Early diagnosis of melanoma: what do we know?

E. F. T. YEE 1, R. M. HOFFMAN 2, M. BERWICK 3

Malignant melanoma is a potentially lethal disease with an excellent prognosis if detected early. In this paper, we review the pathophysiology, clinical presentation, epidemiology, and risk factors for melanoma. We also examine clinical models for predicting risk and survival, and critically appraise data on the effectiveness of screening for melanoma.

KEY WORDS: Melanoma, diagnosis - Melanoma, pathology - Skin neoplasms.

Pathophysiology/clinical features

Melanoma originates from melanocytes, pigment cells that are mostly from neural crest cells or neuroectoderm cells (the latter form retinal pigment epithelium). Melanocyte precursors differentiate and migrate from the neural crest to skin and other tissues during early gestation. More than 95% of melanomas are cutaneous, 1 and while they may be located anywhere on the skin surface, they are often on the trunk (especially the back) in men and legs in women. 2 Primary extracutaneous sites include eyes, gastrointestinal tract, genitourinary tract, leptomeninges, and lymph nodes (unknown melanoma primary). 1 The Clark model describes a stepwise tumor transformation starting with a proliferation of normal melanocytes nesting to form nevi, followed by hyperplasia/aberrant differentiation, atypia and dysplasia, radial growth (intraepidermal proliferation), vertical growth (dermal invasion), and metastasis. 3 While it is thought that most dysplastic melanocytic nevi are terminal lesions that do not progress to melanoma, the frequency of nevi progression to malignancy or nevi regression is not known. 3, 4 Biologically, melanomas may be quite heterogeneous, with variable rates of progression. 5 However, there are no known biologic indicators of indolent melanomas, and the molecular differences between these and clinically important early melanomas are not clear. 6

Epidemiology

Cutaneous melanoma is a public health problem among fair-skinned populations, and the number of cases worldwide is increasing rapidly. 7 Melanoma has ranked as the fourth most common cancer in...
Australia and New Zealand, the seventh most common in the United States and Canada, the tenth most common in Scandinavia, and the eighteenth most common in England, Wales, and Scotland.8 Melanoma incidence is closely associated with skin color and geography.9 Between the early 1960s and the late 1980s, annual incidence rates increased 3-7% in populations of mainly European origins,10 with an estimated doubling of rates every 10-20 years.11 The highest incidence rates (standardized to the world population) are seen in Australia with 40.5 cases/100 000 in males and 31.8 cases/100 000 in females, and New Zealand with 36.7 cases/100 000 in males and 34.9 cases/100 000 in females. By comparison, the standardized rates in the United States are 13.3/100 000 in males and 9.4/100 000 in females. By comparison, the standardized rates in Italy are 4.6/100 000 in males and 5.5/100 000 in females.7, 12, 13

Annual mortality rates vary with the highest rates (standardized to the world population) in males seen in New Zealand: 5.3/100 000, Australia: 4.8/100 000, and Norway 3.3/100 000. The highest rates in females are seen in New Zealand: 3.2/100 000, Norway: 2.7/100 000, and Australia 2.5/100 000.13 Mortality rates increased in most populations of European origin throughout most of the 20th century, but stabilized or even declined in some populations.14 In Sweden, age-standardized mortality rates leveled off since the mid-1980s and declined from 1987 to 1996 in women.15 In Australia, age-standardized mortality rates appear to have peaked in 1985 and then plateaued.16 Mortality rate increases are consistently lower than the increase seen in incidence rates.14 Five-year survival rates have increased from approximately 40% in the 1940s to over 90%,7, 17 Most melanomas are localized (e.g., confined to primary site); US SEER data from 1988 to 2002 show that 82% of cutaneous melanomas were localized at diagnosis, 10% were regional, and 3% had distant metastases.18 Older persons have a higher burden of morbidity and mortality from melanoma,19 especially older men who account for nearly two thirds of all melanoma deaths in Australia and the United States.20 In the United States in 2002-2003, the median age at diagnosis for melanoma was 58 years of age and the majority of cases (77%) were diagnosed in persons aged 45 and older. The median age at death from melanoma was 67 years of age.17

The difference between the trends in incidence and mortality rates may be partly due to the increased diagnosis of thin melanomas. A study of seven populations in the 1980s showed that relative and absolute incidence increased most for the thinnest melanomas and least for the thickest lesions.10 In Central Europe, the median tumor thickness decreased from 1.2 mm in 1986 to 0.8 mm in 1996 (P<0.001), while the percentage of melanomas ≤0.75 mm increased from 29.8% to 46.4% (P<0.001).11 Data from the population-based Queensland Cancer Registry show an increasing age-standardized incidence, with a shift to proportionately more in situ lesions, and stable mortality rates from 1991 to 2002.21

Questions have been raised regarding whether the increased incidence in melanoma reflects a true increase in the disease or is a result of more intensive surveillance and overdiagnosis.22, 23 More intensive melanoma surveillance activity is associated with increased diagnoses of thin melanomas, but not that of advanced tumors. The large increase in melanoma diagnosis without a corresponding increase in advanced tumors and mortality do not fit with a presumed malignant behavior of thin melanomas. Melanoma incidence may be artificially increased by increased melanoma surveillance intensity if histologically “malignant” but biologically benign lesions are excised. There is little available evidence to suggest an actual increase in the frequency of biologically malignant tumors.24

A population based ecological study using Medicare claims data examined changes in skin biopsy rates in persons aged 65 and older to determine the relation to changes in the incidence of melanoma. During 1986 to 2001, the average biopsy rate increased 2.5-fold among people aged 65 and older (2 847 to 7 222 per 100 000 population) while the average incidence of melanoma increased 2.4-fold (45 to 108 per 100 000 population). Most of the increase was due to early stage (in situ and local) rather than late stage (regional and nodal) disease, and mortality due to melanoma did not change appreciably during this period. The authors concluded that melanoma incidence is associated with biopsy rates, and the increased incidence could be attributed to increased diagnostic scrutiny (skin lesions being biopsied that would not have been in the past) rather than an increase in the disease incidence.25 Although this is a plausible hypothesis, the authors were unable to separate biopsies for suspicious melanoma versus
non-melanoma skin cancer. However, this type of misclassification would bias their results to the null rather than inflate the estimates.

Mortality rates and the diagnosis rate for thick tumors (>1.5 mm thick) have not gone up along with the increased rate of melanoma diagnosis. It is not clear whether the increase in diagnosed melanoma cases reflects an actual increase in real disease. Aggressive surveillance might result in the identification of pigmented skin tumors of limited or nonexistent potential for malignant behavior. The improvement in melanoma prognosis may be a consequence of removing biologically benign pigmented tumors that are inadvertently classified as malignant.

Diagnosis of melanoma depends on the examination of a biopsy specimen and the interpretation of a pathologist. Pathology reports lack lexicon and format standardization. Though well-established criteria for diagnosis and staging exist, subjective interpretation can result in discordant diagnoses. Histopathology is qualitative and subjective, and sensitivity in diagnosis melanoma is important due to the risks of missing a cancer diagnosis. Setting a lower diagnostic threshold to increasing sensitivity can result in lower specificity and more false positive diagnoses.

Four histopathologists evaluating 140 slides and classifying lesions as “melanoma” or “other pigmented lesions” agreed on 74% of slides (kappa = 0.61). Eight expert pathologists reviewing 37 slides and classifying specimens as benign, malignant, or indeterminate had unanimous agreement or only one discordant on 62% of slides (kappa = 0.50). A retrospective study of 5,136 specimens reviewed by one pathologist found that 11% had a significant diagnosis change from the referral diagnosis. Critical revisions included 1.2% being changed from malignant to benign, and 1.1% from benign to malignant. The rest, 8.5%, had an upgrade or downgrade in severity sufficient enough to change treatment, prognosis, and research. In a density ratio model, as many as 40% of lesions diagnosed as melanoma in 1988 would not have been so diagnosed in 1978.

### Table I.—Pooled estimates for risk of melanoma.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>RR</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong># Common nevi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole body</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>0-15</td>
<td>1.47</td>
<td>(1.36-1.59)</td>
</tr>
<tr>
<td>16-40</td>
<td>2.24</td>
<td>(1.90-2.64)</td>
</tr>
<tr>
<td>41-60</td>
<td>3.26</td>
<td>(2.55-4.15)</td>
</tr>
<tr>
<td>61-80</td>
<td>4.74</td>
<td>(3.44-6.53)</td>
</tr>
<tr>
<td>81-100</td>
<td>6.89</td>
<td>(4.63-10.25)</td>
</tr>
<tr>
<td>Arms</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>1-5</td>
<td>1.44</td>
<td>(1.29-1.60)</td>
</tr>
<tr>
<td>5-10</td>
<td>2.48</td>
<td>(1.90-3.23)</td>
</tr>
<tr>
<td>11-15</td>
<td>4.82</td>
<td>(3.05-7.62)</td>
</tr>
<tr>
<td><strong>Atypical nevi—all body</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td># of nevi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.60</td>
<td>(1.38-1.85)</td>
</tr>
<tr>
<td>2</td>
<td>2.56</td>
<td>(1.91-3.43)</td>
</tr>
<tr>
<td>3</td>
<td>4.10</td>
<td>(2.64-6.35)</td>
</tr>
<tr>
<td>4</td>
<td>6.55</td>
<td>(3.65-11.75)</td>
</tr>
<tr>
<td>5</td>
<td>10.49</td>
<td>(5.05-21.76)</td>
</tr>
<tr>
<td><strong>Sun exposure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total sun exposure</td>
<td>1.34</td>
<td>(1.02-1.77)</td>
</tr>
<tr>
<td>Intermittent sun exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>— All data</td>
<td>1.61</td>
<td>(1.31-1.99)</td>
</tr>
<tr>
<td>— Aus, US, Can, UK</td>
<td>1.14</td>
<td>(0.90-1.44)</td>
</tr>
<tr>
<td>— Other countries</td>
<td>2.08</td>
<td>(1.55-2.78)</td>
</tr>
<tr>
<td>Chronic sun exposure</td>
<td>0.95</td>
<td>(0.87-1.04)</td>
</tr>
<tr>
<td>Sunburns</td>
<td>2.03</td>
<td>(1.73-2.37)</td>
</tr>
<tr>
<td><strong>Family history, actinic damage, and phenotypic factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family History</td>
<td>1.74</td>
<td>(1.41-2.14)</td>
</tr>
<tr>
<td>Actinic damage:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>— Premalignant/skin CA</td>
<td>4.28</td>
<td>(2.80-6.55)</td>
</tr>
<tr>
<td>— Other actinic indicators</td>
<td>2.02</td>
<td>(1.24-3.29)</td>
</tr>
<tr>
<td>High density of freckles</td>
<td>2.10</td>
<td>(1.80-2.45)</td>
</tr>
<tr>
<td>Phototype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>— I vs IV</td>
<td>2.09</td>
<td>(1.67-2.58)</td>
</tr>
<tr>
<td>— II vs IV</td>
<td>1.84</td>
<td>(1.43-2.36)</td>
</tr>
<tr>
<td>— III vs IV</td>
<td>1.77</td>
<td>(1.23-2.56)</td>
</tr>
<tr>
<td>Eye color</td>
<td></td>
<td></td>
</tr>
<tr>
<td>— Blue vs Dark</td>
<td>1.47</td>
<td>(1.28-1.69)</td>
</tr>
<tr>
<td>— Green vs Dark</td>
<td>1.61</td>
<td>(1.06-2.45)</td>
</tr>
<tr>
<td>— Hazel vs Dark</td>
<td>1.52</td>
<td>(1.26-1.83)</td>
</tr>
<tr>
<td>Hair color</td>
<td></td>
<td></td>
</tr>
<tr>
<td>— Red vs Dark</td>
<td>3.64</td>
<td>(2.56-5.37)</td>
</tr>
<tr>
<td>— Blond vs Dark</td>
<td>1.96</td>
<td>(1.41-2.74)</td>
</tr>
<tr>
<td>— Light brown vs Dark</td>
<td>1.62</td>
<td>(1.11-2.34)</td>
</tr>
<tr>
<td>Skin color Light vs Dark</td>
<td>2.06</td>
<td>(1.68-2.52)</td>
</tr>
</tbody>
</table>

*Sun exposure was defined as: total sun exposure (amount of sun exposure of all kinds); intermittent sun exposure (amount of intermittent pattern of sun exposure); chronic sun exposure (amount of more continuous pattern of sun exposure); sunburns (number of episodes of sunburns). Adapted from Gandini et al. 32-34

## Risk factors for melanoma

A recent meta-analysis assessed major risk factors for melanoma, including number of common nevi on the whole body and the arms, number of atypical nevi on the whole body, sun exposure, family history, actinic damage, and phenotypic factors such as eye color, hair color, and skin photo type (type I-always burn, never tan to type IV-never burn, tan easily). Pooled relative risks (RR) (Table I) demonstrate approximately 6-fold relative risks for...
developing melanoma with a large number of nevi and 5 or more atypical nevi. In addition, a large number of nevi on the arms, actinic damage, and red hair increased risk approximately 4-fold while other important risk factors, such as freckling, light skin type, light skin color, a history of severe sunburns and a family history of melanoma increase risk more modestly, approximately 2-fold. Intermittent sun exposure increased risk while chronic sun exposure appeared to be inversely related to melanoma.\textsuperscript{32,34} Constant sun exposures is postulated to lead to increased tanning and skin thickening conferring a protective effect. Intermittent exposure may stimulate frequent melanocyte activity and lead to more cellular changes resulting in cell transformation than would occur with constant exposure.\textsuperscript{14}

Other proposed risk factors include exposure to tanning beds and sunlamps, obesity, industrial occupation, personal history of skin cancer, higher socioeconomic status, brown hair, male sex, and endogenous hormones have been proposed. However, the evidence for an association between any of these risk factors and melanoma is weak.\textsuperscript{1}

\textbf{Risk models for developing melanoma}

Investigators have evaluated risk for melanoma using either number of risk factors or risk algorithms to form risk prediction models. A Western Australia study\textsuperscript{35} examined the cross-sectional association between the number of skin cancer/melanoma risk factors and the diagnosis of a malignant melanoma. Data were analyzed from 5,950 subjects evaluated between 1996 and 2003 in skin cancer screening clinics targeting high-risk individuals. A questionnaire was used to collect information on 9 risk factors: personal history of malignant melanoma, family history of malignant melanoma, 5 or more moles on the forearm, previous removal of non-cancerous moles, previous skin cancer, a mole/freckle that has changed size, shape, or color, fair skin that always burns, previous blistering sunburn, and non-healing or inflamed skin sores. No relationship was found with logistic regression analyses between the number of risk factors, adjusted for age and gender, and a histopathologically-confirmed melanoma. This study was limited by the small number (18) of confirmed melanomas and the reliance on self-reporting of the number of risk factors which is subject to recall bias and over- or under-statement of participant risk.

MacKie \textit{et al.}\textsuperscript{36} used information from a case-control study of all patients with cutaneous malignant melanoma first diagnosed in Scotland in 1987 to derive a personal risk-factor chart. This was intended for use by medical professionals and the general public. The authors created 4 risk groups for both men and women based on the presence or absence of the 4 strongest risk factors identified by conditional logistic regression: having 20 or more benign pigmented nevi above 2 mm diameter; freckling tendency; having some clinically atypical nevi (over 5 mm diameter and having an irregular edge, irregular pigmentation, or inflammation); and having a history of severe sunburn at any time in life. Patients in the lowest risk group had a relative risk of being diagnosed with melanoma ranging from 1.5, with 95% Confidence Interval (95% CI 0.96-2.2) to 15.6 (95% CI 1.9-130) (compared to patients with none of the 4 risk factors), while patients in the highest risk group had relative risks ranging from 18.2 (95% CI 16.1-54.0) to 587 (95% CI 33.7-10 200).

A prediction model to estimate the 5-year absolute risk of melanoma was developed using case-control data from 718 non-Hispanic white patient cases with invasive cutaneous melanoma from melanoma clinics in Philadelphia, PA and San Francisco, CA, and 945 matched controls. The intent was to help identify high-risk individuals for potential interventions. Sex-specific relative risk models were combined with incidence and mortality rates by United States geographic areas to develop estimates of the absolute risk of developing melanoma within 5 years. The areas under the receiver operating characteristic curves (AUROC) estimated by the models ranged from 0.70 for women aged 50 years and older to 0.80 for men 20 to 49 years of age. Individual absolute risk can be estimated using a program available online at http://dceg.cancer.gov/melanomarisktool. The risk assessment tool factors in geographic residence (northern, central or southern United States), gender, race, age, number of small and large moles on back, complexion (light, medium, or dark), tanning (deeply, moderately, lightly, or not at all) after severe, prolonged exposure to sunlight, the extent of freckling (by comparison to standard photographs), and sun exposure (blistering sunburn, severe solar damage on the shoulders). These projections are not intended to identify current melanoma cases, cannot be used to predict risk in races other than non-His-
panic whites, and are applicable only to those living in the United States.37

Predictors of melanoma survival

A model predicting 10-year survival for patients with primary cutaneous melanoma and no apparent metastatic disease found 4 independent predictors: thickness, site, age, and gender. Adjusted odds ratios were: for lesions ≤0.76 mm thick, OR=50.8, (95% CI 18.5-140), lesions 0.76-1.69 mm, OR=9.5 (95% CI 4.0-22.5), lesions 1.70-3.60 mm, OR=2.9, (95% CI 1.29-6.5), primary lesion on an extremity, OR=4.4 (95% CI 2.3-8.4), age ≤60 years at diagnosis, OR=3.0 (95% CI 1.7-5.3), and female sex, OR=2.0 (95% CI 1.2-3.6). The four-variable model was significantly more accurate than a one-variable model using tumor thickness alone.38 However, a study validating this prognostic model found no significant differences for accuracy between the data sets for the four- and one-variable models.39

An analysis of a multicenter database from 30 450 melanoma patients, found that survival was related to thickness of the melanoma (Breslow depth, measured in millimeters) and regional node status. In localized disease (stage I and II), the strongest prognostic factors were thickness of the tumor and presence of ulceration. In regional metastatic disease (stage III, including regional nodal involvement, in transit or satellite metastases), node status (number, clinical versus occult), and tumor ulceration were important prognostic factors. With distant metastases (stage IV), anatomic site was the most significant factor.40 The 10-year survival rates for stage I tumors ≤1.0 mm are 88% without ulceration and 83% with ulceration or Clark level IV, V invasion. The 10-year survival rates for other stages range from 64%-32% for stage II and 63%-15% for stage III. With stage IV, rates decrease to 16% for skin and subcutaneous lesions, 6% for other visceral lesions, and 2% for lung lesions.41

Other prognostic factors include serum LDH, an independent predictor of disseminated metastases,41 and tumor mitotic rate (TMR), expressed as mitoses/mm². TMR has been shown to be an important prognostic factor in a number of multivariate analyses.42, 43 In a study examining data from 3 661 patients in the Sydney Melanoma Unit database, patients with a TMR of 0 mitoses/mm² had significantly better survival compared to those with 1 mitoses/mm² (P<0.0001). Two different TMR groupings were used for a Cox regression analysis- method A: 0, 1-4, 5-10, and ≥11 mitoses/mm² and method B: 0-1, 2-4, and ≥5 mitoses/mm². TMR was a highly significant independent prognostic factor with both groupings. With method A, TMR was second only to tumor thickness in predicting survival (P<0.0001), and was more powerful than age and ulceration. With method B, thickness was the most powerful predictor, followed by age, ulceration, and TMR.42 In a population-based series of 650 consecutive invasive cutaneous malignant melanoma cases ascertained from the Connecticut Tumor Registry, TMR was a significant predictor, but tumor ulceration was no longer an independent prognostic factor after adjusting for TMR.43

Detecting melanoma

The skin examination

ABCDE AND THE UGLY DUCKLING: OPPORTUNISTIC MELANOMA DETECTION

A visual skin exam is the most commonly used screening method to detect skin cancers.44 Clinical recognition and differentiation of cutaneous melanoma from clinically atypical moles can be difficult.45 Two clinical indicators for visual recognition of melanoma include the ABCDE mnemonic and the Ugly Duckling sign. The ABCD (Asymmetry, Border irregularity, Color variegation, Diameter greater than 6 mm) melanoma screening mnemonic was developed in 1985 to assist lay persons and primary care providers in the early detection of melanomas.46 Evolving lesions (E) was later added to expand the criteria to ABCDE.47 However, a melanoma may be missed if a lesion does not have the typical ABCDE features.44 Another clinical indicator used to recognize melanomas is the “ugly duckling sign.” In a given individual, nevi generally share characteristics. A nevus with different features from other nevi is considered to be an ugly duckling and should raise the consideration of a possible melanoma.48

TOTAL BODY SKIN EXAM: SYSTEMATIC MELANOMA DETECTION

Using the total-body skin exam for screening rather than a partial exam has been advocated because 80%
of melanomas appear in areas of the body normally covered by clothing. In a study of 2,239 persons seen at the Manhattan Melanoma/Skin Cancer Detection Screening Program, patients with complete skin examinations were 6.4 times more likely to have a melanoma detected compared to those with partial examinations (P=0.025). Thirteen out of fourteen melanomas found were on anatomic sites normally covered by clothing.

Important incidental lesions such as melanoma, lentigo maligna, dysplastic nevi, and congenital nevi are found in a significantly higher proportion of patients receiving total body skin exam compared to those receiving a partial examination.

Physician-detected melanomas are usually thinner than patient-detected lesions

Multiple studies have found that primary melanoma is most often detected by the patient, followed by a doctor, spouse or partner, or other source. Physician-detected lesions are usually thinner than patient or spouse-detected lesions, and dermatologist-detected lesions are usually thinner than other physician-detected lesions. Whether that means that physician-detection will be more effective is not proven by these data due to the lack of information about efficacy of screening or early detection in preventing mortality. A population-based study of 3,772 Queensland residents with a histologically confirmed melanoma diagnosed between 2000 and 2003 found that patients detected 44% of lesions while physicians detected 25%. Melanomas detected by doctors were more likely to be thin (<0.75 mm) than those detected by the patient or other layperson. Overall, 81% of physician-detected lesions were thin compared to 62% of layperson-detected lesions. Melanomas detected during a deliberate skin examination were thinner than those detected incidentally. A study of 102 patients with newly detected primary cutaneous melanoma seen at the John Hopkins Melanoma Center found that patients detected 55% of lesions compared to 24% detected by physicians. Physician detection was associated with thinner lesions compared to detection by self or others (median thickness 0.23 mm vs 0.9 mm, P<0.001).

A study of 1,515 consecutive patients with melanoma seen at the University of Michigan, a review of 471 newly diagnosed melanoma patients at Sloan Kettering, and a retrospective cohort analysis of 218 patients with melanoma at the Yale Pigmented Lesion Clinic all found that physician-detected initial lesions were more likely to be thinner than self/non-physician detected lesions. Thin lesions were also associated with detection by a dermatologist compared to other physicians, a finding also noted in a study of 813 patients consecutively diagnosed with melanoma in 11 Italian Clinical Centers. Detection by a dermatologist and skin self-examination were significantly associated with thinner lesions compared to those found by patients or other physicians.

A prospective survey of 590 consecutive melanoma patients in France found that tumor thickness was lower (median 0.94 vs 1.50 mm, mean 1.88 vs 2.82 mm respectively, P<0.001) when first seen by a dermatologist as compared to another physician.

Accuracy of the skin examination

Accuracy by lesion: ABCDE and Ugly Duckling

A review of studies examining the diagnostic accuracy of the ABCDE criteria found that sensitivity and specificity varied depending on whether they were used alone or in combination (Table II). In one study, using all five ABCDE criteria had the highest specificity (100%) but the lowest sensitivity (43%). In other studies, one or more of the ABCDEs had a sensitivity of 92% (95% CI 82-96) and a 3-variable model (the BCD criteria used together) had a sensitivity of 100% (95% CI, 54-00) and a specificity of 98% (95% CI, 95-99).

A prospective French study described the melanoma recognition process by immediately sur-

---

**Table II.** Sensitivities, specificities, and likelihood ratios associated with ABCDE criteria.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Likelihood Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>57</td>
<td>72</td>
<td>2.0</td>
</tr>
<tr>
<td>B</td>
<td>57</td>
<td>71</td>
<td>2.0</td>
</tr>
<tr>
<td>C</td>
<td>65</td>
<td>59</td>
<td>1.6</td>
</tr>
<tr>
<td>D</td>
<td>90</td>
<td>63</td>
<td>2.4</td>
</tr>
<tr>
<td>E</td>
<td>84</td>
<td>90</td>
<td>8.4</td>
</tr>
<tr>
<td>≥1 Criteria</td>
<td>97</td>
<td>36</td>
<td>1.5</td>
</tr>
<tr>
<td>≥2 Criteria</td>
<td>89</td>
<td>65</td>
<td>2.5</td>
</tr>
<tr>
<td>≥3 Criteria</td>
<td>66</td>
<td>81</td>
<td>3.5</td>
</tr>
<tr>
<td>≥4 Criteria</td>
<td>54</td>
<td>94</td>
<td>9.0</td>
</tr>
<tr>
<td>≥5 Criteria</td>
<td>43</td>
<td>100</td>
<td>∞</td>
</tr>
</tbody>
</table>

Adapted from Abbasi et al. and Thomas et al.
vewing 135 dermatologists after they clinically evaluated pigmented lesions (n=4,036) that were subsequently biopsied. Features most predictive of accurately diagnosing melanoma were the impression of overall irregularity of the lesion, the perception that the lesion is an “ugly duckling” compared to the other nevi, and knowledge that the lesion had changed or evolved via patient report. A, C, and D criteria had lower predictive values.

**Correlation between examiners regarding skin examination and risk factors**

A study to determine feasibility of targeted early detection for melanoma found limited agreement between patient and dermatologist assessments of risk factors. The kappa score was best for hair color (0.67), while the kappa scores were 0.22 for prevalence of ≥10 moles on head and neck, 0.36 for reporting of skin type I or II, and 0.13 for reporting moderate or many freckles. Correlation may have been low as patients tended to under-report their risk level. While a high nevi count is an important risk factor for melanoma, patients appeared reluctant to categorize themselves in the most extreme group having a lot of moles. In a study of clinician agreement on clinical characteristics recognition of cutaneous melanoma (number and type of nevi), variation was seen for the index of agreement (calculated as the intra-class correlation coefficient). Agreement was low for freckling on the right forearm (60%) and on the shoulders (69%), and palpable nevi of the arms (36%). Agreement was higher (88%) for total arm nevi (both palpable and non-palpable), as well as total number of atypical nevi on the body (87%), and total number of all types of nevi (92%).

**Accuracy of the total-body skin examination: one or more lesions**

We define a low positive predictive value (PPV) as number of biopsy-proven melanomas divided by number of screens with presumed melanoma diagnosis. A high PPV is defined as the number of biopsy-proven lesions divided by the number of presumed melanoma with biopsy data.

The reported sensitivity of a total-body skin examination in a community-based screening program was 70% (95% CI 51.3-84%), specificity was 98% (95% CI 97.2-97.9), low (PPV) was 11%, and negative predictive value (NPV) was 99.9% for melanomas diagnosed within one year of a screen. The low PPV was 10% if the body site was taken into account. All negative screens were followed through a population-based cancer registry to calculate sensitivity of the screening diagnosis. Screens were conducted by volunteer plastic surgeons and dermatologists. In another community-based screening program, primary care physicians had a reported specificity of 86% (95% CI 86-87), and low PPV of 15% using a denominator of number of screens with presumed melanoma diagnosis, or high PPV of 20% using a denominator of number of presumed melanoma with biopsy data. However, this study did not follow up on negative screens, and the specificity calculations were based on expected number of false-negative cases.

In other studies, the low PPV of a total body skin exam ranged from 7-17%, and high PPV from 20-20% (Table III). One of the largest studies to evaluate screening reviewed 242,374 skin cancer screenings on more than 206,000 Americans who attended the American Academy of Dermatology National Skin Cancer Screening Programs during the period 1992-1994. Investigators contacted 96% of 3,476 screeners with a presumptive diagnosis of melanoma or possible melanoma. Follow-up records were obtained for 73% of screeners, and 363 had histologically proven melanoma. Middle-aged and older men (age ≥50 years) comprised only 25% of screeners but comprised 44% of those with a confirmed diagnosis of melanoma. Men age ≥50 years with a changing mole had a low PPV=22%, and low PPV=28% if they had skin types I and II.

When sensitivities and predicted values are reported in the studies, it appears that only one study, Fritschi et al., attempted to account for body site of the melanoma to improve validity. A reported suspicious lesion may not be the lesion that was biopsied. If a melanoma was found at a different site than what was considered suspicious, then the sensitivity and positive predictive value will be overestimated. Another caution when interpreting these data is that screeners are self-selected, and results might not be applicable to the general population. Sensitivity and prevalence of the preclinical disease influence the PPV of a screening test. The PPV from studies in a community or screener group with a high prevalence of...
YEE EARLY DIAGNOSIS OF MELANOMA

62 GIORNALE ITALIANO DI DERMATOLOGIA E VENEREOLOGIA Aprile 2007

melanoma will be higher than in those with a lower prevalence of melanoma.

ACCURACY OF SKIN EXAM: DERMATOLOGY VS PRIMARY CARE

Studies comparing the accuracy of dermatologists and primary care physicians (PCPs) in identifying pigmented lesions suggestive of melanoma and making the appropriate management decisions regarding performing a biopsy or referring the patient have found mixed results. A systematic review of 32 studies published between January 1966 and October 1999 found that the sensitivity for diagnosing melanoma was 81% to 100% for dermatologists and 42% to 100% for PCPs. None of the studies reported specificity for dermatologists and only one reported a specificity for PCPs of 98%. The biopsy or referral accuracy sensitivity ranged from 82 to 100% for dermatologists and 70 to 88% for PCPs. The specificity ranged from 70 to 89% for dermatologists and 70 to 87% for PCPs. However, authors were unable to detect any significant differences in the areas under the receiver operating characteristic curves for biopsy and referral ability between dermatologists and PCPs.71

A subsequent study assessed differences between dermatologists and PCPs in accurately diagnosing melanoma and appropriately managing (based on decisions to refer/biopsy) using photographs of suspicious pigmented lesions. Dermatologists had better diagnostic accuracy and ability to manage pigmented lesions than PCPs. Investigators used a survey of a random sample of 30 photographs of pigmented lesions with known pathology administered to 101 dermatologists and 115 PCPs. Likelihoods that a photographed lesion was melanoma and that the lesion should be biopsied/referred were scored on a 1 to 10 scale. Accuracy of melanoma diagnosis and appropriateness of pigmented lesion management were compared between dermatologists and PCPs by using the areas under the receiver operating characteristic curves (AUROC). Dermatologists were superior to PCPs in diagnosing melanomas (AUROC 0.89 vs 0.80, P<0.001) and appropriately managing pigmented lesions (AUROC 0.84 vs 0.76, P<0.001).72 Suggestions have been made for additional education and training of PCPs due to concerns regarding a shortage of dermatologists. PCPs have the opportunity to manage pigmented lesions in patients presenting to them. However it is unclear that this would

<table>
<thead>
<tr>
<th>Subjects and setting</th>
<th>Patients</th>
<th>Screening</th>
<th>Examiner</th>
<th>Confirmed melanomas (N)</th>
<th>Presumed melanoma at screen (N)</th>
<th>Biopsies of presumed melanoma at screen (N)</th>
<th>Low PPV (%)</th>
<th>High PPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aitken 2006 66</td>
<td>16,383</td>
<td>TSE</td>
<td>PCP</td>
<td>33</td>
<td>222</td>
<td>161</td>
<td>14.9</td>
<td>20.5</td>
</tr>
<tr>
<td>Fritschi 2006 64</td>
<td>7,436</td>
<td>TSE</td>
<td>Derm or plastic surgeon</td>
<td>23 (1 yr)</td>
<td>201</td>
<td>211 (site specific)*</td>
<td>11.4 (1 yr)*</td>
<td>10.0 (1 yr)*</td>
</tr>
<tr>
<td>Swetter 2003 67</td>
<td>3,747</td>
<td>TSE</td>
<td>Derm</td>
<td>1</td>
<td>14</td>
<td>8</td>
<td>7.1</td>
<td>12.5</td>
</tr>
<tr>
<td>Geller 2002 58</td>
<td>242,374</td>
<td>Partial, TSE, or site specific</td>
<td>Derm</td>
<td>363</td>
<td>3476</td>
<td></td>
<td>10.4</td>
<td></td>
</tr>
<tr>
<td>Engelberg 1999 69</td>
<td>520</td>
<td>Sun exposed or site specific</td>
<td>Derm</td>
<td>1</td>
<td>6</td>
<td>6</td>
<td>16.7</td>
<td>16.7</td>
</tr>
<tr>
<td>Jonna 1998 70</td>
<td>464</td>
<td>TSE</td>
<td>Derm</td>
<td>2</td>
<td>35</td>
<td>13</td>
<td>5.7</td>
<td>15.4</td>
</tr>
</tbody>
</table>

Low PPV: number of confirmed melanomas/number with a presumed or suspected melanoma diagnosis at screen. High PPV: number of confirmed melanomas/number of presumed or suspected melanomas at screen with biopsy results; TSE: total skin examination; Derm: dermatologist; PCP: primary care physician. *: Fritschi’s study=accounting for body-site.
be feasible or useful given the lack of data that early detection of melanoma leads to a decrease in late stage melanoma.6, 72, 73

**EARLY DIAGNOSIS PUBLIC EDUCATION CAMPAIGNS: PROGRAMS TO ENCOURAGE SKIN SURVEILLANCE**

Public education programs to promote early detection have been carried out in Scotland, the United Kingdom (UK), and Australia. In Scotland, the number of referrals to a melanoma clinic and number of melanomas diagnosed increased following an education campaign. Thin tumor percentage increased. The incidence of thick tumors decreased in women, and a decrease in melanoma mortality was seen in women.74 Subsequent studies of a similar education campaign conducted during 1987-1989 in 6 areas of England and one in Scotland showed an increase in referrals to pigmented lesion clinics and an increase in melanoma rates following the campaign. There was an apparent rise in thin melanomas, but the incidence of thick tumors did not appear to decrease.75-79 An analysis of melanoma mortality rates during 1981 to 1996 (adjusted or pre-intervention rates) showed no decrease in cumulative mortality in intervention areas compared to non-intervention areas. As these educational campaigns were not trials, there were no control groups, and the actual effectiveness of the intervention is difficult to measure. Information regarding who received and acted on the health education could not be ascertained, and it is possible that the health education could have reached non-intervention areas and decreased the ability to detect a difference in mortality.80 As these education campaigns were not population screening trials, the results should not be applied to screening.2, 22

**Rationale for screening**

Early stage melanoma is associated with excellent survival and the goal of screening for melanoma is to decrease morbidity and mortality. A case for screening is predicated on the assumptions that melanoma has a long latency period during which it can be treated with a high rate of success, and that early detection prevents progression of early-stage cancers to advanced, lethal stages.81 Melanoma is usually visible on the skin at a curable stage.82 Skin exams are quite feasible and relatively non-invasive, and primary care physicians and the public can be educated to perform them.

**YIELD OF SCREENING PROGRAMS**

Skin cancer screening programs have been conducted worldwide. Helfand et al. reviewed data from 24 screening programs published in papers between 1994 and June 1999. They found that rates for suspected melanoma ranged from 0 to 9 per 100 screenees, referral for follow-up of suspicious lesions ranged from 2 to 34 per 100 screenees, and biopsy ranged from 4 to 31 per 100 screenees. Rates of confirmed melanoma and melanoma-in-situ ranged from 1 to 4 per 1 000 screenees,19 except for one study in Sweden with no melanomas found in 152 suspected cancers in 1 654 people screened,83 and one study in Australia that had 8 confirmed melanomas per 100 screenees.84

A retrospective study of melanoma screening clinics in the United Kingdom examined data from 15 970 physician-referred patients screened from 1997 to the end of 2004. There were 403 primary invasive melanomas detected, and 190 in situ melanomas (37/1 000 screenees).85 The Lions Cancer Institute Skin Cancer Screening Program in Australia has offered screening by dermatologists and plastic surgeons since 1990. Advertisements for attendees target individuals with self-assessed skin cancer risk factors. During 1994 and 2002 there were 7 436 attendees, most had 3 or more risk factors. Thirty-three melanomas were diagnosed within one year of a screen (4/1 000 screenees), and 16 more were diagnosed between 1-2 years after a screen (total 7/1 000 screenees).64 In Northern Italy, a regional program to evaluate the efficacy of early detection of melanoma by general practitioners during 1997-1998 yielded 11 040 examinations. Subsequently, 820 patients (7%) were referred to a dermatologist, and 38 melanomas were detected (3/1 000 screenees).66
A study of 374 veterans in northern California attending free skin cancer screening clinics targeted at a high risk population during 1997-2000 found that dermatologists referred patients (54%) for suspicious lesions, including 14 for possible melanoma versus dysplastic nevi. Of the 101 patients that investigators were able to follow-up, only 1 out of 8 possible melanomas versus dysplastic nevi was confirmed by biopsy (3/1,000 screenees, if data extrapolated to 1,000 screenees). The high referral rate may reflect the highly selected screening population that was mostly male, caucasian, and older (mean age 63). When the database of the more than 242,374 screenings from the American Academy of Dermatology National Skin Cancer Screening Programs during 1992-1994 was analyzed, 3,476 screenees had a presumptive diagnosis of melanoma. The number of confirmed diagnoses of melanomas was 363, giving an overall yield of 1.5 melanomas per 1,000 screenings. In men aged ≥50 years, the yield was 3/1,000 screenees, 4/1,000 if they had skin types I and II, and 5/1,000 if they had a changing mole.

EVIDENCE FOR BENEFIT WITH SCREENING

The gold standard for evidence-based benefits of screening are large-scale, prospective randomized trials demonstrating decreases in cancer-specific mortality in screened versus non-screened populations. There are no such completed randomized controlled prospective screening trials for melanoma. Melanoma is relatively rare in the general population and a randomized clinical trial to test efficacy of screening would be very costly. The Queensland Australia project was the largest randomized trial of population screening for melanoma. 18 communities were randomized to either intervention (community and professional education, and skin screening by a doctor) or control. This project was not funded for follow up, and currently there are no data that provide direct evidence that screening leads to reduced morbidity and mortality. In the absence of such data, we examine observational studies. Care must be taken when interpreting data from observational studies because of inherent biases, and these biases are discussed later. A population-based, case-control study to investigate whether early detection through skin self-examination (SSE), (defined as “conducting a careful, deliberate, and purposeful examination of the skin”) is associated with a decreased risk of lethal melanoma was conducted in 1,199 Caucasian residents of the state of Connecticut. The study included 650 individuals with newly diagnosed cutaneous melanoma, and 549 age- and sex-frequency matched control subjects from the general population. In 5 years of follow-up, there were 110 “lethal” melanomas. SSE was practiced by only 15% of all subjects, and was associated with a reduced risk of the development of melanoma (OR=0.66, 95% CI=0.44-0.99). The results further indicated that SSE may reduce the risk of advanced disease among melanoma patients (unadjusted risk ratio = 0.58; 95% CI=0.31-1.11) and that when combining the incidence reduction with the prevention of mortality, SSE may reduce mortality from melanoma by 63% (adjusted OR=0.37; 95% CI=0.16-0.84). These results are provocative but need to be confirmed with longer follow-up and studies in other populations.

SCREENING SELECTED HIGH RISK POPULATIONS

Screening certain high-risk populations may result in detection of thinner lesions. In a screening study of 555 members of hereditary melanoma kindreds with dysplastic nevi, the average thickness of 28 surveillance-detected melanomas was 0.52 mm, compared to 0.55 mm for 64 non-surveillance incident melanomas (diagnosed before entry into the surveillance program), and 1.44 mm for 48 index lesions (P<0.001). The proportion of cases with level I or II invasion was 61% in the surveillance group, 58% in the non-surveillance group, and 36% in the index group (P=0.002).

COST EFFECTIVENESS OF SCREENING

Studies examining the cost effectiveness of screening for melanoma have found that melanoma screening could be cost effective. Losina et al. used a computer simulation Markov model to evaluate the cost-effectiveness of four types of screening strategies beginning at 50 years of age: background screening only, and screening once, every two years, and annually. Expected outcomes included life expectancy, quality-adjusted life expectancy, and lifetime costs. Cost-effectiveness ratios were calculated in dollars per quality-adjusted life year (US dollars/QALY) gained.
In the general population, screening 1 time, every 2 years, and annually saved 1.6, 4.4, and 5.2 QALYs per 1,000 persons screened respectively. Cost-effectiveness ratios were $10,100/QALY, $80,700/QALY, and $586,800/QALY, respectively. In siblings of patients with melanoma with a relative risk of 2.24 compared with the general population, 1-time, every-2-years, and annual screenings saved 3.6, 9.8, and 11.4 QALYs per 1,000 persons screened. Cost-effectiveness ratios were $4,000/QALY, $35,500/QALY, and $257,800/QALY, respectively. Higher-risk siblings of patients with melanoma with a relative risk of 5.56 cost-effectiveness ratios were $900/QALY, $14,700/QALY, and $99,800/QALY, respectively.

Cost-effectiveness thresholds cited in literature for acceptable interventions range from $50,000-$100,000/QALY gained. Using these thresholds, acceptable strategies include a one-time melanoma screening of the general population older than 50 years, and screening every 2 years in siblings of patients with melanoma. Limitations in this study included possible lead time and length time bias that could influence the survival benefit of earlier detection of melanoma. In addition, the actual rate of melanoma progression is unknown and could impact on results of screening every two years. Non-melanoma skin cancers, particularly basal cell carcinomas with little potential for progression, may be detected by screening and add to costs.

Other analyses have also found that screening could be cost-effective. A computer model simulated a melanoma screening program over a 20-year period, using cohorts of Australians 50 years of age at the start of the program. The sensitivity of the screening test (visual inspection of the skin) was set at 60%. Cost effectiveness ranged from $6,853 (Australian, AUS) per life year saved for screening every five years to $12,137 for screening every two years. For women, it ranged from AUS $11,102 for screening every five years to AUS $20,877 for screening every two years. A decision analysis determined the effectiveness and costs of a one-time visual screen by a dermatologist to diagnose malignant melanoma in high-risk persons ages 20 and older. Using a cost of $30 per screen, a cost-effectiveness ratio of $29,170 per year of life saved (YLS) with screening was found. The cost-effectiveness ratio for screening remained below $50,000/YLS if the prevalence of melanoma in the screened population was at least 0.0009 (9/1,000) and the probability that a melanoma detected in screening was localized was at least 94.8%, or the cost of each screen was below $57. The prevalence of 9/10,000 is highly unlikely at this time.

**Biases in Observational Studies**

Most of the data regarding melanoma screening are derived from observational studies. These designs are subject to a number of potential biases including lead-time, length, and selection/referral. Effective screening for melanoma should diagnose the disease earlier than it would be without screening but would also prevent progression of the disease. Biases could make screening appear beneficial when in actuality it is less or not effective.

Lead time is the period between the detection of melanoma by screening and when it otherwise would be diagnosed once symptoms appeared. Lead time bias occurs when early diagnosis appears to prolong survival because disease is picked up at an earlier stage. Even when early treatment does not increase survival, the apparent survival time following a screening diagnosis will exceed that following a clinical diagnosis. In the only case-control study of screening with skin self-exam (SSE), SSE was shown to be associated with a decreased risk of melanoma mortality (adjusted OR=0.37, 95% CI 0.16-0.84). Lead-time bias could potentially influence the secondary preventive impact of SSE as case subjects who practiced screening discover their cancers earlier in the course of the disease. If SSE results only in earlier diagnosis, but treatment is not effective, then the apparent improvement in survival could be due to lead-time bias. The survival curves showed that the curve for SSE case patients approached a plateau after approximately 2.5 years of follow up, whereas failures (lethality) continue to occur in those who did not practice SSE, lending suggestive evidence that the observed benefit is due to actual survival rather than lead-time bias.

Length bias arises when screening preferentially detects less aggressive disease which may have a longer asymptomatic period and better prognosis. Aggressive disease, which has short asymptomatic period and worse prognosis, is less likely to be detected by screening. Some melanomas may be more indolent than others and survival time is related to

Vol. 142 - N. 2 GIORNALE ITALIANO DI DERMATOLOGIA E VENEREOLOGIA 65
lesion thickness at the time of resection. Length bias is suspected when observational studies of screening report detecting higher proportions of thinner melanomas.24

Clinical studies are very likely biased by the fact that patients are often referred and may not represent the general population. Referral bias is likely to occur in specialty clinics that evaluate pigmented lesions and attract high risk individuals. A high prevalence of melanomas will be seen in this high risk group.93 The variation in positive predictive values may be related to variation in the prevalence of disease, which in turn could be related to the type of patients recruited (high risk or not targeted). Attendees of screening programs were all self-selected, and they may not represent the general population.

Potential risks of screening

Potential adverse effects of screening include being labeled with melanoma which may impact quality of life or an individual’s finances. Mislabeling and misdiagnosis can have similar consequences. Patients may receive an ambiguous result or false positive result and undergo more testing or be treated unnecessarily.19 The diagnosis of melanoma is based on histopathology, and is subjective. Some pathologists will call an abnormality melanoma while others will not.28-30 The concern that pathologists may diagnose borderline lesions as melanoma because of liability issue has been raised.26 Screening can also increase biopsies of benign conditions and can be costly if it leads to more procedures, and treatment that may not be benign.19

There is potential embarrassment or discomfort due to screening. A study of 201 female veterans found that 15% expressed embarrassment about a full body skin exam (FBSE), and 16% would refuse the exam if the primary care provider were of the opposite sex.94 Another downside to screening is that it may distract physicians from issues the patient considers more important. During a clinic visit, limited time may cause physicians to spend less time addressing patient concerns in order to complete screening.95 Finally, while screening may result in an earlier diagnosis of melanoma, a consequent risk is the possibility of increased anxiety as the patient must live with knowledge of the disease for longer.

Consequences of not screening: delayed diagnosis

CONSEQUENCES OF DELAYED DIAGNOSIS

Studies examining the consequences of delayed diagnosis have found inconsistent results.19, 96 In the largest population-based analysis of the role of length of time to diagnosis, Baade et al. examined the relationship between melanoma thickness and reported time from first recognition and from first physician contact to the diagnosis of invasive melanoma in 3,772 participants in Queensland, Australia. They found no significant association between melanoma thickness and reported time to diagnosis for all melanomas combined, superficial spreading melanomas, or nodular melanomas. The exception was a positive association between melanoma thickness and post-presentation delay of physician-detected nodular melanomas. The authors concluded that this may be due to varying biological characteristics of melanomas, as well as the methodological limitations of retrospective studies when trying to measure this association. The investigators did not control for patient knowledge of melanoma symptoms which could have influenced association.97 Seven other studies have reported a null association and two reported an inverse association between delay in seeking diagnosis and lesion thickness.96

Campaigns to reduce delay in diagnosis by a combination of professional and public education have been reported from several centers around the world. The effects of these campaigns in reducing the depth distribution of cutaneous malignant melanoma have been mixed. In the absence of clear evidence of decreased mortality from melanoma due to early detection, opportunistic promotion of early detection may not be cost effective and will fail to reach all of the at-risk population.2

Conclusions

There is no direct evidence that screening for melanoma reduces mortality and studies to date do not support population-based screening. While periodic total skin examination appears to increase the detection of thin melanoma, it is not clear if this detection leads to a reduction in mortality from melanoma. A number of organizations have made recommendations regarding skin cancer screening. The US Preventive Services Task Force (USPSTF)81 concluded
that “the evidence is insufficient to recommend for or against routine screening for skin cancer using a total body examination for the early detection of cutaneous melanoma, basal cell cancer, or squamous cell skin cancer.” Clinical considerations include “being aware of groups at high risk for melanoma including fair-skinned men and women aged >65, patients with atypical moles, and those with >50 moles”. In addition, “clinicians should remain alert for skin lesions with malignant features noted in the context of physical examinations performed for other purposes.” Recommendations from various organizations are summarized in Table IV, and are noted to lack uniformity and consistency.

