MELANOMA CARE OPTIONS™

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FOSTERING AN INTERDISCIPLINARY APPROACH TO MELANOMA CARE

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Update on Advances in Melanoma: Current Progress and Future Promise

Editor's Note...

Dear Colleague,

This issue of *Melanoma Care Options* provides updated information on a number of evolving areas of interest for clinicians caring for melanoma patients. While some data presented here is the result of more recent investigations, all of this information is critically important as we begin to better understand how to predict outcomes and individualize therapy.

The first article by Dr. Susan Swetter summarizes the latest version of melanoma staging guidelines from the American Joint Committee on Cancer, highlighting changes from the prior version issued in 2002. She also discusses which patients are suitable for sentinel lymph node biopsy (SLNB) and potential predictors of SLN positivity.

Next, Dr. Robert Andtbacka picks up on the key role that SLNB plays in staging. He briefly examines the prognostic significance of SLN status before exploring two areas of controversy: whether there is a role for SLNB in patients with prior wide local excision or in those with recurrent satellite/in-transit melanoma.

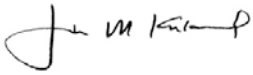
In the third article, Dr. Andtbacka tackles the contentious issue of how best to treat our patients with in-transit disease, an area where there is no current standard of care. Dr. Andtbacka reviews the various local, regional, and systemic treatment options, focusing on the potential advantages, disadvantages, and suitable patients for each approach.

The next article, by Dr. Ahmad Tarhini, examines the current status of adjuvant interferon alpha (IFN-α) for patients with resected stage II/III melanoma, who are at high-risk for recurrence and continue to represent a treatment challenge. High-dose IFN-α, the only adjuvant therapy currently approved by the FDA for these patients, is associated with consistent benefit in terms of relapse-free survival, but is a toxic treatment that has been a subject of controversy. The article concludes by reviewing the latest on biomarkers in melanoma adjuvant therapy, with the hope that they may eventually enable better matching of IFN-α with patients best suited for this treatment.

Last, Dr. Sancy Leachman examines the role that genomics and proteomics have begun to play in melanoma biomarker development. In particular, she discusses the techniques of array comparative genomic hybridization, cDNA microarray expression profiling, and MALDI-ToF mass spectrometry—and their use to improve diagnosis, identify potential therapeutic targets, and develop prognostic biomarkers.

We hope the information provided here will advance your understanding of the latest on melanoma and its optimal diagnosis, staging, and treatment for the various forms of this disease. We welcome your comments and suggestions, and encourage you to participate in other Melanoma Care Coalition programs available at www.MelanomaCare.org.

Sincerely,



John M. Kirkwood, MD

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Target Audience
This activity is directed toward surgical oncologists, general surgeons, oncology nurses, medical oncologists, dermatologists, and other healthcare professionals who screen for and/or treat melanoma.

Statement of Need
Melanoma continues to be a challenging disease to treat. However, recent clinical trials of targeted and immune-based treatments have shown progress and potential for the future. However, practicing clinicians who diagnose and treat melanoma may find it difficult to identify which promising research is truly significant and likely to influence their contemporary practice in the near future. Through expert interpretation of the most current data concerning the pathogenesis, staging, and treatment of melanoma, including discussion of ongoing clinical trials, this program provides participants with a more detailed understanding of key emerging data and how to translate it to a practice setting.

Learning Objectives
After completing this CME activity, participants should be able to:
- Identify changes in the 7th edition of the American Joint Committee on Cancer (AJCC) melanoma staging system compared with the previous edition
- Describe the role of sentinel lymph node biopsy in patients with thin (≤1 mm) melanomas, wide local excision of melanoma, or locally or regionally recurrent melanoma
- Evaluate the role of adjuvant interferon-α therapy and potential markers for treatment response in patients with high-risk melanoma
- Describe different treatment options for patients with in-transit melanoma
- Understand the basic principles and uses of comparative genomic hybridization, cDNA microarray expression profiling, and MALDI-ToF mass spectrometry as they to apply to melanoma research

