results from the Multicenter Selective Lymphadenectomy Trial. They also address the use of tumor thickness to select patients for SLNB and discuss whether SLNB is appropriate for patients with thin melanomas.

As Editor of this issue, I would like to thank you for taking the time to read and consider the opinions expressed herein, and look forward to receiving your feedback.

Sincerely,

Susan M. Swetter, MD

Additional melanoma information available February 2009 at www.MelanomaCare.org or www.MelanomaNurse.org

Clinical Perspectives in Melanoma
A Report on Advances in Melanoma from the 2008 European Society for Medical Oncology (ESMO) Meeting, Stockholm, Sweden, September 12-16, 2008

Insights in Melanoma
Highlights from the Perspectives in Melanoma XII Meeting, The Hague, The Netherlands, October 2-4, 2008

Continuing Medical Education Information

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- Read the newsletter
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Target Audience
This activity is directed toward surgical oncologists, oncology nurses, medical oncologists, dermatologists, and other health care professionals who treat or screen for melanoma.

Statement of Need
Management of all stages of melanoma requires a concerted effort on the part of several specially trained members of a healthcare team. Assessing risk, getting patients into the healthcare system, evaluating prognostic information, analyzing and interpreting new therapeutic information and choosing appropriate therapy, working as a team, and educating, guiding, and motivating the patient remain challenging. A core group of specialists are knowledgeable about the optimal management of this malignancy, and this activity provides their insight for practicing healthcare providers.

Learning Objectives
After completing this activity, participants should be able to:
- Interpret the latest research concerning melanoma genetics and genetic testing for familial melanoma
- Describe conventional and newer histologic and molecular markers of melanoma prognosis
- Discuss the rationale for sentinel lymph node biopsy (SLNB) and potential predictors of SLN positivity, particularly in patients with thin melanomas

Accreditation and Credit Designation
The University of Pittsburgh School of Medicine is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians. The University of Pittsburgh School of Medicine designates this educational activity for a maximum of 1.5 *AMA PRA Category 1 Credits*™. Each physician should claim only those credits commensurate with the extent of his or her participation in the activity.

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Update on Hereditary Melanoma and Genetic Testing

By Sancy Leachman, MD, PhD

Melanoma is caused by an interaction between environmental and genetic factors.[1,2] In most cases, cutaneous melanoma occurs due to sporadic genetic changes in melanocytes. Environmental factors such as ultraviolet (UV) radiation may promote this process through direct mutagenic effects, stimulation of growth factor production, or other mechanisms.[3] Additional factors, including host immunosuppression, further increase the risk of sporadic melanoma development or disease progression.[2,3]

A small percentage of melanomas is familial and linked with germline mutations that are transmitted from parents to their offspring.[1] Germline mutations in melanoma predisposition genes may also interact with environmental factors to greatly increase melanoma risk. This article will discuss causes of hereditary melanoma, ways to identify patients at risk for hereditary melanoma, and the role of melanoma genetic testing in clinical practice. Case studies are used to illustrate points related to appropriate implementation of genetic testing.

Overview of Melanoma Genetics

Studies during the past 20 to 30 years have helped to clarify the genetics of melanoma.[4] It is now clear that melanoma typically develops via stepwise accumulation of mutations, typically in components of major interrelated signaling pathways involved in melanocyte differentiation, proliferation, survival, invasion, and/or progression.[4]

In general, melanoma development involves overlapping risk factors that include environmental factors, pigmentation, ethnicity, and inheritance of high- and moderate-risk predisposition genes. Most of the well-established risk factors for melanoma are related to inheritable factors.[5] It has been estimated that approximately 90% of melanomas are associated with sporadic mutations and only 6% to 12% with familial clustering suggestive of hereditary mutations.[1] Of all established risk factors, the greatest risk compared with the general population is associated with being a member of a melanoma-prone family, with an estimated 35- to 70-fold higher relative risk than the general population. Thus, while familial melanoma syndromes are relatively rare, they are associated with an extremely high risk and therefore demand clinical attention. In addition, a better understanding of genetic alterations associated with melanoma-prone families may shed light on melanoma pathogenesis and ultimately lead to improved therapies.

Germline Mutations Associated With Hereditary Cutaneous Melanoma

High penetrance melanoma predisposition genes are those that confer a high probability of melanoma development when mutated. Germline mutations in 2 high penetrance melanoma-predisposing genes, *cyclin-dependent kinase inhibitor 2A* (*CDKN2A*) and *cyclin-dependent kinase 4* (*CDK4*), have been linked with hereditary melanoma. Of these, *CDKN2A* is the most clinically important melanoma predisposition gene identified to date. Of the roughly 10% of hereditary melanomas, 20% to 40% have been linked with mutations in *CDKN2A*, and only about 1% to 2% with other known single gene mutations.[1] Most cases of hereditary melanoma have not yet been associated with a specific mutation. This is important to remember when performing a genetic test for *CDKN2A* mutations: a negative result does not necessarily mean the tested individual has no melanoma-predisposing gene mutation, since most cases may be associated with as yet unidentified gene mutations.

CDKN2A is located on chromosome 9p21.[6] It encodes a protein generally referred to as $CDKN2A^{INK4a}$ (also known as p16), which functions as a cell cycle regulator.[6,7]

More specifically, CDKN2A^{INK4a} acts as a tumor suppressor by negatively regulating cell cycle progression via the retinoblastoma (Rb) tumor suppression pathway. For individuals carrying a *CDKN2A* mutation, the estimated lifetime risk of developing a melanoma is 91% if they live if Australia, 76% if they live in the United States, and 58% if they live in Europe.[8]

Another high penetrance transcript, generally known as CDKN2A^{ARF}, is encoded at the same genetic locus on chromosome 9p21 as *CDKN2A^{INK4a}*.[7] The protein CDKN2A^{ARF} (also known as p14) is structurally distinct from CDKN2A^{INK4a} but also functions as a tumor suppressor by negatively regulating cell cycle progression through the p53 pathway.[6,7]

CDK4 is the second high penetrance gene that has been linked with increased susceptibility to melanoma. *CDK4* is located on chromosome 12q13 and encodes the protein CDK4, which interacts with cyclin D to regulate cell cycle progression by controlling phosphorylation of Rb.[6,9] CDKN2A^{INK4a} binds to CDK4 and inhibits CDK4-cyclin D-mediated activation of Rb. Hereditary mutations in CDK4 occur in the CDKN2A^{INK4a} binding domain and prevent inhibition of the enzyme by CDKN2A^{INK4a}. *CDK4* mutations account for only a few known cases of hereditary melanoma.[9]

Although the high-penetrance melanoma predisposition genes discussed above lead to extremely elevated risk for melanoma, they are rare. Variants in other low to moderate penetrance genes lead to less of an increase in risk, but are much more common. One such gene is the *melanocortin 1 receptor* (*MC1R*) gene. Germline variants of *MC1R* are fairly common, but exhibit much lower penetrance.[6] The product of the *MC1R* gene, MC1R, is a transmembrane receptor expressed on human skin melanocytes, endothelial cells, and keratinocytes. *MC1R* plays a role in determining skin pigmentation, and certain variants or mutations are associated with red hair color, fair skin, and increased risk of melanoma. The risk of melanoma is increased approximately 2- to 3.6-fold in individuals with particular *MC1R* variants. Risk of melanoma is further elevated in carriers of *CDKN2A* or *CDK4* mutations who also have inherited red hair.[10-12]

A variety of other genes involved in pigmentation processes (eg, the genes encoding agouti signaling protein [ASIP] and tyrosinase [TYR])[13,14] and chromosomal loci (eg, 1p22, 1p36, and 20q11.22)[15-17] have also been implicated in melanoma pathogenesis and are the focus of ongoing investigations.