Implications of screening recommendations

Recommendations of medical organizations have significant implications for the medical and lay communities including medicolegal ramifications through a direct or implied standard of care. Recommending population-based screening for melanoma means that total skin examinations should be performed on all patients in a specific age group. However, there is no direct evidence that such screening would decrease morbidity or mortality from melanoma and such screening may result in a systematic overdiagnosis of melanoma. Selective screening of high risk populations may be the most promising strategy, but there is no consensus on which risk factors to use to define a population that would benefit most from screening. More research is needed to understand how to define high risk individuals, to identify the molecular biology of melanoma tumor progression, and to determine the optimal strategies to improve early detection of melanoma.

Riassunto

Diagnosi precoce di melanoma: cosa sappiamo?

Il melanoma maligno è una patologia potenzialmente letale che tuttavia, se diagnosticato precocemente, ha una prognosi eccellente. In questo lavoro ricercheremo gli aspetti fisiopatologici, la presentazione clinica, l’epidemiologia e i
fattori di rischio per melanoma. Esamineremo anche i modelli clinici per prevedere il rischio e la sopravvivenza e valuteremo criticamente i dati sull’efficacia dello screening per il melanoma.

PAROLE CHIAVE: Melanoma, diagnosti - Melanoma, anatomia patologica - Cute neoplasie.

References


38. Azzola ME, Gaw HM, Thompson JP, Soong SJ, Scolyer RA, Watson GF et al. Tumor mitotic rate is a more powerful prognostic indicator than ulceration in patients with primary cutaneous melanoma: An


90. Ubel PA, Hirth RA, Chernew ME, Fendrick AM. What is the price of life and why doesn’t it increase at the rate of inflation? Arch Intern Med 2003;163:1637-41.


The molecular mechanisms of melanoma tumorigenesis: an update

L. GAO 1, H. ZHAO 2, L. A. CORNELIUS 1

Therapeutic resistance and proclivity for metastasis cast the dismal prognosis of cutaneous melanoma. Although recent discoveries in genetic, epidemiological and genomic studies bring some hope of improved understanding of melanoma pathogenesis, questions regarding underlying molecular heterogeneity of melanoma genesis remain. Mining these complexities will hopefully reveal clues essential to understanding disease pathogenesis, clinical behavior and response to therapy. Current advances in high-resolution genome-wide technologies, as well as gene-specific mutational analysis, in conjunction with genetic and phenotypic analyses, improved animal models, may ultimately help to better define the seminal molecular events contributing to disease pathogenesis and ultimately identify more effective therapeutic targets.

KEY WORDS: Melanoma - MITF protein, human - Genes, p53 - AKT - PI-3K.

Cutaneous melanoma derives from the malignant transformation of melanocytes, the pigment-producing cells that reside in the basal layer of the epidermis in human skin. The incidence of cutaneous melanoma has increased dramatically at a rate greater than that of any other cancer type worldwide, despite increased levels of awareness, and targeted health education. Although cutaneous melanoma accounts for only 4% of all skin cancers, it is among a handful of cancers whose dimensions are reported in millimeters, however, it can demonstrate aggressive clinical behavior and a high mortality rate with metastatic disease. At early stage, melanoma can essentially be cured by surgical excision with wide local margins. Once metastasis develops, however, it is resistant to most current therapies. A median survival of 6-9 month is reported for AJCC Stage IV metastatic disease with only a 14% 5 year survival rate. 1

The etiology of melanoma is complex. As in many cancers, gene-environment interactions are felt to contribute to disease pathogenesis. 2 In fact, epidemiological evidence suggests that melanoma is linked to intermittent sun exposure and a history of sunburns. 3 This association has led to enhanced patient education geared towards ultraviolet (UV) protection, recognition of early signs of skin cancer and consequently, diagnosis of disease at an earlier stage. These efforts may be responsible for the slight improvement in survival rate despite the increased incidence of disease (i.e. diagnosis of melanoma at an earlier stage is more likely to be cured by surgical excision). It has been demonstrated that UV may induce signature mutations (CC → TT), however, they are not significantly found in melanoma tumors. In fact, despite the epidemiologic evidence as sited above, the precise molecular mech-

1Division of Dermatology
Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA 2Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA

Address reprint requests to: L. Gao, Division of Dermatology, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA. E-mail: lgao@im.wustl.edu
mechanisms of the contribution of UV to melanoma pathogenesis has not been clearly defined. In this paper, we will attempt to review the most recent advances that have been demonstrated with respect to the molecular events contributing to melanomagenesis.

**CDKN2A and INK4a/RB-ARF/p53 pathways**

Strong risk factors for melanoma development include a family history of melanoma, multiple benign nevi (>50) or atypical nevi (any number), and a previous history of primary melanoma. Other risk factors include specific phenotypic characteristics, such as fair skin, red or blond hair, blue eyes and a tendency to freckling. Although familial melanoma is not common (~10% of melanoma cases), genetic studies in melanoma-prone families have identified specific molecular events and pathways that are key to the transformation of melanocytes that contribute to both familial and sporadic melanoma development. Traditional linkage studies, combined with cytogenetic and loss of heterozygosity (LOH) analysis, have identified several susceptibility loci predisposing to melanoma. Further studies using positional cloning and sequence analysis have identified **CDKN2A** (p16) at the 9p21 locus and **CDK4** (12q13) as high penetrance melanoma genes and melanocortin 1 receptor gene (**MCIR**) as a low penetrance melanoma gene at 16q24.

The first cytogenetic analysis was performed nearly 20 years ago and demonstrated loss or translocation of 9p21 in a cohort of familial melanoma patients. Homozygous deletions of this region in melanoma suggested the existence of a tumor suppressor gene, that was further supported by germline deletion of this region in a patient with multiple primary melanomas. Subsequent genetic studies in large melanoma-prone families ultimately led to the cloning and identification, from the 9p21 region, of the first high penetrance melanoma susceptibility gene, cyclin-dependent kinase inhibitor (CDKN) 2A tumor suppressor gene. **CDKN2A** is the major high-risk melanoma susceptible gene recognized to date. It is composed of 4 exons: exon 1a, 1b, 2 and 3. It is unique in that **CDKN2A** encodes 2 distinct tumor suppressor proteins, **p16INK4A** and **p14ARF** (**p19ARF** for mouse), via alternatively using the first exons (1a for **p16INK4A** and 1b for **p14ARF**). The shared exon 2 and 3 of the two transcripts are translated in different reading frames, thus encoding two proteins with no amino acid homology. Inherited **CDKN2A** missense mutations in exons 1a and 2 are the most common genetic defects, although deletions and mutations in noncoding regions and in the introns were also found in a small portion of melanoma families. Both **p16INK4A** and **p14ARF** are potent inhibitors of cell cycle progression and act through the retinoblastoma (RB) and p53 pathways, the two critical pathways important in cell cycle control. As illustrated in Figure 1, **p16INK4A** sequesters CDK4/6, abrogates the binding of these kinases to cyclin D and thereby prevents CDK4/6-mediated phosphorylation and subsequent inactivation of RB. Active RB represses E2F mediated gene transcription and results in a G1 to S cell cycle arrest. Loss of this repression, therefore, leads to unregulated cell cycle progression. On the other hand, **p14ARF** inhibits to and inactivates the MDM2 protein, which acts as an E3 ubiquitin ligase to target p53 for proteasomal degradation. Thus, expression of **p14ARF** stabilizes p53 which induces growth arrest, senescence or apoptosis. In addition, loss of the INK4A/ARF locus has also been associated with reduced repair of UV-induced DNA damage and, therefore increased gene mutations. Unlike several other
tumors, including squamous cell carcinoma of the skin, mutations in p53 are uncommon in melanoma, although dysregulation of the p53 pathway may occur due to mutations in its upstream regulator, p14ARF as described above.

The functional importance of CDKN2A locus in the melanoma development has been elucidated extensively in mice during the past few years. Mice harboring deletions in exons 2 and/or 3 of murine CDKN2A, which specifically lack either CDKN2A INK4A (INK4A -/-) or ARF (ARF -/-) both have been generated.25-29 Although these mice have increased predisposition to tumor development, predominantly lymphomas and sarcomas, very few spontaneous melanomas were observed. However, when a constitutively active mutant of HRAS (Tyr-RAS+), an upstream activator of MAPK signaling (see below), was coexpressed in cells of melanocytic lineage on either null background (INK4A -/- or ARF -/-), enhanced melanoma development was demonstrated.29 In addition, INK4A and ARF double null mice are highly prone to cutaneous melanoma, and tumors had a shorter latency and higher penetrance compared with their single gene knockout counterpart mice.30 These studies support the role of p16INK4A and p14ARF as bona fide tumor suppressors in melanoma tumorigenesis although either of these mutations alone does not appear to be sufficient for melanoma development.

As previously stated, approximately 10% of melanomas occur in patients with a family history of disease. Of these, in familial melanoma cohorts studied to date, 50% demonstrate a genetic linkage to the 9p21 locus, and only 20-40% of these have been identified to have germline alterations in CDKN2A. The high linkage to the 9p21 region together with the relatively low detection of CDKN2A mutations supports the existence of additional melanoma susceptibility genes in this region. Analysis of somatic deletions in tumors indicated that at least 2 additional tumor suppressor loci centromeric and telomeric to the CDKN2A are linked to the development of melanoma.31-33 Identification of these genes and characterization of their function will hopefully provide additional insights into the molecular genesis of melanoma.

A second melanoma susceptibility gene is CDK4 located on chromosome 12q13. However, CDK4 mutations have only been documented in a small number of melanoma families to date.34-36 The CDK4 mutation identified so far located in codon 24 of exon 2 and changes a critical arginine residue, crucial for binding to INK4A. As anticipated, this CDK4 mutation is functionally identical to INK4A loss, and renders the CDK4 kinase constitutively active, inactivating RB and leads to unchecked cell cycle progression. Therefore, phenotypic characteristics of the families carrying CDK4 germline mutations are indistinguishable to CDKN2A affected families. Mice that carry the human CDK4 mutation have been shown to be susceptible to melanoma development following a second exogenous carcinogen exposure.37 Despite these findings, a low detection rate of CDK4 mutations have been found in melanoma patients, possibly due to targeted screening performed to date (exon 2), the patient cohorts examined, or an actual low incidence of this mutation in humans. Other investigations have putatively identified other candidate melanoma gene(s) on chromosomes 1p22, 1p36, 11p15, and 4, although the significance of these findings remains unclear.32, 38, 39

A striking recent advance in our understanding of INK4A/ARF and ARF/p53 pathways in human cancer development is the identification of CHD5 (chromodomain-helicase-DNA binding-5) as a tumor suppressor on chromosome 1p36.40, 41 Although its relevance to melanomagenesis is unclear at this time, deletions of 1p36 are widely reported in human cancers, including melanoma.42 However, identification of the 1p36 tumor suppressor has remained elusive to date. By using chromosomal engineering, Bagchi et al. generated mouse strains with a gain and loss of a genomic interval corresponding to human 1p36. Using this powerful model, these investigators identified CHD5 as a tumor suppressor at this locus using a retrovirus-based shRNA strategy. Most interestingly, all CHD5-regulated cellular processes, such as proliferation and apoptosis, were p53 dependent. Furthermore, CHD5 has been shown to function upstream of p53 and most likely RB through regulation of INK4A and ARF expression. Although the data shows that CHD5 may function as a novel tumor suppressor gene, specific mechanisms addressing its affect on p53 and RB pathways remain to be elucidated. Again, whether CHD5 is altered or acts as a tumor suppressor in melanoma has not yet been determined.

A low penetrance melanoma susceptibility gene is the MC1R, located on chromosome 16q24 and has been shown to act as a genetic modifier of melanoma risk in CDKN2A germine mutation carriers.43, 44 MC1R encodes the G-protein coupled receptor (GPCR) for a-
melanocyte stimulating hormone (α-MSH) and plays a central role in regulating melanin synthesis. Ligand binding induces tyrosinase transcription and melanin production. The MC1R gene is highly polymorphic in the Caucasian population, and more than 30 allelic variants have been described. Some of these variants cause loss of gene function (LOF) resulting in the production of phaeomelanin, as opposed to the normal eumelanin, pigment. Specific LOF variants may confer an increased overall risk of melanoma development, even in patients without red hair and blue eyes.

**RAS/BRAF/MAPK/ERK pathway**

As in many other human cancers, aberrant cell growth/proliferation plays a critical role in melanoma tumorigenesis and progression. The mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway has emerged as the central growth-stimulating pathway in melanoma tumorigenesis. RAS and RAF kinases are components of the MAPK/ERK cascade. Activation of this pathway in melanoma can not only occur through RAS/RAS mutations, but can also occur following exposure to autocrine growth factors, such as basic fibroblast growth factor, hepatocyte grow factor (HGF).

Growth factors such as these, certain cytokines and hormones activate RAS through their engagement of their cognate receptors, adaptor proteins or heterotrimeric G-proteins. Once activated, Ras proteins bind to and activate their downstream effectors, such as RAF proteins which are serine/threonine protein kinases. RAF proteins then phosphorylate and activate the MEK protein kinases, which in turn phosphorylate and activate the ERK kinases. ERK is also a serine/threonine kinase that phosphorylate numerous substrates to regulate a wide range of cellular functions, such as apoptosis, migration and cell proliferation.

Although RAS and RAF germline mutations in melanoma have not been found, somatic mutations of NRAS have been demonstrated in approximately 15% of melanoma tumors and this activating mutation drives activation of ERK. With regards to RAF, three mammalian isoforms exist – ARAF, BRAF and CRAF (RAF-1). ARAF and RAF-1 are rarely mutated in melanomas, however BRAF mutations have been determined to have a seminal role in melanoma development. BRAF is mutated in up to 70% melanoma tumors and cell lines. Reported mutations to date have been found in the BRAF kinase domain, encoded by exons 11 and 15. Over 90% of those reported affect one critical amino acid, resulting in a valine to glutamic acid substitution at residue 600 (BRAF V600E), leading to constitutive kinase activation of BRAF, and consequently downstream ERK activation. In support of its role in melanoma formation, studies employing small interfering RNA against mutated BRAF but not CRAF inhibit the transformation of melanocytes harboring this activating BRAF mutation.

Interestingly, NRAS and BRAF mutations are mutually exclusive in melanoma – they have an epistatic relationship. It has been postulated that this mutual exclusivity is due to the ability of each activating mutation to activate the Ras pathway, and consequently downstream ERK, independently, similarly deregulating this common effector pathway. Although the induction of these somatic mutations is not well understood, current evidence suggests that BRAF and NRAS mutations may be instigated by distinct mechanisms. In support of this, genome-wide CGH profiling and targeted re-sequencing of primary melanoma tumors have demonstrated that distinct patterns of genomic alterations, including BRAF and NRAS mutation frequencies, can be identified in melanomas arising at different anatomic sites, different subtypes and with varying UV exposure history (acral melanoma, mucosal melanoma, and histologic evidence of chronic sun damage). As previously noted, this study confirmed that these mutations are not signature UV-induced thymidine dimer mutations. In addition, a recent microarray analysis in melanoma indicates that BRAF and NRAS mutations have overlapping but distinct transcriptional profiles.

Following the determination of the high mutation rate of BRAF in melanoma, several groups investigated BRAF mutational status in benign nevi, in an attempt to determine whether BRAF activating mutations were an early, or late, event, in melanocyte transformation. Although unexpected, it has been determined that BRAF mutation occurs at a similar frequency in benign nevi as in primary and metastatic melanomas, implicating BRAF mutation as an early event in the development of melanocytic neoplasia. In addition, these findings demonstrate that BRAF dysfunction per se is not sufficient to drive melanoma development and that cells of melanocyte lineage in nevi must acquire additional molecular “hits” for transformation to melanoma.
In support of this multi-hit hypothesis, melanocyte-specific expression of BRAFV600E, but not wild-type BRAF, led to increased nevi formation in a zebra-fish melanoma model.52 When combined with p53 deficiency, however, the BRAFV600E induced melanocyte lesions rapidly progressed to invasive melanoma.

Further understanding of the molecular mechanisms underlying the contribution of BRAF activating mutations to the pathogenesis of benign nevi and melanoma may be garnered from studies that have evaluated the effect of the BRAFV600E on cell cycling in melanocytes. Interestingly, sustained BRAFV600E expression in human melanocytes (and consequently prolonged ERK activation) has been found to induce cell senescence by increasing the expression of both p16INK4A and the well recognized senescence-associated marker acidic b-galactosidase. Along these lines, it has been previously demonstrated that although rapid and transient ERK activation stimulates cell proliferation, prolonged ERK activation induces cell senescence in a variety of cell types, including melanocytes and melanoma cell lines. In recent studies, BRAF mutation has been found to predict sensitivity to MEK inhibition, findings that may have implications for melanoma treatment strategies. In this study, small molecule inhibitors of MEK were used in tumors and cells that were either WT for BRAF and NRAS or those harboring gain of function mutations. Compared to WT or cells having a NRAS mutation, V600E activating mutation of BRAF was found to enhance cell sensitivity to MEK inhibition, completely abrogating tumor growth in mutant xenografts.53 In vivo, BRAF mutation status may affect cell response to MEK or ERK specific phosphatases, endogenous and physiologic inhibitors of these kinases.

Another interesting finding is that BRAF mutation alters the stability of BRAF protein.54 Unlike the other RAF isoforms ARAF and CRAF, WT BRAF does not require Hsp90 chaperone for protein stabilization. In contrast, mutated, activated BRAF does bind to an Hsp90-cdc37 chaperone complex, required for its stability and function. Exposure of melanoma cells and tumors harboring this mutation to Hsp90 inhibitors results in the degradation of mutant BRAF and subsequent inhibition of ERK activation, cell proliferation and tumor progression, again, findings that may have implications in melanoma treatment.

Although RAS and RAF proteins are well characterized upstream activators of ERK pathway in melanoma as described above, a recent report has shown enhanced ERK activity despite a low frequency of BRAF and NRAS mutations in one melanoma subtype, acral melanoma. These findings suggest that factors other than BRAF and NRAS gain of function mutations may contribute to constitutive activation of ERK pathway in certain tumors.55 Based upon work in our laboratory, one potential candidate is RAS-associated protein-1 (Rap1), a member of the RAS small GTPase superfamily.56 Like RAS, Rap1 functions as a molecular switch, cycling between an inactive, GDP-bound and an active, GTP-bound form. Rap1 guanine nucleotide exchange factors (GEFs) activate Rap1 by facilitating the replacement of GDP with GTP, whereas Rap1 GTPase-activating proteins (GAPs) inhibit Rap1 activity by enhancing the intrinsic GTPase activity of Rap1 to hydrolyze the bound GTP to GDP. The effect of Rap1 on ERK activation is complex and highly cell type specific. In several cell types, Rap1 attenuates growth factor-induced, RAS-mediated ERK activation by sequestration of CRAF and other RAS effectors in an inactive complex. On the other hand, Rap1 has been shown to stimulate ERK through the direct binding and activation of BRAF in certain, if not all, BRAF expressing cells. Finally, Rap1 has been reported fail to interfere with RAS signaling towards ERK in certain cell types. In support of the role of Rap1 in melanoma development, we have recently shown that Rap1 is activated in both human metastatic melanoma tissues and in several melanoma cell lines. Our in vitro experiments demonstrated that Rap1 positively regulates ERK activation induced by HGF, a growth factor that has been implicated in melanomagenesis and by 8CPT-2Me-cAMP, an activator of Epac, a Rap1 GEF downstream of cAMP (by increasing levels of activated Rap1). Furthermore, Rap1 has demonstrated the ability to bind to BRAF in vitro. Taken together, these data support the model postulated in Figure 2, in which Rap1 couples GPCR and RTK signaling pathways to ERK activation in melanoma cells. In addition to modulating ERK activity, we have shown that Rap1 also induces avb3 integrin activation and consequently increases melanoma cell adhesion and migration, processes previously implicated in tumor progression.57

Accumulating evidence suggests that dysregulation of Rap1 activity, either by Rap1 over-expression or changes in levels of Rap1 activators, such as GEFs, and inhibitors, such as tuberin and Rap1 GAPs, has been
associated with human malignancies. Mice deficient in SPA-1, a principal Rap1 GAP in hematopoietic cells, develop a spectrum of myeloid disorders that resemble human chronic myelogenous leukemia. Rap1-GAP, which is expressed in melanoma cells, maps to chromosomal region 1p36, which is deleted in a wide range of human cancers. CHD5 has been recently identified as a tumor suppressor in 1p36 and functions upstream of p53/RB pathways. Since alterations of the p53 and RB pathways alone do not induce melanoma in several in vivo models, distinct tumor suppressor genes in other pathways must contribute tumor development. We are currently investigating whether mutations of Rap1-GAP may be implicated in this process.

**PTEN/PI-3K/AKT pathway**

A third critical molecular pathway involved in melanoma pathogenesis is the phosphatidylinositol 3-kinases (PI-3Ks)/AKT (PKB) signaling module. Activated AKT phosphorylates and regulates the function of many downstream molecules which control survival/apoptosis, proliferation/cell growth, metabolism and neo-vascularization. AKT exerts its antiapoptotic activity by several distinct mechanisms. First, AKT phosphorylates and inactivates the proapoptotic factors procaspase-9 and BAD, which antagonizes bcl-2 and triggers the release of cytochrome c from the mitochondria, a key step in stress-induced apoptosis. Second, AKT phosphorylates and activates IKK-a, which phosphorylates IKB leading to the release and activation of NF-kB which results in the expression of antiapoptotic genes. Third, AKT phosphorylates the FOXO transcription factors, which results in the nuclear exclusion of FOXO proteins and a decrease of the expression of genes critical for apoptosis, such as the death receptor Fas ligand and proapoptotic Bim. With respect to proliferation and cell growth, active AKT promotes cell cycle progression by phosphorylation and inactivation of GSK-3b to avert cyclin D1 degradation. AKT also directly phosphorylates the cell cycle inhibitors p21WAF1 and p27Kip1, leading to the sequestration of these inhibitors in the cytoplasm, and been shown to terminate FOXO-mediated inhibition of the expression of p21WAF1, p27Kip1, cyclin D1 and D2. Finally, AKT increases MDM2 phosphorylation and the translocation of MDM2 into the nucleus, where it downregulates p53 and antagonizes p53-mediated cell cycle arrest. A major cellular metabolic pathway regulated by AKT lies in mTOR (target of Rapamycin). In this context, AKT phosphorylates TSC2 and prevents its dimerization with TSC1. The TSC1-TSC2 complex functions as a GAP for RAS-like small GTPase, RHEB, which promotes the activity of the kinase mTOR. Therefore, AKT activation inhibits the GAP activity of TSC1-TSC2 towards to RHEB and consequently activates mTOR. Activated mTOR phosphorylates a number of substrates and induces expression of cyclin D1, c-myc, and vascular endothelial growth factor (VEGF).

As illustrated in Figure 3, extracellular stimuli transduced through RTK or GPCR activate PI-3Ks, which phosphorylate phosphatidylinositol 3,4,5-triphosphate (PIP3), generating the second messenger phosphatidylinositol 3,4,5-triphosphate (PIP2), generating the second messenger phosphatidylinositol 3,4,5-triphosphate (PIP2). The high level of PIP3 induces the translocation of the serine-threonine kinase AKT from the cytoplasm to the plasma membrane. Recruitment of AKT by PIP3 results in the activation of AKT. PIP3 can be dephosphorylated to PIP2 by phosphatase and tensin homolog deleted in chromosome ten (PTEN). Thus, PTEN functions as a negative regulator of PI-3K/AKT signaling and, as such, a tumor suppressor.

As outlined above, hyperactivation of AKT is asso-
associated with resistance to apoptosis and increased cell proliferation/growth, events that favor tumorigenesis. It is not surprising that a PI-3K/AKT pathway deregulation occurs in many human cancers, including melanoma. Similar to other tumors, several mechanisms potentially contribute to AKT hyperactivation in melanoma. First, inactivating mutations or deletions of PTEN may lead to AKT activation. LOH at chromosome 10q, which harbors the PTEN locus, has been reported in up to 50% of melanomas. Secondly, amplification and overexpression of PI-3K subunits is observed in certain types of human cancers. More recently, mutations in PIK3CA, the gene that encodes the catalytic subunit p110, and in the regulatory subunit, p85 of PI-3K have been identified in human tumor cell lines and tissues, although not in melanoma to date. Interestingly, activating RAS mutations can activate AKT through direct binding to the p110 catalytic subunit of PI-3K, which may have implications in melanoma in that 15% of melanomas harbor NRAS mutations (as described above), and the previously reported finding that NRAS mutations and PTEN alterations are epistatic in melanoma (see below). Finally, amplification of AKT3 has been identified in melanoma and may be responsible for the high frequency of AKT activation in some tumors. A role for AKT in melanoma progression is demonstrated by the finding by one group that overexpression of AKT in radial growth phase melanoma converts it to an invasive, more advanced vertical growth tumor. Upregulation of VEGF expression and increased production of superoxide ROS were implicated in tumor progression, how-

Figure 3.—PTEN/PI-3K/AKT pathway.
ever, the precise AKT downstream effector pathways involved remain to be elucidated. As previously described, many AKT downstream effectors, such as FOXO and TSC2, function as tumor suppressors in other malignancies, although their contribution to melanoma tumorigenesis has not been demonstrated. A recent study has revealed high level of phospho-AKT in both primary and metastatic melanomas (49% and 77%, respectively)\(^{74}\). Studies by Tsao \textit{et al.} have demonstrated PTEN alterations in approximately 20% of uncultured tumors\(^{75}\), suggesting that factors other than PTEN alterations contribute to constitutively activation of the PI3K signaling pathway. Interestingly, further characterization by Tsao \textit{et al.} demonstrated that melanomas segregate into one of three mutational profiles: a mutation in \textit{NRAS} alone, concurrent \textit{PTEN} and \textit{BRAF} mutations, or \textit{BRAF} mutations alone. From these findings, and the recognition that benign nevi have a low \textit{NRAS} mutation frequency and a relatively high \textit{BRAF} mutation rate, these investigators proposed that \textit{NRAS} acts to stimulate both downstream \textit{BRAF} and PI3K, and consequently MAPK and AKT. Isolated \textit{BRAF} activation may require the cooperation of \textit{PTEN} alterations, possibly a later event in melanoma development, to activate AKT.

### Oncogenic effects of MITF and NEDD9 on melanoma genesis

Melanocytes are derived from neural crest. Microphthalmia-associated transcription factor (\textit{MITF}) is a lineage-specific melanocyte gene and a master regulator of melanocyte development and function. MITF belongs to the MiT family of helix-loop-helix/leucine-zipper transcription factors.\(^{79}\) The binding of \(\alpha\)-MSH to MC1R induces cAMP, which increases MITF expression in a positive regulatory loop. MITF in turn stimulates the transcription of genes governing melanin synthesis. Both activated ERK induced by RTK/RAS/BRAF signaling pathway and \(\beta\)-catenin in the WNT signaling pathway can function to activate MITF (Figure 4). In addition to its role in melanocyte pigmentation, MITF is also essential for melanocyte survival. In fact, mice lacking functional MITF lack melanocytes and are phenotypically albino.\(^{80}\) In humans, MITF mutations result in pigmentary disorders such as Waardenberg’s Type II and Tietz syndrome.

MITF regulates melanocyte survival by transcriptionally activating the \textit{Bcl-2} gene, a key antiapoptotic factor that is characteristically expressed at high levels in melanocytes and melanoma. In normal melanocytes and cells of melanocyte lineage, MITF is required for the induction of cell cycle exit and activation of the differentiation programme via activation of \textit{p16INK4A} and \textit{p21Cip1} genes.\(^{81, 82}\) Another recent finding supports the role of MITF-induced cell cycle arrest in melanocytes where high levels of MITF expression were found to counteract BRAF-stimulated melanocyte proliferation.\(^{83}\)

In contrast to the role of MITF in normal melanocytes of differentiation cell cycle exit, a recent, seemingly contradictory, finding in melanoma has identified \textit{MITF} as a lineage-dependent oncogene amplified in a subset of these tumors.\(^{84}\) Using an integrated analysis of high-resolution single nucleotide polymorphism maps and gene expression databases the authors found that the chromosome region (3p13-3p14) containing the \textit{MITF} gene was amplified in most melanoma cell lines. \textit{MITF} gene was also amplified (from 5 to 119 copies) in about 10% of primary melanomas and up to 20% of metastatic melanomas in
human tissue samples. The MITF amplification is significantly correlated to a decreased five-year survival in patients with advanced melanoma. Moreover, in vitro functional studies showed that MITF together with mutated BRAF transformed immortalized human melanocytes, thereby confirming MITF’s function as an oncogene in melanoma. These findings in melanoma may support a role for MITF as a potential target for treatment. A number of mechanisms may, in fact, support this proposed oncogenic effect of MITF on melanoma cells. First, MITF promotes cell cycle progression and invasiveness of melanoma cells by the suppression of CDK inhibitor p27kip1 via regulating Dia1 and actin polymerization. Second, one of the MITF target genes, Tbx2 (T-Box transcription factor 2), has been recently implicated in proliferation and suppression of senescence in melanoma through its ability to repress ARF and p21 promoter expression. Third, MITF has been shown to regulate cell cycle progression by modulation of CDK2, which is essential for melanoma clonogenic growth. Fourth, MITF binds to the HIF1α promoter and strongly stimulates its transcriptional activities, including the induction of VEGF expression in response to hypoxia. Last, c-Met, the receptor for HGF has been identified as a direct transcriptional target of MITF. HGF/c-Met signaling has been shown to be a key pathway in both melanocyte development and melanoma progression.

Through these mechanisms, MITF may exemplify one of a newly recognized group of lineage survival genes that potentially contribute to tumorigenesis.

Employing genome-wide high-resolution array-CGH of nonmetastatic parental and metastatic variants of murine melanoma cells, a recent study by Kim et al. identified a recurrent focal gene amplification associated with the metastatic cells. The amplified region identified in mouse is syntenic to 6p24-25 (NEDD9) in humans, which is amplified in 36% of metastatic as opposed to primary melanomas and may function as a novel melanoma metastasis gene. It has been proposed that NEDD9 interacts with focal adhesion kinase in mediating cell invasion, and as such, tumor progression.

**Concluding remarks**

In one model of melanomagenesis, Clark proposes the histologic changes accompany the progression from normal melanocytes to melanoma. Five distinct stages have been proposed: common acquired and congenital nevi without dysplastic changes; dysplastic nevi with structural and architectural atypia; radial growth phase melanoma; vertical growth melanoma and metastatic melanoma. This model suggests that certain key molecular events occur at each stage, driving the progression from melanocyte transformation to tumor formation and metastasis. However, with increasing understanding of the molecular pathogenesis of melanoma, tumor heterogeneity is becoming increasingly evident, and with several distinct, and sometimes synergistic molecular mechanisms at play. As has long been recognized, melanomas arising on different body sites exhibit markedly distinct biological and clinical behaviors. Lentigo maligna melanomas are indolent tumors that develop over decades on chronically sun exposed area such as the face. In contrast, acral lentigenous melanomas, which develop on sun-protected regions, are often diagnosed at a later stage, and some propose that they tend to exhibit more aggressive behavior. Breslow thickness clearly has strong prognostic value, however, there exists a subset of thin melanomas that metastasize.

The ‘hallmarks of cancer’ model offers a framework describing the genetic complexity of human cancers. According to this model, detailed dissection of tumorigenic pathways and identification of the ‘molecular signature’ of melanoma at each stage and subtype may aid in our attempt to prevent tumor development and provide insight into treatment strategies. From this review, however, it should be evident that the currently recognized chromosomal alterations and mutational signatures of key melanoma genes do not provide a complete understanding of melanomagenesis. As in most cancers, processes such as epigenetic modification, transcriptional profiling of both coding and noncoding RNAs, and gene-environment interface most certainly contribute to tumor development, and these mechanisms have not yet been clearly defined in melanoma.

**Riassunto**

Meccanismi molecolari della tumoregenesi del melanoma: un aggiornamento

La resistenza alla terapia e la tendenza a metastatizzare spiegano la pessima prognosi del melanoma cutaneo. Sebbene recenti scoperte nel settore della genetica, e studi epidemiologici e gnomici che hanno alimentato una qualche
speranza di migliorare la comprensione della patogenesi del melanoma, restano senza risposta le domande relative all’eterogeneità molecolare sottostante alla genesi del melanoma. La comprensione di queste complessità si spera che sarà di aiuto per identificare gli aspetti essenziali della patogenesi del tumore e per capirne il comportamento clinico e la risposta alla terapia. Gli attuali progressi nelle tecnologie ad alta risoluzione per ampi tratti di genoma, così come l’analisi mutazionale gene-specifica, assieme a quella genetica e fenotipica e ai migliorati modelli animali, possono in ultima analisi aiutare a meglio definire gli eventi molecolari fondamentali che contribuiscono alla patogenesi della malattia e a identificare gli obiettivi terapeutici più efficaci.

**Parole chiave:** Melanoma - Linfonodo sentinella - Biopsia linfonodale - Metastasi linfatiche - Linfoangiogenesi.

**References**


Burley SK, Zimring DC et al. Molecular basis of mouse microphthalmia (mi) mutations helps explain their developmental and phenotypic consequences. Nat Genet 1994;8:256-63.


One of the major goals in the pathologic evaluation of melanocytic tumors is to distinguish benign lesions (nevi) from malignant ones (melanomas). Although this may appear to be a simple task, the assessment of melanocytic tumors can be one of the most difficult of all areas of pathologic diagnosis. Histologic features favoring a benign diagnosis are symmetry, circumscription, a V-shaped silhouette, dermal maturation and absence of mitoses. Poor circumscription, lateral or peripheral pagetoid epidermal spread, expansile dermal growth, high cellularity, necrosis, nuclear pleomorphism and frequent or abnormal mitoses raise the possibility of malignancy. Although most tumors are readily diagnosable with the application of standard criteria, there are unusual variants of both nevi and melanomas that require awareness of pathologic diagnostic pitfalls and careful clinicopathologic correlation to avoid misdiagnosis.

The performance of a complete excision biopsy, wherever possible, is crucial for the optimal pathologic assessment of challenging lesions. For melanomas, the pathology report should include all pathologic factors that are important in determining the patient’s prognosis and management. The use of a formatted synoptic pathology report can facilitate this and present the required information to the clinician clearly. The use of the sentinel lymph node biopsy is now well established in the management of patients with melanoma, as is the usefulness of fine needle biopsy in the assessment of clinically-suspected metastatic melanoma. Determining the prognosis of patients with melanoma requires integration of all pertinent clinico-pathologic features and this may be aided by the use of computer programs in the future. Molecular diagnostic techniques may further refine the diagnosis of challenging lesions and provide more accurate prognostic estimates.

**KEY WORDS:** Nevus, pathology - Nevus, diagnosis - Dermoscopy - Biopsy, fine-needle - Melanoma.

Melanocytic tumors are amongst the most common tumors that occur in humans. Correspondingly, excised skin specimens bearing melanocytic lesions are commonly encountered in the surgical pathology laboratory. They are usually removed either for cosmetic reasons, or for the purpose of making or excluding a diagnosis of melanoma. In the vast majority of instances, the pathologic diagnosis of these lesions is straightforward, but in some cases, diagnosis can be extremely challenging for several reasons. For exam-
ple, the lesion may exhibit a combination of morphologic features that individually are characteristic of either benign nevi or melanoma. The difficulty in assessment can also be compounded by a superficial or incomplete biopsy procedure where only a portion of the lesion can be assessed, where tissue processing is suboptimal or where the nature of the specimen results in suboptimal orientation (Figure 1). The consequences of misdiagnosis can be disastrous for the patient. On the one hand, a false positive diagnosis can result in considerable stress for the patient and unnecessary additional surgery or adjuvant therapy, with possible attendant morbidity. On the other hand, a false negative pathologic assessment can result in delayed definitive treatment and progression of disease, possibly leading to death of the patient. It is therefore not surprising that melanocytic pathology features prominently in discussions of medico-legal issues and diagnostic errors in pathology.

It is imperative that pathologists are familiar with the diagnostic criteria for melanoma. This is especially pertinent as several of the variants of melanomas, e.g. desmoplastic, nevoid and Spitzoid types, all have overlapping features with benign lesions and present potential diagnostic pitfalls. Likewise, knowledge and recognition of the features of benign melanocytic mimics and how they can be distinguished from melanomas is essential. These mimics of melanoma include Spitz nevus, combined nevus, deep penetrating nevus, acral nevus, genital nevus, epithelioid blue nevus and regenerating nevus. Careful clinicopathologic correlation is very important because clinical features such as the age of the patient, site of the lesion, history of trauma, previous excision, pregnancy and intense sunlight exposure may all affect the histologic features and, when available, should be taken into account in the assessment of the lesion by the pathologist. In difficult cases, despite the best efforts of the clinician and pathologist, it may not be possible to render a definitive diagnosis. In such situations, a thorough description of the lesion together with a favored diagnosis should be given in the pathology report, including a comment discussing the areas of difficulty and the degree of uncertainty in the diagnosis. A differential diagnosis and appropriate caveats may also be provided where appropriate. One or more expert opinions are usually advisable if only to avoid potential legal action. A management plan appropriate for the diagnosis and in the best interest of the patient can be developed. Good communication between pathologists, clinicians and patients is of paramount importance in this context.

When a diagnosis of melanoma is made, the pathologist has a responsibility to provide in the pathology report all of the key histologic parameters that are of prognostic importance. This aids the clinician in predicting the likely outcome and in planning subsequent treatment, and communicating this information to the patient. The presence of certain adverse prognostic factors may also qualify the patient for entry into one or more ongoing clinical trials. In view of this, a synoptic reporting template or minimum reporting dataset enables the pathologist to assess and record all the clinically important pathologic data. Other issues of contemporary importance, such as the principles and practice of sentinel lymph node (SLN) biopsy, the role of fine needle biopsy (FNB), correlation of pathologic with dermoscopic evaluation of pigmented lesions and the potential of molecular techniques as aids to diagnosis and prognostication are also addressed in this review.

Nevi that mimic melanoma

The great majority of nevi are easily diagnosed pathologically. Multiple low and high power magni-
fication microscopic features are assessed, enabling a diagnosis to be made in most cases. Features favoring benignity are symmetry, circumscription, presence of maturation, uniform small nuclei, absence of dermal mitoses and absence of pagetoid intraepidermal invasion. Features that raise the possibility of malignancy include asymmetry and poor circumscription, poor maturation, frequent, abnormal or deep dermal mitoses, lateral pagetoid intraepidermal spread, nuclear pleomorphism, expansile or sheet-like cellular growth pattern in the dermis, tumor necrosis and vascular invasion. None of these morphologic features in isolation is diagnostic of a benign or malignant lesion. Definitive pathologic assessment requires a consideration of all the microscopic features in a particular case and a comparison of these with standard diagnostic criteria. The importance of clinicopathologic correlation cannot be overemphasized. For partial biopsies, including punch and some shave biopsies, if the clinical and pathologic features are not compatible, consideration should be given to performing a further biopsy.

Certain characteristic nevi are known to present a diagnostic pitfall to both the clinician and the pathologist as they may exhibit features typical of benign lesions but also possess worrying features seen in malignant lesions. It is important to regard all the features observed in a particular case within the appropriate context of these challenging melanocytic lesions. In such cases a credible diagnosis can only be established following assessment of all architectural and cytologic features, and features of the host response and correlated with clinical data including site of the lesion and age of the patient. It requires a sound knowledge of diagnostic criteria, a keen awareness of potential pitfalls, and finally, the ability to make a logical and well reasoned judgement on the basis of all the information available.

**Dysplastic nevus**

The dysplastic nevus takes up an intermediate position between the common nevus and melanoma in both morphology and underlying genetic changes (Figure 2). The presence of a dysplastic nevus is also a marker for an increased risk of developing a melanoma, especially in the context of the dysplastic nevus syndrome. Clinically, there is some degree of asymmetry, mild border irregularity and color variegation. Histologically, the dysplastic nevus is characterized by 2 major and 4 minor criteria. The major criteria are: 1) a proliferation of atypical and primarily basally-located epidermal melanocytes, and 2) the melanocytic proliferation takes the form of either a lentiginous growth pattern or an epithelioid and nested growth pattern. The presence of papillary dermal lamellar fibroplasia, increased upper dermal vascularity, a lymphocytic infiltrate and fusion of adjacent junctional melanocytic nests are minor criteria. It is usually necessary for the lesion to include a total of at least 2 major and 2 minor criteria for the pathologic diagnosis of a dysplastic nevus.

**Melanoma in situ (MIS)** is pathologically distinguished from dysplastic nevus by the presence of more marked cytologic atypia, more florid intraepidermal pagetoid spread of single atypical melanocytes (often reaching the granular layer) and greater architectural disorder.

**Pigmented spindle cell nevus/pigmented epithelioid cell nevus**

This heavily pigmented nevus is centered in the junctional zone and is another notable mimic of melanoma both clinically and pathologically. There may occasionally be a superficial dermal component as well. Features that are often present and that may cause some concern include the presence of pagetoid intraepidermal spread, mild-to-moderate cellular atypia and heavy pigmentation. The lesion is, however, typified by symmetry, circumscription, absence of marked cytologic atypia, rapid maturation with depth of the dermal component and the absence of frequent or abnormal mitoses.
“Irritated” nevus

The histologic features of a common nevus can be markedly modified by concurrent skin pathology. Nevi that have been subjected to trauma, strong sunlight, or surface irritant agents may show features such as pagetoid intraepidermal spread, mild cellular atypia and occasional mitotic figures. In such cases, correlation with clinical information is often critical in arriving at a correct (benign) diagnosis. Histologically, the presence of parakeratosis, hypergranulosis and epidermal acanthosis provide hints that there was presence of a superimposed irritant etiology. Nevi that are present on skin afflicted by eczema (Meyerson nevus) may also display similar features. Again, clinical correlation is important, however, the diagnosis would be supported by the histologic presence of epidermal spongiosis and an inflammatory infiltrate composed of lymphocytes and some eosinophils.

Regenerating nevus

A regenerating nevus is one that recurs after physical damage in particular following previous incomplete excision. It may have alarming features and has been termed ‘pseudomelanoma’. Such features include pagetoid intraepidermal spread, some degree of cellular atypia and an occasional mitotic figure. An important clue to the diagnosis is the presence of a dermal scar in the vicinity of the regenerating nevus cells. On closer examination, the reactive-appearing regenerating nevus cells can sometimes be seen to interface and merge with the pre-existing banal nevus cells. This is in contrast to melanoma occurring in the context of a preexisting nevus where the malignant cells stand out as a distinct population featuring high cellularity, nuclear pleomorphism, prominent nucleoli and frequent mitoses.

Acral nevus

Nevi occurring on the palms, soles, fingers and toes are often display features that would favor a melanoma when present in a melanocytic tumor occurring at another site. The most notable of these is pagetoid intraepidermal spread (Figure 3). In our experience, this feature can be present in up to 71% of acral nevi. Intraepidermal spread is also seen in acral lentiginous melanoma in situ (MIS), but the pagetoid spread in the latter is usually denser and more haphazard (Figure 4). The pagetoid cells in MIS are usually markedly atypical while those in acral nevi are bland or pyknotic. Other features that favor acral nevi over acral melanomas include greater likelihood to form junctional hests of melanocytes and a paucity of junctional and subjunctional lymphocytes. Many cases of acral nevi also show poor circumscription and asymmetry. The latter two features, how-
ever, may be related to the direction/plane the sections of such skin lesions have been taken for pathologic examination. In a study on acral nevi by Signoretti et al., such features were less frequently encountered when sections were taken perpendicular to the skin dermatoglyphics in contrast to when sections were taken parallel to the markings. The former therefore is the recommended way to process acral skin specimens bearing pigmented lesions.

**Spitz nevus**

This nevus is one of the most notable of melanoma imitators (Figures 5 A, B). Clinically, it presents as a dome-shaped, pale, red or pigmented skin nodule, usually in young patients. Histologically, the tumor is usually a circumscribed junctional or compound melanocytic proliferation of more or less uniform large cells which can be epithelioid or spindle, the latter often orientated in a plane perpendicular to the overlying epidermis, giving rise to a ‘raining-down’ appearance. The overlying epidermis typically shows acanthosis and variable hypergranulosis. Many cases exhibit some nuclear pleomorphism, dermal mitoses and pagetoid epidermal spread, features which raise concern for melanoma. In contrast to melanoma exhibiting Spitz nevus-like morphology (so-called ‘Spitzoid melanoma’), Spitz nevi lack atypical mitotic figures, contain fewer than 2 mitoses per mm² and lack mitoses near the deep edge of the lesion. Pagetoid spread may be seen in the central part of otherwise typical Spitz nevi, but is a cause for concern when occurring at the peripheral edge of the lesion. Other features that support a diagnosis of Spitz nevus are the presence of dermal maturation, clustered and sizable Kamino bodies, terminal junctional nests, cell uniformity in a horizontal plane across the lesion HMB-45 negativity and a low Ki67 proliferative index (<5% of melanocytic cells) in the dermal component of the lesion.

Spitz nevus-like lesions with atypical features (so-called ‘atypical Spitzoid tumors’) show overlapping features between Spitz nevus and melanoma. It is well documented that it is not possible to predict with certainty the biologic behavior of such lesions from their pathologic features. These difficulties were highlighted in a disturbing study involving the assessment of 30 Spitzoid melanocytic tumors by 10 international authorities; 17 cases yielded no clear consensus diagnosis, in only one case did 6 or more of the pathologists agree on the diagnosis, and some lesions that ultimately proved fatal were categorized as benign by most of the pathologists. In a recent study from the Sydney Melanoma Unit, distinction between atypical Spitz tumors exhibiting and lacking sentinel lymph node metastases was not possible on the basis of histologic features (unpublished data). This study found that SLN biopsy offers a means of assessing the metastatic potential of atypical Spitzoid tumors and aids in the management of these patients by selecting patients who may benefit from a regional node field dis-

---

Figure 5.—A) Spitz nevus: at low magnification power, the lesion appears symmetrical, is well circumscribed and shows epidermal hyperplasia and dermal maturation. (H & E, ×20). B) Spitz nevus. At higher magnification power, the cells have enlarged but uniform nuclei and show the typical ‘raining-down’ appearance. (H & E, ×100).
section and those in whom the use of adjuvant therapies could be considered. The recommended clinical management for atypical Spitzoid tumors is for complete excision and careful follow up. At the Sydney Melanoma Unit, patients with such tumors that are >1 mm in thickness are offered sentinel lymph node biopsy as a form of prognostication.

Blue nevus

Dendritic blue nevus and deep penetrating nevus usually present clinically as a heavily pigmented smooth-surfaced nodule. In the cellular variant of blue nevus, the dermal proliferation of plump ovoid and spindled nevus cells are typically admixed with heavily pigmented melanophages and sometimes branched dendritic melanocytes, and forms nodular masses, often with a dumb-bell shaped configuration. It may possess worrying features including high cellularity, nuclear hyperchromasia and the presence of occasional dermal mitotic figures. Given its dermal location, the differential diagnosis includes metastatic melanoma which on rare occasions may be difficult to distinguish. In contrast to cellular blue nevus, melanoma (whether primary or metastatic) can usually be identified by the presence of marked nuclear pleomorphism, frequent mitoses, necrosis, a lymphocytic infiltrate, and with clinicopathologic correlation.