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AJCC Melanoma Staging 2009: Impact on Current Practice

By Susan M. Swetter, MD, FAAD

Cancer staging is critical for devising treatment programs for individual patients to optimize outcomes. The American Joint Committee on Cancer (AJCC) recently revised the staging system for cutaneous melanoma, building on the prior 2002 version and incorporating additional data gathered since then.[1,2] This article provides an overview of the 2009 AJCC melanoma staging guidelines (effective January 1, 2010) and discusses the impact of these changes on clinical practice. This article will also describe the current status of the sentinel lymph node biopsy (SLNB) in cutaneous melanoma staging, discuss the use of SLNB for staging in patients with thin (≤1 mm) melanoma, and identify various factors associated with SLN positivity.

General overview of the 2002 and 2009 AJCC melanoma staging systems

The 6th Edition of the melanoma staging and classification system, published in 2002 by the AJCC and International Union Against Cancer (UICC), was based on a database analysis of prognostic factors involving 17,600 patients.[3,4] The 2002 staging system contained a number of important changes from the 5th Edition, published in 1997. These included redefinition of tumor thickness ranges using whole integers; inclusion of melanoma thickness and ulceration but not level of invasion (Clark's level) in the T category (except for lesions ≤1 mm); use of number of metastatic nodes as well as regional nodal tumor burden (ie, whether nodal metastases were clinically apparent or clinically occult) and presence of primary tumor ulceration in the N category; and incorporation of the site of distant metastases and serum level of lactate dehydrogenase (LDH) in the M category.[3] The 2002 AJCC guidelines also recommended upstaging all stage I-III patients when a primary melanoma is ulcerated and a grouping of microsatellites, local metastases, and in-transit metastases into stage III disease. Of particular note, the 2002 version recommended incorporating SLN results into the definition of pathologic staging, whereas the 1997 version did not.

The 2009 version is based on a multivariate analysis of data from 38,918 patients, including 30,946 with stage I, II, or III melanoma (>27,000 with stage I/II and >3300 with stage III) and 7972 with stage IV melanoma.[1,2] A prognostic factor analysis of nearly 60,000 patients was implemented to validate staging categories and groupings.[2] The 2009 guidelines do not contain many major changes for TNM and stage grouping criteria, with the exception of incorporating mitotic rate (MR) into primary melanoma primary tumor classification (**Table 1**).[1,2]

Table 1. Summary of Changes to the 7th Edition (2009) of the Melanoma Staging System

- No major changes recommended for TNM and stage grouping criteria
- Mitotic rate (MR) of primary melanoma is an independent prognostic factor
  - MR ≥1/mm^2 incorporated into criteria for T1b classification (supplanting Clark's level)
- IHC detection of nodal metastases acceptable (only routine histology used previously)
- No lower threshold of staging for definition of N+ disease
  - ITCs or deposits <0.1 mm now scored as N+
  - N0(i+) no longer used
- Stage IV: Site of distant metastases and elevated serum LDH level confirmed as predictors of survival

MR, mitotic rate; IHC, immunohistochemical; ITC, isolated tumor cells; LDH, lactate dehydrogenase.

In particular, the 2009 staging system identifies the mitotic rate (MR) of the primary tumor as an independent prognostic factor and incorporates a high MR (≥1/mm^2) into the T1b classification.[1,2] Modifications to stage III melanoma in the revised staging system indicate that immunohistochemical (IHC) detection of nodal metastases is acceptable for stage III classification; and that there is no lower threshold for staging N+ disease (ie, the size of isolated tumor cells within the sentinel lymph node is no longer used).[1,2] These changes and other aspects of the new staging system will be discussed in greater detail below.