Phenotypic Features of CDKN2A/CDK4 Mutation Carriers

A common misperception is that all *CDKN2A* or *CDK4* mutation carriers have dysplastic nevi or atypical mole syndrome.[6] While many members of high-risk families do exhibit clinical atypical nevi, this is not true of all members, and the presence of atypical nevi alone (without family or personal history of melanoma) does not reliably predict *CDKN2A^{INK4a}* or *CDKN2A^{ARF}* mutation status. A genetic test is still required to determine whether a particular patient carries a *CDKN2A^{INK4a}* or *CDKN2A^{ARF}* mutation.

In addition to being at increased risk for melanoma, individuals who carry a *CDKN2A* mutation also have an approximately 11% to 17% increased lifetime risk of pancreatic cancer,[6] which is much more difficult to detect than melanoma. It is not yet clear why some families that carry *CDKN2A* mutations tend to develop more pancreatic cancer than others,[18] and this is another area of current study.

Who Should be Tested?

Given that only a small percentage of melanoma patients are likely to be carrying a mutation in *CDKN2A*, how is a clinician to decide who should be tested and who should not? The pros and cons of offering genetic testing to individuals or other members of melanoma-prone families have been reviewed elsewhere.[1,19] In general, genetic testing should be approached cautiously and only performed in a limited number of select cases.[1,5,7]

At the Huntsman Cancer Institute in Utah, a "Rule of Threes" is used as a general guideline to identify patients who are suitable for consideration of genetic counseling and *CDKN2A* testing. According to the "Rule of Threes," a patient is likely to be a good candidate for genetic counseling and possible genetic testing if they fit any of the following:

- *History ≥3 invasive melanomas in a family (any degree of relationship)*: If a patient comes from a family in which 3 or more melanomas (excluding melanoma in situ) have been reported, then the likelihood the patient carries a *CDKN2A* mutation ranges from 11% to 38%, depending on other phenotypic characteristics of the patient.[20,21]
- *History of ≥3 invasive melanomas in the patient*: These individuals have a 7% to 29% chance of carrying a *CDKN2A* mutation.[22,23]
- *History of ≥3 "cancer events" in a family (any combination of melanoma and pancreatic cancer)*: The risk of carrying a *CDKN2A* mutation is about 44% to 72% for patients with a family history that includes any combination of cases of invasive melanoma and pancreatic cancer totaling 3 or higher (at least 1 cancer must be pancreatic and 1 must be melanoma).[24,25]

As mentioned, the "Rule of Threes" is a general guideline and should not be treated as a hard and fast rule for determining candidates for genetic counseling and possible genetic testing. Consideration should be given to age at diagnosis, UV radiation exposure, skin type, and ethnicity, as there may be exceptions to the "Rule of Threes." For example, in a person of dark pigmentation with little UV exposure, a melanoma, and a parent with pancreatic cancer, it might be reasonable to offer testing, even though this patient meets only 2 criteria. The criteria in other countries vary from more stringent (ie, Australia) to less stringent (ie, Spain and Italy).

Finally, it is important to remember that even if a genetic test is negative, an individual in a family known to harbor a *CDKN2A* mutation still has an approximately 2-fold increased risk for developing melanoma relative to the general population, and an even higher risk in the setting of multiple atypical nevi. This is substantially less than the 58% to 91% lifetime risk for *CDKN2A* mutation carriers but warrants ongoing preventive and skin surveillance measures in appropriate patients.

Case 1: Patient with family history but no personal history of melanoma

This case of a 32-year-old woman presenting with numerous clinically atypical nevi

Table 1. Potential Benefits and Risks Associated With Receipt of Positive or Negative Test Results for Mutations in Melanoma Susceptibility Genes

Test result	Potential benefits	Potential risks
Carrier (mutation positive)	• Motivation for surveillance • Lowered threshold for biopsy of suspicious lesions • Earlier detection of primary melanoma • More accurate risk estimate than is possible from family history alone	• Arousal of fear, anxiety, and/or depression • Disruption of family relationships • Over-biopsying • Health insurance and/or employment discrimination
Non-carrier (mutation ruled out in known mutation-carrying family)	• Reduced anxiety • Perceived risk is returned to general population level • Surveillance is returned to general population level • Perceived freedom from feelings of parental guilt	• 'False security' and abandonment of prevention and surveillance • Disruption of family relationships • Survivor guilt • Sustained uncertainty

From Kasparian NA et al. *Psycho-Oncology.* 2007;16:69-78.[29] Copyright © 2007 John Wiley & Sons, Ltd. Reproduced with permission of John Wiley & Sons, Ltd.

should help to illustrate how the "Rule of Threes" applies in a real life situation. The patient has no personal history of melanoma, but does have a confirmed history of invasive melanoma in 2 of 6 siblings and 2 paternal uncles. In addition, her father died from metastatic pancreatic cancer. She has 3 children, ages 6, 8, and 10 years, and 2 of the children have clinical atypical nevi. The patient is NOT interested in participating in an available research protocol for familial melanoma, but she does wish to have *CDKN2A* genetic testing performed. Should genetic testing be offered to this particular patient? She is certainly suitable for genetic counseling, but is she also suitable for genetic testing?

Although the *family* would clearly meet the "Rule of Threes," this family member is not a suitable candidate for genetic testing. There has been no prior testing of the family, so it is unclear whether the family carries a *CDKN2A* mutation. So, in this context, a negative test result for a member who has not had a melanoma would be inconclusive. If it was known that the family carries a *CDKN2A* mutation, then a negative test result would indicate that the patient did NOT inherit that particular mutation. However, since there was no prior testing, a negative test result could also occur because the family is carrying a mutation other than *CDKN2A*.

In this case, the patient did not have a diagnosis or history of melanoma. The situation would be different if an *affected* member of the family was the presenting patient. If the patient had presented with a history of melanoma, should she be tested? The answer here is "probably," assuming that after appropriate genetic counseling and informed consent, the affected member desires testing. Statistically, the patient's family has a >50% chance of carrying a *CDKN2A* mutation, and since her father died of pancreatic cancer, carriers in her family are also likely to be predisposed to pancreatic cancer. Further, if the affected member expresses a desire for testing, she is presumably motivated and interested in knowing the results of testing. If she is not interested in clinical research, there will be little change in the need for ongoing surveillance—depending on the test outcome—but knowledge of status may provide psychological benefits, especially if the test result is negative. In addition, knowledge of mutation status may permit the affected member to make lifestyle changes or decisions that will allow for early detection or prevention of melanoma in herself or other family members, such as her children. In summary, in this scenario, there is little or no risk associated with testing, and there may be benefits associated with knowing the test results. So, in this particular case, an *affected* family member would be tested at our institution, if they so desired.

How Should *CDKN2A* Mutation Carriers be Managed?

For the most part, the management of known carriers of *CDKN2A* mutations is the same as that recommended for other patients with elevated melanoma risk.[5] All patients at elevated risk for melanoma should be educated about the importance of preventive measures, including restricting sun exposure during peak hours, avoidance of sunburns, and use of sunglasses, sun-protective clothing, and sunscreens.

High-risk patients should also be educated about measures for the early detection of melanoma.[5] Beginning at around 10 years of age, *CDKN2A* mutation carriers should ideally have a baseline skin exam and potential photography of the entire body (mole mapping), including dermoscopy of atypical nevi. Patients should be educated about signs suggestive of melanoma and instructed to perform monthly skin self-examinations (SSEs). They should also undergo a clinical skin exam every 6 to 12 months or even more frequently if they have had a melanoma. Studies have shown that physicians are more likely to detect thinner melanomas than patients.[26] Moreover, close surveillance of patients from melanoma-prone families is associated with melanoma diagnosis at an earlier stage of development.[27] A reduced threshold for prophylactic excision of suspicious nevi may also be considered in *CDKN2A* mutation carriers.