The epithelioid blue nevus is a variant associated with Carney myxoma syndrome. Histologically, there are pigmented epithelioid melanocytic cells with rare mitoses admixed with branched dendritic cells. The lesional cells are often HMB-45 positive. The absence of marked nuclear pleomorphism and the lack of frequent or abnormal mitoses argue against a diagnosis of melanoma. Even so, there is some overlap of histologic features between this lesion and two other entities: animal-type melanoma and pigmented epithelioid melanocytoma (vide infra).

Deep penetrating nevus

This lesion is thought to represent a distinct entity but with some overlapping features with blue nevus and Spitz nevus. Deep penetrating nevus is characterized by a wedge-shaped histologic profile with the base towards the epidermis and the apex directed deeply, and composed of epithelioid and spindle-shaped lesional cells with prominent admixed pigment-containing melanophages (Figure 6 A, B). The nuclei are often enlarged and may contain characteristic nuclear pseudoinclusions. The lesion has a tendency to surround skin adnexal structures and neurovascular bundles and may feature a rare mitosis. Immunohistochemically, the lesional cells are typically HMB-45 positive. Awareness of this entity enables it to be differentiated from melanoma which commonly exhibits asymmetry, expansile dermal growth, nuclear pleomorphism and frequent mitoses.

Figure 6.—A) Deep penetrating nevus: at low magnification power, the lesion shows a wedge-shaped profile (H & E, ×20). B) Deep penetrating nevus. The lesional cells display moderate degree of nuclear pleomorphism and occasional intranuclear inclusions. Admixed melanin-containing macrophages are present (H & E, ×100).
Combined nevus

The heterogeneity of the cellular components of this nevus may give rise to a variegated and worrying clinical appearance, prompting its biopsy. Histologically, the lesion contains at least 2 nevomelanocytic components (Figure 7). It typically partners a common nevus with a deep penetrating nevus (62% of cases in one study), blue nevus or Spitz nevus. As a result of the admixture of different populations of nevomelanocytic cells and the asymmetry that often results, this lesion has been a source of histologic misdiagnosis. This is compounded by the increased cellularity and sometimes atypical nuclear features of the secondary component and the presence of an occasional dermal mitotic figure. Knowledge of this entity and the assessment of the lesion using standard criteria should enable it to be accurately classified.

Cellular nodule in congenital nevus

A congenital nevus may occasionally include a localized expansile proliferation of a nodule of tumor cells, featuring enlarged cells with atypia and the presence of mitotic figures. Not unexpectedly, such nodules may be misinterpreted as melanoma supervening in a nevus. Genetic studies of such cellular nodules have shown results supporting the benignity of the lesion. Pathologic features favoring a malignant lesion are the presence of marked nuclear pleomorphism, prominent nucleoli, poor circumscription, abrupt transition between the cells in the nodule and the adjacent banal nevus cells (rather than merging of the two components) frequent and atypical mitoses and the presence of necrosis. Particularly difficult cases with ambiguous morphologic features may be designated as an atypical nodular proliferation within a congenital nevus with a recommendation for complete excision and close follow-up.

Pigmented epithelioid melanocytoma

This is probably a heterogeneous group of melanocytic lesions first characterized by Zembowicz et al. In that series, the entity encompassed lesions originally reported as epithelioid blue nevus and animal-type melanoma. Histologically, the tumor is situated in the dermis and composed of heavily-pigmented epithelioid and spindle cells (Figure 8). Although most cases behaved in an indolent manner, 46% of cases showed metastatic deposits in regional lymph nodes. On histology, it was not possible to segregate the metastasizing and non-metastasizing tumors. A review of similar cases from the Sydney Melanoma Unit also showed overlapping features with epithelioid blue nevus and animal-type melanoma, corroborating the observations of Zembowicz et al., although the fre-
quency of lymph node metastases appeared to be lower. The behavior and clinical outcome of these lesions are yet to be resolved and further studies with long term follow-up are needed to further characterize them, the malignant potential of which is at present, considered to be uncertain.

Potentially problematic variants of melanoma

Although most cases of melanoma show overt malignant features, there are a few notable variants which mimic benign melanocytic and non-melanocytic tumors. Awareness of these entities and their respective features will help in their recognition.

Nevoid melanoma

Nevoid melanoma mimics the common nevus by appearing circumscribed and having a variable, often scant, intraepidermal component. Although dermal maturation appears to be present, closer examination reveals pleomorphic cells in the deep aspect of the tumor with nuclear hyperchromasia and often prominent nucleoli. Mitoses are almost always present and are often frequent and with abnormal forms. There is often expansion of the nests of tumor cells in the dermis. The overlying epidermis may be attenuated with rete ridges that may be elongated or lost.

Desmoplastic melanoma

Desmoplastic melanoma usually presents clinically as a firm skin nodule or plaque in the sun-exposed skin of elderly patients. Histologically, the tumor shows a variably cellular dermal proliferation of often elongated spindle cells with hyperchromatic nuclei and sometimes prominent nucleoli. The spindle cells infiltrate between dermal collagen bundles and in this regard, the tumor closely mimics scar tissue (Figure 9). Closer inspection reveals rare, sparse or frequent mitoses and the characteristic presence of lymphocytic aggregates and perineural invasion. Although not present in all cases, the presence of an atypical junctional melanocytic component, as well as the characteristic lymphoid aggregates are valuable clues to the diagnosis.

As the differential diagnosis of desmoplastic melanoma includes scar tissue, in re-excision specimens for cutaneous melanoma, it is important that the pathologist is made aware if the original tumor was a desmoplastic melanoma. Close examination for the above-mentioned characteristics and use of S-100 protein immunohistochemistry should enable detection of any residual tumor and assessment of the status of the excision margins. However, it is important to note that this variant of melanoma is usually negative for the other traditional melanoma markers such as HMB-45 and Melan-A (unless epithelioid cells are present) and, furthermore, that the fibroblasts in scar tissue may be positive for S100.

Regressing/regressed melanoma

A regressing melanoma may show a paucity of lesional cells, which are often concealed or obscured by a lymphocytic infiltrate and fibroblastic tissue. It has to be distinguished from other regressing cutaneous tumors, e.g. basal cell carcinoma and Merkel cell carcinoma. The presence of an atypical junctional melanocytic component may provide a clue to a regressed melanoma. In this regard, examination of further blocks or levels of tissue may be warranted. If a small residual cellular tumor is present, S-100, HMB-45 and Melan-A immunohistochemistry may help delineate melanoma. Basal cell carcinoma is positive for Ber-EP4 while dot-like positivity for cytokeratin 20 characterizes Merkel cell carcinoma. For tumors that are completely regressed where only fibroblastic tissue and melanin containing macrophages (melano-
phages) remain, it may be impossible to determine the nature of the regressed tumor with certainty since regressed pigmented basal cell carcinomas, melanomas and some other tumors may result in a similar morphologic appearance.

**A diagnostic approach**

The histologic evaluation of a melanocytic lesion must take into account a constellation of pathologic features including a range of architectural and cytologic features and features of the host response. There exists an effective strategy for the pathologist to first assess the histology of a melanocytic lesion without prior knowledge of the clinical history. All the pertinent histologic features are sought and on balance, a provisional diagnosis and relevant differential diagnoses are arrived at. The clinical features are then noted or sought for, correlated with the morphologic appearances, and the diagnosis refined with re-examination of the histology if necessary. This strategy of initial ‘blinded’ histologic assessment without the bias of clinical foreknowledge has been effectively applied in other areas of histopathology, e.g. liver core biopsy interpretation.

There are quite a number of histologic features that need to be examined and taken into account for the overall diagnostic impression of a melanocytic lesion, there is a strong case for an initial objective and comprehensive diagnostic assessment. Final clinicopathologic appraisal then enables a sound diagnosis to be made.

**Handling difficult melanocytic lesions**

Despite the faithful use of established criteria in the diagnostic work-up of melanocytic tumors, there are a small number of cases that will prove difficult even for the experienced pathologist. A practical way of categorizing such difficult melanocytic lesions into two main groups has been proposed by Elder. The first group comprises lesions that are superficially located in the epidermis, junctional zone and superficial papillary dermis. These include dysplastic nevi, pigmented spindle cell nevi, junctional Spitz nevi, nevi of special sites (e.g. acral and genital) and some regenerating nevi. The malignant counterpart of such superficial lesions is the radial growth phase melanoma, including MIS. When standard histologic criteria coupled with clinicopathologic correlation have been utilized and yet a lesion in this category defies definite classification, a label of superficial melanocytic proliferation of uncertain significance may be appropriate. The most adverse outcome of such lesions is that of local recurrence and, thus, complete excision with clear margins and clinical follow-up are recommended.

The second tumor group is dermally-situated and comprise atypical Spitz tumors and some variants of blue nevus, including the cellular and epithelioid types, atypical deep penetrating nevus and pigmented epithelioid melanocytoma. The malignant counterpart of this group is the vertical growth phase melanoma, a tumor that has significant metastatic capability. In tackling an ambiguous or difficult lesion in this category, Elder proposes the term: melanocytic tumor of uncertain malignant potential. In such a situation, following optimal clinician-pathologist and clinician-patient communication, there is a case for the patient to be managed as per the guidelines for a malignant lesion. The pathologist’s report (vide infra) should address all of the parameters normally reported in a synoptic report for a melanoma. Excision with a margin of normal tissue is usually recommended with additional consideration for a sentinel lymph node biopsy for lesions greater than 1mm in thickness.

**The melanoma pathology report**

The melanoma pathology report must contain all of the pathologic information that is deemed to be necessary to determine the optimal clinical management of the patient. Together with clinical staging, a well documented record of the pathologic features and parameters of the excised tumor provides the best available information for the prediction of prognosis. This will help the clinician to counsel the patient appropriately on the need and extent of any further surgery or adjuvant therapy that may be required.

To help in the documentation of all the required prognostic factors, there are a number of minimum data sets or synoptic reporting templates that are available. By providing a checklist, it standardizes the report format and minimizes the frequency with which important information is omitted from the pathology report (unpublished data). In the busy clinics and wards, a synoptic reporting template will also facilitate
the expedient extraction of crucial prognostically-important information from the pathology report by the clinician. An example of a synoptic reporting template is provided (Table I).

### Important issues in melanoma reporting

#### Tumor thickness

The Breslow thickness and Clark level are two of the time-honored and easily assessed prognostic features of primary melanoma. The former is of greater prognostic value and is the primary determinant of the tumor (T) score in the AJCC staging system for melanoma.\(^{66,69}\) Additionally, the Clark level has been shown to stratify prognosis in patients with thin melanomas (less than 1 mm in thickness).\(^{62}\) In a study investigating patients with melanoma 0.6 mm to 1.1 mm thick, Clark level IV tumors had a distinctly poorer prognosis.\(^{63}\)

#### Ulceration

Ulceration is an adverse prognostic feature that reflects the aggressiveness of melanoma.\(^{64}\) It has been shown to be an important independent prognostic factor and has been incorporated into the latest AJCC melanoma staging system.\(^{61}\) Histologically, the ulcer has to be in direct association with the tumor and must be distinguished from secondary ulceration due to trauma or other exogenous factors; such distinction is often difficult, and correlation with the clinical history (e.g., history of trauma or prior topical treatment) may help. The width of the ulcer is also held to be important, with a width of greater than 6 mm being associated with a higher incidence of nodal metastasis.\(^{65}\)

### Dermal mitotic rate

Tumor mitotic rate has been shown to be an important predictor of survival in several studies.\(^{66-69}\) In a study of 3,661 patients from the Sydney Melanoma Unit, the tumor mitotic rate was an independent predictor of survival and emerged second to Breslow thickness and ahead of the presence of ulceration, in its prognostic power.\(^{69}\)

Mitotic rate is a conveniently measured light microscopic parameter. However, as for some other solid tumors, there may be significant intra- and inter-observer variability.\(^{70}\) With a standardized approach, this variability can be minimized. The ‘hot-spot’ method is recommended for assessment of the mitotic rate. The histologic section of the melanoma is first surveyed at low magnification to find areas where mitoses are common. Such areas are examined at high magnification, and mitoses are counted and expressed as a figure per \(\text{mm}^2.\)\(^1\) Using this technique, a recent study from the Sydney Melanoma Unit showed that pathologists with differing backgrounds and varying levels of experience were able to attain excellent interobserver correlation for assessing tumor mitotic rate (intraclass correlation coefficient [ICC] 0.76).\(^{71}\) It is envisaged that with more widespread use of standardized assessment of mitotic rate, this parameter may be considered for incorporation into future revisions of the AJCC staging system.

#### Regression

Tumor regression has been held to be an adverse prognostic factor in melanoma, although not all studies have supported this.\(^{72-74}\) Histologically, regression is recognized by an absence of melanoma cells and replacement by a lymphohistiocytic infiltrate and/or fibrosis. Regression can be graded into early, inter-

<table>
<thead>
<tr>
<th>Pathologic feature</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
</tr>
<tr>
<td>Site</td>
<td>Left forearm</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Melanoma</td>
</tr>
<tr>
<td>Histologic subtype</td>
<td>Superficial spreading</td>
</tr>
<tr>
<td>Vertical growth phase</td>
<td>Present</td>
</tr>
<tr>
<td>Breslow thickness</td>
<td>2.7 mm</td>
</tr>
<tr>
<td>Ulceration (diameter in mm)</td>
<td>Present (3.1 mm)</td>
</tr>
<tr>
<td>Dermal mitotic rate (per (\text{mm}^2))</td>
<td>10</td>
</tr>
<tr>
<td>Clark level</td>
<td>IV</td>
</tr>
<tr>
<td>Vascular or lymphatic invasion</td>
<td>Absent</td>
</tr>
<tr>
<td>Neurotropism</td>
<td>Absent</td>
</tr>
<tr>
<td>Desmoplasia (% of dermal invasive tumor)</td>
<td>Absent</td>
</tr>
<tr>
<td>Satellites</td>
<td>Absent</td>
</tr>
<tr>
<td>Features of regression:</td>
<td>Mild and focal (non-brisk)</td>
</tr>
<tr>
<td>— intermediate (angiofibroplasia +/- TILs)</td>
<td>Absent</td>
</tr>
<tr>
<td>— late (fibrosis and loss of rete ridges)</td>
<td>Absent</td>
</tr>
<tr>
<td>Predominant cell type</td>
<td>Epithelioid</td>
</tr>
<tr>
<td>Associated nevus</td>
<td>Nil</td>
</tr>
<tr>
<td>Nearest lateral margin to in situ component</td>
<td>10.9 mm</td>
</tr>
<tr>
<td>Nearest lateral margin to dermal invasive component</td>
<td>4.1 mm</td>
</tr>
<tr>
<td>Distance from tumor to deep margin</td>
<td>7.1 mm</td>
</tr>
<tr>
<td>Solar elastosis</td>
<td>Mild (1+)</td>
</tr>
</tbody>
</table>
mediated and late. Early changes are essentially the presence of tumor-infiltrating lymphocytes, intermediate changes are characterized by angiofibroplasia and late changes incorporate dermal fibrosis and loss of rete ridges. The reason for regression being an adverse prognostic factor is not entirely clear. One possible reason is that when regression is present in the deep dermal portion of a tumor, it may cause an underestimation of the original Breslow thickness of the lesion and therefore its metastatic risk is underestimated. From a pathogenic point of view, it has been proposed that although a robust immune response to the tumor causes many cells to undergo apoptosis, there is a paradoxical pressure on tumor cells to undergo progressive genetic aberrations, leading to the ultimate generation of more aggressive clones of tumor cells.

Sentinel lymph node biopsy

Sentinel lymph node (SLN) biopsy has emerged as one of the most powerful tools to assess prognosis in melanoma. From a treatment point of view, on the one hand, a negative SLN has been established to be highly predictive of negative lymph nodes in the rest of the draining region, thus sparing such patients a completion lymph node dissection and its associated complications and morbidity. On the other hand, completion regional lymph node dissection (RLND) following detection of metastatic melanoma in the SLN biopsy has been shown to confer a survival advantage when compared with patients who undergo RLND after the occurrence of clinically evident nodal disease. SLN biopsy is currently recommended for patients with melanoma of intermediate thickness (1 to 4 mm). In tumors less than 1 mm thick, SLN biopsy is considered if there is presence of tumor ulceration or regression or if the tumor is in vertical growth phase. SLN biopsy has also been suggested for young patients and those with a high tumor mitotic rate, as these two factors are associated with a higher incidence of SLN positivity.

SLN are most efficiently identified by lymphoscintigraphy combined with injection of methylene-blue dye. Intraoperatively, a hand-held gamma probe is often used to aid the identification of the SLN. In high volume centers, there is a reported failure rate of about 2%, which may either be due to the inability to identify and remove the true SLN or the inability of the histopathologic process to detect metastatic disease within the SLN.

Role of fine needle biopsy

Fine needle biopsy (FNB) is a rapid, convenient and accurate means for the assessment of suspected recurr-
rent or metastatic melanoma. Smears of FNBs of melanoma are characterized by a dispersed population of epithelioid or spindle shaped cells with pleomorphic nuclei, prominent nucleoli and variable presence of melanin pigment. In challenging cases, a cell block specimen can be prepared and the diagnosis of melanoma can be confirmed with the use of immunohistochemistry. In a recent study of 2,204 cases at the Sydney Melanoma Unit, the role of FNB in the evaluation of suspected metastatic melanoma sites was reaffirmed by a high sensitivity of 96% and specificity of 98%. FNB should therefore be regarded as the first-line investigation for the pathologic confirmation of clinically- and/or radiologically-suspected recurrent/metastatic melanoma.

Correlation of dermoscopic features with pathology

Dermoscopy has emerged as a useful adjunct for the clinical diagnosis of melanocytic lesions. Its use has helped to improve the clinical diagnostic accuracy of such lesions and has also been shown to reduce the excision biopsy rates of nevi. The introduction of digital dermoscopic monitoring further allows the detection of subtle changes of a melanocytic lesion over time, thus enhancing the diagnosis of early melanomas. More recently, digital dermoscopic analysis offers automated evaluation of clinically challenging lesions in an attempt to distinguish atypical nevi from early melanomas. The development of these dermoscopic techniques has required close correlation of the clinical, imaging and pathologic features, as histopathologic evaluation is regarded as the ‘gold standard’. Nevertheless, there have been instances where suspicious areas detected on dermoscopy were not sampled during pathologic processing. In this regard, close clinician-pathologist communication is required with the clinician guiding the pathologist to more intensely sample specific areas for histologic evaluation. Such clinician-pathologist cooperation would help to further refine dermoscopic diagnostic criteria. The future trends of refinements to diagnosis and prognostication

Recent advances in molecular biology have enhanced the understanding of the pathology of melanocytic lesions. Using the technique of comparative genomic hybridization, Spitz nevi were shown to have a distinct genetic signature compared with melanomas. Most cases of melanoma have aberrations of chromosomes 9, 10, 7, 6 while most Spitz nevi do not show these aberrations. However, some Spitz nevi show an isolated gain of the short arm of chromosome 11. Gene expression studies have also delineated desmoplastic melanomas, which demonstrate increased expression of neurotropic and extracellular matrix production factors; this at least partially explains the characteristic histologic features of neurotropism and desmoplasia (vide supra). A recent analysis of single atypical melanocytes beyond the histopathologically apparent in situ component of acral melanomas by comparative genomic hybridization showed that these cells often harbor similar genetic changes to those of the melanoma. It is uncertain whether these cells represent the peripheral part of the melanoma or are reflective of a “field effect”, raising the issue whether such tumors may require the consideration of wider excision margins. For assessing prognosis, decreased mRNA expression of the melastatin, a melanocyte-specific gene, has been shown to be an adverse prognostic factor for patients with melanoma. In the effort to increase the sensitivity of SLN biopsies, reverse transcriptase-polymerase chain reactions (RT-PCR) have been used although false positive results remain an issue. The use of multimarker RT-PCR in combination with conventional histology and immunohistochemistry appears promising. It is likely that molecular methods will play an increasing role in the diagnosis and prognostication of patients with melanoma.

The integration of all the relevant clinical, pathologic and molecular features points the way forward towards more effective prognostication of patients with melanoma. There has been development of computer programs that use a selection of salient clinicopathologic parameters to give a recurrence, sentinel node metastasis or survival estimate for patients with melanoma. It is likely that additional pathologic and molecular parameters that are proved to be predictive of outcome will be incorporated into such programs to better refine the prognostication of individual patients.

Conclusions

For a patient with a challenging melanocytic lesion, a chain of events is essential for a correct diagnosis to be reached and appropriate management to be insti-
CURRENT PERSPECTIVES ON THE PATHOLOGIC AND REPORTING MELANOCYTIC TUMORS

TAN

Riassunto

Prospettive attuali sulla diagnosi istologica e sulla referenza dei tumori melanocitari

Uno degli obiettivi fondamentali della valutazione istologica dei tumori melanocitari è rappresentato dalla distinzione tra lesioni benigne (nevi) e quelle maligne (melanomi). Sebbene possa sembrare semplice, la valutazione dei tumori melanocitari è quella più difficile nell’ambito delle diagnosi istologiche. Gli aspetti istologici a favore della benignità sono rappresentati dalla simmetria, dalla delimitazione della lesione, dall’assenza di mitosi. Al contrario, una scarsa delimitazione della lesione, la disseminazione epidermica laterale o periferica, la presenza di necrosi, il pleiomorfismo nucleare e la presenza di mitosi frequenti o anormali aumentano il sospetto di malignità. Sebbene la maggior parte dei tumori siano prontamente diagnosticabili con l’applicazione dei criteri standard, vi sono variabili inusuali sia per i nevi che per i melanomi che richiedono molta attenzione per evitare errate diagnosi istologiche e un’attenta correlazione citopatologica per evitare la mancata diagnosi. L’esecuzione di una biopsia eseczionale completa, laddove possibile, è cruciale per poter fare una valutazione istologica ottimale delle lesioni sospette. Per i melanomi, il referito istologico dovrebbe comprendere tutti gli aspetti istologici che sono importanti per determinare la prognosi e la gestione del paziente. L’utilizzo di un modulo sinottico standard può facilitare questo aspetto e consentire di presentare le informazioni al medico in modo chiaro. Attualmente il ricorso alla biopsia del linfonodo sentinel è ben stabilito nella gestione dei pazienti con melanoma, così come è utile l’agoaspirato in caso di sospetto clinico di mela

nometastatico. La valutazione prognostica dei pazienti con melanoma dipende dall’integrazione di tutti gli aspetti citoistologici pertinenti e questa può essere favorita in futuro dall’utilizzo di programmi informatici. Le tecniche diagnostica molecolare possono ulteriormente affinare la diagnosi delle lesioni sospette e fornire stime prognostiche più accurate.


References

19. Crotty KA, Menzies SW. Dermoscopy and its role in diagnosing...


Dermoscopy is an effective tool for differentiating melanoma from benign melanocytic nevi. A number of score-based and gestalt-based algorithms are available to help accomplish the task of correctly identifying melanoma while at the same time correctly classifying most clinically atypical nevi as benign lesions, which need not be biopsied. We describe a simple and easy approach to learn pattern analysis that can be utilized to help clinicians differentiate melanoma from benign nevi. Most melanocytic nevi manifest one of nine well defined benign patterns. These benign patterns are symmetric, uniform and organized and include the: (1) diffuse reticular network, (2) patchy reticular network, (3) peripheral reticular network with central hypopigmentation, (4) peripheral reticular network with central hyperpigmentation, (5) peripheral reticular network with central globules, (6) globular, (7) peripheral globules with central reticular network or starburst, (8) homogeneous, and the (9) symmetric multi-component pattern. Melanoma on the other hand tend to reveal a pattern that deviates from the above mentioned benign patterns by manifesting asymmetry and an architecture that is disordered. Most melanomas will also contain at least one of the following eight local features: atypical network, streaks, atypical dots or globules, negative pigment network, off center blotch, blue-white veil, and atypical vascular structures. Thus, by simply knowing a set of nine benign nevus pattern and eight local melanoma specific features one can start implementing dermoscopy into the daily routine practice.

**Key Words:** Melanoma, pathology - Dermoscopy - Skin.

**Early diagnosis of cutaneous melanoma remains the best course for ensuring a good prognosis for patients with this malignancy. The added benefit of dermoscopy, above and beyond simple unaided visual inspection, has been firmly established. In the hands of experienced users, dermoscopy boosts diagnostic accuracy by more than 40%.** Additionally, it improves the clinician’s confidence level in the diagnosis being rendered. The ultimate sequel of an improved diagnostic accuracy and confidence level is a decrease in the total number of unnecessary biopsies while at the same time insuring that malignancies are not missed — a fact that translates into an improved benign to malignant biopsy ratio.

The learning curve for dermoscopy, however is steep. The observer needs to recognize a multitude of dermoscopic structures and colors, many of which can at times be subtle. In addition, they need to recognize the patterns created by the placement and distribution of these colors and structures. These patterns can greatly facilitate the clinician in correctly diagnosing a particular cutaneous lesion. A number of diagnostic algorithms and approaches have been described with the aim of teaching novices to correctly interpret dermoscopic primary morphology. In general, the algorithms can be classified as gestalt-based (e.g., pattern analysis) or score based (e.g., ABCD rule of dermoscopy). Menzie’s method or 7-point check list. 
Various studies have demonstrated that there is no single ideal way to diagnose melanoma dermoscopically, and most likely, something can be learned from all these methods. However, pattern analysis is the most frequently used approach by experts. Experience plays a major role in this approach. An expert sees the features and patterns in a lesion, and almost simultaneously he or she compares this lesion with a mental database of dermoscopic images. Usually, in a span of few seconds, the expert reaches a conclusion and renders a diagnosis. The major advantage of pattern analysis over the score-based approach is the speed by which one can reach a diagnosis for most pigmented skin lesions. Since this process requires adequate knowledge and experience, it is perhaps the most difficult method to teach novices. Despite this challenge, we will attempt to describe dermoscopic patterns of melanoma through a three-phase approach. We will first describe the classic patterns of the benign melanocytic nevi (the good), then the typical patterns of frank melanomas (the bad), and finally the patterns encountered in some early melanomas and some atypical nevi (the gray).

The Good: classic patterns of benign melanocytic nevi

Learning the normal patterns of benign nevi is the first step. In medical school, students first learn the normal physiology before attempting to understand the pathophysiology of different disease states. Likewise, to understand the dermoscopic patterns of melanoma, novices first need to replete a mental database with normal melanocytic lesions. Fortunately, benign nevi tend to manifest one of a finite number of well recognized patterns (Table I). If a lesion adheres to one of these benign dermoscopic patterns while at the same time not manifesting any of the local features attributed to melanoma (Table II), then it is highly improbable that the lesion will be a melanoma. Conversely, if a lesion deviates from one of the classic dermoscopic patterns of benign nevi, then the index of suspicion for melanoma should be raised, especially in the presence of one or more of the local features attributed to melanoma (Table II). Hence, by knowing a set of classic dermoscopic patterns encountered in benign nevi and a few local features that occur in melanoma one can easily implement dermoscopy in daily routine practice.

### Table I. Description of nine benign patterns of melanocytic nevi.

<table>
<thead>
<tr>
<th>Benign patterns</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse reticular network</td>
<td>Uniform network distributed diffusely and throughout the lesion. The lines of network represents the presence of melanocytes or pigmented keratinocytes along the epidermal rete ridges. The holes in the network represent the dermal papillae</td>
</tr>
<tr>
<td>Patchy reticular network</td>
<td>Patches of uniform network scattered throughout the lesion. There are structureless areas interspersed among the patches of network.</td>
</tr>
<tr>
<td>Peripheral reticular network with central hypopigmentation</td>
<td>Uniform network is located at the periphery and surrounding a central structureless or light brown homogeneous area</td>
</tr>
<tr>
<td>Peripheral reticular network with central hyperpigmentation</td>
<td>Uniform network is located at the periphery and surrounding a central darkly pigmented and homogeneous area. There may also be a small number of dots present in the center or overlying the network lines.</td>
</tr>
<tr>
<td>Peripheral reticular network with central globules</td>
<td>Uniform network is located at the periphery and surrounding a cluster of globules in the center of the lesion</td>
</tr>
<tr>
<td>Globular</td>
<td>Uniform globules are the predominant feature. The globules correspond to nests of melanocytes. Depending on the depth of these nests, the globules can be either light brown, dark brown or black in color. Close proximity and juxtaposition of these globules can form a “cobble-stoned” appearing pattern</td>
</tr>
<tr>
<td>Homogeneous pattern</td>
<td>Brown homogeneous pigment throughout the entire lesion. On occasion one may see a few globules and/or remnants of a network</td>
</tr>
<tr>
<td>Peripheral globular with central reticular network or centrally homogeneous area (this pattern also includes the starburst pattern)</td>
<td>A rim of uniformly distributed globules surrounding an area of network. This pattern is frequently seen in growing nevi. If instead of seeing globules one sees pseudopods around the periphery of the lesion (starburst pattern) then the most likely diagnosis is a Spitz nevus</td>
</tr>
<tr>
<td>Symmetric multi-component</td>
<td>Combination of three or more structures distributed symmetrically. Multi-component lesions should always be viewed with caution. Only if the structures are completely symmetrically distributed should one consider this a benign pattern</td>
</tr>
</tbody>
</table>
The benign nevus patterns share some common motifs. In general, benign nevi have fewer (<3) colors and fewer numbers of dermoscopic structures. Most importantly, benign lesions tend to be uniform, revealing symmetry in the distribution of dermoscopic structures and colors and they tend to be architecturally organized. Most benign lesions have at least one axis of symmetry. Uniformity is a more elusive concept. It applies to the size and shape of the dermoscopic structures that are present. For example, uniform network is composed of a mesh network with similar sizes of holes and width of lines, and uniform globules are composed of globules of similar shape, size and color. Architectural organization is the most difficult concept to convey in description, though most people have an intuitive understanding of this concept. It is one of the key concepts described in the CASH algorithm. Perhaps the best way to illustrate this idea is by taking a page from the architectural layout of two major cities: New York and Rome. New York streets are laid out in a grid fashion where most of the streets and avenues are straight, aligned either in north–south or east–west directions. In contrast, the streets in Rome are haphazardly, although charmingly, laid out. As we discuss each of the classic benign patterns of nevi, hopefully the concept of architectural order will become clearer.

Specifically, the nine classic dermoscopic patterns of benign melanocytic nevi include: 1) diffuse reticular network; 2) patchy reticular network; 3) peripheral reticular network with central hypopigmentation; 4) peripheral reticular network with central hyperpigmentation; 5) peripheral reticular network with central globules; 6) globular; 7) peripheral globules with central reticular network or starburst; 8) homogeneous and 9) multi-component patterns. A word of caution is warranted here that although a completely symmetric multi-component lesion is usually

<table>
<thead>
<tr>
<th>Structures</th>
<th>Descriptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical network (including branched streaks)</td>
<td>Black, brown or gray network with focal irregular mesh with thick lines and different sized holes. The lines represent the melanocytes and melanin along the rete ridges, and the holes represent the dermal papillae. Branched streaks represent remnants of pigmented rete ridges resulting from bridging of nests of melanocytes at the dermal-epidermal junction</td>
</tr>
<tr>
<td>Streaks (includes pseudopods and radial streaming)</td>
<td>Linear pigmented projections at the periphery of the lesion. When these linear structures terminate with a bulbous projection they are called pseudopods. Histologically they represent confluent junctional nests of melanocytes</td>
</tr>
<tr>
<td>Atypical dots and globules</td>
<td>Black, brown or gray dots or globules, varied in size and distributed haphazardly within the lesions. They frequently occur at the periphery of the lesion and are not associated with the network. Histologically, black dots represent melanin in the stratum cornium, gray dots represent melanin free in dermis or within melanophages, and brown dots represent small nevomelanocytic nests at the tips of the rete ridges. Brown globules represents larger nevomelanocytic nests along the dermo-epidermal junction or in the dermis</td>
</tr>
<tr>
<td>Negative or reverse pigment network</td>
<td>The lines of the network appear lighter (almost white) in color compared to the holes which are darker in color. Histologically this probably represents narrow and hypopigmented rete ridges accompanied by the presence of large melanocytic nests within a widened papillary dermis</td>
</tr>
<tr>
<td>Off center blotch</td>
<td>A blotch is a darkly pigmented area in which one cannot discern any structures. Histologically it represents large concentrations of melanin in the epidermis and/or dermis</td>
</tr>
<tr>
<td>Blue white veil overlying flat (macular) areas and/or the presence of blue-gray granules (peppering)</td>
<td>Irregular, confluent, gray-blue to whitish blue pigmentation overlying flat areas within the lesion. Histologically this represents regression. One can also see peppering which represents melanin or melanophages in the papillary dermis. If the regression is complete then one will see a white scar like area instead</td>
</tr>
<tr>
<td>Blue white veil overlying raised areas</td>
<td>Irregular, confluent, blue-white hazy pigmentation overlying raised areas within the lesion. Histologically it represents melanocytes in the dermis together with compact orthokeratosis</td>
</tr>
<tr>
<td>Vascular structures</td>
<td>Presence of different shaped blood vessels can be seen in melanoma. The most common are dotted, linear irregular and polymorphous vessels</td>
</tr>
</tbody>
</table>
benign, one should always be cautious whenever evaluating such lesions since melanomas frequently possess a multi-component pattern. With that being said, these aforementioned nine benign nevus patterns are derived from three fundamental dermoscopic features, namely reticular network, globules and homogeneous areas. Benign nevi may reveal just one of these three features or may reveal a combination of these three features (Figure 1). The detailed description and schematic illustration of each pattern can be found in Table I and Figure 2, respectively. Dermoscopic images representing each pattern are shown in Figures 3-11. In general, lesions with any of the above nine patterns are uniform and architecturally ordered. Lesions manifesting these benign patterns while at the same time not exhibiting any of the melanoma specific structures mentioned in Table II are highly unlikely to represent melanoma.

**The Bad: classic patterns of frank melanoma**

To a certain degree, learning the dermoscopic patterns of melanoma is easy when one knows the motifs and patterns of benign nevi. Dermoscopic patterns of melanoma should share little to no similarity with those principles and patterns described for benign nevi. Unlike nevi, the motifs in melanomas usually reveal asymmetry, multiple colors (>3) and many dermoscopic structures. The classic dermoscopic patterns of melanoma display asymmetry in at least one axis, and most have two axis asymmetry. They usually reveal non-uniformity in shape and size of the dermoscopic structures. More importantly, the structures are arranged in a haphazard distribution, and there is architectural disorder. On a gestalt level, the dermoscopic patterns of melanoma are defined by chaos and disorganization, and they create a level of unease in the eye of the examiners.

Unlike the nine dermoscopic patterns encountered in benign nevi, there is no finite set of dermoscopic patterns that are characteristic of melanomas. Thus, it is not possible to categorize all the infinite variations of different dermoscopic patterns of melanomas. For novices without extensive training the following guideline may be helpful. Melanomas are lesions that 1) do not fit into any of the aforementioned nine benign dermoscopic patterns associated with nevi; 2) have asymmetry, non-uniformity, and architectural disorder and 3) have any of the local features attributed to melanoma (Table II). Figures 12-16 illustrate the examples of dermoscopic patterns of frank melanomas.

This contrast and comparison between the “good” and the “bad” dermoscopic patterns has also been described as “the beauty and the beast” sign. “Beauty” connotes the dermoscopic patterns of benign nevi revealing symmetry and engendering a sense of ease in the observer. “Beast” refers to the features and patterns of melanomas, most of which reveal asymmetry and engender a sense of unease in the viewer. In essence, the dermoscopic diagnosis of melanoma relies on the understanding of the benign patterns seen in melanocytic nevi. This should not be a surprise, because in nearly all branches of medicine the recognition of pathology requires the understanding of the normal.

**The Gray: the dermoscopic patterns of indeterminate melanocytic lesions**

Biologic systems are complex and often cannot be organized within simple dichotomies. It is almost impossible to neatly classify all lesions as either good...
Figure 2.—Schematic illustration of the nine benign melanocytic nevus dermoscopic patterns includes starbust patterns.

Figure 3.—Diffuse network pattern. There are a few brown dots present within the lesion. However, dots overlaying the network lines is common in benign nevi and they can generally be ignored.

Figure 4.—This lesion has a classic patchy network pattern.
or bad, black or white, beautiful or ugly. In addition, our current understanding of tumor progression and the sensitivity of diagnostic instruments are not adequate to allow us to accurately classify all the melanocytic lesions as “good” or “bad”. For these reasons, there are

Figure 5.—This benign melanocytic nevus reveals a peripheral network with a central hypopigmented area.

Figure 6.—This benign melanocytic nevus reveals a peripheral network with a central hyperpigmented area.

Figure 7.—This lesion has a benign homogeneous pattern.

Figure 8.—This lesion has a peripheral network with centrally placed globules, another benign pattern.

Figure 9.—This lesion has a peripheral rim of globules. A peripheral rim of globules is seen in benign nevi that are in the process of enlarging. Lesions revealing a peripheral rim of pseudopods (streaks) instead of globules usually represent Spitz nevi.
many lesions that fall into the “gray” category, where the diagnosis is not obvious. By and large, most of the lesions in this “gray” category are atypical nevi or early melanomas. Currently, the gold standard, albeit imperfect, for diagnosing melanomas is histology.

The dermoscopic patterns of the “gray” lesions fall between the two extremes (Figures 17-20): the benign nevi and the frank melanomas. These “gray” lesions do not manifest any of the benign dermoscopic patterns of melanocytic nevi. They may have either one or two axis of symmetry, few colors and dermoscopic structures. However, they tend to have varying degree of non-uniformity and architectural disorder. These lesions usually do not manifest any of the ominous dermoscopic structures listed in table II. Accurately diagnosing these lesions on a gestalt level within a span of few seconds can be challenging if not impossible. The use of other score-based systems, such as the ABCD dermoscopy rule, 7 point...
check list, and Menzies method may be helpful for such lesions, but even the score-based systems will usually fail us when it comes to these “gray” lesions. Perhaps the most helpful method, short of performing a biopsy, for correctly classifying these “gray” lesions is via short term mole monitoring.\textsuperscript{13, 14} Short term mole monitoring is based on the premise that biologically relevant melanomas will reveal dermoscopic changes when monitored over a three month period and benign nevi will generally not show any change.

Management of patients with these “gray” lesions depends on a host of factors. The patient’s own history and observation are important elements in the diagnostic consideration. Lesions with symptoms of pain, bleeding, itching or change should be biopsied or monitored closely. Comparison of the lesion with neighboring lesions is another sound practice principle. Lesion that appears different (either via clinical or dermoscopic examination) relative to its neighbors, the “ugly duckling sign”, should be biopsied or monitored closely.

Figure 14.—This melanoma reveals network, streaks, light brown structureless areas and a subtle blue-white veil. The lesion is asymmetric in one axis, has multiple colors and is somewhat disorganized. It clearly does not conform to one of the benign nevus patterns depicted in figure 2.

Figure 15.—This melanoma does not manifest one of the benign nevus patterns. It also has dotted vessels, pink veil (indicating increased vasculature), blue-white veil and light brown structureless areas.

Figure 16.—This melanoma has regression structures, globules and network distributed asymmetrically. The colors and structures are not uniform and are disorganized. This lesion does not conform to one of are the benign nevus pattern.

Figure 17.—This lesion is composed of mostly light brown globules, and it is symmetric in two axes. However, it contains area suggestive of a reverse network. The histologic diagnosis was dysplastic nevus.
In summary, dermoscopy is a valuable tool for differentiating most melanomas from benign melanocytic nevi. For dermatoscopists, learning to recognize melanoma requires knowing the patterns and structures encountered in benign nevi. Physicians should consider melanoma in the differential diagnosis anytime they encounter a lesion that deviates from one of the nine benign nevus patterns (Table I, Figure 2), especially if the lesion also reveals one or more of the ominous dermoscopic structures listed in Table II.

**References**

Melanoma is the most lethal of human skin cancer and its incidence is increasing worldwide. Melanomas often metastasize early during the course of the disease and are then almost intractable by current therapeutic regimens. Consequently, understanding the factors that maintain melanocyte homeostasis and prevent their neoplastic transformation into melanoma is of outstanding interest. Targeted molecular therapeutics are tailored to genetic abnormalities that are associated with tumor progression. In this review, we report about our quest for new diagnostic biomarkers and therapeutic targets, respectively, using different molecular techniques and approaches, including cDNA microarrays, RT-PCR, conventional immunohistochemistry and tissue-microarrays from in vivo and in vitro samples. We will describe our recent findings on the newly discovered markers in detail, among them phosphorylated pRb Ser795, the retinoblastoma protein binding protein 2-homolog 1 (RBP2-H1) and the dipeptidyl peptidase IV (DPPIV), and link them to already known mechanisms of melanoma pathogenesis. Since the reported markers may be causally linked to malignant transformation, their molecular signaling mechanisms deserve to be studied in more detail in future investigations.

KEY WORDS: Melanoma, pathology - Biological markers - Skin neoplasms.

Since the 1960s, melanoma incidence has risen by 5% to 8% per year in the Caucasian population.1 Although early primary melanomas are curable by surgery, treatment of advanced metastatic disease remains futile and the strategies employed in the last 30 years have not significantly improved survival rates, which are 3-16% (10-years survival rate) in stage IV melanoma.2 Thus, advances in understanding the genetic and functional alterations in melanocytic tumors have sustained hopes for a breakthrough in the therapy of disseminated melanoma. A current paradigm is that genetic and epigenetic alterations play an important role in the etiology and pathogenesis of cancer. Apparently, mammalian cells have numerous safeguards that protect against neoplastic transformation, and only when lesions occur in multiple genes, invasive cancer can develop.4

As a consequence of this multifactorial pathogenesis of melanoma, the application of one single biomarker for targeted therapy or diagnosis frequently fails. For example, the recent identification of activating mutations in BRAF in over 60% of cases of melanoma has caused much excitement in the melanoma community and may actually offer the first opportunity for a rational treatment program.2 However, since more than 80% of common benign nevi show the same mutation,3 BRAF can not be applied as a biomarker for differentiation between melanoma and nevi. Particularly, the discrimination of borderline lesions such as dysplastic nevi 6 or deep penetrating nevi 7...
still remains critical at all levels of the diagnostic process, i.e. clinical appearance, dermatohistopathology and possible molecular work-up. Consequently, further molecular markers are highly needed that may also be suitable as therapeutic co-targets.

In this review, we report about our quest for new melanoma biomarkers. We are targeting on both 1) the detection of new markers for discrimination between benign and malignant tumor growth and 2) the therapeutic disclosure of these markers in melanoma development considering functional linkages to already identified melanoma signaling pathways, such as the CDKN2A/CDK/pRb pathway. Different approaches are conceivable for detection of new markers for malignancy. In our group, we usually start from a biologic key process that is involved in melanoma development, as e.g. malignant invasion or disruption of cell cycle control, and either analyze empirically chosen markers in a functional setting or systematically screen for markers. In particular, we will focus on the most promising examples of markers with therapeutic implications, such as phosphorylated pRb Ser795, retinoblastoma protein-binding protein 2-homolog 1 cations, such as phosphorylated pRb Ser795, dipeptidyl peptidase IV, respectively.

Detection of a gene expression profile determining the invasive potential of malignant melanoma

Most melanomas begin to grow within the epidermodermal junction and progress through a period of radial expansion to an invasive phenotype that acquires metastatic capacity possibly in a stepwise fashion. Currently, the genes that orchestrate this transition have not been fully established. In recent years, some PCR- or immunohistchemistry-based approaches could detect a potential association of the invasive character of melanoma cells and the expression of certain singular genes, e.g. metalloproteinase-2 and annexin VII. But, a systematic analysis of gene expression patterns of the invasive margin was lacking, although such an analysis could provide clues for the understanding of this pivotal step in progression. Recent technical advances, the combined application of laser pressure catapulting microdissection with genome wide expression profiling using cDNA microarrays, have opened the gateway to this kind of investigations.

cDNA arrays allow an effective investigation of differential gene expression patterns using a systematic and comprehensive approach. Analysing primary patient tissues, Golub et al. showed that a reliable discrimination between acute lymphoblastic leukemia and acute myeloid leukaemia could not be made based on single genes. In contrast, predictions based on the expression levels of a multitude of genes were highly accurate. For malignant melanoma, it was also demonstrated that a classification based upon differential gene expression might be possible. But, in contrast to Golub’s study, genetic profiling of melanoma was mainly founded on the investigation of cultured cell lines or mouse models. Consequently, predictions about the in vivo situation of human melanomas were still rather limited, because in vitro strategies cannot reproduce the cutaneous environment, which represents a unique determinant for the invading cells. The application of laser microdissection of tissue sections could overcome this problem, because it allows the detection of changes in the transcriptome of cells reflecting intercellular cross-talk. In contrast to former microdissection approaches (e.g. needle microdissection), laser-assisted microdissection allows the isolation of a small number of tumor cells avoiding contamination.

In a previous study, we combined laser microdissection and cDNA array analysis to detect differences in the gene expression profile between the invasive margins and the cores of nine nodular malignant melanomas. For data analysis, we suggested a novel strategy for gene sorting according to the signal-to-noise statistic. In addition, for each of the 9 genes relative expression factors (invasive rim/tumor centre) were calculated for all cases. Using such algorithms, a profile of 9 selected markers was established to distinguish between samples derived from invasive tumor regions and samples from non-invasive regions in a hierarchical cluster analysis. Taken together, 15 of 18 tumor regions (83.3%) were classified correctly. Regarding the differential expression between tumor margins and cores of all melanomas, phosphoenolpyruvate carboxykinase 1 (PEPCK) and two anonymous ESTs (R52541 and N73761) were typically upregulated in the invading edge. PEPCK is a main switch for regulation of gluconeogenesis in order to adjust glucose production to physiologic requirements. In terms of melanoma expansion, the role of PEPCK remains unclear. The upregulation of PEPCK in the invasive margin could reflect the higher degree
of glucose metabolism in those cells, which may need more energy for migration and invasion. New data generated in hepatoma cell lines also showed an upregulation of PEPCK in cells cultured at high density suggesting a possible role of PEPCK in the metabolism of cells competing with each other.24 In contrast to that, Homo sapiens similar to S. cerevisiae SSM4 (TEB4) had higher relative expression ratios in the melanoma centres. In S. cerevisiae, mutations in the SSM4 locus, suppress the cell growth phenotype as well as mRNA instability.25, 26 Therefore, the relative absence of TEB4 activation in the invading edge may increase growth, possibly by an alteration of nuclear mRNA stability. We further measured an increased central expression value of the human homologue of A. nidulans SudD suppressor of bimD6 homologue across 8 of nine melanoma experiments. In A. nidulans, BimD6 mutation is thought to be associated with a failure of chromosomes to correctly attach to the spindle microtubules, resulting in an increase in chromosome loss. SudD is identified to be a suppressor of the bimD6 mutation. Its product is a highly conserved protein that is found in a variety of eukaryotes from fungi to man.27 Accordingly, in humans SudD could represent an important regulator of chromosomal homeostasis, but its exact function in the pathogenesis of melanoma remains to be elucidated. Ribosomal protein L19 was shown to be induced in the tumor centre. In human breast cancer with overexpression of erbB-2, mRNA levels of L19 were also found to be more abundant than in cells with low erbB-2 expression suggesting a potential role in carcinogenesis.28 However, for cutaneous neoplasms no relationship has been found so far. The a subunit of the IL-3 receptor, a very abundant protein in cutaneous neoplasms, was shown to be induced in the invasive edge. The IL-3 receptor subunits a and b on cell cycle regulation and apoptotic pathways have been controversially discussed. For instance in hematopoietic cells, both subunits may be involved in tumor proliferation as well as in growth inhibition. But, in contrast to the b chain, the less characterised a subunit is supposed to be much more ligand specific.29 Hence, an association of IL-3 seems to be conceivable. Inositol 1,4,5-triphosphate 3-kinase isoenzyme, was also found to be upregulated in melanoma cores. Being part of Ca2+-mediated intracellular signalling, this kinase phosphorylates the second messenger mol-ecule inositol 1,4,5-triphosphate (IP3) to inositol 1,3,4,5-tetrakisphosphate (IP4). In contrast to IP3, IP4 mediates slower and more prolonged responses in the cell, but both messengers have important functions in the regulation of cell proliferation.30 Conclusively, an enhanced conversion of IP3 to IP4 as well as described alterations of TEB4, PEPCK, SudD, L19 and I1-3 receptor a subunit might influence cell proliferation and influence melanoma growth, and its infiltrative character, respectively.