Staging for localized (stage I/II) disease

Stage I/II melanoma is characterized by localized tumors of varying thickness without nodal, regional, or distant metastases. The 2009 AJCC TNM classification for cutaneous and metastatic melanoma differs minimally from the prior 2002 version, and uses the same thickness thresholds to classify tumors as T1 (≤1.00 mm), T2 (1.01-2.00 mm), T3 (2.01-4.00 mm), or T4 (>4.00 mm).[1,2] Analysis of the data from patients with stage I/II melanoma in the 2008 AJCC Melanoma Staging Database indicated a progressive decline in survival rates with increasing tumor thickness. In particular, the 10-year survival rates for patients with T1 (n=11,841), T2 (n=8046), T3 (n=5291), and T4 (n=2461) melanomas were 92%, 80%, 63%, and 50%, respectively.[1,2] The general relationship between increasing tumor size and decreasing survival rate also held when examining T1, T2, T3, and T4 substages. For example, the 10-year survival rate for patients with tumors 2.01-3.00 mm thick was 68%, whereas the corresponding rate was only 60% for patients with tumors 3.01-4.00 mm thick.[2]

The 2008 AJCC Melanoma Staging Database also confirmed that the presence of ulceration versus absent ulceration decreased survival rates for all T groupings.[1-3]

An important addition to the 2009 staging system is the inclusion of MR as an additional prognostic factor for staging. This is based on an analysis of more than 10,000 stage I/II melanoma patients, which confirmed that increasing MR is significantly associated with decreasing 5- and 10-year survival rates.[1,2] A multivariate Cox regression analysis of localized cutaneous melanoma identified MR as the second most powerful

predictor of survival, after tumor thickness ($\chi^2 = 79.1$; $P<.001$).[1] A threshold level of ≥1 mitosis/mm^2 was determined to be the most significant correlate of survival in T1 melanoma.[2]

A multivariate analysis of 4861 patients with T1 melanoma identified tumor thickness, MR, and ulceration as significant and powerful predictors of survival.[1] Notably, Clark's level of invasion was no longer a significant predictor when MR was included in the Cox regression analysis. Based on these findings, the 2009 AJCC staging system defines T1b melanomas as tumors ≤1 mm either with ulceration or with ≥1 mitosis/mm^2, and recommends using Clark's levels IV/V to define T1b only in the rare instances when MR cannot be determined in a nonulcerated T1 melanoma.[2]

How should a pathologist measure MR? Recommendations are to locate dermal "hot spot(s)" containing the most abundant mitotic figures and count these figures, extending the counted fields until an area equal to 1 mm^2 (roughly 5 high power fields at 400X magnification in the average microscope) is assessed.[2] Mitoses should be recorded as the number per mm^2. If mitotic figures are not present, the recommendation is to list the count as zero, rather than <1/mm^2. This method of MR measurement has excellent interobserver reproducibility.[2,5]

The change in the staging rule for T1 based on MR may impact performance of SLNB. A recent literature review by Andtbacka and Gershenwald of data from 24 studies suggested MR may be predictive of occult regional nodal disease in patients with thin melanomas.[6] Based on their analysis, the authors tentatively concluded that SLNB can be considered for patients with thin melanomas (≤1 mm) and a MR of ≥1 mitosis/mm^2. Additional data is needed before a more definitive recommendation can be made with respect to use of SLNB for patients with thin melanomas with an accelerated MR.

Staging for regional metastatic (stage III) disease

A Cox multivariate analysis of the 2008 AJCC Melanoma Staging Database confirmed findings from the previous staging system[3] that the 3 most powerful independent predictors of survival in patients with stage III disease are: 1) number of involved nodes, 2) lymph node tumor burden (microscopic versus macroscopic disease), and 3) presence or absence of primary tumor ulceration.[1] These features are retained in the 2009 staging guidelines for the pathologic substaging of patients with stage III melanoma, and different substages were shown to be predictive of survival. The 5- and 10-year survival rates for patients with stage IIIA, IIIB, and IIIC are 78% and 68%, 59% and 43%, and 40% and 24%, respectively.[1,2]

A difference in the AJCC 7th Edition is that histopathologic confirmation of nodal metastases using standard H&E staining is no longer considered mandatory, although it is highly recommended. The 2009 staging guidelines indicate that nodal metastases may be classified solely on the basis of IHC staining of melanoma-associated markers.[1] Currently available IHC staining techniques enable detection of nodal metastases of aggregates of only a few cells. However, some IHC markers (eg, S100, tyrosinase) are sensitive, but not specific, for melanoma. The guidelines state IHC alone is acceptable if the diagnosis is based on "at least one melanoma-associated marker (eg, HMB-45, Melan-A/MART 1) and the cells have malignant morphologic features that can be detected in the IHC stained tissue."[1] Detection of nodal metastases using reverse-transcriptase polymerase chain reaction (RT-PCR) technology remains investigational.