If available, *CDKN2A* mutation carriers should also be offered participation in pancreatic cancer screening trials beginning at age 50 or earlier. See the article by Parker and colleagues for a discussion of recommendations.[28]

Psychological and Behavioral Aspects of Testing

A relatively understudied area of growing interest is the psychological and behavioral impact of genetic testing. **Table 1** lists some of the potential benefits and risks that may arise from genetic testing.[29]

Two recent studies conducted in Australia by Kasparian and colleagues used semistructured interviews to explore various psychosocial or psychobehavioral aspects of genetic testing for melanoma susceptibility.[29,30] The key findings from the studies were as follows:[29,30]

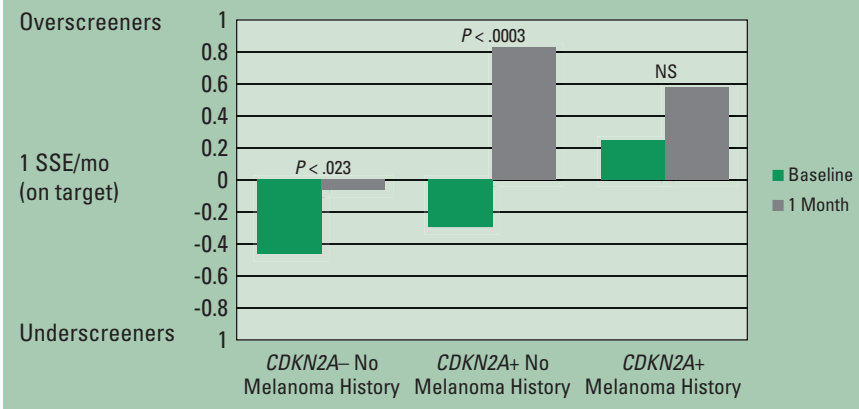
• Familial melanoma patients (high-risk individuals) generally expressed a strong interest in genetic testing.
• They perceived test information as important and valuable.
• Many expected to communicate genetic test results to family members, even if they thought doing so might result in some controversy or negative feelings.
• If the results were positive, most patients anticipated calmly accepting their increased risk, and increasing precaution adoption or maintaining already vigilant behavioral practices with respect to photoprotection.
• If the test results were negative, the majority of participants expected to feel relieved, to maintain current precautionary health practices, and still perceived some risk of developing melanoma (and some were wary that disclosing negative results to family members would reduce their relatives' sun protection practices).

More recently, at Huntsman Cancer Institute, we reported the results of a prospective study evaluating the effect of *CDKN2A* genetic test reporting on melanoma early detection intentions and behaviors in high-risk melanoma families.[31] In this study, questionnaires were used to assess preventive/early detection intentions and behaviors at baseline/prior 6 months, immediately after *CDKN2A* test reporting (intentions), and at 1-month follow-up (behaviors). Assessments were made of photoprotective behaviors (clothing use, sunscreen use, and UV avoidance) and screening (SSE and physician total body skin examination [TBSE]).

Study results were reported for 3 general groups: *CDKN2A*-positive individuals who had no melanoma history, *CDKN2A*-positive individuals who had a melanoma history, and *CDKN2A*-negative individuals with no melanoma history. Of the 45 individuals with a familial history of *CDKN2A* mutations, 19 were non-carriers and 26 were carriers. Forty of the 45 patients reported no downside or negative aspects; the 5 who reported negative aspects indicated that they were very minor, primarily focusing on the relative lack of information concerning pancreatic cancer risk (unpublished data, 2008). Benefits or positive aspects of testing were reported by 43 of 45 members (unpublished data, 2008).

Of note, photoprotective behaviors were maintained by participants who tested negative and, importantly, photoprotective behaviors for patients with no melanoma history improved after receiving positive test results.[32]

Figure 1. Impact of Genetic Test Results on Screening Behavior



SSE, skin self-examination; NS, not significant.

Data from Aspinwall LG et al. *Cancer Epidemiol Biomarkers Prev.* 2008;17(6):1510-1519.[31]

Figure 1 presents the data on the impact of *CDKN2A* test reporting on screening for the 3 categories of patients.[31] Screening behavior significantly improved for patients with no melanoma history following receipt of either a negative or positive result. Receipt of a positive test result dramatically increased screening behavior for patients with no melanoma history. For patients with a melanoma history, a positive test result did not result in a statistically significant change in behavior.[31]

In summary, the findings from this study and the 2 prior Australian studies indicate that reporting of test results is well-tolerated, is appreciated by most melanoma patients, and is generally not accompanied by psychological problems or excess distress. Reporting of positive results in patients without a personal history of melanoma appears to increase compliance with photoprotection and screening recommendations, and reporting of negative results is not associated with development of a false sense of security.

Case 2: Personal history of melanoma and family history of pancreatic cancer

This second case illustrates how the results of genetic testing may impact the psychology/behavior of the affected party as well as that of family members. The patient was a 57-year-old man who presented to the dermatology clinic for an annual TBSE due to a past history of 3 invasive cutaneous melanomas diagnosed at 34, 36, and 39 years of age. The patient had an ongoing history of heightened sun exposure and numerous blistering sunburns. In addition, his family history included a father with pancreatic cancer at age 56 years.

The patient was referred for genetic consultation, after which he stated his desire for genetic testing. Genetic testing of *CDKN2A* was performed, and a mutation in the 5′ untranslated region of the gene was identified. During follow-up, it became clear that the patient was motivated to make lifestyle changes, including decreasing his sun exposure and quitting smoking. He also enrolled in an ongoing pancreatic cancer screening research study, where he is being regularly screened by pancreatic cancer experts.

In addition, the patient's 4 daughters subsequently came in for counseling and ultimately pursued genetic testing. While 1 of the 4 was determined to be positive for the *CDKN2A* mutation, the other 3 were not. Moreover, all 4 daughters—who had never been particularly interested in photoprotective precautions—began to consciously engage in photoprotective behavior and regular screenings. Additional relatives also began to pursue genetic testing and dermatologic screening.

How Should Testing be Done?

First, high-risk patients need to be identified; this can be accomplished by the "Rule of Threes." The next step may be to find some assistance. We recommend referring the patient to a research protocol. If a research protocol is not readily available, or if the patient does not wish to participate in a research protocol, then we recommend consulting a clinical genetic testing center. The location of the nearest center can be obtained at www.nsgc.org or www.cancer.gov/. Testing should be performed at a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory. Details about these laboratories can be obtained at www.genetests.org.

Conclusions

The "Rule of Threes" provides a reasonable guide for identifying candidates for genetic counseling and testing. After complete review of the case and counseling, patients who are deemed appropriate candidates for genetic testing should be tested, if they so desire, and put in touch with appropriate research resources.

Members of melanoma-prone families need to be regularly followed with skin exams starting at age 10 and dermoscopy, if available. Referral to pancreatic cancer screening protocols may also be appropriate for at-risk patients. It is important that members of melanoma-prone families be educated about photoprotection and screening practices, especially on how to do a thorough SSE, why they need a TBSE, and how to engage in optimal photoprotective behaviors.

Update on Histologic and Molecular Markers for Melanoma

By Mohammed Kashani-Sabet, MD

Histologic and molecular markers of melanoma prognosis have been instrumental in current approaches to melanoma staging and treatment. Moreover, continued improvement in understanding of the molecular basis of melanoma development and progression are expected to lead to additional advances in the diagnosis, prognosis, and treatment of melanoma, perhaps enabling more rational design of treatment strategies for individual patients or subsets of patients.[4,7]

While certain prognostic markers are well established and were incorporated into the 6th Edition of the American Joint Committee on Cancer (AJCC) staging system for cutaneous melanoma,[33,34] progress in the field continues. The 7th Edition of the AJCC staging system for melanoma is due in 2009 and is expected to include additional prognostic markers not appearing previously.