Taken together, with this straightforward approach, we could specify some novel differences between cells from the invasive margin and the centre of melanomas that emerge from transcriptome-wide screening. These findings could both foster our understanding of tumor biology and spark new functional investigations. In addition, genes exhibiting an enhanced activation in the invasive margin could potentially serve as prognostic markers.

Dipeptidyl peptidase IV as a new marker for discrimination of malignant melanomas from deep penetrating nevi

In 1989, Seab et al.7 reported a series of highly invasive, but non-metastasizing pigmented melanocytic tumors and invented the term deep penetrating nevus (DPN). DPN mostly appear as darkly pigmented papules or nodules with no or mild epidermal changes and are most commonly found in the face, on the upper trunk or proximal extremities of patients at the age of 10 to 30 years.7, 31 Histopathologic growth patterns are often worrisome showing a wedge-shaped invasive growth extending from the upper dermis into the subcutaneous fat tissue not rarely following preformed structures, e.g. hair follicles or sweat glands.7, 32 Thus, DPN are often mistaken for vertical growth phase nodular malignant melanomas (NMM), both clinically and histologically.7, 32-37 Estimates exist that, depending on the criteria used for classification, misdiagnoses as melanoma occur in 29% to 40% of the cases.7, 31, 32 Beyond the clinical demand for precise diagnosis, the DPN may also represent a valuable natural model for melanocytic invasion without metastatic potential and deserves further elucidation.

In previous studies, several immunohistochemical attempts with standard melanoma markers, such as S-100, failed to differentiate DPN from NMM.7, 38 Before we initiated our experiments, only one study existed
suggesting PCNA (proliferating cell nuclear antigen) as a possible discriminating marker. PCNA represents an accessory protein of DNA δ-polymerase which is increased during the late G1 growth phase and peaks in the S phase of the cellular cycle. However, PCNA never entered routine diagnostics, probably due to a lack of studies with higher numbers of cases. So, we expanded on the search for new discriminating markers analyzing an empirical selection of common candidate markers determining cell proliferation control or melanocytic invasion. However, against our expectations, semi-quantitative assessment of both immunolocalization and immunoreactivity of the proliferation markers MIB-1/Ki-67, retinoblastoma protein (pRb) and its activity-reduced form phospho-retinoblastoma protein Ser795 (p-pRbSer795) revealed no consistent differences between DPN and matched cases of NMM. Moreover, also the invasion-related markers matrix metalloproteinase-1, matrix metalloproteinase-2, membrane-type matrix metalloproteinase-1 and integrin β3 showed no significant differences in expression. According to the highly invasive character of DPN, matrix metalloproteinase-1 and matrix metalloproteinase-2 immunostaining of some DPN even exceeded that of NMM, thereby, confirming their role in tumor infiltration, but, at the same time, questioning their importance for metastasizing.

In addition, we stained for a new marker that was previously shown to affect both invasion and proliferation, the dipeptidyl peptidase IV (DPPIV). Interestingly, in our immunostainings, the mean expression scores for DPN clearly exceeded that of NMM with P<0.001. All DPN stained highly positive, whereas most melanoma samples showed low or no expres-

Figure 1.—DPPIV represents a potential discriminating marker between DPN and NMM (modified from Roesch et al., with kind permission by the Nature Publishing Group). A) DPPIV expression in a DPN with its typical diffuse staining pattern (100x). B) 400x magnification. C) Example of a NMM showing only single positive cells (100x), D) 400x magnification. E) Negative control (100x overview). F) Positive control (sebaceous gland, 400x magnification). G) Immunoreactivity was semi-quantitatively assessed regarding staining quantity and subcellular staining intensity in 11 DPN and 16 NMM. Staining quantity and intensity were summarized to a single expression score (ES) as recently published. The ES values of each sample were grouped into one of 4 ES categories (ES 0, ES 0-40, ES 40-80 and ES >80) and displayed as percentage of all samples of one entity. Differences were statistically significant with higher scores in DPNs (U-test P<0.001).
sion (Figure 1). The intratumoral immunolocalization of DPPIV in both entities showed a diffuse expression pattern with focal accumulation close to cutaneous adnexes or at the tumor periphery. Thus, only DPPIV kept its promise to be a protein that possibly separates infiltrative tumor growth from true melanocytic malignancy. DPPIV (CD26) is a 110-kD, trans-membrane, cell surface peptidase expressed by normal melanocytes and common nevi, but primary and advanced malignant melanomas almost invariably lose or alter their DPPIV expression.40-42 DPPIV has numerous functions including involvement in T-cell activation, cell adhesion, digestion of proline containing peptides in the kidney and intestines, HIV infection and apoptosis, and regulation of tumorigenicity in certain melanoma cells.43 Functionally, DPPIV re-expression leads to re-differentiation and an acquired dependence on exogenous growth factors, but it also favors loss of tumorigenicity and anchorage-independent growth.44 Thus, high DPPIV expression was found correlated with less metastatic potential in vivo.44 The effect of DPPIV appears to be mediated through several mechanisms: 1) by up-regulation of other factors such as E-cadherin and tissue inhibitors of matrix metalloproteinases; 2) by its ability to bind components of the extracellular matrix such as collagen or fibronectin; 44, 46 and, most interesting for melanoma biology, 3) by its inhibition of mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK)1/2 activation.47 Since loss of dipeptidyl peptidase IV may be causally linked to the transition of invasive to metastatic phenotypes, the molecular mechanisms downstream of dipeptidyl peptidase IV deserve to be studied in more detail in future investigations. Taken together, in the case of DPPIV, a strategy that singles out the most promising candidates based on previous evidence led to the establishment of a novel marker also with therapeutic implications.

Hyperphosphorylated retinoblastoma protein represents a possible prognostic marker in the progression of malignant melanoma

The main principle of cell cycle homeostasis is the integrated phosphorylation-dependent activation and deactivation of regulatory proteins upstream of the retinoblastoma protein (pRb), which represents the central cycle-controlling element. At the G1/S transition, the antiproliferative function of active, hypophosphorylated pRb is mediated by its binding capacity to pro-proliferative transcription factors such as E2F or c-Myc.48 Surprisingly, with the exception of retinoblastoma, osteosarcoma and small cell lung carcinoma, in the vast majority of human cancers the overall rate of pRb-mutations is either extremely low or not existent.49 Also in melanoma, the loss of cell cycle control is thought to be due to a lack of pRb-activity and not to lack of expression or mutation.50, 51 Currently, the concept is arguably accepted that persistent inactivation and hyperphosphorylation of wild-type pRb in melanoma is caused by a sustained cyclin dependent kinase activity (CDK2, CDK4, CDK6).52, 53 Substantial experimental evidence shows that this persistent kinase activity can be due to 1) genetic alterations eliminating the activity of cyclin dependent kinase inhibitors (CKIs) such as p16/INK4a; 2) a downregulation of CKIs, e.g. p27/KIP1; 3) the persistent overproduction or mutation of several cyclins (e.g. D1, E, A); or 4) a combination of those factors.54

Due to a lack of studies on pRb phosphorylation status in in vivo samples of cutaneous melanocytic tumors, we analyzed total and phospho-pRb expression in tumor biopsies of progressive and non-progressive melanocytic lesions including common and dysplastic nevi, superficial spreading (SSM) and nodular malignant melanomas (NMM), melanoma metastases and, as control, further neuroectodermal tumors such as glioblastomas, schwannomas and neurofibromas.8 Relative measurements of Rb mRNA expression in microdissected melanocytic tumors biopsies revealed a significant almost 3-fold overexpression of Rb mRNA in melanoma and melanoma metastases versus benign melanocytic nevi. In contrast, expression levels of various non-neuroectodermal malignant control tissues (lung carcinoma, breast carcinoma, colon carcinoma) showed uniformly low expression levels. In accordance with the RT-PCR data, tissue microarray (TMA)-based immunohistochemistry confirmed gross constitutive differences in pRb-expression (Figure 2). Interestingly at phospho-pRb Ser795, we found an almost complete phosphorylation of the progressively overexpressed total pRb, whereas in common nevi only a fraction of total pRb was phosphorylated. Phosphorylation at Ser807/811 generally occurred to a lesser extent, but significantly higher in metastases compared to nevi. In case of Ser780, no significant differential phosphorylation was detectable. To analyse pos-
sible intratumoral heterogeneity that may be missed by TMAs due to limited punch size, we also analyzed sections with preserved full tumor architecture (Figure 3). Expression scoring for total pRb in complete tumor sections was in support of our RT-PCR results, i.e., a progressive increase of pRb in malignant melanocytic tumors. Comparing expression scores from complete sections with TMA-based results, the progressive increase in phosphorylation could be confirmed for all three phospho sites analyzed. Furthermore, the complete tissue sections did reveal considerable intratumoral heterogeneity of total pRb expression and pRb phosphorylation. In SSM, subepidermal vs dermal expression scores were significantly different with the most intense staining result in the subepidermal-lateral portions (P<0.005). In NMM, the invasive part vs tumor centre (P<0.001) and in melanoma metastases outer margins vs centers (P<0.003) also showed significant differences. Other benign neuroectodermal tumors that served as independent controls such as schwannomas and neurofibromas showed a comparably low “melanocytic nevus-like” pRb-expression with no significant intratumoral heterogeneity. In metastases, an accumulation of phospho-pRb staining was mainly detected in the outer margins compared to the tumor centre. In contrast, in benign and dysplastic nevi as well as schwannomas/neurofibromas pRb phosphorylation was almost negligible.

Our data suggest that particularly Ser795 is a good choice for estimating both the presence of pRb and its degree of inactivation. Specifically Ser795 is among those phosphorylation-sites that undergo hyperphosphorylation by CDK2-E in late G1. This results in the release of E2F that finally leads to an enhanced tran-
Figure 3.—Staining patterns of pRb and phosphorylated pRb Ser795 in melanocytic tumors (from Roesch et al., with kind permission by the Nature Publishing Group). First row (200x magnification): negative control with omitted primary antibody (epidermis, A), positive controls (epidermis, B and sebaceous gland, C). Second row (200x magnification): increasing total pRb expression in malignant melanocytic tumors: benign melanocytic nevus (MN, D), nodular melanoma (NMM, E) and melanoma metastasis (MMM, F). Third row: phospho-pRb-staining of a superficial spreading melanoma (SSM). Overview (100x magnification) showing a high regional variance of phospho-pRb staining with a maximum in subepidermal-lateral nests versus dermal melanoma nests (G). Details: 400x magnification of a highly phosphorylated subepidermal melanoma nest (H) in comparison to dermal nests (I). Forth row: phospho-pRb-stained nodular melanoma (NMM). Overview (100x magnification) showing an increase of pRb-phosphorylation in the invasive basal front (J). Details: 400x magnification of the invasive front (K) and almost complete absence of phospho-pRb in central tumor areas (L).
scription of S-phase-genes such as DNA polymerase a and dihydrofolate reductase. In contrast to Ser795, Ser780 and Ser807/811 are exclusively phosphorylated by CDK4-D. Interestingly Ezhevsky et al. reported that CDK4-D-mediated phosphorylation of pRb is a prerequisite for the transition of unphosphorylated inactive pRb into hypophosphorylated active pRb. This suggests that the increase in pRb phosphorylation at Ser780 detected in benign nevi represents active cell cycling suppressing pRb.

To disclose a potential association of the histologically detectable degree of pRb phosphorylation and the prognosis of melanoma patients, we additionally analyzed a panel of long-term survivors with thick, high-risk primary melanoma (TD >3.5 mm). We found a clear trend to higher phospho-pRb expression in short-time survivors versus exceptional long-time survivors in our cohort of matched survivors and non-survivors. Low phospho-pRb expression was significantly correlated with a prolonged survival, whereas high phospho-pRb expression was correlated with early death (log-rank-rest P=0.03). This suggests a possible Breslow-(tumor-thickness)-independent prognostic impact of pRb-phosphorylation.

In conclusion, we could confirm that 1) in cutaneous malignant melanocytic tumors total pRb-expression is progressively upregulated and that 2) simultaneously, augmented total pRb is probably functionally inactive due to protein hyperphosphorylation, particularly at Ser795. Interestingly, a high degree of pRb-phosphorylation can be observed in tumor-regions with expansive growth activity, i.e., the deep invasive front of the vertical growth phase nodular melanomas and the lateral-subepidermal parts of superficial spreading melanomas. Accordingly, the benign counterparts analyzed (melanocytic nevi, schwannomas, neurofibromas) show a moderate, mostly homogeneous expression of pRb and pRb-phosphorylation is diffuse and negligibly weak. Furthermore, Kaplan-Meier analysis of advanced melanomas with long-time follow-up suggested a significant negative impact of pRb-phosphorylation on survival independent of tumor thickness.

**The retinoblastoma binding protein 2-homolog 1: a new tumor suppressive marker downregulated in malignant melanomas**

As described above, the loss of cell cycle control in malignant melanomas is thought to be due to a lack of retinoblastoma protein (pRb)-activity and not to a lack of its expression or mutation. To find new pRb-binding proteins (RBPs) functioning as potential pRb-modulating factors, Defeo-Jones et al. screened a human expression cDNA library with a recombinant Rb probe and identified two novel proteins termed RBP-1 and RBP-2 (alternatively “RBBP1, RBP1; RBBP2, RBP2”). Both proteins contain highly conserved pRb-binding motifs, which show a striking homology with viral oncogenic proteins such as E7, large T and E1A.

In 1999, we have described a novel homolog of RBP-2, termed RBP2-homolog 1 (RBP2-H1, NCB1 genebank Acc. No. AF087481). The corresponding transcript was detected due to its UV-B responsive expression in normal non-transformed human melanocytes using RNA fingerprinting. It encodes a protein with a 54% amino acid identity with RBP-2. Further computerized sequence analyses revealed highly conserved motifs with possible functional implications. Among those, two DNA-binding zinc finger (leukemia-associated protein, LAP) motifs, a rhombotin-2 (RBTN2, LMO2) binding domain and a domain possibly mediating a direct binding and interaction with pRb (non-T/E1A-pRb binding domain) were most remarkable. Since the sequence analysis also found the ancient homeodomain dri (dead ringer protein), it was recently proposed to group RBP-2-homologue proteins into the superfamily of ARID (AT rich interactive domain) DNA binding proteins.

Two more RBP2-H1-homolog transcripts have been described so far that are differentially regulated in breast carcinomas, RBBP2H1a and PLU-1. Two novel RBP2H1a, PLU-1 and RBP2-H1 represent three alternative splicing variants of the PLU-1 gene. RBP2-H1 is distinguished by one extra exon of 108 bp. The RBBP2H1a transcript has a much longer 5′-UTR and an ATG upstream of that used for RBP2-H1 and PLU-1. On cDNA level, RBP2-H1 shows a 98.5% identity to PLU-1 and a 98.0% identity to RBBP2H1a. The comparison of RBP2-H1 with RBP-2 reveals a cDNA homology of 37.8%. On protein level, 54.0% of the amino acids of RBP2-H1 and RBP-2 are identical. Sequence analysis of the three splicing variants revealed further highly conserved
functional motifs. Applying the new HUGO nomenclature,\textsuperscript{64} PLU-1 gene derivate would now be termed as JARID1B variants, \textit{e.g.} JARID1B\_v1 for PLU-1, JARID1B\_v2 for RBP2-H1 and JARID1B\_v3 for RBBP2-H1a according to the respective dates of publication. The corresponding protein isoforms should be denoted as JARID1B\_i1, JARID1B\_i2 and JARID1B\_i3.

Since previous work has gained preliminary evidence that RBP2-H1 mRNA can be downregulated in UV-irradiated melanocytes and possibly in malignant melanomas,\textsuperscript{63} we subsequently investigated mRNA expression of RBP2-H1 by real-time RT-PCR.\textsuperscript{68} Compared to melanocytic nevi, RBP2-H1 mRNA was not significantly decreased in melanomas, but significantly decreased in melanoma metastases. The splicing variant RBBP2H1a also showed the most prominent expression in benign melanocytic tumors. There was a similar, but not significant trend to a deficient expression in melanomas and metastases. Interestingly, similar to melanomas and melanoma metastases, glioblastomas, used as external controls, also showed a relative loss of RPB2-H1 compared to fetal brain. In contrast, epithelial cancers, in particular breast cancer, showed a comparably high RBP2-H1 expression and only marginal RBBP2H1a expression. The progressive deficiency of RBP2-H1 mRNA from common nevi to melanoma and melanoma metastasis could be also confirmed on protein level. TMA were examined to evaluate gross constitutive differences in RBP2-H1-expression between benign melanocytic lesions and malignant melanocytic tumors (Figure 4). In accordance to our RT-PCR data, TMA-based immunohistochemistry confirmed a progressive deficiency of RBP2-H1 expression from nevi (TMA No. 1, n=52) to melanoma (TMA No. 2, n=60) and melanoma metastases (TMA No. 3, n=60). Almost 70% of the spotted nevus samples were classified as “RBP2-H1-expressors”. Strikingly, in melanomas, RBP2-H1 staining was only detected in primary radial growth phase melanomas with a cut-off tumor thickness of $\leq 1.6\text{ mm}$. In this group 31.6\% were positive which adds up to only 10\% of all melanomas spotted on TMA No. 2. In thicker more advanced melanomas (>1.6 mm) no staining signals could be found. However, in melanoma metastases, 30\% of the samples did express RBP2-H1. With regard to the whole tumor architecture, no region-specific distribution (\textit{e.g.} tumor front or tumor core) of RBP2-H1 could be detected in complete tissue sections. However, certain heterogeneity of staining results (number, intensity) across the full sections could be seen. In accordance with our mRNA measurements and the TMA analyses, the highest RBP2-H1 expression scores were found in nevi with a significant decrease in both melanomas and metastases, respectively (Figure 5). Other benign neuroectodermal tumors that served as independent controls such as schwannomas and neurofibromas showed a comparably high “melanocytic nevus-like” RBP2-H1-expression.

Since RBP-2 was suggested to be involved in pRb-phosphorylation control by direct protein-protein interaction,\textsuperscript{62} we addressed the question whether RBP2-H1, the closest homolog of RBP-2, could also bind to pRb. While RBP2-H1 lacks the typical Leu-X-Cys-X-Glu-motif of some pRb-interacting proteins, it bears a homolog-domain of the non-T/E1A-pRb binding domain of RBP-2.\textsuperscript{63} The latter could theoretically confer a direct interaction with pRb and make RBP2-H1 a true pRb-binding protein. Thus, co-immunoprecipitation studies were performed with cells that endogenously express RBP2-H1 at sufficient levels, such as MCF-7 breast carcinoma cells. By this, we could demonstrate that wild type RBP2-H1 can truly bind to pRb suggesting a possible functional interaction of both proteins.\textsuperscript{68}

In a following study,\textsuperscript{69} we re-established the pRb modulating function of RBP2-H1 in highly metastatic A375-SM melanoma cells by re-expressing its C-
term (cRBP2-H1) encoding the non-T/E1A-pRb binding domain. Our results demonstrated that the expressed cRBP2-H1 binds to pRb and, moreover, exerts a continuous hypophosphorylation at pRb Ser795 during the whole G1 phase of the cell cycle, whereas in untreated A375-SM and control virus infected A375-SM cells, phosphorylation at Ser795 slightly increases in late G1. Ser795 has been described to be a phosphoacceptor site which is implicitly required for activation of pRb cell cycle arrest function. As a substrate of cdk4/cyclin D and cdk2/cyclin E, Ser795 is supposed to be among those phospho-sites that undergo progressive hyperphosphorylation from early to late G1 phase and, therefore, might be directly involved in G1/S transition. The observation that in untreated A375-SM cells, Ser795 is partly phosphorylated already in early G1, perhaps due to an additionally uncontrolled cdk4/cyclin D activity generally discussed in melanomas, further reinforces the role of cRBP2-H1 as a stabiliser of hypophosphorylated pRb, since in cRBP2-H1-transduced cells, hypophosphorylated Ser795 was detected already in early G1, too. The exclusively cdk4-targeted phosphoacceptor sites Ser780 and Ser807/811 lacked to show significant differences in phosphorylation in early as well as late G1 in cRBP2-H1-transfected cells. Therefore, we suggested that RBP2-H1 does not act as a cdk-specific inhibitor, but as a pRb phosphoacceptor site-specific stabiliser, although we have not yet investigated the remaining 12 pRb phospho-sites of pRb. A further argument in favor of this hypothesis is that a direct interaction of retinoblastoma binding proteins with cdk5 has never been detected so far.

As demonstrated by cDNA microarrays and confirmed by quantitative RT-PCR, we found a differential expression of various melanoma-associated genes which are, only in part, directly linked to the pRb/E2F pathway; e.g. BMP2, FST and TGFA, downregulated in cRBP2-H1-transduced cells, was shown to be involved in autocrine growth of melanoma cells. FST, also downregulated, inhibits activin-mediated growth reduction and apoptosis in melanoma cells. Finally, BMP-2, upregulated in transduced cells, was shown to reduce the secretion of HGF, which represents a potent pro-proliferative cytokine eliciting mitogenic, motogenic and morphogenic responses in melanoma cells. Consistent with BMP-2 upregulation in cRBP2-H1-transduced cells, HGF was found to be strongly suppressed. Thus, for all four candidates, FST, TGFA, BMP-2 and HGF, we found transcriptional responses typical for a more benign melanocytic phenotype. Beside to E2F downstream target genes, further target genes of other pRb-inhibited transcription factors, such as SPI1 and PP-1alpha2 (not shown), were identified by Ingenuity™.

Since pRb itself was not differentially expressed after transduction, the transcriptional regulation of downstream genes might be caused by a functional modulation of pRb and pRb-inhibited factors on the protein level, e.g. due to an altered E2F release after RBP2-H1/pRb-interaction. However, common E2F-transactivated S phase genes, such as DHFR, TK1, DNA-polymerase alpha were not found differentially expressed. Therefore, further, yet unknown mechanisms, independent from pRb, might be involved, too. One example of cRBP2-H1-activity that is probably independent of pRb is the upregulation of TCF4 which represents another highly melanoma-relevant path-

Figure 5.—Staining patterns of RBP2-H1 in melanocytic lesions (from Roesch et al., with kind permission by the Nature Publishing Group). Immunohistochemical staining of whole tissue sections (200x magnification) reflects the deficiency of RBP2-H1 in malignant melanoma and melanoma metastases. Common melanocytic nevus (A), advanced nodular melanoma (B), and melanoma metastasis (C).
way. Transcription factors of the TCF family are downstream effectors of the Wnt/-catenin signaling pathway which is implicated in developmental processes and progression of some tumors, especially colon carcinoma and melanoma.77, 78 Depending on the interaction with co-regulative factors like -catenin or Groucho (both not regulated), TCF can act as transcriptional activator or repressor. In absence of -catenin, TCF represses the transcription of several target genes, including DCT and TYRP1.79, 80 Next to tyrosinase, both DCT and TYRP1 represent major pigmentation enzymes. Moreover, MITF, strongly suggested that also MITF is a target gene of melanoma and melanoma.81 Recently, Widlund and co-workers suggested that also MITF is a target gene of melanoma.81 MITF, strongly downregulated upon re-expression of RBP2-H1, is discussed as the master gene for melanocytic survival as well as the key transcription factor for regulation of melanocytic protein expression.77, 78 Therefore, the striking downregulation of MITF in our experiments also indicated a more benign gene expression phenotype of cells re-expressing cRBP2-H1 and assigns RBP2-H1 to the genes with tumor-suppressive action.

As a possible result of the observed changes in pRb interaction and gene expression, a block in G1/S transition was detected in cell cycle profile analyses of RBP2-H1-transduced cells. This observation and a recorded decrease in cellular proliferation also support the hypothesis that RBP2-H1 exerts a tumor-suppressive function in melanocytes. In conclusion, our data suggest that the progressive loss of RBP2-H1 might not only serve as a diagnostic and prognostic marker for melanoma, but re-establishing parts of its function in metastatic melanomas may open new avenues of experimental strategies for cure of metastatic malignant melanoma.

Concluding remarks

In the last years, we have detected several new candidate markers of melanocytic tumorigenesis affecting cell cycle control, stroma infiltration and gene transcription regulation. We linked their functions to known tumor pathways and found, especially for RBP2-H1 and DPPIV, a potential involvement in malignant transformation and melanoma progress. We propose that RBP2-H1 and DPPIV deserve further investigations in future projects targeting on both the establishment as diagnostic parameters and the exploration in a possible therapeutic context. However, since it was repeatedly demonstrated that melanomagenesis is not caused by failure of one cellular check point, but by a concert of disrupted regulatory factors, we suppose that marker-targeted diagnosis and therapy of malignant melanoma can only be significantly improved using a multifactorial approach.

Riassunto

Nuovi biomarcatori molecolari per il melanoma

Il melanoma rappresenta il carcinoma cutaneo più letale per il genere umano e la sua incidenza è in aumento in tutto il mondo. Spesso i melanomi metastatizzano precocemente nel corso della malattia e divengono quasi in trattabili con gli attuali regimi terapeutici. Di conseguenza, la comprensione dei fattori che mantengono l’omeostasi dei melanociti e la prevenzione della loro trasformazione neoplastica nel melanoma rappresentano degli aspetti di fondamentale interesse. Le terapie molecolari mirate sono dirette nei confronti delle anomalità genetiche che sono associate alla progressione del tumore. In questa review gli autori prendono in considerazione la ricerca di nuovi biomarcatori diagnostici e di nuovi obiettivi terapeutici, utilizzando diverse tecniche ed approcci molecolari, quali i cDNA microarray, la RT-PCR, l’immunoistochemica convenzionale e i microarray tissutali su campioni in vivo e in vitro. Gli autori inoltre descrivono in dettaglio i loro dati relativi ai marcatori più recenti, quali il pRb Ser795 fosforilato, l’omologo 1 della proteina 2 legante la proteina del retinoblastoma (retinoblastoma protein binding protein 2-homolog 1: RBP2-H1) e la dipeptidil peptidasi IV (DPPIV) e li correleremo ai meccanismi già noti della patogenesi del melanoma. Dal momento che è possibile che i marcatori descritti possano correlare casualmente con la trasformazione maligna, i loro meccanismi di segnale molecolare devono essere studiati più in dettaglio in futuro.

Parole chiave: Melanoma, anatomia patologica - Marker biologici - Cute, neoplasie.

References


Sentinel lymph node biopsy is a highly accurate method for the staging of patients with localized cutaneous melanoma. The status of the sentinel node is the single most important predictor of recurrence and survival in patients with clinically node-negative melanoma. While this procedure has found widespread acceptance in treatment of patients with melanoma >1 mm in thickness, its use in patients with thinner lesions is more controversial. Overall, approximately 5% of all melanoma patients with lesions ≤1 mm are reported to harbor identifiable metastases in the sentinel nodes at the time of diagnosis. There is clearly an unmet clinical need for improved predictive biomarkers of regional metastasis in clinically localized primary melanomas, especially in patients with thin melanomas. This review summarizes the present knowledge regarding the relationship between clinical and histologic prognostic factors related to sentinel node involvement in melanoma, with particular reference to this important group of thin lesions, and introduces the concept of considering host polymorphisms as well as tumor-derived factors as potential biomarkers for refining our understanding of melanoma metastasis.

KEY WORDS: Melanoma - Sentinel node biopsy - Lymphatic metastasis - Lymphangiogenesis.

As the incidence of melanoma continues to rise worldwide, so does the proportion of patients diagnosed with early-stage (Stage I/II) disease. In a recent evaluation, 78% of cutaneous melanomas reported to the US National Cancer Institute’s Surveillance, Epidemiology, and End Results (SEER) cancer registry were American Joint Committee on Cancer/International Union Against Cancer (AJCC/UICC) Stage I disease, with a 5-year survival rate of 97.7%. Yet even though the majority (81%) of these patients have thin tumors (Breslow thickness <1.00 mm), in the SEER database 15% of all melanoma deaths are in these “low risk” patients. Given the large numbers of early stage patients and the worldwide efforts at early detection, this percentage of deaths attributable to thin melanomas is likely to rise considerably in the future. Thus, identification of risk factors for disease progression are perhaps most important in this group of patients.
Sentinel lymph node biopsy is a highly accurate method for the staging of patients with localized cutaneous melanoma. The status of the sentinel node is the single most important predictor of recurrence and survival in patients with clinically node-negative melanoma.\(^3\), \(^4\) While this procedure has found widespread acceptance in treatment of patients with melanoma >1 mm in thickness, its use in patients with thinner lesions is more controversial.\(^5\) Overall, approximately 5% of all melanoma patients with lesions ≤1 mm are reported to harbor identifiable metastases in the sentinel nodes at the time of diagnosis.\(^6\), \(^7\) The incidence of sentinel node positivity in thin melanoma patients has ranged from as low as 0% to as high as 9.7% in most reported series.\(^8\), \(^9\) This variability appears to be due, at least in part, to the differing criteria for selection of patients with melanomas <1 mm for this procedure, as well as to the relatively low numbers of patients (especially node-positive patients) in most studies. There is clearly an unmet clinical need for improved predictive biomarkers of regional metastasis in clinically localized primary melanomas, especially in patients with thin melanomas. The following summarizes the present knowledge regarding the relationship between clinical and histologic prognostic factors related to both sentinel node involvement and clinical outcome in melanoma, with particular reference to this important group of thin lesions.

**Patient age**

Older patients are more likely to present with thick, ulcerated melanomas.\(^10\) Melanoma survival decreases with advancing age, as was demonstrated in a large retrospective study of 17 600 melanoma patients: patients younger than 30 years have a 5-year survival rate of 87% compared to 78%, 71%, and 60% respectively for those in their 60’s, 70’s, and 80’s.\(^11\) The influence of age on nodal status, however, is the inverse of this phenomenon. Sondak \textit{et al.}\ reviewed the University of Michigan experience with over 400 consecutively treated melanoma patients undergoing sentinel node biopsy, with almost all melanomas being at least 1.0 mm in thickness.\(^12\) Their findings revealed that younger age was a significant predictor of an increased likelihood of nodal involvement in patients. In this study, the rate of nodal progression decreased steadily as patient age increased, a finding that has now been confirmed in an extension of this study involving 1130 patients,\(^13\) as well as in other series.\(^14\)-\(^16\)

Less is known about the influence of age on the likelihood of nodal progression in thin melanoma patients. In 2003, a retrospective review by Bleicher \textit{et al.}\ showed that younger age (defined in their study as <44 years of age) was associated with nodal progression in patients with melanomas ≤1.50 mm in depth.\(^6\) A number of recent series have examined the impact of a variety of prognostic factors on sentinel node positivity in thin melanoma patients, but all of these have been negative or inconclusive with respect to the influence of age.\(^7\), \(^8\), \(^17\), \(^18\) except for a recent series from University of Cincinnati. In this series of 64 thin melanoma patients undergoing sentinel node biopsy, patients with sentinel nodes found to be either histologically or PCR positive were significantly younger (mean age 48.3 years) than those with negative sentinel nodes (50.6 years).\(^19\) Several other single-institution series of sentinel node biopsy for melanomas 1.00 mm or thinner have found that patients with nodal progression were confined to those 60 years of age or younger, without finding a direct correlation for age as a continuous variable.\(^7\), \(^18\) However, data presented by Leiter \textit{et al.}\ showed age was significant only in older men with melanomas <0.75 mm in depth.\(^20\) Thus, at present, age alone cannot be considered to be a validated criterion for recommending or avoiding sentinel node biopsy in thin melanoma patients.

**Gender**

Many studies suggest that, overall, men are more likely to die of melanoma than women. While most recent series have not found a significant correlation of gender with sentinel node involvement in thin lesions,\(^7\), \(^8\), \(^18\), \(^20\) Gimotty \textit{et al.}\ found male gender to be prognostically significant for increased risk of progression in thin melanomas.\(^21\) Based on the available evidence, gender alone cannot be considered to be a validated criterion for recommending or avoiding sentinel node biopsy in thin melanoma patients.

**Location**

Several studies have demonstrated a correlation between tumor location and prognosis. In one study of melanomas of all thicknesses, lesions on the extrem-
ity had a significantly improved 10-year overall survival (90%) compared to 70% for those on the trunk, head or neck. In a more recent analysis of 1,130 patients undergoing sentinel node biopsy, trunk or head and neck locations were more predictive of sentinel node involvement than upper and lower extremity locations in both univariate and multivariate models. Although analyses have been limited, tumor location has not been shown to have a significant impact on sentinel node involvement in thin melanomas to date. However, sentinel node biopsy for head and neck primaries is more difficult, riskier and associated with higher rates of false negative findings. This should be taken into account when advising a patient with a thin melanoma regarding sentinel node biopsy.

**Breslow thickness**

Breslow thickness, the tumor depth in millimeters from the granular layer of the epidermis to the deepest point of tumor invasion, is universally considered to be one of the most useful prognostic factors in patients with early stage melanoma. Many studies have demonstrated that aside from sentinel node status, Breslow depth is the most powerful predictor of clinical outcome. In the most recent version of the AJCC/IUCC staging system, the threshold for T2 melanoma (marking the beginning of “intermediate thickness”) was changed from 0.75 to 1.01 mm. Some melanoma centers, including ourselves, continue to use 0.75 mm as the threshold for selecting patients for sentinel node biopsy. But few centers routinely perform sentinel node biopsy for melanomas thinner than 0.75 mm in the absence of other factors indicating an increased risk for progression. While nodal metastasis has been described in melanomas thinner than 0.75 mm, as noted by Stitzenberg et al., it appears to be exceedingly rare. Kesmodel et al. found only one patient with a positive sentinel node among 91 vertical growth phase melanomas <0.76 mm in thickness. In a series of 118 patients with melanomas ≤0.75 mm, Bleicher et al. found only 2 positive sentinel nodes (1.7%). Our own experience is consistent with the two reports just cited: less than 1% of those patients with melanomas <0.75 mm selected for sentinel node biopsy were found to have a positive node (Messina JL, unpublished data). Routine use of sentinel node biopsy for melanomas below 0.75 mm does not appear to be justified based on the available data.

**Clark level**

In the current AJCC staging scheme, nonulcerated invasive melanomas ≤1.00 mm confined to the papillary dermis (Clark level II or III) are classified as T1a, while Clark IV (into the reticular dermis) and ulcerated tumors are classified as T1b. This has led many surgeons to restrict the use of sentinel node biopsy for thin melanomas to patients with Clark level IV or ulcerated Clark level II and III lesions. The evidence to support this approach, however, is lacking. In our recent series at Moffitt Cancer Center of 409 patients with melanomas 0.75 to 1.00 mm in thickness, we found an overall sentinel node positivity rate of 4.9%. In this series, Clark level was not a significant predictor of nodal positivity: 5.7% of Clark IV tumors had at least one positive sentinel node, compared to 4.4% of patients with level II and III lesions, a difference that was not statistically significant and likely reflected the slight preponderance of lesions 0.9 mm to 1.00 mm among Clark IV cases. Greater numbers of thin melanoma patients with Clark level II and III melanomas, in total more node-positive cases were found in the level II and III group than among the Clark level IV cases. In fact, nearly all reviewed reports reveal that Clark level does not correlate with nodal progression in thin melanomas.

**Mitotic rate**

Mitotic rate is generally measured as the number of mitoses per square mm, the area of six 40x high-power microscopic fields. In considering melanomas of all thickness, a cutoff of >6 mitoses/mm² was found to be indicative of a significantly worse prognosis. Investigators at the Sydney Melanoma Unit in Australia evaluated tumor mitotic rate as a prognostic indicator for disease progression. Their findings in a series of 3,661 patients showed that those with a mitot-
ic rate of 0 had a significantly better survival rate than patients with 1 or more mitoses per mm². In their data, mitotic rate was a more significant prognostic factor than ulceration when both were taken into account. Sondak et al. also found mitotic rate to be a significant predictor of nodal progression, and in this series ulceration did not retain statistical significance as a prognostic factor in multivariate analysis once mitotic rate was taken into account. In the University of Pennsylvania experience with patients with vertical growth phase melanomas 1.00 mm or thinner, Kesmodel et al. and Gimotty et al. found that a mitotic rate >0 (so-called “mitogenicity”) correlated with both nodal progression and decreased overall survival. The Indiana University group found that mitoses >6/mm² were significant predictors of sentinel node positivity in a study of 184 thin melanoma patients. However, reviews of small numbers of sentinel node-positive thin melanoma patients from the University of North Carolina and from the Roswell Park Cancer Institute did not suggest that nodal progression was most commonly from higher mitotic rate tumors. Thus, while we believe strongly that tumor mitotic rate is a significant predictor of sentinel node positivity, the optimal cutoffs to use this biomarker to select patients for sentinel node biopsy remains to be defined.

Ulceration

Ulceration is an uncommon pathologic finding in thin melanomas, and when present may be secondary to trauma. While ulceration correlated with increased rates of disease progression in patients with thin melanomas in the large AJCC data set (in which mitotic rate was not evaluated), it has not correlated with nodal progression in most series of sentinel node biopsy for the same patients. In a number of series, including the previously described analysis of the Moffitt Cancer Center data and reports from the University of Pennsylvania and the University of North Carolina at Chapel Hill, no patients with ulcerated thin primary melanomas were found to have evidence of nodal metastases.

Regression

Regression is a pathologic finding suggesting that a melanoma had at one time penetrated to a greater degree than was evident at the time of biopsy and could theoretically identify thin lesions with a particularly high risk of nodal progression. To date, however, no study has found regression to be a statistically significant independent predictor of nodal metastasis either for all patients with melanoma or only for patients with thin melanoma. In fact, it appears that regression may be negatively correlated with nodal metastases: patients with regression were actually less likely to spread to the sentinel node than other melanoma, adjusting for thickness and mitotic rate. Based on the available evidence, the presence of regression should not be used to select patients with thin melanomas for sentinel node biopsy.

Tumor infiltrating lymphocytes

Clark et al. are credited with first evaluating and categorizing tumor infiltrating lymphocytes (TILs) as a prognostic biomarker in primary melanoma. Clark described “brisk” tumor infiltration by lymphocytes as a favorable factor, while “nonbrisk” infiltration was intermediate and “absent or slight” infiltration was unfavorable. A subsequent study by Clemente et al. in a different cohort of patients also established the presence of brisk TILs to be an independent positive predictive factor. A prospective randomized clinical trial of 259 patients with clinically localized melanoma conducted by the Southwest Oncology Group found the presence of brisk TILs (defined as lymphocytes completely surrounding the nodule in a band-like manner, with no defect in the band of greater than 0.30 mm) to be strongly correlated with a favorable outcome. Whether the patient had “nonbrisk” or “absent or slight” TILs was not associated with any difference in outcome. The 10-year survival rate for the 30 patients with brisk TILs was 93%, compared with 58% for nonbrisk and 55% for absent or slight TILs. The relative risk of recurrence in this study for nonbrisk or absent TILs superseded both ulceration and mitotic rate in multivariate analysis. The study stresses the importance of including all known potential prognostic factors in any multivariate model of outcome in melanoma. Much less is known about the implications of TILs for melanoma progression as assessed by regional nodal metastasis, however, as most recent studies have not included this factor. The group at the University of Pennsylvania recently described TILs as an independent prognostic factor for sentinel node metastasis in patients...
with stage I and II melanoma, with patients with absent TILs having a three-fold increased relative risk of nodal metastasis. They found that the presence or absence of TILs was second only to tumor thickness in a regression tree analysis for predicting risk of sentinel node metastasis in melanomas greater than 1 mm. These findings, which have yet to be validated in an independent data set, support a potential but as yet incompletely defined role of TILs as a biomarker for melanoma regional progression.

**Growth phase**

The first step in melanoma progression is referred to as radial growth phase, which can be found either in non-invasive (in situ) or early invasive melanomas. As originally described by Clark et al. in radial growth phase lesions, melanocytes in the dermis are present as nests smaller than those in the epidermis, without mitotic activity. The onset of vertical growth phase, which heralds increased potential for metastatic behavior, is characterized by either mitotic activity (“mitotic vertical growth phase”) or dermal tumor nests larger than those in the epidermis (“tumorigenic vertical growth phase”). Several groups have demonstrated that growth phase is an independent prognostic indicator of both sentinel node positivity and outcome.

**Histologic type**

Generally speaking, histologic type does not correlate directly with the likelihood of finding a positive sentinel lymph node. It has been suggested that patients with “pure” desmoplastic melanomas have a very low incidence of nodal metastasis and sentinel node biopsy may be unnecessary. Our experience has been that desmoplastic melanomas have a lower rate of nodal metastasis adjusted for tumor thickness, but that sentinel node biopsy is still indicated for desmoplastic lesions ≥1.00 mm in thickness. The role of sentinel node biopsy for the very rare thin desmoplastic melanoma is undefined.

**The relationship of tumor and host factors**

Although this discussion has focused primarily on those clinicopathologic prognostic factors found in the primary melanoma which predict risk of metastasis, it is highly likely that melanoma development and metastasis is influenced by both “seed” and “soil.” That is, host factors undoubtedly play a key role as well as tumor factors. For example, the development of melanoma is related to either acute or chronic ultraviolet light exposure, or both, as well as the host’s ability or inability to respond to that exposure in a protective manner. This has newly appreciated clinical relevance as well: melanomas that arise in the setting of solar elastosis (a “host” response to chronic ultraviolet exposure) appear to have a better overall outcome, as well as distinct genetic abnormalities compared to other melanomas. The tumor and host molecular factors underlying lymphangiogenesis in melanoma are at present almost totally unknown, but there is increasing preclinical and clinical evidence that nodal metastasis is associated with lymphangiogenesis.

Lymphangiogenesis can now be visualized and quantified by observing definable lymphatic endothelial cells in the region of the tumor, and it appears to correlate with melanoma nodal metastasis.

Over the next several years, we believe that improved biomarkers of melanoma nodal metastasis can be found through an improved understanding of the biologic processes involved in tumor growth and the host response that growth incites. For example, our current research has focused on STAT3 as a potential new biomarker. STAT3, phosphorylated to an activated form, leads to the transcription of a number of proteins important to cancer cell survival, and its activation is common in melanoma. When some of the potential consequences of STAT3 activation – increased tumor proliferation (increased mitotic rate), decreased host immune response (diminished or absent tumor infiltrating lymphocytes), and increased angiogenesis/lymphangiogenesis – are considered in the context of known predictors of melanoma metastasis, it becomes apparent that STAT3 activation could serve as a unifying biomarker explaining several observations. Incorporating an understanding of the host responsiveness to the effects of STAT3 activation potentially could allow this predictive model to be refined even further. To cite two concrete examples, it is now known that STAT3 activation leads to the production of VEGF (an angiogenesis/lymphangiogenesis factor) and IL-10 (an immunosuppressive cytokine). Polymorphisms in these proteins and/or their receptors are known to occur, and to influence the degree of host response to malignan-
cy their expression will generate.50,51 Thus, one could envision a model of tumor progression where primary melanomas with low levels of STAT3 activation have a low risk of metastasis and manifest concordant pathologic predictors: low mitotic rate, brisk tumor infiltration by lymphocytes and limited or no lymphangiogenesis, but primary melanomas with high levels of STAT3 activation have a variable risk of metastasis – and potentially manifest discordant pathologic predictors – based on host polymorphisms affecting sensitivity to the tumor-derived factors produced as a consequence of that activation. This important concept of tumor and host factors both contributing to biomarker utility deserves to be carefully evaluated in a large-scale, well characterized cohort of patients to determine if it has potential clinical relevance, independent of the factors currently known to be associated with sentinel node metastasis.

Riassunto
Fattori prognostici nel melanoma cutaneo localizzato
La biopsia del linfonodo sentinella rappresenta un metodo altamente accurato per la stadiazione dei pazienti con melanoma cutaneo localizzato. Lo stato del linfonodo sentinella rappresenta il predittore singolo più importante per quanto riguarda la recidiva del tumore e la sopravvivenza dei pazienti con melanoma clinicamente linfodema-negativo. Mentre questa procedura ha incontrato un ampio consenso nel trattamento dei pazienti con melanoma >1 mm di spessore, il suo utilizzo nei pazienti con lesioni più sottili è più controverso. In generale, in circa il 5% di tutti i melanomi con lesioni ≤1 mm è possibile identificare metastasi nei linfonodi sentinella al momento della diagnosi. Esiste un chiaro e non ancora soddisfatto bisogno clinico di avere a disposizione dei biomarcatori predittivi migliori per le metastasi regionali nei melanomi primitivi clinicamente localizzati, specialmente nei pazienti con melanomi sottili. Questa review riassume le attuali conoscenze circa la correlazione esistente tra i fattori prognostici clinici e istologici e il coinvolgimento del linfonodo sentinella nel melanoma, con particolare attenzione a questo gruppo di lesioni sottili e introduce il concetto dei polimorfismi dell’ospite così come dei fattori tumore-derivati quali biomarcatori potenziali per il miglioramento della nostra comprensione delle metastasi del melanoma.


References


32. Cook MG, Spatz A, Brocker E, Ruiter DJ. Identification of histological features associated with metastatic potential of thin (<1.0 mm) cutaneous melanoma with metastasis; a study on behalf of the EORTC Melanoma Group. J Pathol 2002;197:188-93.


It is an undoubted fact that melanoma may arise in melanocytic nevi, either of acquired or of congenital type. However, the magnitude of this risk is debated controversially. The decision towards a prophylactic excision, especially in the case of larger congenital melanocytic nevi (CMN), importantly depends on the assessment of the melanoma risk. In this article, scientific data from the literature are summarized to determine the exact role of CMN as melanoma precursors. First, clinical studies providing a systematic follow-up of patients with CMN are analysed. It is shown that those mostly smaller studies reporting a high incidence of melanoma probably underly selection bias. Furthermore, the melanoma risk strongly depends on the size of CMN and is highest in very large, so-called “garment” nevi. Second, the significance of histological studies focussing on remnants of “congenital” nevi in melanoma specimens is critically evaluated.

KEY WORDS: Melanoma, diagnosis - Melanoma, pathology - Nevus, pigmented.

CMN represent neural crest-derived hamartomas that are visible as pigmented lesions of the skin at or shortly after birth.\(^1\) The diameter of these lesions varies between a few millimeters and large parts of the body surface. The most common classification discriminates small (<1.5 cm), medium (1.5-20 cm), and large CMN (>20 cm), according to their largest diameter.\(^2\) CMN are found in 0.2% to 2% of newborn infants, mostly being very small. These small CMN later may hardly be distinguishable from melanocytic nevi acquired during childhood or adolescence. The incidence of larger CMN is much lower. Until now, only one study provided data on a cohort large enough to estimate this incidence. Castilla \textit{et al.} identified nevi \(\geq 4\) cm in 15:100 000 and nevi \(\geq 10\) cm in 5:100 000 South American newborns.\(^3\) In another population-based study, Berg and Lindelöf gave incidences of “small” and “large” CMN without properly defining these terms.\(^4\)

In 1963, Pers from Denmark published the first retrospective series of 110 patients with “giant” CMN, in which three cases of melanoma were found during 23 years of follow-up.\(^5\) Since then, numerous epidemiological studies, either prospective or retrospective, have been performed, reporting a melanoma risk of up to 42% in CMN.\(^6\) Moreover, several authors tried to assess this risk by determining the frequency of remnants of “congenital” melanocytic nevi in histological melanoma specimens. The goal of this article is to critically review the published data in order to better define the risk of melanoma in CMN.