Consistent with the use of IHC to detect microscopic disease, the concept of isolated tumor cells (ITCs) as node-negative disease [N0(i+)] is no longer used.[1] The 2009 guidelines do not recognize a lower threshold of staging N+ disease and recommend that any positive cell(s) meeting the criteria for histologic or IHC detection of melanoma be scored as N+. In other words, there is no definitive evidence for a lowest threshold to define N+ disease as was previously employed (ie, micrometastasis <0.2 mm in the SLN).[7] The bottom line is that it remains unclear whether ITCs in the regional nodes are of clinical significance, but the concept of "clinically insignificant nodal disease" is unproven.

As in 2002, the 2009 guidelines merge satellite metastases or microsatellites with in-transit lesions in the N category and use them for substaging stage III disease.[1] Patients with intralymphatic metastasis (in-transit metastases, microsatellites, or satellites) without metastatic involvement of the regional lymph nodes are defined as stage IIIB N2c melanoma. For the first time, analysis of the 2008 AJCC Melanoma Staging Database enabled a prospective determination of the survival rate for these patients. The 5-year survival rate of 69% for these patients was higher than that for patients with combined intralymphatic metastases and nodal metastases (stage IIIC N3 disease; 46%) or stage IIIB disease (59%), but lower than that for patients with stage IIIA (78%) melanoma. The literature also indicates that patients with microsatellites in the primary melanoma have comparable survival outcome to those with clinically detectable satellites). The presence of microsatellites is highly predictive of locoregional recurrence and lower disease-free survival, even if lymph nodes are negative. Microsatellites have been more precisely defined in the 7th Edition of the AJCC Cancer Staging Manual as any discontinuous nest of intralymphatic metastatic cells >0.05 mm in diameter, clearly separated by normal dermis from main invasive melanoma component by at least 0.3 mm.[2]

Value of SLNB for staging

While the impact of SLNB on overall survival remains controversial, its value in melanoma staging is indisputable.[8,9] Regional lymph nodes are the most common site of melanoma metastases, and patients with clinically occult disease (micrometastases identified through SLNB) now make up the vast majority of patients who present with stage III melanoma.[2] Moreover, there is large variability in survival outcomes between clinical and pathologic stages with stage III disease, and greater prognostic accuracy is obtained when combining pathologic information from both the primary melanoma and regional lymph nodes (particularly the SLN).

The 2002 AJCC melanoma staging system incorporated SLNB results into the definition of pathologic staging, and noted that SLN positivity versus negativity in patients with clinically-negative nodal metastases was a significant predictor of lowered 5-year survival rates for all T stages and substages (except T4b).[3] The 2009 AJCC staging system further confirms the prognostic utility of SLNB status for survival outcomes.[1,2] Currently, both the AJCC and National Comprehensive Cancer Network (NCCN) encourage SLNB as a standard staging procedure in clinically appropriate patients.[1,10] The question then becomes, what defines a "clinically appropriate patient"?

Who should undergo SLNB? Both the 2009 AJCC staging and 2010 NCCN practice guidelines recommend consideration of SLNB for staging of melanoma patients with clinically node-negative T1b to T4 disease

(ie, clinical stage IB or II disease).[1,10] The NCCN guidelines, published prior to the AJCC staging system, state that discussion of SLNB should be considered for patients with stage IA melanomas (T1a) that have adverse prognostic features such as thickness >0.75 mm, high MR, and young patient age, but stage 1A patients with MR ≥1/mm^2 or ulceration are automatically upstaged to stage IB T1b in the 2009 AJCC staging system. The NCCN guidelines also indicate that factors such as lymphovascular invasion or positive deep margins may be considered indications for SLNB on an individual basis.[10] A decision not to perform an SLNB for an otherwise suitable patient is valid and may be based on significant patient comorbidities, patient preference, or other factors.