This presentation examines the latest findings on histologic and molecular markers in melanoma and describes research strategies intended to improve general understanding of the molecular basis of the disease.

Histologic Markers

Numerous studies have documented the prognostic significance of tumor thickness and ulceration for overall survival in patients with melanoma.[33,35-39] The 6th Edition of the AJCC staging system designated tumor thickness as a primary determinant for tumor staging in the tumor node-metastasis (TNM) classification, with breakpoints of ≤1 mm, 1.01 to 2.0 mm, 2.01 to 4.0 mm and >4 mm, and included presence of ulceration as a second determinant of T and N staging categories.[33] Increasing tumor thickness correlated significantly with metastatic risk, while ulceration signified a locally advanced lesion and served as a critical prognostic factor for Stage I, II, and III groupings.

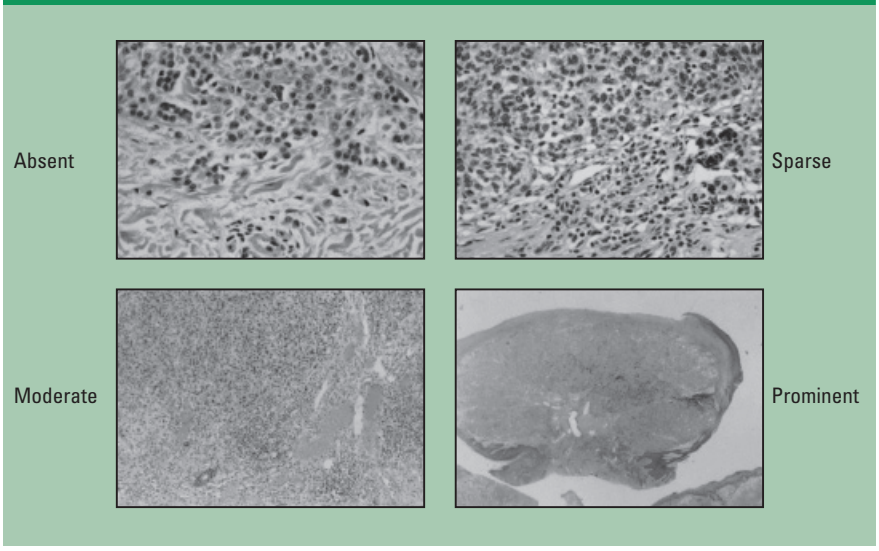
Other histologic variables associated with worsened prognosis in cutaneous melanoma include higher Clark level, sentinel lymph node (SLN) involvement, absence of tumor-infiltrating lymphocytes, higher mitotic rate, and presence of tumor vascularity, lymphovascular invasion, microsatellites, or regression.[40-42] Of these, Clark level, SLN status, and microsatellites are included in the current AJCC staging system.[33] A number of these factors are examined in greater detail below.

Ulceration. With the incorporation of ulceration into the AJCC staging classification for melanoma, identification of biologically meaningful cases of ulceration becomes important. A recent study by our group at the University of California in San Francisco (UCSF) examined the prognostic significance of extent of ulceration in cutaneous melanoma.[43] Data from 235 patients with cutaneous melanoma who had undergone an SLN biopsy (SLNB) were analyzed for the correlation between percentage ulceration and (1) overall survival (n=235) and (2) SLN status (n=218). Ulceration was defined as absence/separation of the epidermis overlying the vertical growth phase (VGP) of the primary tumor, and ulceration percentage as the diameter of ulceration relative to the diameter of the VGP.

We studied the relationship between the extent of ulceration and SLN status and overall survival.[43] For SLN status, 2% ulceration was the best cutoff. Twenty-six percent of cases with ≤2% ulceration had positive SLN status, compared with 40% of cases with >2% ulceration (P=.022). For overall survival, patients with 5% or less ulceration experienced longer survival than patients with >5% ulceration (P=.0006). Hence, knowing the ulceration percentage of a primary cutaneous melanoma improves the ability to predict SLN status and overall survival. The results suggest that no more than minimal ulceration is required to have prognostic impact for both of these outcome measures.

Because ulceration is clearly an im-

Figure 2. Degrees of Tumor Vascularity in Melanoma Tissue



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portant prognostic factor, it would be useful to understand more about its etiology. Current hypotheses are that ulceration is associated with increased thickness and mitotic rate and/or increased or decreased tumor vascularity.[33,44] As discussed below, our group at UCSF has evaluated the prognostic significance of tumor vascularity to try to achieve a better understanding of the relationship between degree of tumor vascularity, ulceration, and clinical outcomes.[45]

Tumor vascularity. Tumor vascularity is the formation of new microvessels in the dermis underlying an invasive melanoma.[42] Tumor vascularity has been linked with the development of the VGP in thin (≤1 mm) melanomas[46] and with metastasis[47] and recurrence[48] in melanomas of intermediate (1-4 mm) thickness. Moreover, various studies have identified tumor vascularity as a significant independent predictor of overall survival in melanoma.[49,50]

We conducted a study at UCSF to explore the relationship between the degree of tumor vascularity and clinical outcomes.[45] Tumor vascularity was evaluated in tissue from 417 melanoma patients with at least 2 years follow-up or first relapse, and graded histologically using routine stains. Four general vascularity patterns were recorded prospectively at the time of diagnosis (**Figure 2**):

- *Absent:* no apparent difference from the normal vascular plexus in or about the tumor;
- *Sparse:* a few additional small vessels, usually located at the base of the thickest part of the tumor (VGP);
- *Moderate:* pattern of both small and dilated vessels observable at intermediate magnification; and
- *Prominent:* widely dilated large vessels in easily recognized increased numbers within and at the base of the VGP, usually apparent at low magnification.[45]

Analysis of the data showed that the mean tumor thickness was significantly associated with tumor vascularity (1.55 mm for cases with absent vascularity vs 4.01 mm for cases with prominent vascularity; $P<.00005$).[45] Increased tumor vascularity was also associated with significantly shorter median overall survival (65 months with absent vascularity vs 35 months with any degree of vascularity; $P<.02$), median relapse-free survival (20 months with absent vascularity vs 10 months with any degree of vascularity; $P<.0034$), and other key clinical outcomes, including relapse and metastasis (**Table 2**).[45] A multivariate Cox regression analysis including several well-recognized histologic factors showed tumor vascularity to be the most important determinant of overall survival (relative risk [RR], 2.47; $P=.0006$), surpassing tumor thickness.

The prevalence of ulceration increased with increasing vascularity (**Table 3**).[45] Although prior reports had established ulceration as a significant prognostic factor for survival in melanoma, relatively little is known about factors involved in the ulcerative process. This was the first study to suggest a link between increasing degree of tumor vascularity and ulcer development.