\section*{Epidemiological studies}

The plenitude of scientific articles dealing with the melanoma risk of CMN makes it necessary to identify those articles bearing epidemiologically relevant
data. It is often difficult to define whether an article represents a selection of individual cases or a systematic case collection in the sense of a retrospective or prospective study. In a systematic analysis of the literature, we therefore decided that studies with less than 20 patients or with a mean follow-up of less than 3 years should be regarded as epidemiologically less significant. As the result of a literature search in Medline (1966-October 2005), 14 articles meeting these criteria were chosen for further analysis.4, 8-20

The studies varied significantly with respect to study design (source of cases; retrospective vs prospective analysis), age of patients, follow-up time, and nevus size. The proportion of melanomas in the 14 studies ranged from 0.05% to 10.7% of the CMN cases. Taken together, 49 melanomas were reported in 46 out of 6 571 patients (0.7%). The mean follow-up ranged between 3.4 and 23.7 years. The mean age of the patients at entry into the study ranged between 0 and 19 years. The sample size of the studies ranged between 39 and 3 922 patients. Importantly, smaller studies reported higher incidences of malignant transformation (P<0.0001). This finding strongly indicates selection bias. Cases from referral centres and retrospective case collections are likely to result in highly selected cohorts of patients that are at an increased risk for melanoma.

Melanoma of childhood is a very rare disease. It has long been reported that, in patients with CMN, a significant percentage of melanomas arises in early childhood.21, 22 In the 46 melanoma patients of our literature analysis, the mean age at diagnosis was 15.5 years (median: 7 years). This seems to confirm a maximum risk for melanoma in childhood and adolescence. However, most studies only included information between childhood and early adulthood; therefore, younger cases might have been relatively over-reported. By comparison with age-matched data from the Surveillance, Epidemiology, and End Results database (SEER) we calculated that patients with CMN carry an approximately 465-fold increased risk of developing melanoma during childhood and adolescence.

However, these general data are of limited value for the counselling of parents or patients regarding their individual risk. Assuming that the melanoma risk is probably influenced by the size or localisation of the nevus, it appears necessary to analyse subgroups of CMN. Some studies demonstrated that the vast majority of melanomas in their samples arose in very large CMN,8, 23 so-called “garment” nevi. Therefore, we analysed the data from the 14 selected studies with regard to the CMN sizes. For this subgroup analysis, we counted the numbers of large CMN (>20 cm diameter; LCMN) and garment nevi (GN) which we defined as a nevus situated on the trunk which measures >40 cm in largest diameter or is expected to reach this size in adulthood. In those studies that gave sufficient information on the CMN size (9/14 studies), 39 melanomas were reported in 1 539 LCMN (2.5%). The number of GN was stated in only 3/14 studies.8, 9, 18 In these three studies, the proportion of melanomas was 3.1% (20/636). Compared to the overall incidence of 0.7% in all 14 studies, this obviously shows a higher incidence of melanoma in LCMN and GN. Additionally, we analyzed the size of the underlying CMN in the melanoma cases. Sufficient clinical information was available in 41/46 melanoma cases. In 30/41 cases (73.2%), the patients had a GN; in only 5/41 cases (12.2%), the patients had a non-garment LCMN. These figures underscore that the risk of malignancy is markedly elevated in GN in comparison to non-garment LCMN.

Although the above-mentioned data suggest a correlation between nevus size and risk magnitude, the risk of small CMN remains to be systematically evaluated. Additionally, it remains to be investigated whether this risk exceeds that of acquired nevi of the same size. Earlier reports that suggest relatively high incidences of melanoma in small CMN certainly require revision because their data are based on histological rather than epidemiological findings.

Given that melanoma in CMN patients may also develop in deep structures or at extracutaneous sites, removal of the CMN is no guarantee to protect the patients against melanoma. Moreover, larger CMN may be too large to be completely excised. Therefore, the impact of prophylactic surgical or other therapeutic measures, e.g., dermabrasion, on the risk of melanoma is difficult to assess. Each technique that removes significant numbers of melanocytic cells certainly reduces this risk. However, a quantitative analysis is hampered by the heterogeneity of the studies concerning mode and extent of prophylactic therapy.

**Histological studies**

CMN are defined as melanocytic nevi clinically detectable either at birth or, at the latest, during the first weeks of postnatal life. Histological criteria for the
diagnosis of CMN irrespective of their size have been originally suggested by Mark et al.26 These include: 1) presence of nevus cells in the lower two thirds of the reticular dermis or in the subcutis; 2) disposition of these deep nevus cells between collagen bundles singly or inIndian files; 3) involvement of skin appendages, nerves and vessels (i.e. presence of nevus cells inside the epithelium, perineurium or vessel wall). In their study, the first two of these criteria were encountered in 59 out of 60 anamnestically congenital lesions and only in 2 out of 60 acquired nevi from the control group. Similar findings were presented by Walsh et al.27 Walton et al. in 1976, were the first to demonstrate that smaller CMN often lack the above-mentioned histological characteristics.28 Accordingly, Barnhill and Fleischli 29 demonstrated a direct correlation between the size of the lesion and the depth of nevus cell infiltration. Other groups confirmed that: 1) acquired nevi often exhibit congenital histological features; 2) congenital nevi may present with predominantly junctional histological changes.32-39 In contrast, when only the subgroup of larger CMN is regarded, histological findings are much more sensitive and specific, and provide some evidence that these nevi are based on a distinct pathogenic background. Recently, Saida proposed that the pathogenesis may be essentially the same in small congenital and acquired melanocytic nevi, because both develop from melanocytes situated at the dermo-epidermal junction, whereas larger CMN are derived from migrating neural-crest cells, so-called melanoblasts.40 A common molecular finding in small congenital and acquired melanocytic nevi is the presence of BRAF-mutations, which are much less frequent in larger CMN.41 Similarly, Zalaudek et al., based on dermoscopic findings (globular/cobblestone pattern), have proposed that small CMN and acquired melanocytic nevi detectable during childhood should be regarded as a pathogenic entity.42

Taken together, despite certain typical histological patterns in (mostly larger) congenital nevi, the diagnosis of congenitalness is not possible solely on histological grounds. Regarding the assessment of melanoma risk, a histological rather than clinical definition of CMN has produced significant confusion. Most importantly, several authors counted congenital features of nevus remnants in specimens of malignant melanoma and concluded that congenital nevi, irrespective of their size, carry an increased risk for the development of melanoma.24, 25 It is an undoubted fact that a melanoma may arise in congenital (and acquired) nevi of any size.43 However, as shown above, a statistically increased risk of malignancy has only been proven for larger CMN. The risk of malignant degeneration in small and medium-sized congenital nevi is definitely not estimable by histological studies of malignant melanoma showing remnants of congenital nevi. Unlike other authors,44 we believe that the mere fact of the congenital presence of a small or medium-sized melanocytic nevus does not justify treatment decisions.

Conclusions

Epidemiologically, the rarity of (large) CMN and the heterogeneity of studies only allow a rough assessment of the risk of melanoma. However, the overall risk seems to be lower than it has long been suspected. The higher incidence of melanomas in smaller studies indicates selection bias. The risk strongly depends on the size of the CMN and is highest in those nevi traditionally designated as “garment” nevi. In these nevi, preliminary data point to a maximum risk in childhood and adolescence. Regarding the relatively short follow-up in most of these studies, conclusions on the lifetime risk of melanoma should always be drawn with caution.

The fact that nearly 3 of 4 melanomas in our systematic analysis appeared in very large, garment CMN, leads us to propose that larger CMN should be subdivided into distinct groups for clinical handling and for further studies. One big advantage of the Kopf classification is that it is very easy to assess the largest diameter (although the measurement of the nevus area would be more accurate to describe the size). Therefore, we agree with Ruiz-Maldonado,45 who recently recommended to classify CMN according to their largest diameter as follows: small <1.5 cm; medium 1.5-10 cm; large 11-20 cm; giant G>20 cm; G1 21-30 cm; G2 31-40 cm; G3 >40 cm (patients with giant nevi and with more than 50 small or medium-size satellite nevi should be classified one group above their corresponding size classification).

It is not possible to deduce the risk of melanoma in CMN from histological studies on melanoma specimens. Eventually, similar studies will get feasible when better molecular markers for CMN and CMN subgroups, respectively, have been defined. Until then, the term “congenital nevus” comprises a heteroge-
Rischi di melanoma nei casi con nevi melanocitici congeniti di grandi dimensioni. La decisione di ricorrere ad una escissione profilattica dipende strettamente dalla valutazione del rischio di comparso di melanoma. In questo articolo vengono riassunti i dati presenti nella letteratura scientifica, per determinare l’esatto ruolo dei CMN quali precursori del melanoma. Anzitutto sono stati analizzati gli studi clinici che hanno avuto un follow-up sistematico dei pazienti con CMN. Si è dimostrato che gli studi, spesso con scarsa numerosità, che hanno evidenziato un’elevata incidenza di melanoma, presentavano probabilmente degli errori di selezione. Inoltre, il rischio di melanoma dipende strettamente dalla dimensione del CMN ed esso è maggiore per i nevi molto grandi, i cosiddetti nevi “giant pigmented nevi”. In secondo luogo è stato valutato criticamente il significato degli studi istologici focalizzati sui resti dei nevi “congeniti” nei campioni biopatici di melanoma.

Parole chiave: Melanoma, diagnosi - Melanoma, anatomia patologica - Nevi pigmentati.

References
32. Rhodes AR, Silverman RA, Harrist TJ, Melski JW. A histologic com-


The incidence and mortality of melanoma is steadily increasing and therapies for advanced melanoma lack efficacy. Melanoma has been proposed to originate via a series of stepwise genotypic and phenotypic changes—these stepwise changes create targets for chemoprevention. Investigation of multiple novel agents that block UV radiation, prevent activation of oncogenes and oxidative stress, exploit apoptosis, and boost the immune system suggest promising strategies for melanoma chemoprevention. A better understanding of cancer biomarkers of toxicity and drug efficacy, improved chemoprevention trial design, and heightened public awareness of the benefits of cancer prevention will facilitate efficient identification of useful chemopreventive agents. Because no agent yet emerges as a clear choice for effective melanoma chemoprevention, advising patients to avoid excessive sun exposure remains the mainstay of melanoma prevention for persons at high risk. This review summarizes recent evidence regarding potential melanoma chemopreventive agents and discusses current barriers to providing chemoprevention to patients at high risk of developing melanoma.

**KEY WORDS:** Melanoma - Chemoprevention - Photoprotection - Hydroxymethylglutaryl-CoA reductase inhibitors - Diet - Immunologic factors - Retinoids - Vaccines - Neoplasms, prevention and control.

---

**Funding**—This review was supported by the Dermatoepidemiology Research Unit of the Department of Dermatology at the University of Colorado, by the University of Colorado Cancer Center, by grant K-07 CA92550 (R.P. Dellavalle) from the National Cancer Institute, and by grant T32 AR07411 (S. Freeman) from the National Institutes of Health, Bethesda, MD.

**Conflicts of interest**.—Dr. Dellavalle owns 100 shares of Merck common stock in a retirement account. Merck makes the statin drug lovastatin.
with men over 50 counting for around two thirds of these deaths. The incidence rate for melanoma in the United States has more than doubled from 5.7% to 14.3% in the past 30 years.

Risk factors strongly associated with development of melanoma are personal history of melanoma or multiple benign or atypical nevi, presence of associated genetic abnormalities and family history of melanoma. Other risk factors include skin phototypes I and II, intermittent UV light exposure, blistering burns during childhood, large congenital nevi over greater than 5% of the body surface area, immune suppression, older age, male gender, higher socioeconomic status, equatorial latitudes, and inheritance of rare DNA repair defects (Xeroderma Pigmentosum).

Epidemiologic observations suggest that chronic or low-grade exposure to UV light induces protection against DNA damage, whereas intense, intermittent exposure causes genetic damage.

Patients with thin melanomas who receive early treatment experience excellent outcomes, with 10-year metastasis-free survival rates between 91% and 100%. Treatment modalities for advanced melanoma are largely ineffective; they provide modest survival benefit and can be associated with serious side effects.

The results of multivariate analyses using a database derived from records of 17,600 patients with melanoma from 13 cancer centers and cooperative groups from North America, Europe, and Australia identified low survival rates for patients with advanced stages of melanoma. Melanoma patients with nodal metastases had five-year survival rates ranging from 69% for patients with nonulcerated melanomas who had a single clinically occult nodal metastasis to 13% for patients with ulcerated melanomas who had four or more clinically apparent nodal metastases. Survival rates for patients with metastatic disease, unfortunately, is often measured in months; few survive beyond one year.

Educational and melanoma screening programs have been proposed to improve melanoma outcomes. While these systems are appealing, worldwide health initiatives including physician directed and patient self-assessment screening programs have so far failed to provide conclusive data on effectiveness. The steady increase in melanoma incidence and mortality coun-

---

### Table I.—Possible melanoma chemoprevention agents and proposed mechanisms of action.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Photoprotection</th>
<th>Anti-oxidant</th>
<th>Anti-inflammatory</th>
<th>Pro-apoptosis</th>
<th>Anti-proliferative (Cell cycle arrest)</th>
<th>Anti-angiogenesis</th>
<th>Immunomodulatory</th>
<th>Oncogene inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunscreen</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forskolin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMG-CoA reductase inhibitors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPAR-alpha inhibitors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorafenib</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cox-2 Inhibitors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRM *</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perillyl alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGC2*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-carotene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lycopene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resveratrol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apomine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curcumin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFMO **</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betulinic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genistein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4NV °°</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Immune response modifiers; Imiquimod and Resiquimod; °Epigallocatechin 3-gallate; **Difluoromethylornithine; °°T4 endonuclease V.
pled with the meager efficacies of current interventions for advanced disease has spawned great interest in prevention of this disease.

Chemoprevention

The Clark model of melanoma details the cellular changes that occur as normal melanocytes progress in a stepwise manner toward malignancy. The proliferation of melanocytes resulting in the formation of nevi, and the subsequent development of dysplasia, hyperplasia, invasion, and metastasis observed in the growth phases of melanoma is hypothesized to correspond to the accumulation of genetic mutations. The presence of such stepwise histologic changes are linked to specific biologic and molecular changes within the transforming melanocytes that have created new targets for therapeutic intervention. Melanoma chemoprevention is being explored as one such intervention aimed at targeting these molecular events.

Chemoprevention is a novel approach to cancer management and can be defined as the use of natural or synthetic agents to reverse, suppress, or prevent molecular or histologic premalignant lesions from progressing to invasive cancer. The first approved medication for the prevention of breast cancer in women at high risk was Tamoxifen, though other chemopreventive agents have followed including Celecoxib for Familial Adenomatous Polyposis syndrome, Diclofenac for actinic keratoses, retinoids for head and neck cancers and Imiquimod for actinic keratoses and superficial basal cell carcinoma. Gardesil, a vaccine proven 89% effective in guarding against 4 common strains of human papillomavirus, was recently approved for use in the United States. This vaccine could prevent many of the 300 000 cervical cancer related deaths that occur worldwide each year.

Recent discoveries in melanoma biology have illuminated many of the biochemical pathways this cancer depends upon for malignant transformation, local invasion and metastasis and have identified a myriad of potential targets of interest from a chemoprevention standpoint. A variety of natural and synthetic agents that block ultraviolet radiation, prevent activation of oncogenes and oxidative stress, exploit apoptosis, and boost the immune system have been suggested as promising strategies that may delay, reverse, suppress, or prevent pre-malignant lesions from progressing to cancer.
This review summarizes recent evidence regarding potential melanoma chemopreventive agents (Tables I, II) and discusses current barriers to providing chemoprevention to patients at high risk of developing melanoma.

**Melanoma chemoprevention agents and mechanism of action**

**Sunscreen**

Sunscreen is frequently recommended as a UV protective compound with presumed skin cancer protective properties, though its association with melanoma is unclear. Meta-analyses of observational studies have failed to show an inverse relationship between sunscreen use and melanoma. Results from individual studies included in the meta-analyses are inconsistent, with some studies reporting positive, negative or no association between sunscreen use and melanoma risk. The lack of conclusive quantitative evidence is not surprising given the retrospective nature and large methodological discrepancies of many of the included studies. Variability in sunscreen application practices, sun exposure duration and frequency, and inferior UV protection provided by the sunscreens studied likely impacted the results of these studies.

In spite of this controversy, photoprotection is currently a mainstay of melanoma protection, with appropriate sunscreen use and peak hour sun avoidance being ideal photoprotective measures. Emphasis should also be placed on comprehensive sun protection with UV protective clothing, sunglasses and use of shade structures while outdoors. It is currently unclear whether UVA, UVB, or both forms of radiation are significant in melanoma, though a recent mouse model suggests that UVB may be most important. Sunscreens protecting against both UVA and UVB are strongly recommended and agents including Parsol 1789 and titanium dioxide are effective in providing this coverage. Both animal and human studies have demonstrated that sunscreens reduce UV-induced immunosuppression, and animal studies have been shown to protect mice from p53 mutations. The association between sunscreen use and the development of nevi has not been confirmed and results of several studies have reported conflicting data. It is reassuring that a recent randomized controlled trial found that children wearing broad-spectrum SPF30 sunscreen developed fewer nevi compared to control group children, particularly since nevi are a surrogate for melanoma risk.

**Forskolin**

Decreased ability to tan in response to UV light is associated with an increased susceptibility to melanoma. Melanin synthesis and distribution controls pigmentation and alpha-melanocyte stimulating hormone (α-MSH) is the main physiologic regulator of skin pigmentation. Binding of α-MSH to the melanocortin 1 receptor (MC1R) leads to increased intracellular signal transduction through the cyclic-AMP pathway (c-AMP), causing increased expression of enzymes responsible for generation of melanin. Alterations in melanin synthesis that result in defective UV-induced tanning response have been documented in many persons with fair skin. Although melanin synthesis can be disrupted at any step, functional changes in the MC1R have been shown to have a strong association with fair skin and weak tanning responses to UV light exposure. Forskolin, a labdane diterpene produced by the Indian Coleus plant Plectranthus barbatus can affect melanin synthesis and is being studied as a potential chemopreventive agent. Forskolin resensitizes melanocortin-1 receptors by activating the enzyme adenylyl cyclase which increases the intracellular levels of cAMP and ultimately leads to increased melanin synthesis. A recent study showed that mice with inactivating mutations in MC1R that were unable to utilize UV to form pigment became pigmented after topical application of forskolin. Further investigation with mice carrying xeroderma-pigmentosum-complementation-group-C-deficient (Xpc-/-) revealed that mice treated with forskolin had a decreased incidence of tumorigenesis. Forskolin holds chemopreventive promise should it induce melanogenesis in human skin without promoting melanoma.

**Lipid lowering medications**

The oncogenes N-RAS and BRAF are strongly associated with melanoma development and somatic mutations of these genes can cause abnormal activation of the mitogen-activated protein kinase (MAPK) pathway and stimulate tumorigenesis through the constitutive activation of serine-threonine kinases.
This signaling pathway represents an important target for melanoma chemoprevention. Medications known as statins inhibit the rate-limiting enzyme Hydroxymethylglutaryl-coenzyme A Reductase (HMG-CoA) in the cholesterol synthesis pathway, thereby lowering cholesterol. These medications are used to treat hypercholesterolemia and prevent cardiovascular diseases. Surprisingly, randomized controlled trials studying cardiovascular disease reported a statistically significant decrease in melanoma and other cancers in patients taking statins. Subsequently a multitude of studies have reported pro-apoptotic, anti-inflammatory, immunomodulatory and anti-angiogenic effects of statins on tumor cells. For example, statins affect the RAS signaling pathway by preventing the activation of RAS proteins. The oncogenic properties of RAS proteins depend on isoprenylation—they must have a farnesyl and geranyl groups attached to accelerate cell growth and cell cycle progression. The cholesterol synthesis pathway is integral to the isoprenylation of RAS proteins and by inhibiting HMG-CoA Reductase, statins prevent the attachment of key pro-tumor control groups.

No consensus exists on the effectiveness of statins as antineoplastic agents, either as a therapeutic or chemopreventive. Recently statins have been proposed by a limited case control study as possibly exacerbating bladder cancer. In the case of melanoma, a randomized control trial of lovastatin found a decreased incidence of melanoma in patients exposed to the drug, while a case-control study reported no association. Additionally, meta-analyses of randomized controlled trials of melanoma found no association between melanoma and standard low doses of statins.

In vitro and animal studies of statins provide strong evidence of the melanoma killing potential of statins and further investigation is needed to reveal their role in melanoma prevention. Large randomized control trials of lipid lowering medications have also found decreased incidence of melanoma in patients exposed to fibrate medications. These medications inhibit peroxisome proliferator-activator receptors (PPAR) but the mechanisms by which they may perhaps retard or prevent the development of melanoma are largely unknown. Meta-analyses of studies of these medications have failed to identify an association between fibrates and melanoma.

Other agents that exhibit chemopreventive properties in melanoma by acting on similar pathways include perillyl alcohol and apomine. Perillyl alcohol, which is found in fruits and vegetables, has been shown to inhibit the farnesylation of RAS in vitro. Apomine is a member of the family of cholesterol synthesis inhibitors known as 1,1-bisphosphonate esters. This compound has been shown to increase the rate of HMG-CoA Reductase degradation, decrease proliferation and induce apoptosis in tumor cells at lower concentration than statins.

Sorafenib

BRAF mutations lead to constitutive activation of serine-threonine kinases in the MAPK pathway and are detected in up to 66% of melanomas. The high prevalence of BRAF mutations highlights their central role in melanoma development and makes all associated pathways appealing targets for chemoprevention. Sorafenib is an oral multikinase inhibitor that inhibits Raf kinase and receptor tyrosine kinases, thereby specifically targeting the Raf/extracellular signal-regulated kinase (ERK) kinase (MEK)/ERK signaling pathway. Inhibiting Raf/MEK/ERK signaling leads to apoptosis in melanoma cells and clinical data on sorafenib demonstrates significant activity against neovascularization and tumor progression.

Epigallocatechin-3-gallate

Epigallocatechin-3-gallate (EGCG) is a polyphenolic compound present in green and black teas and grape seeds and possesses both antioxidant and sunscreen activity. EGCG has been shown to decrease cell proliferation, increase apoptosis, and inhibit the colony forming ability of multiple melanoma cell lines, and has little effect on healthy melanocytes. Multiple murine models report strong photoprotective properties, especially against UVB radiation, and have shown that EGCG reduces the incidence of skin cancers in mice exposed to UVB radiation.

COX-2 inhibitors

Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit cyclo-oxygenase (COX) enzymes (COX-1 and COX-2), thereby preventing the formation of
prostaglandins and other pro-inflammatory molecules.\textsuperscript{17, 78} UVB radiation has been shown to increase COX-2 expression and subsequent prostaglandin production like prostaglandin E2 (PGE-2) in the epidermis and studies have demonstrated a strong correlation with increased PGE-2 levels and the development and metastasis of cancer.\textsuperscript{17, 79-82} The COX pathway may prove to be a key component in melanoma tumor development, thus making NSAIDS potential candidates for melanoma chemoprevention.\textsuperscript{17, 78, 83} Mouse models have shown the selective COX-2 inhibitor celecoxib to be effective both at increasing skin cancer latency and limiting tumor multiplicity.\textsuperscript{24} Case-control and retrospective studies in humans demonstrate decreased incidence, recurrence rates, and metastasis of melanoma in patients prescribed a COX-inhibitor.\textsuperscript{84}

\textbf{Retinoids}

Retinoid is a term used to describe vitamin A (retinol) and all of its derivatives.\textsuperscript{24} Retinoids bind to all-trans retinoic acid receptors (RAR) and rexinoid receptors (RXR) in nuclei, and control epidermal differentiation, proliferation, and apoptosis.\textsuperscript{85} Clinical trials of oral retinoids in patients at moderate to high risk for nonmelanoma skin cancer have demonstrated significantly lower risk of developing a first new squamous cell carcinoma (SCC) when compared to placebo group.\textsuperscript{24, 79} Due to melanoma’s relative resistance to apoptosis, retinoids have been proposed as possible chemoprevention agents.

\textbf{Difluoromethylornithine}

Difluoromethylornithine (DFMO) is an irreversible inhibitor of ornithine decarboxylase (ODC).\textsuperscript{24} ODC is the rate-limiting enzyme in the synthesis of polyamines, a process thought to be critical to the formation and survival of several cancers.\textsuperscript{24, 86} DFMO studies in mice have shown this agent effective in preventing epidermal tumors, and Phase I studies in humans are underway.\textsuperscript{24, 87}

\textbf{Resveratrol}

Resveratrol is a plant polyphenol found in grapes, red wine, berries, and peanuts inhibits the growth of various tumor cell lines, including melanoma and squamous cell carcinoma.\textsuperscript{88} The mechanisms through which resveratrol acts are thought to be linked to cell cycle arrest and angiogenesis suppression.\textsuperscript{88, 89} Besides chemopreventive effects, resveratrol also appears to exhibit therapeutic effects against melanoma cells in vitro.\textsuperscript{90} Resveratrol has been shown safe in humans and analogues of this drug are being studied with emphasis on improved bioavailability.\textsuperscript{17, 88}

\textbf{Vaccines}

Tumor-associated antigens (TAAs) include non-mutated, overexpressed, or inappropriately expressed tissue differentiation antigens and are increasingly being targeted in laboratory and clinical cancer research.\textsuperscript{17} Melanoma cells are immunogenic and TAAs have been identified allowing for the specific targeting of malignant cells.\textsuperscript{17, 91} The aims of current vaccine systems in melanoma include enhancing cellular and humoral responses, improving melanoma antigen presentation to antigen presenting cells like dendritic cells and combat local immunosuppressive effects that melanoma has on tissues.\textsuperscript{92} Melanoma vaccines being studied are diverse, including polyclonal agents created from autologous and allogeneic tumors, antigen specific vaccines and DNA based vaccines.\textsuperscript{92} Challenges facing melanoma vaccines include immunologic recognition escape common to malignancies and development of effective \textit{in vivo} vaccine delivery systems.\textsuperscript{92, 93} Multiple phase I, II and III clinical trials have been performed utilizing various vaccination approaches.\textsuperscript{92, 94} Despite encouraging success in phase I and II trials, the outcomes from phase III trials have largely been disappointing, though additional studies are ongoing.\textsuperscript{17}

\textbf{Immune response modifiers}

Another approach to preventing and treating cutaneous malignancy is the use of Immune Response Modifiers (IRMs). IRMs include imiquimod and resiquimod and these agents are able to stimulate both the innate and acquired immune responses leading to the local production of interleukins (ILs), interferons (IFNs) and tumor necrosis factor alpha (TNF-α).\textsuperscript{44} Subsequently, monocytes, macrophages, toll-like receptor-7 bearing dendritic cells, and cytotoxic T-lymphocytes are activated and this activity is thought to increase the skin’s ability to fight cancer cells.\textsuperscript{44, 95} Imiquimod is a member of the imidazoquinoline family and the 5\% cream preparation is currently approved for the treatment of actinic keratoses, squamous cell
carcinoma in situ, superficial basal cell carcinoma and genital warts.\textsuperscript{96, 97} Imiquimod has been successful in 2 small studies at treating lentigo maligna (LM), a precursor to melanoma.\textsuperscript{98, 99} The largest of these studies was an open-label case series that included 30 patients with histologically proven LM. After daily application of 5% imiquimod for 3 months, 93% of subjects experienced complete clinical and histological clearance.\textsuperscript{44, 99} A smaller study (6 patients) looked at the effectiveness of topical imiquimod 5% at treating LM when used once daily for 13 weeks. There was a 100% response rate in all 6 subjects and no recurrences at 18-month follow-up.\textsuperscript{98}

Resiquimod is an analogue of imiquimod that is being investigated for treatment of actinic keratoses.\textsuperscript{44} Like imiquimod, this medication stimulates the production of ILs, IFNs and TNF-$\alpha$, but unlike imiquimod, resiquimod has not been shown to induce apoptosis in basal cell carcinoma and melanoma cells in vitro.\textsuperscript{44}

**T4 endonuclease V**

The accumulation of UVB-induced pyrimidine dimers in DNA is carcinogenic and can lead to the malignant transformation of epidermal cells.\textsuperscript{24} T4 endonuclease V (T4NV) is a bacterial DNA repair enzyme proven to accelerate the removal of harmful photoinduced DNA adducts and is currently being studied in humans as a potential chemopreventive agent. Topical T4NV effectively decreases skin tumors in UVB exposed mice, and a study in patients with xeroderma pigmentosum found decreased incidences of both actinic keratoses and basal cell carcinomas in patients exposed to this medication topically.\textsuperscript{24, 40, 41} An ongoing phase II clinical trial is underway to measure the chemopreventive efficacy of topical T4NV in renal transplant patients.\textsuperscript{24}

**Other agents**

Many agents exist that have limited evidence in melanoma chemoprevention. These include vitamin E, beta-carotene, selenium, lycopene, curcumin, genistein, vitamin D, flavonoids (found in fruits and vegetables), silymarin (found in milk thistle) and betulinic acid (found in bark and roots of certain flora). Of these compounds, vitamin E, beta-carotene and selenium have been studied specifically for their roles in photodamage and photocarcinogenesis and found ineffective.\textsuperscript{24} Lycopene, which gives some fruits and vegetables a red color, is an antioxidant that exhibits antitumor properties in mice exposed to UVB.\textsuperscript{24} Curcumin (diferuloylmethane) is found in many foods and is derived from the root of the *Curcuma longa* plant. While studies in mice suggest that this compound has antitumor properties, its mechanism of action is largely unknown and studies in humans are lacking.\textsuperscript{24} Genistein is a phytochemical antioxidant found in high concentration in soybeans. This compound is chemopreventive in mice exposed to UVB and at least one small study suggests that it is photoprotective in humans.\textsuperscript{24}

The role of vitamin D in cancer prevention is a controversial issue. Studies demonstrating decreased risk of non-Hodgkin’s lymphoma and improved survival in patients with non-metastatic melanoma in which chronic sun damage was present have led many to cogitate that vitamin D is responsible.\textsuperscript{100-102} Data from an Australian study suggests that sun exposure may have a positive effect on melanoma outcome, but linking this to vitamin D is premature.\textsuperscript{17, 103}

**Discussion**

Melanoma incidence and mortality is increasing and current therapies for advanced disease are largely ineffective.\textsuperscript{15} Molecular studies have described the step-wise genotypic and phenotypic transformations that takes place in UV-induced melanoma and have identified potential molecular targets for chemoprevention.\textsuperscript{11} Effective prevention strategies could save countless lives, though no agents currently exist to fill the need. While some of the agents targeting these pathways were reviewed in this article, most are still in the preclinical stages of investigation. Studies of melanoma agents in humans have been, for the most part, limited to retrospective studies which are subject to recall bias and other confounding factors.\textsuperscript{17}

While policy makers remain open to chemopreventive strategies and understand the potential benefits these agents may confer to populations at risk for melanoma, they require more conclusive evidence.\textsuperscript{18} Efficient generation of convincing evidence for an agent’s chemopreventive effect will require careful attention to the hurdles preventative medicine faces. Current research environments focus mainly on therapeutic interventions for established diseases, not disease prevention, and the public has very little understanding of the importance and potential of cancer preventive science.\textsuperscript{21} The lack of public knowledge...
and the relatively small investment in chemopreventive research at industry and government levels further impede cancer preventive research because the studies needed to prove benefit are complex, expensive, long and require many subjects. Campaigns aimed at improving the public knowledge of cancer prevention science should be initiated in order to ensure research study recruitment and aid in dissemination of important trial findings.

Clear designs for clinical research models should be established. Of great importance is safety. Given that cancer prevention trials by nature will include healthy individuals followed for considerable lengths of time, the tolerance for adverse events associated with trial drugs is very low. Effort must be made to establish safe, effective dosing regimens based on information collected from transgenic murine models. Ideal chemoprevention trials should utilize molecular safety biomarkers that allow close surveillance of drug associated toxicity and disease specific biomarkers that allow for identification of those at risk and are linked to disease occurrence or progression.

Such biomarkers need to be identified and validated. Better understanding and identification of those at risk for developing melanoma should allow for smaller studies that still possess the power to generate accurate assessment of drug benefit. Chemoprevention of cancer, including melanoma, is in its infancy, but significant progress is being made. Evidence from in vitro, animal, and epidemiologic studies continue to identify key melanoma pathways and raise the possibility that a chemopreventive agent is feasible. New insights into the normal physiologic tanning response to UV radiation may help identify a new class of chemopreventive agents, but until a melanoma chemoprevention agent is found, advising patients to avoid excessive sun exposure remains the most reasonable tool available to prevent melanoma.

Currently, there is no evidence proving that provider or patient skin examinations prevent melanoma but recent studies show these interventions are cost-effective, logistically possible and effective at targeting at-risk populations.

Riassunto

Chemoprevenzione del melanoma

L’incidenza e la mortalità del melanoma sono costantemente in aumento e le terapie per il melanoma avanzato per-dono efficacia. È stato proposto che il melanoma sia il risultato di una serie di modificazioni successive di tipo genotipico e fenotipico; queste modificazioni successive possono diventare dei bersagli per la chemioprevenzione. La ricerca su nuovi e diversi agenti che bloccano la radiazione UV, che prevengono l’attivazione degli oncogeni e lo stress ossidativo, che sfruttano l’apoptosi e che amplificano la risposta immunitaria, ha suggerito strategie promettenti per la chemioprevenzione del melanoma. Una migliore comprensione dei biomarcatori cancerosi di tossicità e di efficacia farmacologica, il miglioramento del disegno degli studi clinici sulla chemioprevenzione e l’attenzione pubblica sempre più alta circa i benefici della prevenzione tumorale faciliteranno l’identificazione degli agenti chemiopreventivi più efficaci. Dal momento che attualmente non è ancora stato identificato un agente chiaramente efficace per la chemioprevenzione del melanoma, l’avvisare i pazienti di evitare di esporsi eccessivamente alla luce solare resta la forma principale di prevenzione del melanoma per le persone ad alto rischio. Questa review riassume le evidenze relative ai potenziali agenti chemiopreventivi del melanoma e discute le attuali difficoltà nel mettere in atto la chemioprevenzione in pazienti ad alto rischio di sviluppare melanoma.

PAROLE CHIAVE: Melanoma - Chemoprevenzione - Fotoprotezione - Statine - Dieta - Fattori immunologici - Retinoidi - Vaccini - Neoplasie, prevenzione e controllo.

References


78. Harris RE, Beebe-Donk J, Doss H, Burr Doss D, Aspinir, Ibufrofen, and other non-steroidal anti-inflammatory drugs in cancer preven-

79. Wright T, Spencer JM, Flowers FP. Chemoprevention of non-


84. Ramírez CC, Ma F, Federman DG, Kirsner RS. Use of cyclooxy-

85. Hansen LA, Sigman EC, Andreola F, Ross SA, Keloff JG, De Luca LM. Retinoids in chemoprevention and differentiation therapy. Car-

86. Weiss RL, Calhoun KH, Ahmeal AE, Stanley D. Ornithine decar-


The clinical experience of cancer vaccines for malignant melanoma

M. S. BLOCK 1, S. N. MARKOVIC 2

Much effort has been made to improve systemic treatment of advanced melanoma. However, clinically meaningful prolongation of survival in patients with metastatic melanoma remains elusive. Anecdotal reports of spontaneous cancer regressions as well as durable remissions following immune modulating therapies (e.g., interleukin-2, interferon alpha, anti-CTLA4) continue to support the effort and hope that effective immune therapy of melanoma is an achievable goal. One approach in this effort has been the attempt to improve immune destruction of melanoma through active immunotherapy via vaccination against melanoma antigens. A broad range of strategies have been employed, from single peptide vaccinations to injections of modified dendritic cells; many of these vaccines have been tested in clinical trials in melanoma patients. Herein, we review the breadth of clinical trials conducted to date using vaccine based therapy of melanoma.

KEY WORDS: Melanoma - Skin - Vaccines.

The incidence of malignant melanoma is increasing at an alarming pace, faster than any other form of cancer.1 When diagnosed early, melanoma is curable by simple surgical resection. However, when diagnosed after skin lesions have invaded more than 1 mm beyond the granular layer of the epidermis (T2 and greater), melanoma has a high propensity to metastasize to regional lymph nodes and subsequently to visceral organs. When metastatic, melanoma is among the most difficult of cancers to treat, as it is generally resistant to all current forms of chemotherapy.2 This lack of effective forms of chemotherapy has spurned investigators to turn to novel strategies in melanoma treatment.

While metastatic melanoma is nearly impossible to cure with chemotherapy, primary tumor sites of cutaneous melanoma frequently demonstrate histologic and clinical evidence of spontaneous partial regression. Complete regressions are rare, but documented full regressions of both primary cutaneous melanoma and metastatic melanoma have been reported. A common histologic feature of clinically regressing cutaneous melanoma is the presence of cytotoxic T lymphocytes in the regressing lesions.3 Likewise, autoimmune phenomena such as vitiligo frequently accompany spontaneous regression of melanoma. This suggests that patients’ immune systems are capable of destroying malignant melanocytes. These data in addition to a voluminous preclinical literature demonstrating the responsiveness of murine melanoma tumor models to immunomodulatory/vaccine therapies serve as the basis for the ongoing clinical trial efforts in...
developing effective immune/vaccine based therapy for melanoma in humans.

Several factors must be considered in the development of an effective melanoma vaccine. First, the vaccine must provide tumor-associated antigens (TAA) that the patient’s immune system can recognize. TAA used in therapeutic vaccines may be highly defined peptide or glycolipid moieties, or may be a host of proteins found in cell lysates or irradiated cells. Peptides generally stimulate cellular (T cell-mediated) immunity, while glycolipids (commonly gangliosides) stimulate humoral (antibody-mediated) immunity; protein antigens may generate either cellular and/or humoral immunity. Second, the TAA must be presented in a manner leading to generation of a potent immune response. This may be accomplished by the use of immune stimulants (adjuvants), coadministered cytokines, or by providing antigen presenting cells (APC) such as dendritic cells (DC) as part of the vaccine treatment. Finally, an effective vaccine depends on the ability of the patient’s immune system to mount an effective immune response, as well as on the tumor being susceptible to immune-mediated destruction. Patients who have lost immune responsiveness will have a poor clinical response to vaccination even when given an optimal vaccine. Thus, for a vaccine against melanoma to be clinically effective, appropriate TAA must be presented in an adequate immune-stimulatory context to a patient capable of mounting an effective immune response.

**Vaccines that use endogenous antigen presentation**

**Tumor lysates**

Among the first antigen sources used in clinical trials of melanoma vaccines were lysed melanoma tumor cells. Tumor lysate-based vaccines provide multiple TAA from killed melanoma cells. Early trials of whole lysates or cell membranes that were lysed and formed into liposomal preparations showed little efficacy, with few clinical responses. Thus, tumor lysates alone have not generally been regarded as effective vaccines.

Adjuvants have been used in an attempt to enhance the immunogenicity of tumor cell lysates. One preparation known as Melacine® combines lysates from two melanoma cell lines with a modified Freund’s adjuvant. Phase III trials in stage IV patients comparing Melacine® to standard chemotherapy with BCNU plus tamoxifen or to interferon-α-2b monotherapy failed to demonstrate a survival benefit for patients receiving vaccine therapy. When Melacine® was compared to observation in the adjuvant setting, no significant difference in progression-free survival was found, although retrospective analysis showed benefit in patients with certain HLA alleles. Unfortunately, discouraged by the overall results of Melacine® in clinical testing, further development has been abandoned.

Because viral infections generate significant cellular immune responses, several trials have been conducted in which melanoma cell lines were incubated with viruses prior to lysis. In phase III trials in stage II and stage IIB and III melanoma patients, adjuvant immunization with lysates from allogeneic melanoma cell lines incubated with vaccinia virus was compared to placebo. No significant difference in progression-free survival or overall survival was seen, although both studies demonstrated a trend towards increased overall survival in those receiving the cell lysate vaccine.

Lysates from cell lines incubated with Norwalk disease virus (NDV) have also been studied. Survival of patients treated with NDV lysates was prolonged compared to historical controls, and patients who relapsed after treatment had improved survival if they were reinmunized. However, a small randomized study of an NDV lysate vaccine in stage III patients failed to demonstrate a benefit in relapse-free survival for patients immunized with the NDV lysate vaccine. An additional strategy used to enhance lysate-based vaccine efficacy involves affixing lysed membranes to silica microbeads. A phase I trial conducted in stage IV patients showed little toxicity from the microbead-membrane preparation; however, only 2 of 19 patients studied showed mixed or partial treatment responses. While tumor lysates, either alone or with additional immune stimulants, would seem to have great potential as melanoma vaccines, they have been largely unsuccessful in clinical trials.

**Irradiated tumor cells**

Vaccines prepared from melanoma cells that have been irradiated are among the most studied vaccine types in melanoma clinical trials. Broadly speaking there are two types of irradiated tumor cell vaccines: autologous and allogeneic. An autologous vaccine is generated by culturing tumor cells from a patient and...
then vaccinating the patient with the cultured cells, often after they have been modified to improve immunogenicity, while allogeneic vaccines use one or more “off the shelf” cultured human melanoma cell lines. Autologous vaccines have the advantage of being identical in HLA type and TAA to the patient’s tumor, while allogeneic vaccines are far easier to prepare and modify. One major reason by which both autologous and allogeneic vaccines have been modified to enhance their immunogenicity has been through the use of genetic engineering.

The earliest irradiated tumor cell vaccines were comprised of a mixture of several different allogeneic cell lines. After irradiation, cells were injected with or without cyclophosphamide, used as a vaccine adjuvant. In phase II trials, clinical responses to vaccine were seen in a minority of patients. One study showed complete responses in 3 of 40 and partial responses in 6 of 40 patients. Subsequent allogeneic vaccines have used other vaccine adjuvants such as bacillus Calmette-Guérin (BCG), or recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF). Measurable immune responses were demonstrated by delayed type hypersensitivity (DTH) testing in many cases, and several phase II studies have shown a modest improvement in 5 year survival relative to single-institutional historic controls.

To improve immunogenicity of allogeneic irradiated melanoma cell vaccines, several investigators have transfected the vaccine cells with genes encoding immune-stimulating molecules. The earliest such trial, a phase I trial in advanced melanoma patients, involved allogeneic cells transfected with IL-4. Only 1 of 12 patients mounted a measurable immune response to autologous tumor, and no patient had significant tumor regression. Both IL-4 and IL-2 have been tried in different allogeneic tumor cell vaccines, with occasional clinical responses seen in early phase clinical trials. However, when in vitro immune responses were measured, one trial demonstrated immune responses only to allogeneic and not to autologous melanoma cells.

Because autologous vaccines require patient melanoma cells, excised tumor is required for their production. As a result, the majority of clinical trials of irradiated autologous tumor cell vaccines have been done in patients with stage IV disease. With allogeneic tumor cell vaccines, the vast majority of trials have been nonrandomized trials with small numbers of patients. Adjuvant strategies used in autologous vaccines have included: 1) modifying the tumor cells with the hapten dinitrophenyl (DNP); 2) using BCG; 3) modified Freund’s adjuvant; 4) co-administering cytokines such as interferons, and/or GM-CSF. Many of the trials yielded responses in a minority (often 10-20%) of patients, with a few complete responses and long-term survivals reported; however, no randomized trials have been conducted to verify a beneficial clinical effect.

Autologous tumor cells have also been transfected with numerous cytokines and other immune proteins in an attempt to enhance antitumor immune responses. The majority of trials conducted thus far have been small phase I trials. Transfected genes have included IL-2, interferon gamma (IFN), IL-12, IL-7, GM-CSF, and a Th1/NK cell stimulating molecule known as d-SLIM, and an innate immune protein called tag7/PGRP-S. The majority of these trials report immune stimulation as measured by in vitro assays in a significant proportion of patients (over half in some cases). However, clinical responses, when seen, were present in a minority of patients.

**Peptides**

Peptide-based vaccines provide a highly defined antigen to which the patient immune response can easily assessed. Furthermore, peptide vaccines are relatively easy to produce compared to vaccines requiring patient tumor or immune cell harvesting. For these reasons, peptide vaccines are among the most studied types of melanoma vaccines. One of the limitations of peptide-based vaccines, however, is that they require a particular HLA allele for peptide binding. Because of this, many trials of peptide vaccines are performed only in HLA-A2-positive patients. Clinical trials to date have all been phase I or II trials; these have focused on types of peptides, the use of HLA class II-binding peptides, and the use of adjuvants and other immune stimulants.

Melanocytes use multiple unique proteins not produced by other cell types. As such, many of the peptides used in melanoma vaccines are specific to melanocytes, and immune responses to peptide vaccines would be expected to be restricted to tumor cells and melanocytes. Among the earliest peptides used in clinical trials were derived from the gp100 protein. Peptides from MART-1/Melan A and from the
enzyme tyrosinase have also been widely used. MART-1 and gp100 peptides have generally been found to be more immunogenic than tyrosinase-derived peptides. Many vaccines utilize combinations of peptides to enhance and broaden the immune response. Additional peptides that have been used in trials include NY-ESO-1, MAGE-A1, and multiple nonspecified peptides complexed with heat shock proteins. Few immune responses have been seen with peptides other those derived from MART-1, gp100, MAGE-3, and tyrosinase.

While initial trials focused exclusively on HLA class I-binding peptides that stimulate cytotoxic T cells, the importance of T helper cells in immune responses has prompted trials involving the use of class II-binding peptides. Some trials have utilized class II-binding peptides from melanoma-associated proteins, such as gp100, MART-1, and NY-ESO-1; others have used unrelated peptides such as tetanus vaccine-derived peptides. While one trial reported frequent immune responses to a class II-binding peptide, one reported no effect on class I-restricted immune responses, and one actually reported worsening of the class I-restricted response with concomitant use of a class II-binding peptide. It is difficult to draw conclusions from these limited data. However, it appears that the addition of class II-binding peptides do not seem to provide significant improvement to peptide-based vaccines.

As peptide vaccines provide a defined antigen and thus an easily measurable immune response, trials of peptide vaccines frequently test novel adjuvants and immune stimulants. One early trial used the cytokines IL-2, GM-CSF, or IL-12, and found that each supported a clinical tumor response in a significant minority of patients. However, a later trial saw fewer immune responses with concurrent versus delayed use of IL-2. Stimulants of the innate immune system such as Toll-like receptor seven ligand (TLR7L) and CpG oligonucleotides appear to enhance immune responses in early trials; however, improvement in clinical responses has not been clearly shown. Finally, blocking antibodies to the immune inhibitor CTLA-4 have been added to a multi-epitope peptide vaccine, with a significant minority of patients (11%) experiencing objective clinical responses. Albeit not overly successful in phase II clinical testing, some peptide vaccines have advanced to randomized phase III clinical trials. Most notable of these studies is E4697 where patients with surgically resected advanced stage III and IV melanoma were treated with a mixture of three HLA class-I binding peptides (MART-1, gp100, tyrosinase) with/without adjuvant GM-CSF administered at a dose of 125 μg/m² for 14 days of a 28 day treatment cycle. The vaccine was compared to GM-CSF alone and placebo. We eagerly await the results of the study.

**Proteins**

Whole protein vaccines have seen little use in melanoma therapeutic trials, despite their widespread use in other applications. Compared to peptide vaccines, they provide the theoretical advantages of being applicable to patients with different HLA types and of simultaneously providing HLA class I and class II-binding antigens capable of stimulating both cytotoxic T cells and helper T cells. The first protein vaccine trial for melanoma used recombinant MAGE-3 as the protein antigen given in adjuvant as a series of intramuscular injections. Humoral responses to MAGE-3 were elicited, but clinical responses were limited to partial and mixed responses in a minority of patients. A second trial studied the effects of immunization with recombinant NY-ESO-1, a cancer-testes antigen present in melanoma and sarcoma patients. Immunization with NY-ESO-1 produced highly potent immune responses. The trial was not designed to assess clinical outcomes, but showed a favorable trend toward increased relapse-free survival in patients receiving NY-ESO-1 plus adjuvant. Protein vaccines, although they are little studied in melanoma, represent a viable means of immunization against TAA, and further study to determine their clinical efficacy is warranted.

**Gangliosides**

Gangliosides are a class of glycosphingolipids that contain sialic acid. They are found on the plasma membrane of cells. Gangliosides are involved in many cellular functions including cell adhesion and signaling responses to receptor-ligand interactions. Certain gangliosides that are overexpressed in a number of different malignancies, including melanoma, have been implicated in enabling tumors to grow and metastasize. As such, they are attractive therapeutic targets, since elimination of cells with altered ganglioside expression would theoretically limit a tumor’s ability to grow and metastasize. Several groups have attempt-
ed to target gangliosides as antigens in cancer vaccines. In an early phase III clinical trial comparing immunization of stage III melanoma patients with the ganglioside GM2 plus BCG versus BCG alone, no difference was seen in progression-free survival or overall survival; however, a post hoc analysis revealed differences in both progression-free survival and overall survival in those patients without prior immunity to GM2. Various vaccine preparations have been tried, and GM2 is most immunogenic when complexed to the carrier protein keyhole limpet hemocyanin (KLH). However, a phase III clinical trial comparing GM2-KLH to high-dose IL-2 in stage IIIB/III patients demonstrated superiority of IL-2 over ganglioside vaccine in overall and progression-free survival. Other ganglioside vaccines have been tried including GD3, GD2, and GM3; however, none of these have demonstrated significant success in generating clinical responses. Thus, while ganglioside vaccines appear to be immunogenic and can generate clinical responses, they are clinically inferior to conventional immunotherapy thus far.

Anti-idiotype antibodies

One potential barrier to vaccination against melanoma and other cancers is that TAA are inherently “self” antigens. As such, TAA often promote immune tolerance, limiting the immune system’s ability to effectively eliminate TAA-bearing cells. Furthermore, some potential TAA such as gangliosides are difficult to prepare in vaccine form, as they are difficult to generate synthetically. For these reasons, investigators have tried anti-idiotype vaccine strategies. Briefly, in order to generate an anti-idiotype vaccine, a TAA is used to immunize a host animal. A monoclonal antibody against the TAA is generated and is in turn used to immunize a second host animal. A new monoclonal antibody, specific for the antigen binding portion of the first antibody (anti-idiotype) is formed. The antigen binding site of this second antibody closely resembles the original TAA. The anti-idiotype antibody, itself a potent immunogen, is used as a therapeutic vaccine. Patient immune responses to the anti-idiotype antibody are cross-reactive with the TAA and can promote tumor immunity. Phase I studies have confirmed the safety of anti-idiotype vaccines against tumor associated proteoglycans and gangliosides. However, randomized trials demonstrating the efficacy of anti-idiotype vaccines have yet to be reported.

Nucleic acid vectors

Vectors that contain genes coding for TAA have several attractive features that have prompted interest in their use as melanoma vaccines. First, proteins produced in eukaryotic cells frequently undergo post-translational modification, including glycosylation. Synthetic peptides and prokaryote-derived recombinant proteins lack these modifications, but vector-driven eukaryotic (patient cell) expression generates proteins in a manner allowing for normal post-translational modification. Second, many vectors such as viruses induce potent immune responses and thus serve as their own adjuvants. Because of these qualities, investigators have attempted to use several different types of vectors to deliver TAA and other genes to patients. The first attempt to use gene delivery to immunize patients against melanoma antigens was with an adenovirus-based construct with genes encoding MART-1 or gp100. Although the vaccine was administered safely, high pre-existing levels of neutralizing antibodies to adenovirus precluded the generation of an effective immune response to the TAA. An early plasmid-based vaccine also failed to generate immune responses. Subsequent trials have employed different poxviruses including vaccinia, canarypox, and fowlpox. In addition to delivery of genes encoding TAA, viral vectors have been used to generate expression of costimulatory molecules, including CD80 and CD86 (B7-1 and B7-2). One strategy has involved priming patients with canarypox-TAA constructs, then boosting with free peptides. In general, the use of poxvirus vectors has promoted the generation of immune responses to most TAA, although the tyrosinase antigen has been poorly immunogenic in this setting. Despite this, clinical responses to vaccination with vectors encoding TAA have thus far been modest at best.

Vaccines that provide antigen presentation

The above vaccine strategies all involve injecting patients with TAA in a manner designed to promote effective antigen presentation by the patients’ APC. These modes of vaccination depend on patients’ ability to absorb antigen and present it in a manner supportive of a protective immune response. Alternative strategies have been developed in which the vaccine includes both TAA and APC. This circumvents the
need for endogenous antigen processing and presentation. Most vaccines that provide APC employ DC. DC are specialized APC that are especially proficient in stimulating naïve T cells. Immature DC are capable of phagocytosing large amounts of antigen, while mature DC can present antigens in the context of a host of costimulatory molecules. Two groups of vaccines employing APC have been used: 1) those in which the APC are loaded with a limited number of defined antigens; and 2) those in which APC are loaded with a multitude of antigens that are not defined.

**Antigen presenting cells with defined antigens**

Most APC-based vaccines employ DC because of their ability to prime immune responses with naïve T cells. However, DC are not present at significant numbers in the circulation, and, therefore, must be mobilized prior to harvest or generated in vitro. There are numerous techniques for culturing DC from peripheral blood mononuclear cells (PBMC). Most commonly, GM-CSF and IL-4 are used to generate immature DC from cultured PBMC. Other investigators have used type I interferons to generate DC, but DC generated by type I interferons have not produced immune responses as efficiently in patients as those generated by GM-CSF and IL-4. After they are generated, DC are often incubated with additional cytokines to make them “mature” (upregulate expression of costimulatory molecules). Commonly used maturation cytokines include IL-3, IL-6, IL-12, IL-15, IL-18, GM-CSF, IL-4, and CD40 ligand. An alternative method to culturing DC in vitro from PBMC is to mobilize DC in vivo by injecting the patient with the kinase Flt3, then directly harvesting DC from blood. Finally, PBMC have been used as APC without in vitro maturation into DC, and have been shown to generate anti-tumor immune responses when injected along with IL-12.

Investigators using peptide-pulsed APC in clinical trials have used a diverse array of peptide antigens. Some trials employ DC pulsed with a single peptide antigen, such as MAGE-3, MelanA/MART-1, or NA17.A2. Others use an array of tumor-associated peptides, making tumor immune escape more difficult. In addition to TAA, some investigators have used unrelated peptides/proteins to assess patient immune competency and to compare antitumor responses with those against other foreign antigens. These include KLH, tetanus toxoid, and influenza matrix protein (Flu-MP). Immune responses to each of the above tumor-associated peptides have been demonstrated, albeit with different immunization efficacy.

At this time, most clinical trials of peptide-pulsed APC vaccines have been conducted in patients with metastatic melanoma (stage IV). Despite this, many trials demonstrate frequent robust immune responses to TAA, as measured by DTH and in vitro assays. Clinical responses are seen at a lower frequency in patients with advanced disease. A number of phase I clinical trials have recorded clinical responses, and complete responses have been documented. Further evidence that peptide-pulsed APC vaccines generate an effective antitumor response is seen when residual tumor after a partial response is examined. Several investigators have noted that remaining tumor following a partial or mixed response to vaccination shows evidence of antigen loss. To date, there is published data from only one phase III clinical trial using peptide-pulsed DC vaccine in patients with metastatic melanoma. Patients were randomized to receive either DC pulsed with a cocktail of peptides bound by various HLA alleles or dacarbazine. No difference was seen in overall survival, progression-free survival, or objective response. A few trials have been done in the adjuvant setting in patients with surgically resected melanoma. One such trial in stage II patients demonstrated frequent durable immune responses but did not assess clinical outcomes. In conclusion, peptide-pulsed APC vaccines show promise in that they frequently induce measurable immune responses to TAA in advanced melanoma patients; however, they have not yet been shown to be clinically superior to “conventional chemotherapy” in the setting of advanced disease.

**Antigen presenting cells with undefined antigens**

Peptide-pulsed APC vaccines share the main limitations of peptide vaccines in that any given peptide requires a particular HLA allele for its proper presentation, and that individual tumors may lose expression of a peptide antigen either prior to or in response to therapy (antigen escape). Investigators have used several vaccine strategies that exploit the T cell priming capability of DC in a manner that provides a broad range of patient-specific antigens. Early trials used DC that had been pulsed with lysed tumor cells. Subsequent trials have used autologous DC with both
autologous \cite{97, 112} and allogeneic \cite{113-115} melanoma cell lysates. Other investigators have used DC pulsed with irradiated tumor cells as an antigen source.\cite{116-118} Yet another strategy has been to fuse autologous tumor cells with allogeneic DC with the goal that immune responses to allogeneic HLA molecules in the fusion cells would stimulate or augment TAA-directed immune responses. Finally, investigators have used gene transfer strategies to transfect DC with mRNA from autologous tumor tissue, causing the vaccine DC to produce and present TAA.\cite{123, 124} As with peptide-pulsed APC vaccines, the various multiantigen vaccine strategies have shown evidence of TAA-directed immune responses in a majority of patients, but clinical responses are seen in a significantly lower percentage.

In one early clinical trial of DC-based vaccines, investigators used either multiple peptides or tumor cell lysates as a source of TAA.\cite{94} Immune responses were monitored by measuring DTH skin responses to intradermal injections of DC pulsed with control antigen (KLH) or a cocktail of tumor-associated peptides. No exogenous maturation cytokines were given. Both tumor antigen-specific immune responses and clinical responses were seen in subsets of patients treated with either peptide-pulsed or lysate-pulsed DC. This early study was not designed to determine whether peptides or lysates are more effective as antigens in DC-based immunotherapy. A second clinical trial compared immunization with DC loaded with a cocktail of HLA class I-binding peptides \textit{versus} melanoma cell lysates.\cite{97} Here, TAA-directed immune responses as measured by DTH were seen in 5 of 15 patients treated with lysate-pulsed DC and in approximately half the patients treated with peptide-pulsed DC. However, no clinical response was seen in the group treated with peptide-pulsed DC, while 3 partial responses and one mixed response were seen in the group treated with lysate-pulsed DC. Although patients were not randomized, these data argue for increased efficacy of lysates over peptides as a source of TAA in DC-based vaccines.

Conclusions

A vast range of therapeutic vaccine strategies against melanoma have been developed and tested in clinical trials. Tested antigen sources include autologous and allogeneic melanoma cell lysates, irradiated tumor cells, synthetic peptides and proteins, gangliosides and anti-idiotypic antibodies, and nucleic acids encoding protein and peptide antigens. Methods to increase the immunogenicity of vaccines consist of chemical and biological adjuvants, cytokines, viral vectors, APC including DC, and blocking antibodies to regulatory molecules. Trials have taken place in patients from stage II through stage IV melanoma, with the majority of trials being in patients with metastatic disease. Numerous clinical trials have produced promising results in terms of objective evidence of tumor antigen-specific immune responses, at times in the majority of patients immunized. Clinical responses are seen less frequently. Despite this promising data, no phase III trial to date has demonstrated superiority of melanoma vaccines over standard cytokine therapy or chemotherapy.

Clinically useful vaccine treatment of melanoma requires the use of effective TAA presented in appropriate immune-stimulatory context to a host capable of generating a robust response. Failure of effective vaccine antigen selection is seen in immune escape, which has been documented in several clinical trials. Immune escape occurs when the immune response selects out variant tumor cells that lack the TAA used in the vaccine. Strategies to overcome immune escape include the use of more than one TAA in the vaccine regimen; however, tumors that loose expression of HLA class I molecules may evade even multiantigen vaccines. When a vaccine fails to generate an immune-stimulatory context, a clonal expansion of TAA-specific T cells may be produced, but the cells are incapable of immune destruction of the tumor, either due to anergy (tolerance) or activation of inappropriate functions. Based on measures of \textit{in vitro} functionality of T cells (cytotoxicity and cytokine secretion assays), many strategies appear to generate appropriate immune responses. The disparity between robust immune functionality and inconsistent clinical responses may be due to local or systemic effects of the tumor on immune cell effector function \textit{in vivo} that are not detectable \textit{in vitro} (immunosuppressive tumor micro environment). Finally, some patients fail to generate immune or clinical responses to vaccines that have appropriate antigens presented with appropriate immune stimulation. This includes patients who fail to mount a response to control antigens such as Flu-MP or KLH. In these patients, the melanoma itself and/or chemotherapy may be causing global
immune dysregulation such that effective immune responses cannot be generated.

Clinical trials of melanoma vaccines thus far have seen sporadic success, but have not demonstrated a consistent patient benefit. Overcoming this will require investigators to understand tumor antigen escape mechanisms as well as mechanisms of tumor immunosuppression both local in the tumor micro-environment (IL10, TGFβ overproduction at the tumor site) and systemic (regulatory T cell mediated immune suppression).

Riassunto

L’esperienza clinica dei vaccini per il melanoma maligno

Sono stati fatti molti sforzi per migliorare il trattamento sistemico del melanoma avanzato. Tuttavia, l’allungamento clinicamente significativo della sopravvivenza nei pazienti con melanoma metastatico resta deludente. Le comunicazioni aneddotiche relative a remissioni aneddotiche relative a regressioni spontanee del carico tumorale in pazienti con melanoma metastatico rimangono sporadiche, ma non hanno dimostrato una benefica influenza sull’outcome. La somministrazione di vaccini contro gli antigeni del melanoma si dimostra un obiettivo raggiungibile. In questo senso, un approccio è stato il tentativo di migliorare la distruzione cellulare del melanoma tramite un’immunoterapia attivante mediante una vaccinazione diretta contro gli antigeni del melanoma. È stato impiegato un ampio spettro di vaccini, sia locali che sistemici, che sono stati stati testati negli studi clinici eseguiti su pazienti con melanoma maligno. In questo lavoro vengono sintetizzati gli ampi studi clinici condotti sinora sulla terapia del melanoma basata sui vaccini.

Parole chiave: Malanoma - Cute - Vaccini.

References

23. Osanto S, Schiphorst PP, Weijl NI, Dijkstra N, Van Wees A, Brouwen-


Molecular targeted therapy of melanoma

R. HOUBEN, S.ORTMANN, D. SCHRAMA, E.-B. BRÖCKER, J. C. BECKER

The increasing understanding of the molecular pathology of malignancies will have impact on future therapeutic approaches. Effective, non-toxic intervention in a broad range of cancers including melanoma may potentially be provided by targeted therapeutics which are tailored towards the molecular abnormalities that cause tumour progression. a) Constitutive activation of the Ras/MAPK- and PI3K/Akt-signal transduction pathways, b) epigenetic deregulation of gene expression due to drastic changes in DNA methylation and histone modification and c) loss of stability of tumour suppressor proteins have been demonstrated to be involved in melanomagenesis. Small molecular weight inhibitors interfering with these deregulated events, as well as the results of first clinical trials applying these substances are covered in this review.

KEY WORDS: Melanoma, diagnosis - Melanoma, drug therapy - Skin.

The current standard therapy of inoperable metastasised melanoma is largely based on the use of cytotoxic and cytostatic substances.1 These substances, which unselectively target all proliferating cells in the body, have improved significantly cure and survival rates for several malignancies. However, for the treatment of malignant melanoma, the outcome is poor.2 In contrast to this cessation concerning therapies, tremendous progress has been made in understanding the mechanisms of melanomagenesis by applying modern biochemical and molecular techniques.3 Now, the development of drugs targeting the molecular abnormalities that cause melanoma has started, hopefully leading to effective and specific therapeutics. Clinical research however, is still at the beginning and time will tell whether targeted therapy can fulfil these hopes.

Activation of the MAPK pathway in melanoma

Raf kinases have been shown to be components of an evolutionarily conserved signalling cascade that links receptor activation at the cell membrane to the modification of cytoplasmic or nuclear targets which are required for the execution of developmental programs as well as for cell survival and proliferation.4 An important linker coupling receptor activation with the induction of Raf kinase activity is the small G protein Ras that can switch between an inactive GDP bound and an active GTP bound form. GTP-Ras recruits Raf to the plasma membrane initiating a complex activation process.5 Active Raf (MAP kinase kinase kinase) phosphorylates and activates MEK (MAP kinase kinase), and MEK phosphorylates and activates ERK 1/2 (p44/p42 MAP kinases). While Raf and MEK appear largely restricted to only one class of substrates, ERK targets more than 70 substrates including mem-
brane, cytoskeletal, cytoplasmic, nuclear and even mitochondrial proteins.\(^6\)

The Raf family of serin/threonine kinases consists of three isoforms: A-, B- and C-Raf. In 2003, the cancer genome project revealed B-Raf to be affected by activating mutations in several cancers most frequently in melanoma.\(^7\) The incidence of B-Raf mutations in melanoma varies between different subtypes but a mean frequency of 46% in 2 338 melanoma samples analysed (Catalogue of somatic mutations in cancer, [COSMIC], http://www.sanger.ac.uk/genetics/CGP/cosmic/), demonstrates the exceptional importance of this alteration. Additional activators of the MAP kinase cascade which are found to be mutated in melanoma are N-Ras (mutated in 19% of melanoma according to the COSMIC database) and the stem cell factor receptor Kit.\(^8\)

For B-Raf the exchange of a single amino acid the valine at position 600 to a charged residue (glutamic acid, aspartic acid, lysine or arginine) accounts for more than 90% of the B-Raf mutations found in melanoma. These exchanges render B-Raf constitutively active\(^9\) and it is believed that this is due to mimicry of activating phosphorylations at nearby positions in this part of the protein which is termed the kinase activation segment. In B-Raf, the threonine at position 599 and the serin at position 602 both require phosphorylation to achieve maximal kinase activity and both are indeed phosphorylated upon recruitment of B-Raf to the plasma membrane by activated Ras.\(^10\)

For N-Ras the activating mutations are paradoxically active\(^9\) and it is believed that this is due to mimicry of activating phosphorylations at nearby positions in this part of the protein which is termed the kinase activation segment. In B-Raf, the threonine at position 599 and the serin at position 602 both require phosphorylation to achieve maximal kinase activity and both are indeed phosphorylated upon recruitment of B-Raf to the plasma membrane by activated Ras.\(^10\)

For N-Ras the activating mutations are paradoxically active\(^9\) and it is believed that this is due to mimicry of activating phosphorylations at nearby positions in this part of the protein which is termed the kinase activation segment. In B-Raf, the threonine at position 599 and the serin at position 602 both require phosphorylation to achieve maximal kinase activity and both are indeed phosphorylated upon recruitment of B-Raf to the plasma membrane by activated Ras.\(^10\)

The Raf family of serin/threonine kinases consists of three isoforms: A-, B- and C-Raf. In 2003, the cancer genome project revealed B-Raf to be affected by activating mutations in several cancers most frequently in melanoma.\(^7\) The incidence of B-Raf mutations in melanoma varies between different subtypes but a mean frequency of 46% in 2 338 melanoma samples analysed (Catalogue of somatic mutations in cancer, [COSMIC], http://www.sanger.ac.uk/genetics/CGP/cosmic/), demonstrates the exceptional importance of this alteration. Additional activators of the MAP kinase cascade which are found to be mutated in melanoma are N-Ras (mutated in 19% of melanoma according to the COSMIC database) and the stem cell factor receptor Kit.\(^8\)

For B-Raf the exchange of a single amino acid the valine at position 600 to a charged residue (glutamic acid, aspartic acid, lysine or arginine) accounts for more than 90% of the B-Raf mutations found in melanoma. These exchanges render B-Raf constitutively active\(^9\) and it is believed that this is due to mimicry of activating phosphorylations at nearby positions in this part of the protein which is termed the kinase activation segment. In B-Raf, the threonine at position 599 and the serin at position 602 both require phosphorylation to achieve maximal kinase activity and both are indeed phosphorylated upon recruitment of B-Raf to the plasma membrane by activated Ras.\(^10\)

For N-Ras the activating mutations are paradoxically active\(^9\) and it is believed that this is due to mimicry of activating phosphorylations at nearby positions in this part of the protein which is termed the kinase activation segment. In B-Raf, the threonine at position 599 and the serin at position 602 both require phosphorylation to achieve maximal kinase activity and both are indeed phosphorylated upon recruitment of B-Raf to the plasma membrane by activated Ras.\(^10\)

For B-Raf the exchange of a single amino acid the valine at position 600 to a charged residue (glutamic acid, aspartic acid, lysine or arginine) accounts for more than 90% of the B-Raf mutations found in melanoma. These exchanges render B-Raf constitutively active\(^9\) and it is believed that this is due to mimicry of activating phosphorylations at nearby positions in this part of the protein which is termed the kinase activation segment. In B-Raf, the threonine at position 599 and the serin at position 602 both require phosphorylation to achieve maximal kinase activity and both are indeed phosphorylated upon recruitment of B-Raf to the plasma membrane by activated Ras.\(^10\)

For N-Ras the activating mutations are paradoxically active\(^9\) and it is believed that this is due to mimicry of activating phosphorylations at nearby positions in this part of the protein which is termed the kinase activation segment. In B-Raf, the threonine at position 599 and the serin at position 602 both require phosphorylation to achieve maximal kinase activity and both are indeed phosphorylated upon recruitment of B-Raf to the plasma membrane by activated Ras.\(^10\)

For N-Ras the activating mutations are paradoxically active\(^9\) and it is believed that this is due to mimicry of activating phosphorylations at nearby positions in this part of the protein which is termed the kinase activation segment. In B-Raf, the threonine at position 599 and the serin at position 602 both require phosphorylation to achieve maximal kinase activity and both are indeed phosphorylated upon recruitment of B-Raf to the plasma membrane by activated Ras.\(^10\)

For N-Ras the activating mutations are paradoxically active\(^9\) and it is believed that this is due to mimicry of activating phosphorylations at nearby positions in this part of the protein which is termed the kinase activation segment. In B-Raf, the threonine at position 599 and the serin at position 602 both require phosphorylation to achieve maximal kinase activity and both are indeed phosphorylated upon recruitment of B-Raf to the plasma membrane by activated Ras.\(^10\)

Inhibitors of the Ras-MAPK signal-transduction pathway

A relatively specific inhibition of the different intracellular signal transduction cascades was enabled by the development of a set of low molecular weight kinase inhibitors. Among those are Raf inhibitors such as sorafenib (Bay43-9006) and Raf 265 (CHIR-265), MEK inhibitors such as PD184352, CI-1040 and U0126 and tyrosine kinase inhibitors including Gefitinib (Iressa\(^\text{TM}\)), Erlotinib (Tarceva\(^\text{TM}\)) and Imatinib (Glivec\(^\text{TM}\)). The kinase inhibitor molecules work in many cases through competitive binding to the ATP pocket displacing ATP which is necessary to provide the phosphate for the kinase reaction. This common mechanism explains why the specificity of many kinase inhibitors is limited. Sorafenib developed as C-Raf inhibitor acts on B-Raf isoforms as well but is also inhibitory to more distantly related kinases including vascular-endothelial growth factor receptors (VEGF-2 and VEGFR-3), platelet-derived growth factor receptor (PDGFR), Flt-3 and Kit.

Only one year following the publication in “Nature” reporting activating B-Raf mutations in melanoma, first results of a phase-I/II study demonstrating therapeutic activity of sorafenib in combination with carboplatin/placitaxel were presented by Flaherty et al at the VIII Perspectives in Melanoma Meeting in Berlin in September 2004. 28 of 35 patients showed either regression or stable disease.\(^12\)\(^13\) Currently there are 8 ongoing clinical trials evaluating sorafenib in combination with different chemotherapeutics for the treatment of advanced melanoma (http://www.clinicaltrials.gov) and we can expect a lot of information from these studies. However, the results reported so far, are not confirming the initial impact on disease progression (http://www.medicalnews.today.com/medicalnews.php?newsid=58112).

Sorafenib was also evaluated as mono-therapy for advanced melanoma. However, since no benefit for the treated patients could be demonstrated\(^13\) sorafenib as a single agent is not further under investigation. Interestingly, sorafenib failed also as a therapeutic in some animal models while application of the MEK inhibitors U0126 or CI-1040 was successful. In a C-Raf lung adenoma as well as in a B-Raf\(^\text{V600E}\) melanoma metastasis mouse model inhibition of MEK led to tumour reduction and inhibition of metastasis, respectively, while sorafenib did not show any effect.\(^14\)\(^15\) Therefore, although there is still hope that next gen-
eration Raf inhibitors like Raf-265, which is in vitro 50- to 100-fold more potent than sorafenib,11 will improve Raf targeted therapy, it may arise that MEK is the better target for treating melanoma.

The MEK inhibitors UO126 and CI-1040, however, due to bad pharmacokinetics and lack of anti-tumour activity observed in patients with several advanced malignancies are considered insufficient to warrant further development.16, 17

In contrast, PD0325901, a derivate of CI-1040 that blocks MEK at much higher potency, still holds promise for the use of the compound as a therapeutic agent. In a phase I/II study enrolling 41 patients (27 melanoma) possible anticancer activity has been evaluated. Two partial responses were observed in melanoma patients, while 8 patients (5 melanoma, 2 non small cell lung cancer [NSCLC] and 1 colon cancer) achieved stable disease lasting 3-7 months.

ARRY-142886 (AZD6244), another novel and highly-selective oral MEK inhibitor, is also under study in clinical trials currently. A study including melanoma patients as well as patients with other advanced cancers has completed enrollment and first results of this study were reported at the EORTC-NCI-AACR Symposium on “Molecular Targets and Cancer Therapeutics” in November 2006. Sixteen of the 20 patients with melanoma completed at least one cycle of treatment. Twelve had stable disease after completion of cycle two, with stable disease persisting for at least five months in six patients (range, 5-13 months; median, 6.5 months) compared to 3 of 19 patients with other malignancies.17

In both above mentioned studies the therapeutic impact of the MEK inhibitors seems to be more pronounced for melanomas than for other cancers. B-Raf mutant melanoma cell lines have been demonstrated to be particularly sensitive to MEK inhibition compared to cells with wt B-Raf (including cells with N-Ras mutation).18 The genotype of the treated tumours was not reported yet. It will be interesting to see whether in patients there is also a prevalence of MEK inhibition being more effective in B-Raf mutant tumours.

The lack of specificity of protein kinase inhibitors may sometimes be of advantage. Sorafenib developed as a Raf inhibitor has now been approved by the US Food and Drug Administration for the treatment of patients with advanced renal cell carcinoma or kidney cancer. However, since in several models sorafenib inhibited tumour growth without affecting MAPK signalling in the tumour cells, it is now believed that sorafenib acts by exerting potent antivascular effects via inhibition of VEGF receptors.19

**PIP3 signalling**

A second downstream signalling pathway of Ras and many growth factor receptors which is thought to be one of the major survival pathways, involves class 1 phosphoinositide-3 kinases (PI3Ks) and the Akt kinase. PI3Ks constitute a large family of lipid kinases and class 1 PI3Ks phosphorylate phosphatidylidyinositol 4,5- bisphosphate (PIP2) to produce phosphatidylinositol 3,4,5-trisphosphate (PIP3), a key lipid second messenger that controls a wide range of cellular responses via downstream effectors such as adaptor proteins, protein kinases (most important the phosphoinositide-dependent kinase 1 (PDK-1) and Akt/PKB) and nucleotide-exchange factors or GTPase-activating proteins (GAPs) for GTPases of the Rho, Ras and Arf families.20 Growth factor receptors drive activation of PI3Ks and thereby PI3P formation either through direct binding and phosphorylation or via associated tyrosine kinases, heterotrimeric G proteins or Ras.20 The antagonist of the PI3Ks down regulating PIP3 levels is the phosphatase and tensin homologue deleted on chromosome 10 (PTEN). PTEN is a phosphatidylinositol phosphate phosphatase which is frequently inactivated in human cancers including melanoma.11

Akt contains an NH2-terminal pleckstrin homology domain, which interacts with PIP3 synthesized at the plasma membrane. Akt recruitment to the plasma mem-
brane results in a conformational change enabling phosphorylation by PDK-1, which also requires PIP3 for activation and by a second kinase whose identity is not yet clear. Established antiapoptotic modes of action of Akt are phosphorylation and inactivation of the proapoptotic protein Bad, phosphorylation of pro-caspase-9 rendering it resistant to processing and activation, phosphorylation of proapoptotic Forkhead transcription factors and activation of the NF-κB pathway possibly through phosphorylation of I-κB. Akt has also been implicated to be involved in driving the cell cycle via a couple of direct and indirect targets including Myc, Mdm2/p53, the Retinoblastoma protein, cyclin D and cyclin dependent kinase inhibitors (see tumour suppressor section). Moreover, Akt plays a role in angiogenesis, cell migration and metastasis formation. This multitude of cancer related functions explains why inhibiting PI3K or Akt are regarded as some of the most promising molecular targets for future therapies.

**Targeting PI3K dependent signalling**

Unfortunately, most of the inhibitors developed so far display only restricted specificity. Nevertheless, preclinical studies applying the PI3K inhibitor Ly294002 in xenotransplantation or transgenic mouse tumour models demonstrated high antitumoural activity following topical application. The Akt inhibitor triciribine has been tested in phase I/II clinical trials for the treatment of advanced breast cancer. The outcome of this study was a lack of activity and toxicity at higher doses. However, at that time triciribine was tested due to its ability to inhibit DNA and protein synthesis without the knowledge of triciribine being an Akt inhibitor. Our days patients with metastatic cancer whose tumours must be shown to be phospho-Akt positive are recruited for a triciribine study (http://www.clinicaltrials.gov).

Further downstream the PI3K/Akt pathway the protein kinase mTOR (mammalian target of rapamycin) gets activated. mTOR is a member of the recently identified family of PIKKs (PI3K and related kinases) which are involved in cell cycle regulation. Activation of mTOR can be induced by hypoxia, which results in two independent cellular responses. On the one hand proliferation is accelerated, on the other hand via the activation of hypoxia inducible factor (HIF)-1α genes get activated which adopt the tissue to oxygen deficiency. Both mTOR induced responses are essential for hypoxia induced neoangiogenesis and enable thereby the so called angiogenic switch of solid tumours. Interestingly, according to the data base of the American Organ Procurement and Transplant Network/United Network for Organ Sharing (OPTN/ UNOS) the risk to develop cancer post transplantation for patients treated by mTOR inhibitors is markedly reduced compared to patients treated with traditional calcineurin-inhibitors. Indeed, immunosuppression by e.g. cyclosporine or tacrolimus can be associated with high cancer rates and can be the cause for the subsequent death of the transplant recipient. Analogs of rapamycin, like CCI-779 (temsirolimus), AP23573 and RAD001 (everolimus) are relatively specific inhibitors of mTOR which block G1/S transition and activation of HIF-1α. In several phase II clinical trials (4 melanoma) the effectivity of CCI-779 as monotherapy and in combination with other agents (combination with sorafenib in 2 melanoma studies) is currently evaluated. In a study including 109 patients with advanced breast cancer CCI-779 demonstrated a modest but clear cut activity with one third of the patients having partial remission or stable disease lasting for more than two months. It should be noted that the number of patients bearing tumours with PTEN-mutation or Her2/neu overexpression – which both should lead to activated PI3K/Akt signalling – was increased among the responders. The conclusion however, of a study with 31 patients with advanced melanoma treated with CCI-779 as a single agent was that CCI-779 is...
not sufficiently active in melanoma to warrant further testing as a single agent. So far CCI-779 was tested in clinical trials for its direct effect on tumour cells. As described above inhibition of mTOR should also attenuate neoangiogenesis and thereby indirectly tumour growth. Therefore, currently conducted studies combining CCI-779 with other potentially antiangiogenic substances may provide interesting results.

**Tumour suppressor genes**

Tumour suppressor genes code for proteins for which loss of function, frequently achieved by repressed expression, contributes to carcinogenesis. The first tumour suppressor to be identified is the product of the Retinoblastoma gene (Rb) which discovered in retinoblastoma is also inactivated in many other cancers. Rb is a transcriptional repressor inhibiting entrance into the cell cycle. It can be inactivated by phosphorylation through cyclin D/cyclin dependent kinase (cdk) complexes. A cellular defence mechanism against oncogene driven deregulated Rb phosphorylation is expression of the cdk inhibitor p16ink4a. In melanoma the Rb pathway is frequently affected by loss of expression of p16ink4a and this seems to be mediated mainly by so called epigenetic mechanism.

PTEN the above mentioned antagonist of PI3Ks is a further example of a tumour suppressor whose expression is frequently down regulated in melanoma. This is associated with PTEN promoter methylation,
and demethylation using the demethylation agent 5-aza-2’-deoxycytidine leads to PTEN reexpression.35

**Epigenetic silencing**

Cancer is viewed today as a genetic as well as an epigenetic disease. DNA methylation and histone modifications are two of the most important epigenetic mechanisms through which hereditary variants in gene expression without alteration of the DNA sequence occur.36

Histones are proteins that, tightly associated with the DNA, build up the nucleosomes. Three dimensional arrangements of the nucleosomes form the so called chromatin and the structure of chromatin plays a critical role in gene expression. Post translational modifications (acetylation, methylation and phosphorylation) of histone tails lead to architectural changes of nucleosomes and chromatin remodelling. Chromatin remodelling may finally enhance or repress gene transcription.37 Histone acetylation takes place at the N-termini of the histones that have been highly conserved throughout evolution and contain a number of lysine residues which are the targets for acetylation. Two opposite enzymatic activities, histone acetyl transferase (HAT) and histone deacetylase (HDAC), regulate the level of histone acetylation. Imbalances between the activities of HAT and HDAC, leading to histone hypo-acetylation and repression of gene expression are regarded as a necessary step in human carcinogenesis.38

DNA methylation occurs at cytosines within CpG-rich DNA regions and is catalysed by DNA methyltransferases.39 Methylation of DNA in promoter regions can prevent binding of the basal transcriptional machinery and ubiquitous transcription factors to their cognate DNA sequences.40 Therefore, DNA hypermethylation is a hallmark of gene silencing and was described in melanoma and other cancers for the promoters of many relevant genes.41, 42

**Derepression of silenced genes**

Enzymes that regulate DNA methylation and histone modifications have been successfully targeted by a variety of small molecule drugs including the DNA methyltransferase inhibitor decitabine (5-aza-2’-deoxycytidine) and the HDAC inhibitors suberoylanilide hydroxamic acid (SAHA), valproic acid and the bezamid-derivative MS275.43, 44 These drugs can potentially revert the epigenetic modifications and reactivate tumour suppressor genes or genes that increase the sensitivity of target tissues to anticancer drugs. Following a hierarchical model of cancer development, cancer stem cells represent the main therapeutic target, as only they have the ability to initiate and maintain tumour growth.45 Current therapies, however, are often largely aimed at reducing the tumour mass, primarily affecting cells undergoing cell division. Slowly-proliferating or dormant cells, which presumably include cancer stem cells, are spared. Modulation of epigenetic imprinting offers the possibility of also targeting the cancer stem cell population.

The methyltransferase inhibitor decitabine has been evaluated in a phase I clinical trial in combination with high doses of the immune stimulator IL-2 for the treatment of melanoma and renal cell carcinoma. The treatment was well tolerated and 5 of 16 melanoma patients responded with partial regression suggesting that decitabine may enhance the activity of IL-2 in melanoma (response rate 15%).46 Two phase I studies evaluating decitabine as mono therapy for melanoma treatment have not yet been reported. Most clinical trials on HDAC inhibitors in humans are also still in phase I/II. The HDAC inhibitor SAHA has shown significant anticancer activity against both hematologic and solid tumours at doses well tolerated by patients.47 A drug application was approved by the US Food and Drug Administration for SAHA for the treatment of cutaneous T-cell lymphoma.48 For the treatment of
melanoma evaluation of SAHA has just started; one study applying SAHA is recruiting patients. Valproic acid, which has been used for decades in the treatment of epilepsy, has also been recognized as HDAC inhibitor.\textsuperscript{49} The discovery of its inhibitory effect came after thorough evaluation of its main side effect of causing birth defects such as defective closure of the vertebral canal and disproportionate facial bone formation. The embryotoxic effect was found to be caused by inhibition of HDAC. Other side effects, and unfortunately also antitumour effects of this substance class, are relatively moderate, and it can be presumed that sufficient therapeutic effectiveness can only be realized by suitable combination therapies, \textit{e.g.}, with methyltransferase inhibitors, cytostatics, differentiation inducers or proteasome inhibitors (see following section). For example a recently reported phase I/II clinical trial combining the DNA methylation inhibitor decitabine with valproic acid for the treatment of patients with advanced leukaemia demonstrated that the combination of epigenetic therapy in leukaemia was safe and active (12 objective responders (10 complete remissions) within a group of 54 patients), and was associated with transient reversal of aberrant epigenetic markers.\textsuperscript{50}

### Table I.—Examples of the described therapeutic agents.

<table>
<thead>
<tr>
<th>Active principle</th>
<th>Agent</th>
<th>Substance Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAPK pathway inhibitors</td>
<td>Sorafenib (Bay 43-9006)</td>
<td>Raf-, VEGF-Inhibitor</td>
</tr>
<tr>
<td></td>
<td>RAF-265</td>
<td>RAF-Inhibitor</td>
</tr>
<tr>
<td></td>
<td>CI-1040</td>
<td>MEK inhibitor</td>
</tr>
<tr>
<td></td>
<td>U0126</td>
<td>MEK inhibitor</td>
</tr>
<tr>
<td></td>
<td>ARRY-142886</td>
<td>PI3K inhibitor</td>
</tr>
<tr>
<td>PI3K/Akt pathway inhibitors</td>
<td>Ly294002</td>
<td>PI3K inhibitor</td>
</tr>
<tr>
<td></td>
<td>Triciribine</td>
<td>Akt inhibitor</td>
</tr>
<tr>
<td></td>
<td>CCI-779 (Temsirolimus)</td>
<td>mTOR inhibitor</td>
</tr>
<tr>
<td></td>
<td>RAD001 (Everolimus)</td>
<td>mTOR inhibitor</td>
</tr>
<tr>
<td>Derepression of tumour suppressor genes</td>
<td>SAHA</td>
<td>HDAC inhibitor</td>
</tr>
<tr>
<td></td>
<td>Valproic acid</td>
<td>HDAC inhibitor</td>
</tr>
<tr>
<td></td>
<td>FK228</td>
<td>HDAC inhibitor</td>
</tr>
<tr>
<td></td>
<td>Decitabine</td>
<td>Methyltransferase inhibit</td>
</tr>
<tr>
<td>Protein stabilization</td>
<td>Bortezomib</td>
<td>Proteasome inhibitor</td>
</tr>
</tbody>
</table>

Proteasome structure and function

A proteasome is a highly selective, multicatalytic proteinase complex that degrades and thereby inactivates intracellular proteins.\textsuperscript{51} Proteasomes consist of two complex components, a cylindrical 20S core complex (or 20S proteasome) formed by 28 subunits and the 19S caps, docked on the ends of the core. The entire complex, referred to as 39S proteasome, is viewed as the biologically active unit within the cell. For proteasomal degradation proteins are marked by conjugation with several ubiquitin molecules, which are small ubiquitously expressed 76 amino acid proteins. Ubiquitin is among the evolutionary most highly conserved proteins – yeast and human ubiquitin differ only by three amino acids. Ubiquitin is initially activated in an ATP dependent manner, prior to its binding to one of hundreds of ubiquitin ligases, which specifically recognize the proteins to be degraded. The process is repeated several times to form an ubiquitin chain of at least four residues. The longer the chain, the more rapid is the degradation of the ubiquitinated protein. Marked proteins are bound by the 19S complex, unfolded energy dependently, and drawn into the lumen of the 20S proteasome cylinder where they are cut into peptides of variable size (3–22 amino acids).\textsuperscript{52}

The proteasome as a therapeutic target

Protein degradation by proteasomes is an important physiological process ensuring exact regulation of the breakdown of metabolic enzymes, transcription factors, and proteins regulating cell cycle and cell death, such as cyclins, CDK inhibitors or p53. Proteasomes are targeted in the treatment of malignant tumours, since the inhibition of their activity reduces the degradation rate of proteins with anti-tumour activity, leading to their accumulation. The main targets for proteasome inhibition are thought to be primarily the NF-κB pathway and to a lesser extend p53 degra-
dation and some other proteins like heat shock proteins, Jnk and caspases. The tumour suppressor p53 is responsible for the transcriptional activation of a series of proteins involved in cell cycle control, apoptosis and senescence. Activation of p53 occurs mainly through protein stabilization, i.e. decreased ubiquitination by its specific ubiquitin ligase mdm2 and decreased proteasomal degradation. NF-κB is a transcription factor that activates the expression of a number of genes whose products mediate cell proliferation and the prevention of apoptosis. NF-κB is activated by proteasomal degradation of its cytoplasmic binding partner I-κB which prevents NF-κB nuclear translocation. Increased levels of I-κB in the cytoplasm as a result of proteasome inhibition reduces proliferation of cancer cells, as well as resistance to cell death programs, and moreover increases the proapoptotic effect of conventional chemotherapy regimes and radiation therapy.

Proteasome inhibitors

Bortezomib (Velcade™), a dipeptidyl boronic acid, is a synthetically manufactured highly specific proteasome inhibitor. It inhibits growth in vitro in a number of cancer cell lines and has also demonstrated growth-inhibitory effects on xenotransplanted human tumours in mice; in some models it has been associated with a notably longer survival rate of laboratory animals. In in vitro studies, hypoxia enhanced the proapoptotic effect of bortezomib. In addition, in vivo activity was partly due to the pronounced anti-angiogenic effect of the proteasome inhibitor. A number of phase I clinical trials have shown activity of bortezomib against advanced-stage melanoma, and several studies are currently evaluating its efficacy as a monotherapy or in combination with cytotoxic substances (http://www.clinicaltrials.gov). One bortezomib/melanoma study however, was already closed due to early evidence of insufficient clinical efficacy following an interim analysis after treating 27 patients. In contrast, for the treatment of multiple myeloma bortezomib has entered clinical practice as third- and second-line treatment and is currently under evaluation as first-line treatment in patients with newly diagnosed multiple myeloma in combination with melphalan/prednisone in comparison to standard melphalan/prednisone therapy.

Perspectives

Therapy of stage IV melanoma has not been fundamentally improved over the last years. Therefore, there is a pressing need for new therapeutic approaches. Although the first attempts of targeted therapy of advanced melanoma did not fulfil the great expectations, the success of some targeted therapeutics in the treatment of other malignancies maintains the hope that a critical molecular target and the optimal agent to interfere with will be discovered in the near future. Moreover, targeting multiple cancer pathways by the combination of inhibitors or combination of targeted with traditional therapeutics may provide the future breakthrough. However, since the enormous multitude of possible combinations of agents can not be evaluated in patients meaningful preclinical test system are warranted.

Riassunto

Terapia target molecolare per il melanoma

La crescente comprensione della patologia molecolare dei tumori maligni avrà un impatto sui futuri approcci terapeutici. L’intervento efficace, privo di tossicità, per un ampio gamma di neoplasie, compreso il melanoma, può potenzialmente derivare dai farmaci mirati nei confronti delle anomalità molecolari che provocano la progressione del tumore: 1) L’attivazione costitutiva della cascata legata alla traduzione del segnale Ras/MAPK- e PI3K/Akt; 2) la deregolazione epigenetica dell’espressione genica dovuta a mutazioni radicali della mutilazione del DNA e a modificazione dell’istone; e 3) la perdita della stabilità delle proteine sopprimenti il tumore sono eventi per i quali è stato dimostrato il coinvolgimento nella melanomagenesi. In questa review vengono presi in considerazione gli inibitori a basso peso molecolare che interferiscono con questi eventi deregolati, così come i risultati dei primi studi clinici relativi a queste sostanze.

PAROLE CHIAVE: Melanoma, diagnosi - Melanoma, terapia farmacologica - Cute.

References

MOLECULAR TARGETED THERAPY OF MELANOMA

Houben


Melanoma is an enigmatic cancer that can be deadly in any and all of its forms if left unchecked or undiscovered. The incidence of melanoma is rapidly increasing over the last decade, the reasoning being multi-factorial combined with several known risk factors within the environment as well as personal behaviors. Although often overlooked in terms of importance, it cannot be stressed enough that we will have the most impact upon overall survival if we re-focus our efforts on the early detection and prevention of melanoma. Once melanoma has spread to the lymphatic system or hematogenously, the overall survival of all patients dramatically decreases in comparison to the outstanding survival of those patients who undergo the appropriate margins of excision for melanoma in situ. Indeed, the surgical management of melanoma has changed dramatically within the last 20 years. We have been guided by the results of well-designed prospective, randomized trials addressing the optimal surgical margins of excision of the primary melanoma, able to modify and refine the way that we surgically manage such patients. Further studies have definitively addressed the efficacy of the elective lymph node dissection, with recent studies showing the central role of selective lymphadenectomy in the management of the draining lymph node basin(s). We will discuss all of these issues and more, providing evidence-based data to support (or refute) current surgical thought and decision making. Some issues still remain controversial due to a paucity of evidence-based data to support their use. Other ongoing studies may further guide us in the surgical management of melanoma. Lastly, we will revisit the past surgical advances for melanoma and how we can learn from them. In turn, this will guide us as to how we will proceed and advance this field in the future.

**KEY WORDS:** Melanoma - Metastases - Sentinel lymph node biopsy - Lymphoscintigraphy - Lymph node dissection - Multidisciplinary cancer care - Surgery - Cytoreductive surgery.

**The history of surgical treatment for melanoma**

There are but a few diseases that are capable of eliciting a true sense of fear in individuals, with melanoma renowned for being such a deadly disease. Some of this fear is real, while other long-held beliefs are but the result of folklore and tales passed down from one generation to the next. The surgical management of melanoma can be generally divided into 3 eras: the 1st period defined as the John Hunter era, with the surgical philosophy of “small cancer, large operation”. The 2nd period, often described during the late 1800’s, is defined by the work of Dr. H.E. Snow, who published his observations advocating extensive surgery for melanoma, consisting of a large excision of the primary melanoma with concomitant excision of the draining lymph nodes. The 3rd period began in
the early 1960’s and continues through today, defined by the design and conduction of experimental clinical trials based upon hypothesis driven ideas as to the optimal surgical management of melanoma.

Our history reveals that cutaneous melanoma has afflicted humans as far back as paleopathologists are able to examine, first noting rounded, melanotic masses on the skin of pre-Columbian Incan mummies, now present day Peru. These mummies were estimated to be 2,400 years old by radiocarbon dating, with the calveria and long bones revealing metastatic tumor deposits. Melanoma was also described in the writings of Hipposcrates in the 5th century B.C. and by the Greek physician Rufus of Ephesus, circa 60-120 A.D.2 Over the centuries, there have been only a few well-described cases of pigmented malignant lesions, with the first published account discussing the surgical treatment of melanoma by John Hunter in 1787.3 Although he never described the excised tumor in any great detail, the actual specimen, the lower mandible of a male with melanoma, remains well preserved as Hunter’s specimen #219 in the Hunterian Museum of the Royal College of Surgeons in England. Hunter described his specimen as “…a cancerous fungous excrescence….part white and part spongy, soft and black”.

In 1806, it was Renee Laennec, more famous for his invention of the stethoscope, who first described melanoma as “la melanose” to the Faculté de Medicine in Paris, France.4 He later coined the term “melanosis” (derived from the Greek word meaning “black”) in 1812.5 In 1820, William Norris described the first case of melanoma in the English literature, later publishing the first comprehensive study of melanoma entitled, “Eight cases of melanosis with pathological and therapeutic remarks”.6 7 This landmark article was the first observational analysis of a group of patients with melanoma, accurately describing many of the epidemiological, clinical and pathological features of patients with melanoma, of which many of his observations remain true today (Table I).