Vascular invasion. Vascular involvement is defined as tumor cell abutting the dermal microvasculature (incipient invasion) or present inside the vessel lumen (vascular invasion).[42] A 1998 study by Spatz and associates reported that lack of vascular invasion was one of the histopathologic factors associated with long-term survival in patients with thick (>5 mm) melanomas.[51] More recently, our group used Cox regression analysis to identify vascular involvement as significant independent predictors of overall survival in melanoma patients.[52,53] Other studies have also identified vascular invasion as a significant independent predictor of overall and disease-free survival in patients with melanoma.[39,54]

In our initial study, we obtained tissue and clinical records from 526 patients with primary cutaneous melanoma and at least 2 years of follow-up or documented first relapse, and conducted multivariate analyses of the relative contributions of various high-risk prognostic factors for overall and relapse-free survival.[52] Multivariate analyses identified vascular involvement as the second most potent predictor of relapse-free survival (hazard ratio [HR], 1.87; $P<.001$) and overall survival (HR, 2.22; $P<.001$), after tumor thickness. Vascular involvement and ulceration had virtually identical impacts on relapse and survival in this study. Additional analyses revealed that the presence of vascular involvement was associated with significantly greater regional nodal involvement and distant metastases, and significantly shorter relapse-free and overall survival.[52]

In a follow-up study using the same dataset of 526 patients, we examined a model of melanoma progression based on vascular factors (tumor vascularity and

Table 2. Relationship Between Degree of Tumor Vascularity and Outcome

	Degree of Tumor Vascularity			
Outcome	Absent	Sparse/ Moderate	Prominent	*P*-value
Relapsed, %	33.1	50.9	68.5	<.00005
Regional nodal metastases, %	23.7	42.2	51.1	<.00005
Distant metastases, %	16.9	26.7	56.1	<.00005
Dead, %	12.3	25.2	42.4	<.00005

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Table 3. Relationship Between Degree of Vascularity and Presence of Ulceration

Degree of vascularity	Percent with ulceration	
Absent	9.7	
Sparse	16.2	*P*<.00005
Moderate	22.3	
Prominent	48.9	

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vascular involvement) and interactions between various prognostic factors.[53] In particular, we used matched-pair analysis to compare the expression of the transcription factor NF-κB in cases with vascular involvement and tumor vascularity and in cases lacking these factors. Prior animal studies had implicated this molecule in melanoma metastasis, possibly mediated through the enhancement of tumor invasiveness or the promotion of tumor-related angiogenesis. [55,56]

Multivariate analyses identified vascular involvement and tumor vascularity as the strongest predictors of overall survival.[53] Moreover, increased tumor vascularity appeared to be an earlier step in melanoma progression. In addition, both tumor vascularity and vascular involvement appeared to be important factors driving ulceration. As tumor vascularity increased, so did the prevalence of vascular involvement: vascular involvement was present in 4.6%, 14.7%, 23.5%, and 33.7% of cases with absent, sparse, moderate, and prominent vascularity, respectively (*P*<.00005). Ulceration was observed in only 9.5% of cases lacking either tumor vascularity or vascular involvement, 22.1% of cases with either factor present, and 53.9% of cases with both factors present (*P*<.00005). Lastly, the matched-pair analysis involving NF-κB expression demonstrated a significant correlation between overexpression of NF-κB and the presence of tumor vascularity or vascular involvement.[53]

Taken together, these findings are consistent with a hypothesis that tumor vascularity is an early step in melanoma progression, promoting the development of vascular involvement, which acts together with increased tumor vascularity to promote ulceration. Ulceration can thus be considered a surrogate marker for events involving the tumor vasculature. Further, the data are consistent with the notion that NF-κB contributes to melanoma progression by facilitating interactions between melanoma cells and the tumor vasculature. Additional studies are required to further explore and test these ideas.

Microsatellites. Microscopic satellites or microsatellites are defined as nests of melanoma cells separated from the main body of the tumor by a layer of normal dermal collagen or subcutaneous fat.[42] Microsatellites are believed to be a microscopic sign of the metastatic potential or aggressiveness of the primary tumor. Presence of microsatellites has been associated with higher rates of nodal metastases[57] and local clinical recurrence[58] as well as reduced disease-free and overall survival.[39,59,60]

Our group at UCSF recently examined the impact of microsatellites on clinical outcomes in 504 patients with a history of primary cutaneous melanoma and 2 years of follow-up or documented first relapse.[61] A multivariate analysis identified presence of microsatellites as a significant independent predictor of relapse-free survival, but not overall survival. Interestingly, microsatellites were associated with significantly increased rates of locoregional metastases but not distant metastases. In addition, presence of microsatellites was significantly correlated with a number of histopathologic factors, including increasing tumor thickness, higher Clark level, higher mitotic rate, ulceration, tumor vascularity, and vascular involvement.

In the 6th Edition AJCC staging system, the presence of satellites (including microsatellites or in-transit metastases) "upstages" the patient—those with satellites in the absence of nodal involvement are staged in the same classification as those with 2 to 3 metastatic nodes (N2c; pathologic Stage IIIB), and those with any number of metastatic nodes and microsatellites are staged in the same classification as patients with 4 or more metastatic nodes (N3; pathologic Stage IIIC).[33] The 7th Edition of the AJCC staging system will more carefully define satellites according to size criteria, distance from the main tumor mass (measured histologically), and other variables.

Novel Molecular Markers

In addition to NF-κB, a number of other molecules are being explored as potential prognostic indicators in patients with cutaneous melanoma.[62,63]

Nuclear receptor coactivator 3. Nuclear receptor coactivator 3 (NCOA3; also known as AIB1 or SRC-3) is a member of the steroid receptor coactivator family. Its gene, which maps to region 20q12, is frequently amplified in human breast cancers and correlated with poor outcome.[64,65] Increased copy numbers of 20q sequences have also been observed in melanoma tissues and cell lines.[66]

We recently used immunohistochemical analysis of a tissue microarray containing melanomas from 343 patients to assess NCOA3 and further examine its potential as an independent marker of melanoma outcome.[67] Statistical analyses demonstrated that NCOA3 overexpression was significantly predictive of SLN metastasis. Overexpression of this protein was also significantly associated with reduced relapse-free (*P*=.021) and

disease-specific survival (*P*=.030), and was an independent predictor of these events. In fact, NCOA3 was the most powerful predictor of disease-specific survival in this study. These findings suggest NCOA3 may be a novel, independent marker of melanoma outcome.

Osteopontin. This secreted, integrin-binding protein, also known as secreted phosphoprotein-1 (SPP1), has been implicated in progression of various solid tumors.[68,69] Several gene expression profiling studies have correlated osteopontin overexpression with melanoma progression and have highlighted its potential role as a prognostic biomarker for melanoma.[70-72] Another recent study suggested osteopontin expression may also be able to differentiate melanoma cells from benign nevi in SLNs.[73] Currently available data suggest that osteopontin may promote melanoma progression through interactions with NF-κB.[74-76]

Using immunohistochemical analysis of a tissue microarray containing melanomas from 345 patients, our group recently demonstrated significant associations between osteopontin expression and increased tumor thickness (*P*=.037), Clark level (*P*=.035), and mitotic index (*P*=.046), and showed that increased osteopontin expression was associated with reduced relapse-free survival (*P*<.03) and disease-specific survival (*P*=.05).[77] Furthermore, multivariate regression analyses identified osteopontin expression as a significant independent predictor of SLN status (*P*=.015) and disease-specific survival (*P*=.049). The data from this study point to the potential import of osteopontin as a prognostic marker for melanoma, and suggest that osteopontin expression may be able to predict nodal status in patients with melanoma.

Regulator of G-protein signaling 1. *Regulator of G-protein signaling 1* (*RGS1*) encodes a protein that modulates G-protein signaling. *RGS1* resides at 1q31, and gains or amplifications of this locus have been linked with several cancers. In a recent study conducted at UCSF, we used immunohistochemical analysis of a melanoma tissue microarray containing primary cutaneous melanomas from 301 patients to assess the potential impact of RGS1 as a molecular prognostic marker for melanoma.[78] High RGS1 expression was significantly correlated with tumor thickness (*P*=.0083), mitotic rate (*P*=.04), and vascular involvement (*P*<.02), and Kaplan-Meier analyses showed a significant association between elevated RGS1 expression and reduced relapse-free survival (*P*=.0032) and disease-specific survival (*P*=.018). Multivariate Cox regression analyses identified RGSI expression as a significant independent predictor of relapse-free survival (*P*=.0069) and disease-specific survival (*P*=.0077).[78] Furthermore, RGS1 overexpression was significantly correlated with SLN node metastasis (*P*=.04). Taken together, these results suggest that RGS1 expression may be a novel prognostic marker for melanoma, although further studies are required to establish its import.

Comparative Genomic Hybridization

Comparative genomic hybridization (CGH) is a genetic technique used to screen the entire genome of tumor cells for genetic variations in DNA copy number (losses, gains, amplifications) compared with a reference sample.[79] In the typical approach, total genomic DNA isolated from the tumor (test) and reference (nontumour) cell populations are differentially labeled with fluorescent dyes and then mixed with unlabeled human DNA that binds to repetitive sequences and removes these potentially confounding sequences from the analysis. The remaining labeled DNA is hybridized to a representation of the genome. This allows researchers to differentiate sequences that bind to various genomic locations and to assess alterations in DNA copy number. In the original technique, chromosomes were used to represent the genome, but modern approaches typically replace chromosomes with DNA microarrays containing elements that can be mapped directly to the genome sequence.[79]

In recent years, CGH has begun to establish its utility as a technique to screen melanoma and normal cells (eg, benign nevi) for altered DNA/gene copies that are associated with melanoma and may be candidates for further evaluations of function and prognostic significance.[80,81] A genomic ratio (tumor:reference) of 1 means the genomic regions and DNA copies of the tumor and reference are equal, whereas a ratio >1 indicates an increase in copy number in the genomic region of the tumor, and a ratio <1 indicates that genomic loss occurred in the tumor. The degree of increase in genomic ratio provides a measure of the degree of gain or amplification.

CGH analyses of DNA derived from melanocytic nevi and melanomas indicate that melanoma DNA displays frequent chromosomal aberrations, whereas melanocytic nevi have few or none. Melanocytic nevi that do show chromosomal aberrations are almost exclusively Spitz nevi. The identified patterns of chromosomal aberrations could potentially be utilized as a diagnostic tool, particularly in cases with ambiguous histopathology.[81]

CGH may someday be used clinically to classify melanocytic tumors in conjunction with histopathologic evaluation alone. Currently, it should be considered an experimental test with a substantial cost that is generally not covered by insurers. Use of CGH in the clinical setting represents an "out of pocket" expense for patients. Hence, for the time being, it should probably be considered as a very promising, but as yet unproven research tool.

Summary and Conclusions

A number of histologic and serum markers predictive of overall survival are well established and were incorporated into the 6th Edition of the AJCC staging system for cutaneous melanoma. Since then, research has continued to evaluate other histologic or molecular markers that may be used to improve management of melanoma patients, as well as to advance knowledge of the disease process. Some of the more promising markers include tumor vascularity, vascular involvement or invasion, and expression of NCOA3, osteopontin, and RGS1. Subsequent advances in the treatment of melanoma will no doubt be intertwined with an enhanced understanding of the impact of histologic and molecular markers on melanoma development and progression. In addition, an improved knowledge of these prognostic factors may be useful in predicting outcomes and planning therapies for individual patients with melanoma.

Using Pathologic and Histopathologic Information to Predict Sentinel Lymph Node Status and Survival

By Vernon K. Sondak, MD, and Jane L. Messina, MD

It has been recognized for years that melanoma has a predilection to spread to the regional lymph nodes, and that patients with lymph node metastases have worse outcomes than those without lymph node involvement. This has raised concerns over how best to deal with clinically negative regional lymph nodes: whether to remove normal-appearing nodes on the premise there might be microscopic metastases present, or to observe the nodes and manage lymph node metastases when and if they occur.

In the early 1990s, Donald Morton and colleagues introduced a technique for lymphatic mapping and sentinel lymph node biopsy (SLNB); this procedure has established a "middle-ground" approach commonly used in the United States for the staging and management of patients with primary cutaneous melanoma.[82] Patient selection for SLNB is an important aspect of the procedure, and identification of potential predictors of SLN positivity is an area on ongoing research.

The Rationale for SLNB

SLNB is based on the anatomic concept that for every location on the skin, there is a corresponding lymph node(s) that lymph from this skin drains into, and that this "sentinel node" serves as an indicator of the status of the remaining nodes in the regional basin. If the SLN is free of metastatic disease, all subsequent nodes in the regional basin are likely to be free of metastatic disease, and the patient should be at low risk of distant metastases. Conversely, if the SLN is positive, melanoma cells have metastasized to the regional lymph nodes and all the lymph nodes in that basin need to be considered suspect. A positive SLN has been found to be the strongest predictor of disease recurrence and death from melanoma.[41]

By injecting blue lymphangioscintigraphic dye and/or a radio-labeled colloid into the primary tumor site, the SLN can be identified at the time a wide excision (WE) is performed to treat the primary melanoma.[83] SLN tissue can then be examined for evidence of metastatic disease. SLNB is thus a low morbidity staging procedure that allows clinicians to obtain information on lymph node status at a time when the patient is already undergoing an operation.

As the technique for performing SLNBs has improved, pathologists have become more adept at analyzing the SLN, and now often find smaller and smaller metastatic deposits within these nodes. The SLN is typically analyzed by staining multiple sections of the lymph node with standard hematoxylin and eosin (H&E), as well as with immunohistochemical (IHC) stains. This approach enables pathologists to identify extremely small deposits of metastatic melanoma that previously would have gone undetected. One of the current controversies in SLNB (beyond the scope of this article) is whether all of these IHC-detected metastases have clinical significance.

There is also some debate concerning how best to evaluate results obtained using newer molecular techniques to detect metastatic disease, such as reverse transcriptase-polymerase chain reaction. At this time, these techniques should be considered experimental approaches that are best evaluated within a clinical trial setting.[84,85]

SLNB is First and Foremost a Staging Procedure

It is important to recognize that SLNB was never intended to be a procedure that, by itself, leads to a survival advantage. It was envisioned as a staging technique that would help clinicians manage their melanoma patients, since SLNB can provide information useful in making subsequent decisions about treatments that may themselves be associated with survival advantage.

Following the results from a SLNB, patients with a negative finding can be provided with the good news that their risk of recurrent disease and unfavorable long-term outcomes is low, although certainly not zero. For those with a positive SLN, the news is not as good, but the result does provide patients and their physicians with information that can be used to plan further treatment strategies to obtain the best outcomes currently possible, including the option of adjuvant therapy or clinical trial enrollment. The patient with a positive result would at least have the benefit of earlier surgical intervention in the lymph nodes than with a "watch and wait" approach. This affords them a better chance of having durable regional control and avoiding extensive nodal recurrences that are sometimes seen with the "watch and wait" approach.[84]

Even a negative SLN is not an absolute guarantee that the patient is cured and that the melanoma will never return or spread.[84,86] The result may be falsely negative due to limited sensitivity of current technologies to identify existent metastases and even all true SLNs. Also, not every melanoma metastasizes via a nodal pathway. There are some patients who never have any evidence of nodal disease who develop distant metastases, presumably due to hematogenous spread. In various studies, false-negative rates (ie, nodal recurrence in patients with negative SLNs) have been less than 6%.[87-89]

What Does SLN Status Tell Us?

Numerous studies have shown that SLN positivity is associated with significantly reduced disease-free and overall survival for patients with melanoma of various thicknesses.[33,40,41,90-97] Moreover, Cox multivariate regression analyses have demonstrated that SLN positivity is a significant independent predictor of overall survival, and often the strongest or most important prognostic factor for this outcome.[40,90,93-95] SLN status also plays an important role in identifying patients suitable for more aggressive surgi-

cal treatment and possibly adjuvant therapy or enrollment in a clinical trial.