In 1834, David Williams may have been the first to comment on the vertical and horizontal growth phases of a superficial spreading melanoma. He describes a spilus (tumor), that “increase(ed) in size and …continued to spread gradually and after the spilus had attained the circumference of a shilling, an excrescence, similar in color to itself, began to rise in its center”.8 Oliver Pemberton published his observations of 60 cases of cutaneous and ocular melanoma in 1858, by far the largest series of patients with melanoma for the time period, noting the post-mortem findings in 33 cases.9 One of his reported cases is that of a 29 year old male from Madagascar, the first described case of cutaneous melanoma in a black person. He reported the site as along the side of the man’s foot, an uncommon site for caucasians. Pemberton was also one of the first surgeons to note the futility of many treatments for advanced disease and was a strong advocate of surgical management of melanoma with wide excision of the primary and extensive resection and removal of the draining lymph node basins. However, such radical concepts of surgical management were not uniformly accepted, shunned by many in favor of traditional local therapies with salves and other medicinal treatments passed down from previous generations.

Over time, the excision of the primary lesion with wide margins was slowly gaining favor with a small group of surgeons. In 1903, Frederick Eve described a case series of 45 patients with melanoma treated at the London Hospital, commenting that ~80% of the melanoma cases had originated from pigmented moles on the skin.10 He strongly expressed his views on the surgical management of melanoma, stating in his lecture, “The treatment of melanoma can be given in a few words, free excision or amputation, in accordance with the position and extent of disease...The removal of the nearest chain of lymphatic glands, whether palpably involved or not, should never be omitted; for it may be taken as a matter of certainty that in the great majority of cases they are infected.”

<table>
<thead>
<tr>
<th>TABLE I.—Observations on melanoma (Dr. William Norris, 1857).7</th>
</tr>
</thead>
<tbody>
<tr>
<td>— There is a correlation between moles and melanoma</td>
</tr>
<tr>
<td>— The majority of patients have light-colored hair and hair complexities</td>
</tr>
<tr>
<td>— Family history of melanoma in some cases and probably a hereditary predisposition to the disease/cancer may run in families</td>
</tr>
<tr>
<td>— Although often black in color, the degree of pigmentation varied, with some amelanotic</td>
</tr>
<tr>
<td>— Tumor were often nodular and pedunculated/satellite tumors can develop anywhere</td>
</tr>
<tr>
<td>— Widespread dissemination could involve the lungs, liver, bone, heart</td>
</tr>
<tr>
<td>— They were more often men and in heavy smokers</td>
</tr>
<tr>
<td>— They usually remained in good health until a very late stage of the disease</td>
</tr>
<tr>
<td>— Fever was not a feature, in contrast to tuberculosis</td>
</tr>
<tr>
<td>— Local lesions can recur with minimal excisions</td>
</tr>
<tr>
<td>— Wide excision is essential for long-term survival (reported an 8-year survivor with this treatment)</td>
</tr>
<tr>
<td>— Neither medical or surgical treatment was effective when the disease was widely disseminated</td>
</tr>
</tbody>
</table>

172 GIORNALE ITALIANO DI DERMATOLOGIA E VENEREOLOGIA Aprile 2007

RIKER
THE SURGICAL MANAGEMENT OF CUTANEOUS MELANOMA
During his research fellowship at Middlesex Hospital in London 1907, William Sampson Handley studied the lymphatic spread of a melanoma in individuals. He summarized his results in a Hunterian Lecture entitled “Melanotic growths in relation to their operative treatment,” in which he strongly supports the views of Frederick Eve, advocating wide excision of the primary melanoma in combination with elective regional lymph node dissection or possibly amputation in selected cases. In this manuscript, he accurately notes that the “permeation of the lymphatics is the principle agent in this local centrifugal spread” of melanoma, recommending a circular incision of about one inch from the edge of the tumor, and another two inches into the subcutaneous fat. Handley’s work, published in *The Lancet* in 1907, is of great historical significance as his recommendations became the basis for the surgical management of melanoma for the next 75 years (Figure 1).

Until the 1960s, invasive melanoma was considered a high-risk disease that required an extensive local excision for all tumors. Wallace H. Clark, Jr., a pathologist at the Massachusetts General Hospital, had a passion for the morphology and biology of neoplastic pigment cells, becoming renowned for his descriptions of melanocytes and melanosomes seen with electron microscopy. In 1969, he used the microscopic morphology of melanoma cells as a basis for classifying tumors into various stages of development. His studies revealed the progression of melanomas from a radial growth phase to that of a nodular, or vertical growth phase. Clark also recognized variant patterns of the radial growth phase, further developing a classification system (“Clark’s level”). He classified primary cutaneous melanoma samples based upon the extent of tumor invasion related to the anatomic layers of the skin, correlating the level of invasion to that of overall survival. This system constituted the first widely used prognostic model for melanoma, followed shortly thereafter by Alexander Breslow who added a second method of measurement, based upon the true vertical thickness of the tumor, measured in millimeters. This system provided a more accurate and reproducible method of measurement than previous methods, providing an excellent correlation with overall 5-year survival. A comparison of the two systems along with other histologic parameters reveal that the tumor thickness, measured in millimeters, was a better predictor of metastasis and overall survival compared to the Clark’s level of tumor invasion.

In the early 1970s, Donald L. Morton observed that the body developed serum antibodies against tumor cell surface antigens in melanoma patients. His pioneering discoveries led to a major program in vaccine development against melanoma antigens. Additionally, he added to our understanding of the lymphatic system and how it relates to the drainage of melanoma cells from the primary lesion, developing the sentinel lymph node concept in 1990. He conclusively showed that sentinel lymph node mapping was able to accurately identify the first draining lymph node that was connected to the primary tumor site, further revealing that extensive microscopic analysis of a single lymph node was possible and predictive of the status of the remaining nodal basin. The utility of sentinel lymph node mapping has resulted in a true paradigm shift in our surgical approach to staging patients with melanoma.

Steven Rosenberg continues to spend most of his career, which has lasted more than 3 decades to date, focused upon the immunotherapy of cancer, in particular melanoma. He was the first to utilize interleukin-2 to treat patients with metastatic melanoma, currently the only FDA approved agent to treat such patients. His group within the surgery branch of the National Cancer Institute continues to develop groundbreaking treatments for patients with advanced melanoma. They have recently developed a novel approach, termed adoptive cell transfer (ACT) therapy, which involves the isolation, and *in vitro* growth of large numbers of highly active, melanoma-specific autologous T cells. This is followed by infusion of these reactive T-cells back into the same patient, usually given in combination with high dose IL-2 as well. This approach represents a promising therapy for the treatment of advanced melanoma. The initial studies using cloned melanoma-antigen specific T cells, with or without IL-2, seemed to be relatively ineffective at inducing an objective anti-tumor response. Others

![Figure 1.—A depiction of Handley’s original diagram advocating wide local excision for primary melanoma. The dotted lines (arrowheads) represent his recommended margin of excision.](image-url)
approaches utilized tumor-infiltrating lymphocytes (TIL), which has hypothetical advantages over other approaches due to the diverse effector population comprised of both CD4+ and CD8+ T cells. In clinical studies where TIL were used in conjunction with high dose IL-2, objective tumor regressions were seen in 33% of the patients treated, unfortunately, these clinical responses were of short duration with the persistence of the transferred TIL only transient.\textsuperscript{22, 23}

To enhance the efficacy of ACT utilizing TIL, a lymphodepleting, non-myeloablative pre-conditioning chemotherapy regimen consisting of cyclophosphamide and fludarabine was given before the administration of highly reactive melanoma-antigen specific TIL combined with high dose IL-2.\textsuperscript{24} The initial study involved 13 patients with progressive melanoma despite multiple previous treatments, including high dose IL-2, 6/13 patients achieving an objective clinical responses and 4/13 exhibiting a mixed response with regression of some lesions but with progression in other lesions.\textsuperscript{24} In a recent follow-up study, cancer regression in patients with refractory metastatic melanoma with large, vascularized tumors was found in a striking 18/35 patients (51% response rate), including 4 patients with a complete regression of all metastatic disease.\textsuperscript{25} To date, this immunotherapeutic approach is the highest reported response rate ever reported for the treatment of patients with stage IV melanoma.

Recently, the Rosenberg group examined the utility of autologous peripheral blood lymphocytes transduced \textit{ex vivo} with a retrovirus encoding a T-cell receptor for the MART-1 antigen.\textsuperscript{26} This experimental regimen was administered to a total of 15 patients in conjunction with high dose IL-2 in a lymphodepleting, non-myeloablative setting. A total of 2/15 patients achieved objective clinical responses that are ongoing after a year, demonstrating for the very first time the efficacy of gene therapy combined with a multi-modal approach (IL-2, non-myeloablative chemotherapy) to treating stage IV melanoma patients.

It is important to recognize the important contributions of past physicians and surgeons, learning from their experiences in the clinical management of patients with melanoma. Their exceedingly insightful observations have paved the way for improvements in our present day surgical management. Other such observations and tenets have since been dismissed based upon the results of several well designed multi-institutional and cooperative prospective, randomized clinical trials. It is clear that we must continue down the pathways of our predecessors and strive to improve the quality of surgical care for all melanoma patients. The future holds great promise for the development of novel treatment strategies for those patients with advanced disease, many of which do not require the scalpel. However, the basic tenets for the surgical management of primary melanoma and regional nodes have been forged from previous trials, while questions still remain as to the optimal management of patients with late stage, advanced disease.

### Early detection and prevention of melanoma

It is imperative that the diagnosis of cutaneous melanoma be made as early as possible, as this directly correlates with long-term outcome. For decades, physicians have utilized the clinical examination of the skin as the primary screening modality for detect-

---

**Figure 2.**—The ABCDE’s of melanoma diagnosis. A: asymmetry. B: border irregularity. C: color change. D: diameter increase. E: elevation.
ing melanoma. Yet, the ability of the clinician to accurately identify those lesions that are melanoma is highly variable in most cases, making the correct diagnosis in only about 65% of all cases. Current methods of diagnosis depend heavily upon the physician’s ability to identify the classic signs of a cutaneous melanoma, primarily the A-B-C technique (Figure 2). Although many of newly diagnosed lesions may have one or more of these signs, some may not, such as the amelanotic melanoma that is void of any pigmented change (Figure 3). It is very common to have the patient diagnose their own melanoma, often coming in to see the physician after noticing a recent change in the lesion or an episode of bleeding. Sometimes, it is the spouse that first notices a distinct change in a skin lesion. Regardless, we currently recommend that everyone perform full body, naked skin exams on a monthly basis at home, in combination with a yearly full body skin examination by their physician. All lesions that are deemed suspicious on physical examination should undergo a biopsy, with the continued observation of such suspicious lesions discouraged.

The accuracy rate of detection can be improved by 10-20% with the addition of other imaging tools, such as epiluminescence microscopy and sequential full body photography. However, no matter what observational threshold is being followed, many lesions that are deemed suspicious for melanoma will ultimately undergo a biopsy to obtain a definitive diagnosis. Obtaining a tissue sample by means of whatever method of biopsy, followed by histological examination of the tissue is still considered the “gold standard” for accurately making the diagnosis of primary cutaneous melanoma.

Biopsy techniques

The majority of clinical management guidelines recommend that a pigmented lesion or mole that is deemed suspicious undergo an excisional biopsy as the preferred method of biopsy, obtaining a margin of normal skin of 1-2 mm. The depth of the biopsy should encompass the subcutaneous fat, with complete removal of the lesion in order to provide an unencumbered histologic examination that is inclusive of the entire lesion. This will provide an accurate Breslow’s depth of invasion in addition to other important prognostic determinates. The definitive surgical procedure of the primary melanoma should be deferred until the final histologic diagnosis has been made, even for suspected thin melanomas such as melanoma in-situ. It is imperative for the clinician to be cognizant of cosmically sensitive areas when performing a biopsy as this will dictate the type of biopsy performed and the necessity for possibly specialty surgical consultation. Definitive excision of such areas must be deferred until the final diagnosis has been com-
pleted, as often the pathological diagnosis yields a benign result that does not require any further surgical intervention.41, 42

If a punch biopsy is performed, one should obtain the sample from the thickest portion of the lesion, avoiding areas of crusting, ulceration or necrosis that may grossly underestimate the overall thickness of the tumor (Figure 4). It is not recommended to perform a punch biopsy at the edge of the lesion, as this may markedly underestimate the true thickness of the lesion and may not be representative of the sample as a whole. If the surrounding architecture is to be included, then a deep shave (scallop) biopsy of the entire lesion should then be performed (Figure 4). Although the preferred method of biopsy is the excisional biopsy, many physicians will perform a deep shave, or saucerization, of a lesion suspected of being melanoma. This is usually done with either a scalpel or a single-edged razor blade held in a semi-curved position.27 A saucerization is essentially a modified shave biopsy that adequately samples the deeper dermis, and is achieved by pinching the skin around the lesion while curving the razor blade.43

One potential drawback of either method is that there remains the possibility of transecting the base of the lesion, thus resulting in a deep margin that is involved with melanoma. This is problematic in that the true Breslow’s thickness is not known, creating a diagnostic dilemma for the surgeon in terms of the decision-making for the appropriate surgical margins and whether the draining lymph node basin needs to be evaluated. A second consideration as to the type of biopsy performed is the cosmetic outcome. A shave biopsy site will heal by secondary intention, often taking several weeks before completely filled in with scar tissue. This type of healing process is inferior in terms of cosmetic outcome compared to other techniques, such as a punch biopsy with primary closure. However, the biggest advantage of performing a punch biopsy is that the specimen can be accurately measured for true depth of invasion. The Breslow’s depth of melanoma invasion is what will ultimately dictate the appropriate course of surgical management for the majority of patients. The resulting defect after a punch biopsy is easily closed primarily with 1 or 2 interrupted sutures, resulting in a superior cosmetic outcome compared to a shave biopsy that heals by secondary intention. The largest available punch biopsy is 6 mm in greatest diameter, thus for larger primary lesions, this may not be able to completely remove such lesions. This may limit the amount of adjacent normal skin collected and thus limit the interpretation of the histologic architecture of the entire specimen, which in combination with other cytological features, is of particular importance when diagnosing melanoma.44

Additionally, there are several other important features that require special attention in order to obtain an accurate diagnosis of melanoma, such as the presence of asymmetry, the lack of circumscription and the presence (or absence) of scattered atypical melanocytes throughout the epidermis and adnexal epithelium. Such features may not be present if a small punch biopsy only is performed and the type of biopsy must be taken into account by the dermatopathologist.43 In cases of inadequate sampling, it may be necessary to completely remove the lesion with an excisional biopsy in order to confirm the diagnosis of a suspected melanoma.

**Excision of the primary melanoma and surgical margins**

The surgical management of cutaneous melanoma must always begin with the proper identification and treatment of the primary lesion. When melanoma is diagnosed in its earliest stages, over 90% will be essentially cured by surgical excision alone.45, 46 It is important to understand that removal of the appropriate surgical margins with the excision of a primary melanoma is instrumental in minimizing the most important potential complication associated with surgical excision, that of local tumor recurrence. Local recurrence of disease may be due to the lack of excising adequate surgical margins, with the possibility of tumor cells present outside of the margins of excision (Figure 5). It is
TABLE II.—Local recurrence and associated mortality.

<table>
<thead>
<tr>
<th>Primary tumor thickness</th>
<th>Recurrent rate</th>
<th>5-year survival rate</th>
<th>10-year survival rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ng et al.57</td>
<td>&lt;1 to &gt;4 mm</td>
<td>7%</td>
<td>61%</td>
</tr>
<tr>
<td>Dong et al.58</td>
<td>0.76 to 4.0 mm</td>
<td>—</td>
<td>51%</td>
</tr>
<tr>
<td>Soong et al.59</td>
<td>&lt;1 to &gt;4 mm</td>
<td>5%</td>
<td>41%</td>
</tr>
<tr>
<td>Urist et al.50</td>
<td>0.76 to 4 mm</td>
<td>5%</td>
<td>50% (3-yr)</td>
</tr>
<tr>
<td>Reintgen et al.61</td>
<td>0.76 to 4 mm</td>
<td>—</td>
<td>55%</td>
</tr>
<tr>
<td>Kalady et al.62</td>
<td>&lt;1 mm</td>
<td>15%</td>
<td>60%</td>
</tr>
</tbody>
</table>

The standard operative approach in the past usually included a 3-5 cm margin of normal skin measured from the outer edge of the melanoma in all directions, with most patients requiring a split-thickness skin graft to cover the resulting defect. This extensive surgical procedure resulted in a prolonged hospital stay and associated perioperative complications such as wound infection and skin graft necrosis. Fortunately, the extent of surgical resection and margins began to be questioned, allowing for the completion of several prospective, randomized trials that have addressed this issue. The first trial addressed the efficacy of 2 versus 4 cm margins for primary melanomas between 1 and 4 mm in Breslow’s thickness. The Intergroup Melanoma Committee found a local recurrence rate of 0.8% for patients who had 2 cm margins compared to 1.7% for those who had 4 cm margins taken at the time of surgery. The differences in local recurrence were not statistically significant. There was, however, a significant difference in the number of skin grafts needed, with 46% of the 4 cm group requiring a skin graft but only 11% of the 2 cm group. This same trial was recently updated to provide 10-years of follow-up, again revealing no significant differences in the local recurrence rate, disease-free or overall survival. This trial clearly demonstrated that a 2 cm margin is safe and effective compared to a 4 cm margin for primary melanomas between 1 and 4 mm, with a marked decrease in the need for skin grafting. There have been two other trials that have examined 2 cm vs 5 cm margins for intermediate thickness primary melanomas <2 mm in Breslow’s thickness, with both studies showing no difference in local recurrence rates or overall survival.

Several randomized trials have firmly established that the overall thickness of the primary melanoma dramatically influences the likelihood of a local recurrence. The first trial to address this issue was the World Health Organization (WHO) Melanoma Group study by Veronesi et al., which prospectively randomized patients with primary melanomas ≤2.00 mm in Breslow’s thickness to 1 cm versus 3 cm surgical margins. There were no local recurrences at all among patients with primary melanomas of <1 mm, regardless of whether a 1 cm or 3 cm margin was taken. In those patients with primary melanomas between 1 and...
2 mm, there were 4 local recurrences, all occurring within the group that had received 1 cm margins. However, there were no differences noted in either group in terms of disease-free or overall survival. This trial has been recently updated with 15-year follow-up, and there were still no difference noted in disease-free or overall survival. This important study provides a clear demonstration that a surgical excision margin of 1 cm is safe and provides excellent local control for melanomas ≤1.00 mm in Breslow’s thickness.

For primary melanomas with a tumor thickness between 1-2 mm, current NCCN guidelines suggest that the margin of excision can be between 1 and 2 cm, depending upon the anatomic circumstances. In most cases, it will be possible to perform a 2-cm margin of excision with a smaller 1-cm margin acceptable if placement of a skin graft or an excessively high amount of skin tension will result from taking a larger 2-cm margin. Recently, Thomas et al. prospectively examined the excision margins in a defined “high-risk group” of patients with primary melanoma, considered >2 mm in Breslow’s thickness in this study. All patients were randomized to either 1 cm or 3 cm margins of excision, finding that a 1 cm margin of excision for melanomas of at least 2 mm in Breslow’s thickness was associated with a significantly greater risk of combined (local and regional) recurrence when compared to a 3 cm margin. It is important to note that this high risk group included all primary lesions >2 mm in thickness (median tumor thickness was 3 mm), and therefore the results and conclusions of this trial cannot be directly applied to those patients with only thick (>4 mm) primary lesions. Regardless, this is an important trial because it is the first time that a randomized trial examining surgical margins of excision has demonstrated a significant increase in combined locoregional recurrence with a narrower 1-cm margin. However, there was no statistically significant difference noted in the death rate from melanoma associated with a narrow (1 cm or less) margin of excision for thicker melanomas.

The data addressing the recommended surgical mar-
gins for thick melanomas (>4 mm) have been addressed with both retrospective and prospective analyses. A multi-institutional retrospective review of surgical margins and prognostic factors in patients with thick (>4 mm) primary melanomas was performed on 278 patients, showing no difference in local recurrence rate, disease-free survival or overall survival if margins larger than 2 cm were taken.66 Indeed, this is addressed by Ng et al., who analyzed 1155 patients who had their primary melanoma excised, finding 84 (7%) patients overall with local recurrences, and 33 of those 84 (39%) patients dying of their disease.57 The 5-year survival for all patients with local recurrence was 59%, compared to 86% for those without a local recurrence (P<0.0001). Other studies examining local tumor recurrence and its impact upon long term survival have yielded similar results.58-61

Even patients with thin melanomas (≤1 mm in thickness) deserve an appropriate surgical margin, as recurrence does occur in this group and is a true harbinger of very poor prognosis and outcome.62 Although it is recognized that a 2-cm margin of excision is adequate for melanomas >1 mm, there may not be overwhelming support for a trial that wishes to address the value of a smaller margin, such as a 1 cm margin compared to a 2 cm margin of excision for primary melanoma. This would certainly be interesting to conduct such a trial for cosmetically sensitive areas such as the face and head and neck, however, this would require a tremendous amount of resources in order to answer this question. Rather, our efforts may be better directed towards research into developing better prognostic markers of outcome, whereby we can apply such markers to specific subgroups of melanoma patients. This may allow us to select those patients that will benefit the most (and exclude those that will not) from a given procedure, such as SLNB for thin or thick melanoma. The current recommendations for excision margins for primary cutaneous melanoma are outlined in Table III. In terms of the need to remove (or not remove) the deep muscular fascia, there does not appear to be any clear advantage (or disadvantage) to removing the deep muscular fascia as part of the definitive excision of the primary melanoma. Several studies have addressed this issue and it does not appear that there is any significant difference in recurrence rates, locally or distant, when the fascia was either left in place or removed as part of the definitive surgery.63, 64

---

**Table III.—Recommendations for excision margins of primary cutaneous melanoma.**

<table>
<thead>
<tr>
<th>Location</th>
<th>Tumor Thickness</th>
<th>Margins</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trunk/proximal extremity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanoma in-situ</td>
<td>0.5 cm</td>
<td></td>
</tr>
<tr>
<td>≤1 mm</td>
<td>1 cm</td>
<td></td>
</tr>
<tr>
<td>1-2 mm</td>
<td>1-2 cm</td>
<td></td>
</tr>
<tr>
<td>&gt;2-4 mm</td>
<td>2 cm</td>
<td></td>
</tr>
<tr>
<td>&gt; 4 mm</td>
<td>2 cm</td>
<td></td>
</tr>
<tr>
<td><strong>Head/neck, distal extremity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(or cosmetically sensitive area)</td>
<td>≤1 mm</td>
<td>1 cm</td>
</tr>
<tr>
<td>&gt;1 mm</td>
<td>at least 1 cm</td>
<td></td>
</tr>
</tbody>
</table>

---

**Mohs micrographic surgery**

The primary goal of the surgical management of primary cutaneous melanoma is to completely remove the entire tumor in order to minimize the chance of a local recurrence. However, removal of the optimal surgical margins may be compromised when dealing with melanomas in cosmetically sensitive areas, such as the head and neck. The utility of Mohs micrographic surgery (MMS) in treating melanoma has developed over the years to become a recognized form of surgical therapy in select patients with melanoma. For example, MMS may provide some advantages in treating head and neck melanoma, such as with the removal of the lentigo maligna subtype. The utility of MMS in this situation has been demonstrated to have a low local recurrence rate (0.2% for MMS vs 9% for conventional surgery) and smaller tissue defect due to the minimization of the skin margin removed.65 Additionally, MMS for primary cutaneous head and neck melanomas may contribute to favorable outcomes where extensive sub-clinical spread can be found in many cases.

However, it should be recognized that when MMS is applied to melanoma lesions, it is important to examine 100% of the surgical margin for each tumor, accomplished by either frozen section or standard histological analysis. If frozen section analysis is to be performed at the time of MMS, it is essential that the Mohs surgeon be adequately trained to interpret such results accurately and that a trained and experienced histotechnician be available for the proper preparation of thin marginal analysis without undo processing artifact.66 Although there may be some advantages to performing MMS on primary melanomas of the head and neck due to the minimization of removing excess skin, its role in truncal and most extremity melanomas is limited. In general, there is only a small difference
in excess tissue removed with MMS compared to that of standard surgical excision based upon Breslow’s tumor thickness. Regardless, MMS requires both meticulous surgical and pathological analysis in order to optimize patient outcomes and minimize local recurrence rates.

Truncal and extremity melanoma

The surgical management of truncal and extremity melanoma is fairly straight-forward, with the basic tenets of surgical therapy to remove the primary melanoma with the appropriate surgical margins. However, certain situations and anatomic locations may alter the surgeon’s approach to management, such as melanomas located along the forearm, leg, and digits. In particular, a melanoma >2 mm in Breslow’s thickness on the forearm will require a 2-cm circumferential excisional margin with a resultant defect of at least 4 x 4 cm. Due to the anatomic limitations of skin mobility in such areas, it is often necessary to utilize a split-thickness skin graft (STSG) for adequate coverage, often taken from the anterolateral aspect of the thigh. Other possible donor sites may include a full thickness skin graft from the lower quadrant of the abdomen with primary closure of this defect, thereby sparing the patient the increased pain and discomfort associated with a STSG from the thigh.

The majority of primary melanomas located on the back can be treated with the appropriate excision margin followed by skin edge approximation and primary closure (Figure 7). The skin on the back is generally thicker and has more laxity compared to other areas of the body, with the resulting defect successfully closed primarily without the need of skin grafting. In order to minimize the amount of tension along the mid-portion of the defect, attention should be given to the optimal orientation of the surgical excision related to the optimal lines of skin tension in order to minimize the need for extensive undermining of the surrounding skin edges. Occasionally, the surgeon may encounter an undue amount of skin tension and this situation is best treated with the placement of a STSG or possibly one of several plastic reconstructive options such as a rotational, advancement or rhomboid skin flap.

The surgical management of head and neck melanoma

Special attention should be paid to the patient with a primary melanoma of the head and neck due to the added anatomic complexity posed within this region. Although the established guidelines are generally followed whenever possible, melanomas arising within aesthetic areas of the face often require a compromise in such margins. Every attempt should be made to obtain the appropriate surgical margins and concomitantly achieving the best cosmetic outcome with the lowest possible chance of local recurrence. It is imperative that a thorough discussion of the planned excision be made with the patient, outlining the operative plan and any associated reconstruction being performed. The risks, benefits and expected cosmetic outcomes should be carefully discussed with the patient, discussing any unrealistic expectations of any surgical procedure.

The surgical treatment of the primary tumor of the head and neck includes planning the complete excision...
The surgical management of cutaneous melanoma

The surgeon should be well-versed in the anatomy of the face, considering the relaxed skin tension lines and functional aesthetic units of the entire face. Special consideration should be given to primary melanoma excisions that involve overlying lymph node-bearing areas, such as the parotid gland and neck. A pre-auricular vertical incision followed by the development of an anterior cervico-facial flap is able to adequately expose the parotid gland or periauricular and upper neck lymph nodes. In the neck, an upper-neck transverse incision or a mid-neck posterior vertical incision provide optimal exposure to the appropriate cervical lymph node basin.

The method of reconstruction of the primary melanoma excision site depends upon several factors such as the location and size of the defect, the functional and aesthetic requirements and the overall medical condition of the patient. There are numerous possible reconstructive options such as the utility of a STSG, local vascularized and regional tissue flaps as well as myocutaneous flaps (Figure 8).

Common surgical excision of a primary scalp melanoma involves the removal of the appropriate skin margins and underlying subcutaneous fat down to the galea. The underlying periosteum is well-vascularized and provides a good base for the proper healing of a STSG. For smaller excisions, local random rotational skin flaps may be suitable in lieu of skin grafting. In rare cases, extensive surgical excision of the primary melanoma with a large residual defect may require a free flap for adequate wound closure, possibly obtained from several sites such as the rectus, latissimus or radial forearm muscle.

Small excisions of the cheek can usually be closed within the exaggerated “smile lines” on the face. For larger defects involving the medial portion of the cheek, an inferiorly-based cervico-facial rotation advancement flap may provide the optimal aesthetic result. For upper lip defects that are lateral to and above the vermillion border, we utilize a cheek advancement flap for good cosmetic results. Defects along the medial and central upper lip are best suited for a lower lip-cross lip flap. For lower lip defects, local rotation flaps

Figure 8.—Intraoperative photograph of an excision and sentinel lymph node biopsy of a intermediate thickness melanoma of the cheek. A) The excision margins and planned flap have been marked. The sentinel node is located with the gamma probe. B) The lesion has been widely excised and the rotational flap has been raised. C) The skin flap has been retracted and the sentinel node located and excised. D) The completed rotational flap reconstruction.
are often utilized, bearing in mind that if the defect is a result of a complete excision of the lip, muscle, and mucosa, then one of several lip advancement techniques can be employed. The orbicularis oris myocutaneous flap can be an excellent reconstructive choice for lip excisions that have removed between 25% and 75% of the lower lip. If the entire lower lip must be excised, utilization of a radial forearm free flap may be necessary as part of the reconstructive process.

The role of elective lymph node dissection

The evaluation and management of the draining lymph node basins for patients with clinically localized melanomas >1 mm in Breslow’s thickness has evolved over the last 20 years. In the past, we relied upon the staging information gained from performing an elective lymph node dissection (ELND). Although such information on staging was obtained, several prospective, almost all of the randomized trials examining the therapeutic role of ELND have failed to show an overall survival advantage for this technique. However, there is one trial of special note, the Intergroup Melanoma Surgical Trial, reporting 10-year survival results for those patients undergoing ELND. A prospective, stratified subgroup of patients were found to have a significant decrease in their overall mortality, namely those with non-ulcerated melanomas (30% reduction), tumor thickness between 1-2 mm in Breslow’s thickness (30% reduction), age <60 years old (27% reduction) and location of the melanoma on an extremity (27% reduction). Regardless, an elective lymph node dissection provides durable local control and accurate staging for most patients with occult lymph node metastases. A therapeutic lymph node dissection, performed for patients with clinically evident regional lymphadenopathy, provides locoregional control of disease and a chance of cure, with 5-year survival rates of 20-40%. Meyer et al. performed 144 therapeutic lymph node dissections in 140 melanoma patients (14 cervical, 49 axillary, 73 groin) and found a 5-year survival for all patients of 30%.

Secondly, there is some evidence of a potential survival benefit for early evaluation and management of the draining lymph nodes from the WHO trial that compared ELND to observation. This trial randomized those patients with a primary melanoma >1.5 mm into either observation versus an immediate ELND. Although there was no evidence of an overall survival benefit, the group who underwent an immediate ELND and found to have positive lymph nodes had a 5-year survival of 48% compared to the observation arm and delayed CLND with an overall 5-year survival of only 27% (P=0.04). A second trial by Morton et al. compared the survival of matched groups of patients undergoing immediate (after a positive SLN) versus delayed (after nodal recurrence) CLND. They found a significantly higher survival rate (at 5, 10 and 15 years) in the group that underwent immediate CLND following a positive SLN compared to the latter group that underwent a delayed procedure.

The role of sentinel lymphadenectomy for the surgical staging of melanoma

Intraoperative lymphatic mapping and selective lymphadenectomy was originally developed to provide a minimally invasive staging procedure that reduced the associated morbidity and expense of an ELND. The minimally invasive technique of sentinel lymph node biopsy (SLNB) for appropriately selected patients with melanoma has become part of the standard discussion of operative intervention, with the available data showing that SLNB is the best available diagnostic test to date in detecting nodal metastases. Obvious advantages of SLNB over ELND include a less invasive procedure, smaller skin incisions, reduced peri- and post-operative complications, less extensive dissection of the nodal basin, and improved staging information for both the physician and patient. The concept of SLNB is based upon the hypothesis that cancer cells from the primary lesion gain entrance into the lymphatic channels and travel to the nearby draining nodal basin. The SLNB technique is further supported by a large amount of data that clearly indicates that the identification of a tumor-free SLN will translate into a remaining draining nodal basin that is without evidence of metastatic cancer cells. The evaluation of the draining lymph node basins for patients with clinically localized melanoma has resulted in a true paradigm shift in our surgical management.

It is vitally important that we continue to learn from past trials in order to improve upon the way that we treat patients with melanoma presently and in the future. There are four major reasons to perform SLNB, all driven to provide the most accurate information available. The first reason is to improve the accuracy of staging. The second is to facilitate early thera-
patients that present with palpable regional disease. Regard-
lessness, it would seem illogical to think that a staging
test that provides a significant improvement in the over-
all sensitivity, specificity and accuracy of identifying me-
tastatic disease within the SLN of the draining nodal basin.
Few would argue that SLNB provides the most accurate stag-
ing information available for melanoma patients. How-
ever, there are those that will continue the discussion as to the
utility of SLNB, insisting that it must also provide a therapeu-
tic advantage before being accepted into standard surgical
practice. It is akin to the argument for the develop-
ment and utility of the PET scanner, a similarly new
technology developed as a staging tool. McMas-
ters et al. eloquently describes this parallel develop-
ment and reinforces the notion that we never should have
to raise the bar any higher for a diagnostic procedure
that was never meant to show or give an overall survival
benefit to patients.75 Thus, although SLNB is not 100%
accurate, it is a far better test than clinical staging of
regional lymph nodes, and it would seem illogical to re-
vert back to a delayed nodal dissection for those patients
that present with palpable regional disease.77

Others have strongly argued that since this procedure
does not provide a survival advantage, “that it should be abandoned immediately, without question”.78, 79

Additionally, those opposed to SLNB argue that the
involvement of the lymph nodes (sentinel or not) with
melanoma is a “harbinger of distant spread of disease
and associated poor outcome in all cases, with the only uncertainty being when the metastatic disease will manifest itself”.78,79 The current available evidence would strongly refute this notion, faced with several lines of evidence showing that surgical thera-
py alone can result in long-term cures of patients with
stage III melanoma. Young et al. recently evaluated the role of definitive surgical management for patients
with stage III disease.80 At a maximum follow-up of
386 months (32 years) for the total population of 1,422
patients in the trial, the 15-, 20- and 25-year melanoma-
specific survival rates were 36%, 35% and 35%, respec-
tively. Additionally, they found that survival rates were
significantly lower if the regional nodes were palpable.
Although some risk factors decreased the likelihood of
long-term survival, the high overall survival rates in all
groups clearly supports the utility of surgery as the
primary treatment option for regional metastatic
melanoma.

The Multicenter Sentinel Lymphadenectomy Trial
(MSLT-I) is a prospective, randomized, multination-
al trial specifically designed to address the possible
therapeutic utility of SLNB. In this study, a total of
1,269 patients with intermediate thickness melanomas
(1.2 to 3.5 mm) were randomized to either wide exci-
sion only followed by observation (no SLNB) or to
wide excision and SLNB. In the observation only
group, complete lymphadenectomy was performed only when there was clinical evidence of nodal recur-
rence (delayed), while the SLNB group underwent a
complete (immediate) lymphadenectomy if nodal
micrometastases were detected within any of the
SLN’s. The results from this trial were first reported in
December 2004, updated in May 2005 and most recent-
ly published in September 2006 based upon the 3rd
interim analysis.81, 82 They found that the mean esti-
mated 5-year disease-free survival rate was signifi-
cantly higher in the SLNB group compared to the
observation only group (78.3% vs 73.1%, respective-
ly, P=0.009). Although 5-year melanoma-specific sur-
vival rates were similar in the two groups, the presence
of metastatic disease within the SLN was found to be
the single most important prognostic factor predictive
of overall survival. The 5-year survival rate was 72.3%
in those patients with tumor-positive SLN’s and 90.2%
in those with tumor-negative SLN’s.

Interestingly, the incidence of SLN micrometas-
tases was 16% as compared to a very similar rate of
nodal relapse of 15.6% in the observation only group,
strongly suggesting that occult micrometastases in the
sentinel node usually progress to aggressive regional
or distant disease, rather than remaining clinically
insignificant within the SLN [82]. Furthermore, the
mean number of clinically detectable tumor-positive
lymph nodes in the delayed CLND group was 3.3,
compared to 1.4 lymph nodes within the immediate
CLND group. This would indicate that disease pro-
gression is indeed occurring during the observational

---

THE SURGICAL MANAGEMENT OF CUTANEOUS MELANOMA

RIKER

---

Vol. 142 - N. 2
GIORNALE ITALIANO DI DERMATOLOGIA E VENEREOLOGIA

183
period, with a significant difference noted between the two groups in both disease-free survival and the rate of nodal relapse (4.0% in patients with tumor-negative SLN’s and 15.6% in the observation only group). It would appear that such results support the notion that observation of metastatic lymph nodes allows enlargement and adjacent spread to other nodes, thereby increasing the chances of the patient developing distant metastatic disease and a poorer long-term survival.82 This is most evident when a direct comparison is made between the immediate vs. delayed CLND groups, revealing a melanoma-specific survival of 72.3% for the immediate CLND compared to 52.4% in the delayed CLND group. Thus, this recent data is quite compelling in support of performing a SLNB in those patients with an intermediate-thickness melanoma as compared to observation only (no SLNB). Immediate CLND after the finding of SLN-positive disease prolongs survival compared to a delayed CLND performed only upon the discovery of clinically evident disease.81, 82

We believe that it is in the patient’s best interest to be fully informed as to the possible diagnostic and therapeutic tests available to them. A thorough discussion as to the risks and benefits of SLNB should be included for those patients deemed appropriate candidates. It has been our experience that the vast majority of melanoma patients who are candidates for SLNB will chose to undergo this procedure in order to gain useful information about their case. The discussion is not focused on the argument of whether we are providing a survival benefit, but rather centered on if we are providing a diagnostic procedure capable of answering the question of a primary melanoma cell metastasizing to the draining lymph node basin(s). Thus, the available data supports a role for SLNB, able to accurately identify the first draining lymph nodes in a basin and to predict the outcome and staging for melanoma patients. Currently, we utilize a dual technique, combining the pre-operative radiolymphoscintigraphy with intra-operative injection of vital blue dye (Lymphazurin, 1% isosulfan blue dye), resulting in a high accuracy rate for the proper identification of the SLN. (Figure 9).

Figure 9.—Techniques of preoperative and intraoperative lymphatic mapping. A) Preoperative Tc-99 lymphoscintigraphy of a chest wall melanoma demonstrating lymphatic uptake in both axillae. B) Preoperative lymphoscintigraphy of a trunk melanoma demonstrating lymphatic uptake in the ipsilateral axilla and contralateral groin. C) Technique for injection of a primary melanoma lesion with vital blue dye for intraoperative lymphatic mapping. Note the uptake of dye within the afferent dermal lymphatics (arrow). D) Intraoperative appearance of a blue sentinel node.

Sentinel lymph node mapping for thin melanoma (<1 mm)

The risk of regional nodal involvement for thin melanomas (<1 mm in Breslow’s thickness) is in the range of 0-9.7%, with an average risk of about 5%.85 For those primary melanomas with other higher risk factors or between 0.75 mm and 1 mm, the risk of nodal involvement is slightly higher to about 5-8%. It is well recognized that patients within this group also are heterogeneous in nature, with some patients at a much higher risk as compared to others that may have minimal to no risk. There are several factors which may be considered as higher risk when discussing the risks and benefits of SLNB in the patient with a thin melanoma. The presence of a vertical growth phase, Clark’s level of invasion IV or V, ulceration, regression, high mitotic rate and a younger age are all considered high risk features for those with thin melanoma.46, 86-90 There is strong evidence that indicates that tumor mitotic rate and a younger age are more powerful predictors than most, if not all, other prognostic factors available today.88-90 The focus of
our discussion for patients with thin melanomas is centered upon issues of whether the incidence of nodal progression of ~5% warrants the added morbidity and cost of SLNB and if there are specific prognostic/predictive risk factors that could be utilized to determine the need for SLNB among thin melanoma patients.91

There are many patients and physicians who consider a 5% likelihood of nodal metastasis an adequate risk in order to justify the relatively low morbidity of SLNB. However, one cannot forget the intrinsic false-negative rate of about 3% with the procedure itself, with the caveat that such statistics are only meaningful for those patients who indeed have positive lymph nodes, providing a minimal true benefit to most of the overall patient population with a thin melanoma. Thus, a thorough discussion is necessary describing the risks and benefits of SLNB for patients with thin melanoma. Clearly, there are certain recommendations that can be made for melanomas that are very close to 1 mm in Breslow’s thickness, with a strong argument made for performing a SLNB for those melanomas within a few tenths of a millimeter of 1 mm. Although we all have a few anecdotal cases, it is extremely rare to have a truly thin melanoma <0.75 mm metastasize via the lymphatic channels or hematogenously. Such patients are unlikely to benefit from a SLNB and it is fairly safe to recommend to the vast majority of patients (if not all) with melanomas <0.75 mm that they may be safely and adequately treated with a 1 cm margin of excision without a SLNB.92 A recent analysis of 223 patients with thin melanomas who underwent a SLNB reveals that there was a 3.6% rate of nodal metastasis, all in patients with a primary melanoma >0.75 mm in Breslow’s thickness.85 Furthermore, Thompson et al. reported on 187 similar patients with thin melanomas from the Sydney Melanoma Unit, finding a positive SLN rate of 5%, with all patients having a primary melanoma >0.9 mm in Breslow’s thickness.92

It is important to take an evidence-based approach when considering the role of SLNB for thin melanoma patients due to the increasingly large number of patients within this subset. Many have tried to define a specific population of patients that will benefit the greatest from this staging procedure. The biopsy specimens of those patients within the so-called “grey zone” (primary melanoma between 0.75 and 1 mm) must be examined very carefully histologically for all of the previously described pathological features which may sway the surgeon to proceed with a SLNB. We approach such patients by first re-examining all of the pathological slides from the original biopsy or excision, utilizing the expertise and experience of a dermatopathologist. The presence or absence of all pathological features should then be carefully examined and a full and detailed report issued.

It has been our practice to fully explain the risks and benefits involved of the SLNB and weigh them against the small risk of nodal metastasis of about 5%. In particular, if a patient is found to have any one (or more) of the previously considered poor prognostic features, we will thoroughly discuss the role of SLNB with each patient. For those patients with no evidence of such prognostic features, we will still have an in-depth discussion of performing the SLNB with the full understanding of the risks and benefits of the procedure. It has been our experience that the overwhelming majority of patients within this subset will choose to undergo the SLNB after carefully examining their options of either excision of the primary (alone) versus combining this with SLNB.

Role of sentinel lymph node mapping for thick melanoma (>4 mm)

The patient with a thick primary melanoma (>4 mm) has an overall poor prognosis, with an average 5- and 10-year survival of 45% and 39%, respectively.45, 46 For this reason, many question the utility of performing a SLNB in this group of patients, since it is presumed that the patient will already have evidence of either microscopic or macroscopic systemic disease. However, the role of SLNB in this group of patients remains a valuable tool for the most accurate staging and associated survival data based upon clinical and pathological stage. Although there is a clear consensus that such patients have a much poorer long term survival, it is of benefit to perform the SLNB in this setting in order to gain the most accurate surgical staging and to achieve early locoregional control of disease. The best opportunity to provide locoregional disease control is at the earliest possible time of diagnosis, which often represents an earlier time in the progression of the disease process. It is fairly well recognized among surgical oncologists that locoregional control of microscopic disease is associated with a markedly decreased morbidity with a much simpler surgical procedure as compared to surgical excision of bulky regional lymphadenopathy, which is fraught with intra-
operative and postoperative complications ranging from nerve dysfunction, chronic pain, decreased functional ability and life-long lymphedema.75

The final decision to undergo a SLNB for a primary cutaneous melanoma must be made by the patient. We believe it is our obligation as physicians and surgeons to have an objective, data-driven and understandable discussion with our melanoma patients addressing the risks of nodal involvement and what this actually means to the patient. This is equally weighed against the morbidity and possible adverse outcomes of the SLNB procedure, to include a discussion about the costs, false-negative rates and the current paucity of data in regards to any proven survival benefit from this staging procedure.

Role of sentinel lymph node mapping for head and neck melanoma

In general, primary cutaneous melanoma of the head and neck has an overall worse prognosis compared to those located in other locations, such as the trunk and extremities, and therefore it is of particular importance to obtain the most accurate staging information available which will then dictate the adjuvant course of management. The lymphatic drainage of the head and neck is variable and involves a higher number of lymph nodes within the five levels of the cervical region. There are also added concerns in regards to aesthetic outcome that can often be overlooked for head and neck melanoma in comparison to other locations such as the trunk and extremities.

Due to the relatively small anatomic region of the cervical triangles within the head and neck, sentinel lymph nodes may be located very close to, and occasionally directly beneath, the primary site. This may pose some difficulties in visualization as to the exact location of the sentinel node(s) as they may be obliterated by the “shine through” effect of the Te-99 injection around the primary site as seen on preoperative radiolymphoscintigraphy. It is important to perform a thorough exploration of the area directly beneath the primary tumor site for evidence of SLN’s, especially in the area of the pre- and post-auricular face and neck. An additional tool that may be helpful is the utilization of a small amount of vital blue dye.
injected around the primary melanoma site. It is only necessary to utilize a small amount of dye, usually of 0.5 ml. or less, as larger amounts can diffuse into the surrounding skin of the face and neck, possibly resulting in the permanent discoloration of the area outside of the margins of excision. The excision of the primary tumor site should be done simultaneously with the lymphatic mapping and sentinel node removal, thereby eliminating the background radiation from the primary tumor site (Figure 10).

Once the primary melanoma has been completely removed, sub-platsymal skin flaps are raised for identification and localization of the SLN utilizing the hand-held gamma probe to pinpoint the exact location. Careful blunt and sharp dissection is critical within all anatomic nodal levels of the cervical region as many vital structures are at risk for injury and transection. Utilizing the combined techniques of pre-operative radiolymphoscintigraphy and intra-operative vital blue dye injection is the most accurate method of SLN identification, with an accuracy rate of >90%.75

One caveat of performing SLNB in the head and neck is the essential inclusion of “side-view” three-dimensional pre-operative images that note whether the sentinel nodes are within the posterior versus anterior triangle or deeper portions of the cervical region (Figure 11).

There are several structures within the neck that demand particular attention. The greater auricular nerve, external jugular vein, spinal accessory nerve, and branches of the facial nerve should be spared from division during the sentinel lymph node excision. The great auricular nerve to the ear should be identified and can be retracted anteriorly and posteriorly to remove sentinel lymph nodes in the jugulodigastric area. The spinal accessory nerve is often identified deep in the jugulodigastric area along the posterior border of the sternocleidomastoid muscle during sentinel lymphadenectomy. The marginal mandibular branch of the facial nerve must be carefully protected and is particularly prone to a retraction injury against the hardened surface of the mandible. Blunt dissection around the submandibular gland spares any sharp division of the facial nerve at this site.

Lymphatic mapping of the scalp will usually identify sentinel lymph nodes within the occipital and postauricular area, or along the surface or within the superficial portion of the parotid gland. The parotid gland is exposed through a pre-auricular, vertical skin incision followed by retraction and raising of a facial flap. The parotid gland can be fully visualized and careful blunt dissection is utilized to identify and remove the SLN that may be within the parenchyma of the superficial lobe of the parotid gland. The hand-held gamma probe is very helpful in guiding the surgeon towards the identification of a radioactive SLN that has a hint of blue staining within it. The SLN in this area can often be found between the parotid gland fascia and the auricular cartilage, while others may be identified along the posterior surface of the facial vein that lies within the tail of the parotid gland.

If micrometastatic melanoma is found within the SLN of the head and neck, a completion neck dissection should be performed. This will usually involve a modified radical neck dissection in order to remove all of the lymph node-bearing tissue within the various cervical regions. If micrometastatic disease is found within a SLN removed from the parotid gland, a superficial parotidectomy with preservation of the facial nerve is also performed in combination with a modified radical neck dissection.

**Complete and selective lymph node dissection**

Patients with melanoma may often present with more advanced regional disease. The surgical management of bulky lymphadenopathy of the axilla or groin is best managed with a thorough and complete operative removal of levels I and II within the axilla, with level III lymph nodes carefully palpated and
examined in order to determine if further dissection needs to be performed. If there is evidence of palpable adenopathy within level III, they should be removed, as it is very possible that this represents extension of disease into this region. If necessary, transection of the pectoralis muscle is performed in order to improve the overall exposure of this area. Operative removal can be problematic as matted adenopathy of the axilla can be associated with involvement and possibly encasement of adjacent structures such as the thoracodorsal neurovascular bundle, long thoracic and intercostobrachial nerve, axillary vein and even the brachial plexus. Dissection may result in injury of these structures and attention to detail and anatomic localization of the thoracodorsal and long thoracic nerves is essential; sacrifice of either of these nerves should be reserved for cases when the tumor is intimately involving these nerves. Within the groin, complete surgical resection of disease can be equally challenging in the presence of bulky lymphadenopathy, with the overall complication rate with regional lymphadenectomy relatively high.93, 94

Observations about Cloquet’s node

For palpable inguinal lymphadenopathy, the mainstay of surgical therapy will usually include a superficial inguinal lymphadenectomy with inclusion of the deep pelvic region for either clinical evidence of disease (palpable pelvic lymph nodes), radiographic or intra-operative evidence of obvious lymph node involvement. Intra-operative pathologic analysis of clinically suspicious lymph nodes may be necessary in order to determine the presence of metastatic disease and possibly more extensive nodal dissection. Hughes et al. describes 132 patients presenting with clinically palpable inguinal lymphadenopathy, with the extent of dissection determined by pre-operative radiographic studies, clinical suspicion of pelvic nodes, patient morbidity and performance status and evaluation of Cloquet’s node.95 They found that those patients that underwent a superficial and deep nodal dissection (N=72) had a lower regional recurrence rate compared to those whom underwent a superficial dissection only (N=60), with no statistical difference in overall survival for either group.

Cloquet’s node is defined as the highest lymph node within the superficial inguinal node basin, often located at the entrance of the femoral triangle at the inguinal ligament, sometimes adjacent and medial to the femoral vein or within the femoral canal (Figure 12). This lymph node is considered by many to be the last (highest) lymph node within the superficial inguinal region prior to entrance into the deep iliac and obturator nodal system. Thus, it has been hypothesized that the status of Cloquet’s node can be predictive of metastatic involvement of the deep nodal basin, thereby selectively performing a deep pelvic lymphadenectomy only in those patients with a positive Cloquet’s node. The sensitivity and predictive value of Cloquet’s node is variable, ranging from 44-90%.96-98 Recently, Essner et al. have updated their previous experience with Cloquet’s node and have reported a positive predictive value of 66% (negative predictive value of 97%) when basing the decision to perform a deep groin dissection upon the status of Cloquet’s node being positive or negative.99 They found that the pathologic status of Cloquet’s node was superior to the clinical status of the superficial groin nodes for predicting occult iliac node metastases. However, although most surgeons will agree as to the utility of performing a superficial inguinal lymphadenectomy for metastatic disease within this nodal basin, it remains
controversial as to the utility and benefits of performing a deep pelvic lymphadenectomy, regardless of the status of Cloquet’s node. In summary, surgeons should use all possible available data in order to determine whether a patient should undergo further dissection and removal of deep pelvic lymph nodes.

Isolated limb perfusion/infusion for melanoma

The technique of isolated limb perfusion/infusion (ILP) was originally developed by Creech and Krementz at Tulane University in 1958, as a way to deliver higher concentrations of regional chemotherapy compared to systemic administration, but without the systemic side effects. In about 10% of extremity melanoma cases, the recurrence of melanoma is confined to the same extremity where the previous excision was performed, referred to as in-transit disease or satellitosis. Although surgical resection should be considered as the first line of treatment in all cases if possible, there are those patients where resection is not a viable option, either due to an overwhelming tumor burden within the extremity or possibly the disease is spread out over a large area and thus precluding a surgical option. In such situations, ILP may be a favorable therapeutic option as a limb-sparing regional treatment modality. The surgical technique of ILP is a complex and highly invasive procedure that is associated with a number of possible intra- and post-operative complications. However, although complex and requiring a considerable amount of experience from the surgeon, ILP has an established role for patients with in-transit and satellite metastases of the extremity. The addition of hyperthermia (mild hyperthermia, 39-40°C) to the technique of ILP has further enhanced the overall response rates, while limiting the toxicity associated with higher temperatures (40-43°C) (Figure 13).

The reported complete response (CR) rates for melphalan-alone, mild hyperthermic ILP range from 40-82%, with a median response rate of 54% in a large retrospective meta-analysis. The addition of tumor necrosis factor-α (TNF) to melphalan was previously thought to enhance the complete response rate to 60-85%, with suspected increased therapeutic value in patients with large, bulky lesions or recurrent disease after a previous ILP. However, a recent randomized, multi-center trial conducted through ACOSOG has compared hyperthermic ILP with melphalan alone to melphalan + TNF. The results revealed that in locally advanced extremity melanoma treated with ILP, the addition of TNF to melphalan did not demonstrate a significant enhancement of short-term response rates (3 month follow-up) compared to melphalan alone. Furthermore, the addition of TNF to melphalan was found to significantly increase the overall complication rate. Other excellent reviews have described the clinical experience with melphalan alone, or in combination with several other agents, such as TNF, interferon-α (IFN) and chemotherapeutic agents, concluding that ILP has earned a permanent place in the treatment of patients with unresectable or recurrent melanoma of the extremity.

Despite the clear evidence for the effectiveness of ILP, it is a technique that is only utilized in a select few hospitals around the world. Although the technical concept is simple and elegant, it continues to challenge surgeons as a technically complex, labor-intensive and time-demanding procedure that remains costly. For these reasons, Thompson et al. developed the isolated limb infusion (ILI) technique in 1994 as a simpler and less invasive technique compared to ILP. Similar in concept to ILP, ILI was designed as a method with broader applicability, such as in the elderly or those patients with co-morbid conditions that would make an ILP prohibitive. The overall response rates of ILI with melphalan and actinomycin D for treatment of recurrent or advanced melanoma of the extrem-
Reappraising the role of surgical intervention for advanced melanoma

It is well established that the overall prognosis for patients with advanced stage III and IV melanoma is poor. Most patients who experience recurrence with regional nodal disease and subsequently undergo a complete lymphadenectomy will have a 5-year survival of about 25%, while those that recur at any other distant site carry a much poorer prognosis of about 5%. The surgical options for patients with metastatic melanoma will generally fall into three categories: curative, palliative or immunotherapeutic. It is very important that detailed discussions are undertaken with the patient in order to address the ultimate intent of surgical intervention with a special focus upon the realistic view of expected complications, outcomes and goals.

Curative attempts with surgery for metastatic melanoma should carefully weigh the risks of the surgery against the potential benefits. Recent data on the surgical management of metastatic melanoma note that certain factors are associated with an improved overall survival: 1) ability to achieve a complete surgical resection with negative margins; 2) location of the initial site of metastasis; 3) the extent of metastatic disease (single or multiple sites); 4) disease-free interval after surgical removal of the primary melanoma and 5) stage of initial disease. Favorable sites of resection include the skin, subcutaneous tissue, lymph nodes, lung, and gastrointestinal tract, with unfavorable sites associated with metastasis to the brain, adrenal, and liver. Skin and subcutaneous sites had the best long-term outcome with a 20-30% 5-year survival and a median survival of 48 months. Among the patients with metastases to unfavorable sites, there...
were no 5-year survivors, with a median survival of only 18.2 months. In reality, the overall survival and possible long-term outcomes for most patients with stage IV disease seems to ultimately depend upon the intrinsic biologic activity and nature of the melanoma itself.

Often, surgical intervention for metastatic melanoma is performed for palliation, with the primary goal to relieve identifiable symptoms directly caused by growing and metastatic tumor deposits. For instance, patients will often present with bulky, recurrent lymphadenopathy of the axilla or groin that cause severe pain in addition to motor and sensory limitations of the involved extremity (Figure 14). Other sites for successful surgical palliation may include a single metastatic deposit within the gastrointestinal tract that is causing either bleeding or a high-grade obstruction. Single metastatic deposits within the brain can also be resected for palliative intent, as waiting may result in surrounding brain edema that may be fatal if left untreated. However, the outcome for such patients has been uniformly dismal, with a median survival of <1 year. Careful consideration should be given to any palliative surgical intervention, weighing the benefits of surgery in alleviating the symptoms against the true morbidity and complexity of the surgical procedure.

The last type of surgical intervention to be performed for patients with stage IV melanoma can best be described as cytoreductive immunotherapeutic surgery. An immunotherapeutic surgical approach has been described, whereby complete cytoreductive surgery is hypothesized to play a central role in eliminating the tumor burden. This, in turn, allows for an improved overall function of the host anti-tumor immune response. It is thought that removing the tumor burden, or “tumor cell factory” as described by Morton et al, through complete cytoreductive surgical resection may allow the host immune system to overcome tumor-induced immunosuppression. Indeed, Morton has recently described his results from the premature closure of the onamelatucel-L (Canvaxin) trial, designed to assess the efficacy of this vaccine in both stage III and IV melanoma patients.

Although both trials were closed to further accrual early due to an interim analysis that revealed no probable efficacy over placebo, some very interesting results were nonetheless found in stage IV patients. The design of the Canvaxin trial for stage IV patients required that all patients receive definitive surgical removal of all metastatic disease prior to entry into the trial. Although there was no advantage to receiving the Canvaxin vaccine over placebo, they found that a remarkably high 40% of all patients (in both arms) were alive at 5 years, suggesting that prolonged survival may be due to the vaccine, but instead to complete surgical resection of metastatic disease. It is clear that our understanding of the tumor biology of melanoma remains limited, however, there is a suggestion that complete surgical resection of metastatic disease may play an important role in long-term survival in selected patients with stage IV disease.

### Multidisciplinary care of the melanoma patient

The multidisciplinary care of the melanoma patient begins with the initial diagnosis. Once the definitive biopsy has been performed, usually in the office, every attempt should be made for a subsequent follow-up visit to thoroughly discuss the pathology results and to examine the biopsy site for signs of infection and the overall healing process. We believe it is in poor form to provide such results over the telephone, often delegated to a member of the office staff that may be ill-prepared for questions and or emotional outbursts as a result of a patient receiving the diagnosis of cancer over the telephone. Every attempt should be made by the treating physician to sit down with the patient (and family members) in a quiet and comfortable setting and thoroughly review the entire pathology report in detail, allowing the patient to comprehend the results and possibly ask pertinent questions about their situation.

The care of the patient with melanoma can be complex, often requiring the opinions of peers from multiple specialties. The management of a thin primary melanoma that simply requires a 5 mm or 1 cm excision is fairly straightforward. However, a considerably higher level of expertise and complexity are added to the patient with an intermediate or thick primary melanoma. The discussion must be understandable to the patient, no matter what their educational background, and encompass a global perspective on what lies ahead for them. This, of course, should include a thorough discussion as to the surgical treatment options as well as the possible pathological outcomes and its implications in regards to further surgical or medical therapy. In regards to the planned surgical intervention, all options should be discussed, focusing on the cur-
rent data and literature that support the procedure. A detailed explanation of SLNB and other such staging procedures should be given, including all of the possible risks and benefits of the procedure.

We encourage the participation of all family members in these discussions, often providing an extra “set of ears” and to ask intuitive questions that the patient may not ask. Ample time should be given for the patient and family to ask questions about the surgery and also about the “bigger picture” of their disease. Such detailed conversations are very important to alleviate the fears and to dispel many of the common myths associated with cancer surgery. It is important to remember that the patient with melanoma, or any cancer for that matter, can be emotionally distraught over such news and may not be focused on the discussion at hand. Receiving the diagnosis of cancer is a life changing event and it is imperative that the surgeon be cognizant of the emotional issues at hand, providing for a compassionate and supportive environment for the patient and family members.

The patient’s case will often be presented in detail at a weekly multidisciplinary cutaneous oncology conference, attended by the radiologist (radiographic interpretation of studies), dermatopathologist (review of all biopsy slides), medical oncologists (adjuvant options for appropriate patients), surgical oncologists (expert surgical opinions), radiation oncology (expert opinion on adjuvant radiation therapy) and dermatologist (whom may have done the original biopsy). Other important members of the multidisciplinary team should include the clinical and research nurses, social workers, physician extenders and a recorder for each meeting that will provide written documentation of each weekly proceeding.

Many hospitals and institutions will not have the luxury of such coordinated care of the melanoma patient. Indeed, there are only a handful of places in the United States able to provide such comprehensive care within a single institution, while the vast majority of melanoma patients will be seen outside of such a setting. It is our recommendation that patients that are diagnosed with intermediate and thick primary melanomas be referred to an institution with an established record of multidisciplinary care of the melanoma patient, such as an NCI-designated cancer center. If the geographic location does not allow for this, referral to an academic medical center may benefit the patient as most institutes will have at least a few physicians with a special interest and training for melanoma patients. Optimally, this would include a surgical oncologist who is well versed in the latest surgical treatment options and who is up-to-date on the most recent literature in regards to the surgical management of melanoma.

Conclusions

Receiving the diagnosis of melanoma is a life-changing event for the patient as well for all of the family members involved. The spectrum of mental distress related to this diagnosis is highly variable, ranging from acceptance and rapid treatment to overt stages of anger and depression for more advanced cases. We must never forget the melanoma patient in all of this discussion, for it is impossible for those of us who are “cancer-free” to experience the emotional rollercoaster that many, if not all, will experience. In regards to the utility of SLNB in treating patients with melanoma, it is clear that they do not accept a “wait and see” approach supported by other physicians. Patients should clearly understand that the primary role is one of accurate staging, with most patients overwhelming choosing to undergo this procedure. Regardless of such deep-seeded beliefs about SLNB, the diagnostic utility of SLNB is unmatchable, firmly established as a necessary part of the discussion that we have on a daily basis with our patients. Lastly, the surgical management of the melanoma patient continues to evolve as we improve our understanding of the complex interactions that are occurring within the tumor microenvironment and the host immune system.

Riassunto

Gestione chirurgica del melanoma cutaneo

Il melanoma è un carcinoma enigmatico che può essere mortale in qualsiasi sua forma se non identificato o misconosciuto. Negli ultimi 10 anni l’incidenza del melanoma sta aumentando rapidamente, essendoci una multifattorialità combinata con diversi fattori di rischio noti sia ambientali che comportamentali. Sebbene spesso sovrastimato in termini di importanza, non si può dimenticare che il maggior impatto che riusciremo ad avere sulla sopravvivenza globale sarà il risultato di nuovi sforzi tesi all’identificazione precoce e alla prevenzione del melanoma. Dal momento che il melanoma si diffonde per via linfatica o ematogena, la sopravvivenza globale di tutti i pazienti diminuisce drammaticamente.
rispetto a quella notevole di quei pazienti che si sottopongono all’asportazione del melanoma in situ con ampi margini di escissione. Infatti, la gestione chirurgica del melanoma è mutata radicalmente negli ultimi 20 anni. Siamo stati guidati dai risultati di studi clinici ben disegnati, prospettici, randonizzati, che hanno evidenziato l’importanza dell’estensione dell’asportazione chirurgica del melanoma primitivo che, se ottimale, è in grado di modificare e di raffinare il modo con il quale gestiamo chirurgicamente tali pazienti. Studi ulteriori hanno definitivamente sottolineato l’efficacia dell’asportazione linfonodale selettiva nella gestione del drenaggio linfonodale. Gli autori discutono tutti questi aspetti ed altri, fornendo dati basati sull’evidenza a favore del loro utilizzo. Altri studi in corso potranno guidarci ulteriormente nella gestione chirurgica del melanoma. Infine, gli autori prendono in considerazione i progressi chirurgici del passato e come possiamo imparedare da essi. Tutto questo guiderà su come procedere e avanzare in futuro nell’ambito di questo settore.


References

73. Cascinelli N, Morabito A, Santinani M, Mackie RM, Belli F. Imme-
75. Johnson TM, Sondak VK, Bichakjian CK, Sabel MS. The role of sen-
78. Medalie NS, Ackerman B. Sentinel lymph node biopsy has no bene-
79. Medalie NS, Ackerman B. Sentinel lymph node biopsy has no benefi-
tive patients with primary cutaneous melanoma metastatic to a
lymph node: An assertion based on comprehensive, critical analysis.

Can surgical therapy alone achieve long-term cure of melanoma meta-

81. Morton DL. Interim analysis of the multicenter selective lymphade-
necctomy trial (MSLT-I) in clinical stage I melanoma. Presented at
the American Society of Clinical Oncology, May 14th, 2005.

82. Morton DL, Thompson JF, Cochran AJ, Mozziello N, Eshoff R,
Essner R et al. Sentinel-node biopsy or nodal observation in melanoma.

83. Wick MR, Patterson JW. Sentinel node biopsies for cutaneous mela-

84. Gershenwald JE, Thompson W, Mansfield PF, Lee JE, Colome MI,
Tseng CH et al. Multi-institutional melanoma lymphatic mapping
experience: the prognostic value of sentinel lymph node status in 612

85. Wong SL, Brady MS, Busam KJ, Coit DG. Results of sentinel lymph
node biopsy in patients with thin melanoma. Ann Surg Oncol

86. Kalady MF, White RR, Johnson JL, Tyler DS, Seigler HF. Thin mel-
anomas: predictive lethal characteristics from a 30-year clinical expe-

87. Stitzenberg KB, Groben PA, Stern SL. Indications for lymphatic map-
ping and sentinel lymphadenectomy in 204

al. Mitotic rate and younger age are predictors of sentinel lymph node
positivity: Lessons learned from the generation of a probabilistic

89. Kesmodel SB, Karakousis GC, Bothyld JC, Canter RJ, Lewis RT, Wahl
PM, et al. Mitotic rate as a predictor of sentinel lymph node positivity

90. Azzola MF, Shaw HM, Thompson JF, Soong SJ, Sculter RA, Watson
GF et al. Tumor mitotic rate is a more powerful prognostic indicator than
ulceration in patients with primary cutaneous melanoma: an analysis of

91. Pulleco CA, Messina JL, Riker AI, Glass LF, Nelson C, Crate CW et
al. Sentinel node biopsy for thin melanoma: Which patients should be

92. Thompson JF, Shaw HM, Is sentinel lymph node biopsy appropriate
in patients with thin melanomas: Too early to tell? Ann Surg Oncol

93. Thompson JF, Shaw HM, Is sentinel lymph node biopsy appropriate
in melanoma patients with palpable inguinal lymph node metastases

et al. Operative morbidity associated with groin dissections. Surg
Today 2004;34:413-8.

95. Hughes TM, A’Hern RP, Thomas JM. Prognosis and surgical mana-
gement of patients with palpable inguinal lymph node metastases

DL. Is the node of Cloquet the sentinel node for the iliac/obturator

97. Cott DG, Brennan MF. Extent of lymph node dissection in melanoma

al. The value of Cloquet’s node in predicting melanoma nodal metastases

99. Essner R, Scher I, Kanavanagh M, Torisu-Itakura H, Wanek LA, Mor-
ton DL. Surgical management of the groin lymph nodes in melanoma
in the era of sentinel lymph node dissection. Arch Surg
2006;141:877-84.

100. Kalady MF, White RR, Johnson JL, Tyler DS, Seigler HF. Thin mel-
anomas: predictive lethal characteristics from a 30-year clinical expe-

101. Beitsch P, Balch C. Operative morbidity and risk factor assessment
in melanoma patients undergoing inguinal lymph node dissection. Am

102. Cossich O, Kremetz ET, Ryan RF, Winblad JN. Chemotherapy of can-
ter: regional perfusion utilizing an extracorporeal circuit. Ann

103. Grunhagen DJ, deWilt JHW, vanGeel AN, Eggemont AMM. Isola-
ted limb perfusion for melanoma patients-a review of its indications

et al. Randomized multicenter trial of hyperthermic isolated limb perfu-
sion with melphalan alone compared with melphalan plus
tumor necrosis factor: American College of Surgeons Oncology

BBR. Isolated limb perfusion: What is the evidence for its use? Ann

106. Noorda EM, Vrouwenraets BC, Nieweg OE. Isolated limb perfusion

107. Thompson JF, deWilt JHW. Isolated limb perfusion in the manage-
ment of patients with recurrent limb melanoma: An important but limi-

108. Thompson JF, Kam PC, Waugh RC, Harman CR. Isolated limb infu-
sion with cytotoxic agents: A simple alternative to isolated limb per-

109. Thompson JF, Kam PC. Isolated limb perfusion for melanoma: A sim-
ple but effective alternative to isolated limb perfusion. J Surg Oncol

Sentinel lymph node biopsy for melanoma: therapeutic procedure or diagnostic test?

C. VEMURI 1, M. S. SABEL 2

Although sentinel lymph node (SLN) biopsy is considered standard of care by many surgical oncologists and dermatologists, it remains controversial among others. Clinical practitioners in both surgery and dermatology have used the same available evidence to both support and refute the sentinel node hypothesis and the role SLN biopsy should play in the management of cutaneous melanoma. Much of the disagreement centers on whether one views SLN biopsy as a therapeutic intervention meant to improve survival or a diagnostic test meant to stratify risk and select patients for further therapy. This article will review the available data, including the most recent data from the Multicenter Selective Lymphadenectomy Trial-I (MSLT-I), the first prospective randomized study of SLN biopsy in melanoma.

KEY WORDS: Melanoma - Biopsy - Sentinel lymph node biopsy.

Although it is still the greatest source of debate among physicians who treat melanoma, the importance of the regional nodal basin in the management of melanoma was recognized in the late 1800’s. Today, the great majority of patients with primary cutaneous melanoma present with clinically negative (nonpalpable) regional lymph node basins, but 1/5th of these patients harbor occult regional metastases. Prior to the introduction of lymphatic mapping and sentinel lymph node (SLN) biopsy by Morton et al., this posed a clinical dilemma. Patients and their physicians were faced with two choices. With the understanding that primary melanomas often spread to regional nodal basins before metastasizing widely, some surgeons advocated elective lymph node dissection (ELND). This approach hinged on the idea that early clearance of tumor deposits in the regional nodal basin could prevent subsequent dissemination and improve survival, an idea supported by retrospective studies. Elective node dissection, though, exposed the 80% of patients who were node-negative to the morbidity of a nodal dissection.

The alternative approach was that of limiting node dissections to those patients with documented metastases, the therapeutic lymph node dissection (TLND). Clinically node negative patients underwent wide excision alone. If they developed clinically palpable nodal disease, but were without evidence of distant disease, they underwent TLND. This spared the node negative patients the morbidity of a lymph node dissection, but risked the possibility that in the time period between the primary excision and when the nodal recurrence became evident, melanoma cells may have metastasized systemically from the node, losing the chance for cure.
This dilemma ultimately led to several randomized trials, each of which showing no overall survival advantage to elective node dissection, but perhaps some benefit among certain subsets of patients. The Intergroup Melanoma Surgical Program randomized 740 stage I and II melanoma patients to ELND or observation. While there was no difference in survival between the two groups overall, in a subgroup analysis, ELND was seen to confer a survival benefit in patients with nonulcerated melanomas and in patients with tumor thickness between 1 and 2 mm. This data suggested, although by no means proved, that there did exist a portion of patients for whom the early removal of microscopic disease from the regional nodes would improve survival. The results of these trials prompted many surgeons to abandon ELND while others chose to use ELND selectively. As there was no way to specifically identify patients with microscopic disease, surgeons based the decision to perform ELND on clinical features such as patient age, gender, tumor location, tumor thickness and the absence of ulceration.

This point became moot with the introduction of lymphatic mapping and SLN biopsy, a minimally invasive procedure capable of identifying that very subset; patients with melanoma harboring synchronous occult microscopic disease in the lymph nodes. With this procedure, the management of melanoma changed swiftly, allowing node-negative patients to avoid unnecessary lymphadenectomies without sacrificing accuracy staging. Today, SLN biopsy is considered standard of care by most surgical oncologists for staging the regional lymph nodes of patients with primary cutaneous melanomas ≥1 mm thickness. Patients with thin melanomas (<1 mm) have a low incidence of regional metastases, and so SLN biopsy is not routinely recommended. In some cases, however, the presence of other adverse features (ulceration, mitotic rate, young age, or Clark’s level IV or V tumors) may prompt SLN biopsy in patients with melanoma <1 mm.

Despite the clinical acceptance of SLN biopsy, its application is not without controversy and there is broad disparity of opinions regarding SLN biopsy among dermatologists. While some of this debate may be fueled by the shift in who is responsible for the treatment of melanoma (although this is only a minority of melanoma patients as most do not fall within the current guidelines for SLN biopsy), there are many unanswered questions and legitimate concerns that had not been addressed by current trial data. Should SLN biopsy be accepted as the standard of care in the management of melanoma? To best answer this question, one must address several issues surrounding the procedure and ask not only whether SLN delivers what is expected of it, but what precisely is expected of it.

The SLN technique is designed to identify the lymph node or nodes that accurately represent the status of the draining nodal basin. The concept is not a new one. In the mid-19th century, Virchow described the concept of lymphatic drainage from a given body site to a specific lymph node. Based on studies in cats and humans with vital dye, Braithwaite first described the “glands sentinel” as the lymph node which drains a particular area. In 1960, Gould described a “sentinel node” that directly drained the parotid gland and proposed that a radical neck dissection should be performed if this node contained micrometastatic disease. And, in 1976, Cabanas suggested that the sentinel node of the penis could be used to determine the need for regional node dissection for penile cancer. However, the use of intraoperative lymphatic mapping to identify the sentinel node was truly brought forward by Donald Morton for the treatment of malignant melanoma.

The hypothesis underpinning SLN biopsy is that while the mechanism by which melanoma cells metastasize to the lymph nodes is a complex and difficult to predict process, the manner in which they metastasize is orderly and definable by mapping the lymphatic drainage from the site of the melanoma. Typically, two methods are employed for identifying the sentinel node; a blue dye and a radiolabelled colloidal solution. The radiolabelled colloid is injected 1 to 4 h preoperatively and the blue dye is injected intradermally at the site of the primary tumor a few minutes before the sentinel node biopsy incision is made. The surgeon then uses a hand-held gamma probe to identify the “hot spot” marking the location of the sentinel node, thereby minimizing the size of the skin incision needed. Once the incision is made, the surgeon identifies the sentinel node by either following blue-stained lymphatics or by finding the areas with the highest signal.

The prevailing argument is that identification and removal of the SLN will accurately stage the patient. This presumes two things. First, the lymph node(s) that take up the tracers are truly the nodes most likely to harbor micrometastases if present. In other words,
it is highly unlikely that if the SLN is negative (has no identifiable micrometastases) there are melanoma cells in other lymph nodes. The second presumption is that the identification of melanoma cells in the SLN portends a poor prognosis as compared with patients who are SLN negative. In other words, is this a true staging procedure that stratifies patients by projected outcome? If these two presumptions are true, then the next question is whether there are any interventions available to improve outcome among SLN positive patients. If not, then outside of accurate staging for research purposes, SLN biopsy provides minimal benefit to the patient and is not justified outside of a clinical trial. If so, the final question is whether the potential benefit to the patient is worth the cost and morbidity of the procedure.

Does the sentinel lymph node accurately reflect the status of the regional basin?

The SLN biopsy typically begins with the injection of a radiolabeled colloid tracer in 4 quadrants around the melanoma or biopsy scar. Lymphoscintigraphy then demonstrates the anatomic locations of the SLN(s) in the draining basin(s). The patient comes to the operating room where a fat-soluble blue dye is injected in a similar manner. The hand-held gamma probe is used to identify the vicinity of the SLN within each basin by means of elevated counts and a small incision is made in the skin. Any lymph node within the basin that is blue or has blue-stained lymphatics, or has high counts on the gamma probe is excised and labeled a “sentinel node.” Any lymph node that is clinically suspicious on digital examination of the basin is also excised. The basin is then demonstrated to the surgeon for pathologic examination. The procedure is completed when all blue nodes have been removed and counts have dropped to 10% of the highest node count \textit{ex vivo}. This approach will identify the SLN in over 97% of cases.\textsuperscript{16,18} The incision is closed and the sentinel node(s) are sent to pathology. An equally important aspect of the SLN biopsy is the pathologic analysis of the specimens. Step sectioning of the harvested nodes increases detection of micrometastases. If step sectioning and routine hematoxylin and eosin (H&E) staining is negative for metastasis, then immunohistochemical staining for melanoma markers such as S-100, Melan-A, and HMB-45 is performed.\textsuperscript{19,22} Does the SLN truly reflect the lymph node status? As one can imagine, there are several missteps that can occur during the procedure that can lead to a false-negative finding- calling the patient node-negative when in reality spread to the regional nodes did occur (Table I). Initial studies of the false negative rate for SLN biopsy were pathologic in nature. Patients who underwent lymphatic mapping and SLN biopsy had a complete node dissection so that the status of the SLN could be compared to the status of the nonsentinel lymph nodes (NSLN). Multiple studies have demonstrated that, when the SLN is negative, the likelihood of finding disease in any NSLN is quite low.\textsuperscript{16,23,24} This finding, however, assumes that the $^{99m}$Tc-labeled colloid sulfur and lymphoscintigraphy fail to identify all draining basins. For example, if a flank melanoma metastasized to the inguinal nodes, but the lymphoscintogram only showed drainage to the axillary basin, then the absence of disease in the axillary sentinel and nonsentinel nodes does not mean that this is a true negative.

The second measure of the accuracy of the SLN in predicting the nodal status is the regional recurrence rate among patients who are SLN negative. Several single-institution series have demonstrated a relatively low false-negative rate in this situation, although follow-up for some of these series has been limited.\textsuperscript{23,28-27} The most recent data comes from the Multicenter Selective Lymphadenectomy Trial-I (MSLT-I); a prospective, randomized trial comparing wide local excision alone to wide local excision and SLN biopsy, with complete lymph node dissection for SLN positive patients.\textsuperscript{28} In this trial, the false negative rate was 3.4% at 5 years. The regional recurrence rate, however, does not

\begin{table}
\centering
\caption{Possible mechanisms of false negative sentinel lymph node (SLN) biopsy.}
\begin{tabular}{l}
\hline
- $^{99m}$Tc-labeled colloid sulfur and lymphoscintigraphy fail to identify correct basin(s). \\
- Tracer travels to correct basin but moves past SLN to second-tier lymph nodes (may be related to time between injection and procedure). \\
- Tracer travels to correct basin but collects in NSLN (serial versus parallel drainage). \\
- Surgeon fails to remove all true sentinel nodes (background counts do not drop to <10% of highest node \textit{ex vivo}). \\
- Tumor emboli within lymphatics block flow of tracer into the SLN. \\
- Clinically involved nodes, without tracer uptake, missed by surgeon. \\
- Lack of thorough pathologic evaluation of the SLN (no step sectioning, lack of immunohistochemistry) \\
- Crush or cautery artifacts preclude identification of micrometases. \\
- Microscopic disease in lymph node not identified despite appropriate methods. \\
\hline
\end{tabular}
\end{table}
absolutely define the accuracy of the SLN procedure either. For patients who have micrometastases limited to the SLN but missed by the pathologist on histologic examination, they would be false negatives but would not clinically recur because the SLN was excised. In one series, 4.1% of patients who developed a nodal recurrence after a negative SLN had re-evaluation of the SLN and 80% had evidence of missed occult metastasis.\(^{27}\) It is certainly feasible that a portion of patients who did not recur also had missed occult metastases. It is also possible that some false-negative cases may have residual microscopic disease in a NSLN that simply didn’t recur, possibly kept in check by the immune system or capable of spread but incapable of growth and survival. On the other hand, patients who suffer a regional recurrence may have been truly node negative at the time of their SLN biopsy, but in-transit disease in the lymphatic vessels may have reached a node subsequent to that procedure.

Therefore, the precise accuracy of SLN biopsy could be questioned for either method of assessing the false negative rate. However, when one looks at the compilation of data, combining both the low rate of identifying disease in the NSLN on completion dissection after a negative SLN with the low regional recurrence rate after a negative SLN biopsy not followed by CLND, the evidence to date strongly supports the SLN hypothesis.

**Does finding a positive sentinel lymph node imply a worse prognosis?**

While the evidence strongly suggests the SLN is the most likely to harbor micrometastatic cells if they exist, this is meaningless if these cells are clinically insignificant. If the SLN procedure finds melanoma cells that are not indicative of the metastatic potential of the cancer, then it is of little benefit. One example of this might be if a great number of the foci identified within the SLN were there secondary to mechanical dislodgement, incapable of spread on their own. Another example would be if a high percentage of patients had melanomas capable of true spread to the lymph nodes but incapable of further growth or hematogenous spread. In these cases, SLN positive patients would have a prognosis not too dissimilar to SLN negative patients.

As with the accuracy of the procedure, the prognostic worth of the procedure is also strongly supported by the literature. There is an overwhelming preponderance of evidence that SLN status is the most significant factor for clinical outcome and is a critical component of melanoma staging.\(^ {29-33}\) Despite the fact that SLN biopsy is often restricted to patients with tumors between 1 and 4 mm in depth, studies have validated the prognostic significance of the SLN biopsy in both thin (<1 mm) and thick (>4 mm) melanoma.\(^ {34-38}\) In the MSLT-I trial, the status of the SLN was the most important prognostic factor, with the 5 year survival dropping from 90% among SLN negative patients to 72% among SLN positive patients.\(^ {28}\)

While the literature supports that the SLN procedure is both accurate and provides the most important prognostic information available, this still does not in itself justify the procedure (outside of the context of a clinical trial or for research purposes). Prognostic signs offer an accurate assessment of the likelihood of recurrence and death so physicians and patients make choices as to the relative risks and benefits of either surgery or adjuvant therapy. But what if there are no choices for additional therapy? While one might argue that having accurate prognostic information is beneficial to patients, the true benefit of prognostic and predictive markers lies in our ability to intervene in those patients with potentially poor outcomes. Simply knowing patient A has a worse outcome than patient B is relatively meaningless unless we can offer patient A something to improve that outcome.

When one talks of staging cancer patients, it is typically with adjuvant therapy in mind. While their options are limited, high-risk melanoma patients do have one adjuvant therapy available to them: high-dose interferon (HDI) with interferon alpha-2b (Intron-A). Adjuvant HDI is a controversial topic. While three studies have clearly demonstrated a benefit to disease-free survival for HDI, only two of these three studies demonstrated an overall survival benefit.\(^ {39, 40}\) While one found no survival advantage.\(^ {41}\) Furthermore, an ideal subset of high-risk melanoma patients who benefit the most from HDI has not been identified. A discussion of the relative pros and cons of adjuvant IFN is beyond the scope of this paper.\(^ {42-45}\) However, if after reviewing the data one agrees that the evidence supports at least offering patients adjuvant HDI, then the prognostic information provided by the SLN procedure is crucial.

The other avenue by which SLN biopsy may provide a survival benefit to patients is through additional
surgery, specifically a completion lymph node dissection (CLND) in patients found to harbor micrometastases. If one excludes any potential improvements in survival obtained with HDI, then the true benefit of identifying node-positive patients is if survival is improved by the early eradication of that disease, as compared with delaying surgery until those patients would recur.

Is the sentinel lymph node biopsy a therapeutic procedure that improves survival?

There are several arguments that one can make in favor of sentinel node biopsy irrespective of whether overall survival is improved. SLN biopsy will provide accurate staging for appropriate counseling, decision-making and prognostication. In this way, surveillance patterns may be adjusted accordingly, increasing surveillance of high risk individuals while the 80% of patients who are found to be node negative can be spared some of the anxiety (and the health care system can be spared some of the cost) of an intensive surveillance schedule. There is indirect evidence that SLN biopsy and immediate CLND will provide better regional control than delayed CLND.27, 46, 47 In addition, the CLND for a positive SLN has decreased complications as compared with CLND for palpable disease.48 As patients who recur after wide local excision alone often have more advanced regional disease (multiple involved nodes, extranodal extension), they often require radiation therapy to optimize regional control.49 The use of SLN biopsy would decrease the need for this, and the associated cost and morbidity. These relative advantages can be argued back and forth, however the most important question, and argument for the routine application of SLN biopsy, is whether identifying this disease at an early stage and removing it before it is clinically apparent improves survival.

Another way to frame this question is whether, in the interim of time between when the primary melanoma is treated and regional metastases are identified, could melanoma cells have spread from the nodes to other sites and recur as distant metastases that would have been prevented had the nodes been excised at the time of the primary wide excision? If the answer to that question is yes, the next question is how large a subset of patients would this represent? Patients with clinically node negative melanoma essentially fall into 4 categories, only one of which would realize a survival benefit:

1. Patients with no metastases to the regional nodes. Obviously these patients would not experience any survival advantage to the SLN biopsy, and they represent the overwhelming majority of patients (approximately 75% to 80%).

2. Patients with microscopic disease in the SLN who have not yet metastasized but will in the time it takes those regional mets to be clinically detectable. This is the group that does benefit from SLN biopsy and the subsequent CLND.

3. Patients with microscopic disease in the SLN who still have no distant disease when they suffer their regional recurrence and undergo a TLND.

4. Patients with microscopic disease in the SLN who already have distant disease when their primary melanoma is discovered. This is the biggest question mark. If almost all patients with microscopic disease in their nodes already harbor distant metastases, then groups 2 and 3 would represent such a small fraction of the SLN positive patients that it is unlikely that SLN biopsy and CLND for node positive patients would impact survival in any more than a negligible way. However, clinical evidence does not support this notion, as multiple prospective studies have demonstrated a significant percentage of long-term survivors with stage III disease.

The relative distribution of patients into these four categories is dependent upon multiple factors. First and foremost is the biology of melanoma and those factors that may favor lymphatic versus hematogenous spread. These fractions will also change with earlier diagnosis of melanoma, the accuracy of SLN biopsy and our ability to detect regional and distant metastases on imaging. Therefore, the role that SLN biopsy may play in the management of melanoma is in a constant state of flux, and could be impacted tomorrow by improvements in early diagnosis, imaging studies or adjuvant therapies.

As for today, is SLN biopsy, with CLND for node positive patients, a therapeutic surgical procedure? If one considers SLN biopsy a therapeutic procedure, one must seek a survival advantage among all patients to whom the procedure is applied. Using this threshold, which many have done in arguments against the use of SLN biopsy, then the answer is clearly no. Prior to the onset of SLN biopsy, the overriding question in melanoma surgery was whether ELND would
improve survival over delaying lymph node dissection until there was clinical evidence of recurrence. Despite retrospective data supporting ELND, four prospective randomized trials failed to demonstrate any survival advantage to the ELND versus a watch and wait approach. Would we expect SLN biopsy to change that? It seems unlikely. It is true that the SLN biopsy procedure will identify aberrant lymphatic drainage pathways and intercalated nodes (also known as ectopic or inter nal nodes) that would have been missed otherwise. However, this would be a small benefit, offset by a false negative rate resulting in node-positive patients not undergoing node dissection. The SLN biopsy primarily serves to limit the morbidity of the node dissection to those patients harboring microscopic disease. Its direct impact on survival is unlikely to be significantly different than ELND as 80% of the patients are still going to be node negative and not realize any survival benefit from the procedure. The survival benefit obtained from early eradication of nodal microscopic metastases would have to be large to demonstrate that benefit in a randomized study (or the study would have to accrue a number of patients beyond what is feasible to achieve statistical significance). As expected, there has to date been no survival advantage to SLN biopsy compared with observation alone in MSLT-I (with a 5-year melanoma-specific survival rate of 86.6% in the observation group and 87.1% in the biopsy group, P=0.58).

Many who argue against SLN biopsy use that data to support their arguments that SLN biopsy should never be done outside a clinical trial as it infers no survival benefit to the patient population as a whole. However, is this the correct yardstick by which we measure SLN biopsy?

Is the sentinel lymph node biopsy a diagnostic procedure that identifies patients who may benefit from further surgery?

Let us say hypothetically that we had a serum test that identified patients at a high likelihood of harboring regional metastases. The test was inexpensive and had minimal morbidity, save those complications associated with phlebotomy, and was accurate in about 95% of cases (with both a high sensitivity and specificity). If performing CLND on those patients who had a positive serum test was shown to improve their survival, would you order the test? Obviously the answer is an overwhelming yes. We order similar tests for a variety of malignancies every day. Upon diagnosis melanoma patients often undergo chest X-rays, a host of blood tests, CT scans and PET scans even though none of these have been shown to impact survival. It is, therefore, hard to imagine there is any practitioner who would not order our imaginary blood test if it truly identified patients for whom survival might be improved by further surgery.

Following this logic, the question is whether SLN biopsy functions in the same manner, as a diagnostic test meant to identify a portion of patients who may benefit from intervention. If the answer is yes, then the only remaining question is whether the costs and side effects of this surgical diagnostic test (obviously more substantial than a blood test or an X-ray) are justified by the benefit to the patient.

To assess the performance of a diagnostic test, one must ask whether it accurately identifies a patient population who derives benefit from further intervention, in this case a survival benefit. As a diagnostic procedure, SLN biopsy is meant to identify node-positive patients, so one must ask whether survival is improved in this subset by CLND. This benefit must be further tempered against the false negative rate of the procedure.

Prior to the MSLT-I trial, there was significant evidence that subsets of patients might benefit from early removal of nodal metastases. As discussed previously, the Intergroup Melanoma Surgical Program, while demonstrating no overall survival difference between ELND and observation, did show in subgroup analysis a survival benefit to ELND in patients with nonulcerated melanomas and in patients with tumor thickness between 1 and 2 mm. Further evidence comes from the World Health Organization (WHO) Melanoma Group Program 14 Trial, which randomized patients with truncal melanoma to wide excision plus ELND or wide excision plus observation, with subsequent lymph node dissection if patients recurred. Again, there was no overall survival benefit, but when survival of patients with microscopic disease on ELND were compared with those who had regional recurrences, the survival was significantly improved in the ELND group (48.2% vs 26.6%, P=0.04). While this data is strongly suggestive, it certainly does not prove that even among node positive patients the node dissection provides that degree of
benefit, as there may be patients who were node positive on ELND who would not have recurred had the nodes been left in place.

This all leads to the recently reported interim results of the Multicenter Selective Lymphadenectomy Trial I (MSLT-I) which is the first prospective randomized trial to specifically address the survival benefit of the SLN biopsy. As stated, to date there has been no significant difference in survival between the patients randomized to SLN biopsy versus those randomized to excision alone. However, when one compares the melanoma specific survival among those patients who were SLN positive to those patients who recurred after excision alone, there was a significant improvement in survival in the SLN group. Among the node positive patients, the 5-year survival rate with CLND for a positive SLN was 72.3% versus 52.4% for patients who underwent CLND for a recurrence (HR 0.51, 95% CI, 0.32 to 0.81; P=0.004). When one includes the false negative patients (those patients with a negative SLN biopsy who had a regional recurrence) in the SLN biopsy group, there is still a significant improvement in survival (66.2% vs 54.2%; HR 0.62; 95% CI 0.40 to 0.95; P=0.02).

Certainly, the same criticisms of the WHO Program 14 data could be applied here; that there may have been a significant number of patients positive on SLN biopsy who would not have recurred had they not undergone SLN biopsy. The initial report, in fact, did suggest more patients who were SLN positive than recurred. However, with longer follow-up, there were nearly the same numbers of patients who were either SLN positive or recurred after a false negative SLN as there were patients who had a regional recurrence after wide excision alone. The cumulative incidence of regional metastases in both the observation group and the biopsy group were equal by 10 years of follow-up (about 20% in both groups). This suggests a very small number, if any, of patients who had nodal metastases that would not ultimately suffer a regional recurrence.

This data, therefore, would strongly suggest that SLN as a diagnostic procedure will accurately identify patients who will experience a survival benefit from further intervention, specifically completion node dissection (and possibly further benefit from adjuvant HDI). It is hard to argue against its effectiveness as a staging procedure. Thus, the argument surrounding the appropriateness of SLN biopsy shifts from whether there is a benefit (there clearly is) to whether the costs and morbidity of a surgical staging procedure are justified. Again, if this were a simple blood test or X-ray meant to identify patients who would experience a significant decrease in mortality with further intervention, there would be no argument as to its worth.

The morbidity of the SLN procedure is low. Several studies document postoperative complications after SLN biopsy in the range of 5%, 46, 52, 53 Complications are relatively minor, primarily consisting of wound infection, seroma or hematoma. Allergic reaction to the blue dye is rare but potentially serious. Lymphedema after SLN biopsy is uncommon, and despite early concerns, SLN biopsy does not increase the likelihood of in-transit recurrences, 28, 54 The cost is another question. Much of the controversy surrounding the use of SLN biopsy centers on the increased costs of a procedure that benefits a small subset of patients. For patients deemed appropriate candidates, surgical therapy shifts from an office-based procedure, which can be done for approximately $1,000 to $1,750, to one where i.v. sedation or general anesthesia is utilized, nuclear medicine is involved, and a time-consuming pathologic evaluation of the sentinel nodes is necessary. This raises the cost of treating melanoma to between $7,150 and $15,223. 55, 56 Are these costs justified? In previous cost analyses of SLN biopsy, the answer was yes, but this was only if one considered the survival benefit associated with HDI or compared to ELND. 57, 58 This final outstanding question; whether SLN biopsy is worth the cost, should be subjected to a cost-analysis taking into account the most recent information gained from the MSLT-I study, and compared to other diagnostic or therapeutic practices in oncology.

Conclusions

The SLN procedure for melanoma patients should be thought of and evaluated as a staging procedure as opposed to a therapeutic procedure. Attempts to judge the merits of the procedure as a therapeutic intervention will always come up lacking, as 80% of these patients are node negative and will derive no direct survival benefit from the procedure. However, as a diagnostic procedure, SLN biopsy fulfills all of the criteria. It is highly accurate in identifying a subset of patients who 1) have a significantly worse prognosis and 2) will benefit from further intervention. Certainly there are outstanding questions. Whether the cost and
morbidità di questo procedimento di staging possono essere giustificate se debattute, ma possono solo essere veramente basate su un beneficio di costo-beneficio analisi che prenda in considerazione il più recente disponibile. È impossibile, a pieno titolo, separare aspetti diagnostici dell'intervento dal terapeutico, dunque si considera l’SLN l’unico punto di riferimento dato che nessun nodo secondario non sia sottoposto a controllo di laboratorio. Avere un nodo secondario positivo è assolutamente accessibile. Aumentare la discussione.


tutti i pazienti per una terapia ulteriore. Questo articolo riassumerà i dati disponibili, compresi quelli più recenti forniti da Multicenter Selective Lymphadenectomy Trial-I (MSLT-I), il primo studio prospettico randomizzato sulla biopsia del linfonodo sentinel nella melanoma.

**Parole chiave:** Melanoma - Biopsia - Linfonodo sentinel.

**Riassunto**

**Biopsia del linfonodo sentinel nella melanoma: procedura terapeutica o test diagnostico?**

Sembra che la biopsia del linfonodo sentinel venga considerata il gold standard da parte di molti chirurghi oncolo- gisti e dermatologi, essa continua ad essere oggetto di discussione da parte di altri. I clinici, sia chirurghi che dermatologi, hanno utilizzato la stessa evidenza disponibile sia a favore che contro l’ipotesi del linfonodo sentinel-la e sul ruolo che la sua biopsia dovrebbe giocare nella gestione del melanoma cutaneo. Molto del disaccordo nasce da un diverso punto di vista della biopsia del linfonodo sentinel, se considera-

**References**

11. Medalie N, ACKERMAN AB. Sentinel node biopsy has no benefit for patients whose primary cutaneous melanoma has metastasized to a lymph node and therefore should be abandoned now. Br J Dermatol 2004;151:298-307.
56. Carlson GW, Murray DR, Hestley A, Staley CA, Lyles RH, Cohen C.