An important question is whether SLNB followed by immediate complete lymph node dissection(CLND) for positive patients is associated with better outcomes than delayed CLND for patients who develop clinical nodal disease (the "watch and wait" or observational approach). The Multicenter Selective Lymphadenectomy Trial (MSLT) is a large, prospective, multicenter, international clinical trial that was designed to evaluate the contribution of SLNB to outcomes in patients with newly diagnosed melanoma.[88] In this study, 2,001 patients with primary cutaneous melanoma were randomized to receive either (1) WE and postoperative observation of regional lymph nodes, with CLND if nodal relapse occurred; or (2) WE and SLNB with immediate CLND if nodal micrometastases were detected on biopsy. Early results for the primary stratum of 1,269 patients with melanomas 1.2 to 3.5 mm in thickness were published in 2006.[88]

For patients randomized to the SLNB group, the presence of SLN metastases was the most powerful prognostic factor for death from melanoma, with a 5-year melanoma-specific mortality of 28% for patients with a positive SLNB and 10% for those with a negative SLNB (hazard ratio [HR], 2.48; 95% confidence interval [CI], 1.54-3.98; *P*<.001).[88] SLN status was also the most powerful predictor of disease recurrence in the SLNB group. The estimated 5-year disease-free survival rate was 78% for patients in the SLNB group and 73% for those in the observation group (HR, 0.74; 95% CI, 0.59-0.93; *P*=.009). The 2 groups did not significantly differ in melanoma-specific survival rates (87.1% vs 86.6%). The 5-year survival rate was significantly higher for patients with nodal metastases who underwent immediate CLND (in the SLNB group) than for those who underwent delayed CLND (in the observation group) (72% vs 52%; HR, 0.51; 95% CI, 0.32-0.81; *P*=.004).[88]

These results highlight a number of important points. First, even a negative SLN is not a guarantee of cure. Within the first 5 years postsurgery, 10% of patients in the SLNB group with a negative biopsy still died from melanoma and 17% relapsed. So, while a negative SLNB is generally deemed a favorable result and would preclude routinely offering aggressive surgical procedures and adjuvant therapy to these patients, patients still require follow-up. Alternatively, a positive SLNB is clearly associated with poor outcomes, but it is not an absolute indicator of imminent death. Only about half of the patients with a positive SLN status experienced a relapse in the first 5 years postsurgery, and only about a third died within the first 5 years. That is, however, a high enough risk to justify aggressive surgical procedures and adjuvant therapy. This large prospective study demonstrated that SLN status was a strong and reliable predictor of outcome and could be relied on to make important treatment recommendations, specifically, whether to proceed with a CLND and whether to consider adjuvant treatment. Finally, known node-positive patients fared significantly better with immediate CLND after SLNB than with delayed CLND after nodal recurrence.[88]

Long-term follow-up is required to better understand whether every single SLN-positive patient would have gone on to develop clinical nodal disease if he or she had simply been observed. In other words, do the microscopic findings always have clinical significance, or are some of these patients destined to never experience nodal recurrence? Long-term data are needed to answer this question because some patients with a positive SLN might require years to develop a clinically palpable nodal mass. Of note, the first longer-term follow-up from this study was published in a letter to the *New England Journal of Medicine* in 2007, and it strongly suggested that an increasing number of patients in the observation group developed positive nodes as time passed.[98] At 10 years, the proportion of patients with a positive lymph node in the observation arm was almost identical to the proportion of SLN-positive plus false-negative patients in the SLNB arm (20.5% vs 20.8%).[98]

Using Tumor Thickness to Select Patients for SLNB

Tumor thickness (Breslow thickness) is the primary histopathologic feature used to identify melanoma patients likely to have a positive SLN and therefore suitable for SLNB.[99-101]

Intermediate and thick tumors. The MSLT focused on the intermediate-thickness group because this group is favorable for demonstrating statistically significant benefits from SLNB. Thicker tumors are more likely to be associated with a positive SLN, but there is also a greater likelihood of distant metastases and early death, which may decrease the total value that can be expected from SLNB. Conversely, with thinner tumors a positive finding may have great value, but the overall frequency of positive nodes is lower than with thicker tumors, making it more difficult to demonstrate a statistically significant benefit of SLNB.

In evaluating the data collected over the years at the Moffitt Cancer Center in Florida, we were struck by the fact that even patients with thick tumors did not always have a positive node. In fact, only about 30% of patients with melanomas >4.0 mm have a positive SLN. Looking at the impact of a positive SLN, we found that, as in the MSLT with tumors of intermediate thickness,[88] the presence of a positive SLN in patients with thick tumors was associated with significantly worse outcomes.[102] Other investigators working at different institutions have also reported that a positive SLN status is predictive of significantly poorer relapse-free and overall survival in patients with primary tumors >4.0 mm.[40,93,94]

At the Moffitt Cancer Center, we generally consider patients with thick primary melanomas to be very good candidates for SLNB. Many of these patients are going to return fairly quickly with palpable nodal disease if we do not perform SLNB. Furthermore, patients with a positive SLN and thick tumor are those typically offered the option of adjuvant therapy. "Watch and wait" is therefore not the best strategy for most patients with thick melanomas.

Thin primary melanomas. The use of SLNB in patients with thin melanomas is an area of controversy. Thin melanomas (≤1 mm thick) are increasingly coming to the forefront with the current emphasis on early detection and increasing awareness and knowledge among family and primary care physicians, dermatologists, and even patients themselves about detection of melanoma and other skin cancers. Various studies have reported a rate of SLN positivity of between 3% and 7% for patients with melanomas ≤1 mm thick,[96,103-106] highlighting a small but real risk of regional nodal involvement in this patient population. Nodal status has clear prognostic significance in patients with thin melanomas; SLN positivity is associated with significantly lower disease-free survival and overall or melanoma-specific survival.[96,106]

The proportion of patients with a positive SLN is extremely low in very thin melanomas (<0.76 mm thick). In one study of patients with thin melanomas, the overall rate of positive SLNs was 3.6%, but no positive SLNs were identified in patients with melanomas <0.76 mm thick.[106] Another study reported an overall SLN-positive rate of 6.5% for patients with thin melanomas, but subgroup analyses found that nodal status was heavily dependent on tumor thickness in these patients. The SLN-positive rate was 10.2% for patients with melanomas 0.76 to 1.0 mm thick and only 2.3% for those with melanomas <0.76 mm thick.[96] Similarly, Bleicher and colleagues reported that only 1.7% of patients with melanomas ≤0.75 mm had SLN metastases.[103] At our institution, we observed that the risk of a positive SLN was <1% for patients with melanomas <0.76 mm thick.[107]

At the Moffitt Cancer Center, we generally do not consider SLNB to be justified in patients with melanomas ≤0.75 mm thick, regardless of other histologic factors. There may be some other factor that eventually will identify a subset of those patients that should be offered SLNB, but as of today there is no clear evidence that such a factor or subset exists.

At our institution, melanomas between 0.76 and 1.0 mm thick are routinely recommended for SLNB because we cannot reliably identify a high- or low-risk subset on the basis of histologic parameters we currently have. We inform patients that there is an approximately 5% chance that their SLN will be positive, and if it is, this finding will provide important information that may improve outcomes. With our current knowledge, it is not possible to identify the 5% of patients who will have a positive SLN from the 95% who will not. For some patients, a 5% chance of finding a positive SLN is not enough to motivate them to have the procedure; for others, it is. If a patient is elderly or has a lot of comorbidities, we ourselves may decide that a 5% chance may not be a sufficient risk to recommend the procedure.

Are there predictors of SLN positivity with thin melanomas? It would be very helpful to have markers that could be used to identify which subset of patients with thin melanomas are most likely to benefit from SLNB, particularly if there is a subset with melanomas <0.76 thick that may warrant the procedure. Unfortunately, although a number of histologic factors have been considered, studies to date have found only a few that may potentially be of use for this purpose. Our experience at the Moffitt Cancer Center indicates that Clark level and tumor regression are not useful predictors of positive SLN in patients with thin melanoma.[108] In fact, histologic regression has been correlated with reduced SLN positivity and disease recurrence in several recent analyses.[109,110]

Age, general health/comorbidities, and mitotic rate are 3 factors that may have value when considering which patients with thin melanomas are suitable candidates for SLNB. The first 2 generally go together, since older patients are also more likely to be in frail health and have multiple comorbidities. Older patients also have fewer years to live to manifest the consequences of a thin melanoma. In our experience, many elderly patients are less concerned with what might happen to them in 4 or 5 years, and more concerned about the potentially negative consequences of a near-term surgery. These considerations may also be relevant for SLNB in thicker melanomas. Interestingly, younger age has been reported to be a significant predictor of positive SLNs in patients with melanoma, including those with thin melanomas.[97,101,111] As younger patients have a higher likelihood of nodal involvement, more years to worry about nodal recurrence, and are relatively healthy and generally able to recover quickly from the modest morbidity associated with SLNB, we believe that younger patients with thin lesions (0.76 to 1 mm thick) should be preferentially considered for SLNB. Exactly what the risk of finding a positive SLN is in this population and what constitutes "young" are questions that have yet to be answered, and issues that should be discussed with each patient on an individual basis.

The other factor that may play an important role in patient selection for SLNB is the mitotic rate of the tumor. A 2003 study in 419 patients with primary cutaneous melanomas of various thicknesses identified mitotic rate, along with age and Breslow thickness, as significant independent predictors of a positive SLN.[111] The higher the mitotic rate, the more likely the patient was to have a positive SLN. These data suggest that the risk of SLN positivity may be higher than expected for many patients with thin melanomas if they are also young and have tumors with a high mitotic rate. A more recent study also identified mitotic rate and tumor thickness as significant independent predictors of SLN positivity for patients with thin (≤1.0 mm) melanomas.[112]

In the past 5 years at the Moffitt Cancer Center, we have been increasingly interested in mitotic rate as a predictor of positive SLN in patients with thin melanomas. So far, our unpublished data are promising and suggest that mitotic rate may ultimately turn out to be an important predictor of SLN positivity, and hence a key factor to consider when selecting patients with thin melanomas (0.76-1.00 mm) for SLNB.

Future Considerations

Clinicians and patients would clearly benefit from improved predictors of melanoma SLN status and prognosis. More refined prognostic factors would be particularly helpful in the management of patients with thin melanomas. It would also be very useful to have markers that identify patients with negative SLN status who are at high risk of dying from hematogenous spread. New biomarkers, predictors of metastasis, and signatures of patient outcomes will be required to take our prognostic capabilities to the next level.

Until such predictors become available, we are still left with the fact that a positive SLN is the most powerful prognostic factor we have in clinically node-negative melanomas, regardless of tumor thickness. We know SLNB is a relatively low morbidity procedure that reliably identifies patients at increased risk for disease recurrence and poor long-term survival. We at the Moffitt Cancer Center routinely recommend SLNB to our patients with melanomas ≥0.76 mm thick. Our patients with both negative and positive SLNs continue to say they are glad they underwent the procedure. At this point, there is no sign on the horizon of an imaging test capable of replacing SLNB for the purpose of diagnosing a positive node in early-stage melanoma.

So, for the time being, SLNB is here to stay. We and other members of the melanoma community continue to actively participate in research intended to improve SLNB, and to better understand the predictive factors that will enhance our ability to select patients for this procedure.

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POSTTEST Pathogenesis and Predictors of Prognosis in Melanoma

For each question or incomplete statement below, please indicate your answer or completion in the space provided on the evaluation form on page 16.

1. The estimated relative risk of melanoma for a person who is a member of melanoma-prone family is as high as _____.
A. 2 to 3 B. 12 to 15
C. 20 to 40 D. 35 to 70

2. What percentage of the total population of melanoma cases may be classified as having hereditary or familial melanoma?
A. 5% B. 10%
C. 20% D. 30%

3. Which of the following is the most common high penetrance melanoma-predisposing gene associated with hereditary melanoma?
A. *CDKN2A* B. *CDK4*
C. *MC1R* D. *BRAF*

4. Which of the following statements is TRUE concerning genetic testing?
A. Familial melanoma (high-risk) patients generally express a lack of interest in genetic testing and do not perceive test information as very important for their lifestyle.
B. Most individuals say they would expect to reduce their current precautionary health practices if they received negative results from testing.
C. Persons with no melanoma history who receive a positive test result dramatically increase screening behavior, to levels commensurate with that of persons with a prior history and positive test result.
D. Most individuals who receive a positive result from genetic testing report substantial negative downside effects associated with that knowledge, including significant anxiety and depression.

5. Which of the following is NOT TRUE concerning the presence of tumor vascularity in melanoma as detailed in the 2002 study by Kashani-Sabet and colleagues?
A. Tumor vascularity versus absence of vascularity was associated with significantly shorter relapse-free survival.
B. Multivariate analysis showed tumor vascularity was the second most powerful independent predictor of overall survival, just behind tumor thickness.
C. Mean tumor thickness is significantly correlated with tumor vascularity.
D. The prevalence of ulceration increases with increasing vascularity.

6. The overexpression of which biomarker has been shown to be significantly correlated with the presence of tumor vascularity or vascular involvement?
A. Osteopontin B. NCOA3
C. NF-κB D. RGS1

7. Which of the following has been reported to be significantly associated with sentinel lymph node positivity and a significant independent predictor of disease-specific survival?
A. RGS1 B. Osteopontin
C. NCOA3 D. All of the above

8. In the Multicenter Selective Lymphadenectomy Trial (MSLT), the 5-year death rate for patients in the SLNB group with nodal metastases was _____, compared with 10% for those in the group with a negative SLNB.
A. 28% B. 36%
C. 44% D. 52%

9. In the MSLT, the 5-year survival rate for patients with nodal metastases who underwent immediate CLND was _____, compared with a rate of 52% for those in the observation group who underwent delayed CLND.
A. 38% B. 54%
C. 72% D. 86%

10. Which of the following patient populations appears to generally be the least suitable for SLNB?
A. Patients with tumors ≤0.75 mm thick
B. Patients with tumors 0.76–1.0 mm thick
C. Patients with tumors 1.2–3.5 mm thick
D. Patients with tumors >4.0 mm thick

EVALUATION FORM Pathogenesis and Predictors of Prognosis in Melanoma

Please use the scale below in answering these questions. Fill in the circle completely. You may use pen or pencil.

Very low Low Moderate High Very High

1. To what degree will you apply the following objectives of the educational activity in your practice and/or professional responsibilities?
   - A. Interpret the latest research concerning melanoma genetics and genetic testing for familial melanoma ❍ ❍ ❍ ❍ ❍
   - B. Describe conventional and newer histologic and molecular markers of melanoma prognosis ❍ ❍ ❍ ❍ ❍
   - C. Discuss the rationale for sentinel lymph node biopsy (SLNB) and potential predictors of SLN positivity, particularly in patients with thin melanomas ❍ ❍ ❍ ❍ ❍
2. To what extent were you satisfied with the overall quality of the educational activity? ❍ ❍ ❍ ❍ ❍
3. To what extent was the content of the activity relevant to your practice or professional responsibilities? ❍ ❍ ❍ ❍ ❍
4. To what extent did the program enhance your knowledge of the subject area? ❍ ❍ ❍ ❍ ❍
5. To what extent did the program change the way you think about clinical care and/or professional responsibilities? ❍ ❍ ❍ ❍ ❍
6. To what extent will you make a change in your practice and/or professional responsibilities as a result of your participation in this educational activity? ❍ ❍ ❍ ❍ ❍
7. To what extent did the activity present scientifically rigorous, unbiased, and balanced information? ❍ ❍ ❍ ❍ ❍
8. To what extent was the activity free of commercial bias? ❍ ❍ ❍ ❍ ❍

Posttest Answer Sheet

1. ❑ 2. ❑ 3. ❑ 4. ❑ 5. ❑ 6. ❑ 7. ❑ 8. ❑ 9. ❑ 10 ❑

If you wish to receive credit for this activity, please complete the form below and:

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JANUARY 2009

Issue 1: Pathogenesis and Predictors of Prognosis in Melanoma

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