Potential predictors of SLN positivity. A number of factors have been evaluated for their ability to predict SLN positivity in melanoma. For example, a multivariate analysis in 2007 by Paek and colleagues of 1130 consecutive melanoma patients in a prospective database who underwent SLNB, identified a number of factors significantly associated with SLN positivity, including younger age, increasing MR (especially in younger patients), increasing Breslow depth (especially in older patients), lymphovascular invasion, and trunk or lower extremity location of the primary tumor.[11]

• Age. Some investigators have questioned whether older age should be a reason for foregoing staging with SLNB. The study by Paek and colleagues indicated that age independently affects SLN positivity and interacts with MR and Breslow depth.[11] In particular, results from this and other studies suggest that older age is associated with higher risk adverse features of tumor histology (thicker and more ulcerated tumors) and generally worse prognosis, but lower rates of SLN positivity[11-14]—leading to questions about the utility of SLNB as a staging technique in elderly patients. Findings such as these also raise questions as to whether the biology of melanoma is different or whether diminished host immunity adversely affects outcomes in elderly patients. It has also been proposed that older patients may have delayed lymphatic spread or may be more likely to have their melanoma disseminate via hematogenous rather than lymphatic routes.[11,14]

In any case, given the current state of knowledge, discussion of SLNB should occur on a case-by-case basis in appropriate patients, regardless of age, when the risk of regional nodal metastasis is high, comorbid conditions are not prohibitive, and the patient is interested in pursuing pathologic staging of the regional lymph nodes. Whether older age should affect the decision to perform SLNB will become an increasingly important issue, as the incidence of melanoma in this patient population is expected to dramatically increase in the coming decades.

• Tumor thickness. SLNB strongly correlates with primary melanoma tumor thickness and increasing disease stage.[2] However, the use of SLNB in very thin melanomas without ulceration or MR≥1/mm^2 does not appear to be cost-effective. Only 3.9% of patients with stage IA melanoma in the most recent analysis of the AJCC Melanoma Staging Database exhibited SLN positivity.[15] Hence, neither the AJCC nor the NCCN recommend the routine performance of SLNB for staging of patients with stage IA melanoma.

• Lymphatic invasion. Lymphatic invasion or lymphovascular invasion may also be an important predictor of SLN positivity in melanoma, although it is not currently incorporated into the 2009 AJCC staging guidelines. It is now possible to differentiate lymphatic invasion (LI) from vascular invasion (VI) through the use of lymphatic-specific endothelial markers such as LYVE-1 and D2-40 (podoplanin), and the distinction might have important staging implications. A recent analysis by Doeden and associates of LI versus VI frequency in melanoma sections from 94 patients with a mean 3-year clinical follow-up showed LI occurred more frequently than VI (16% vs 3%, *P*=.001) and was significantly correlated with higher AJCC stage at diagnosis.[16] Moreover, LI appeared to be associated with increased propensity to develop SLN metastases, and the combination of LI with presence of intratumoral lymphatics had higher positive and negative predictive values for risk of SLN metastases than VI and routine histology.

The commercial availability of lymphatic-specific markers may make assessment of lymphatic or lymphovascular invasion in primary tumors more accessible to pathologists. This, in turn, may lead to better characterization of this factor as a possible predictor of SLN positivity. Depending on the outcome of further studies, lymphovascular invasion may well be incorporated in the next version of the AJCC staging system.

Staging for distant metastatic (stage IV) disease

The 2009 melanoma staging guidelines contain no changes from the prior version with respect to stage IV melanoma. Analysis of the 2008 AJCC Melanoma Staging Database confirmed the significance of both the site(s) of distant metastases and elevated serum LDH level as predictors of survival.[2] Multivariate Cox analysis of the 2008 database also confirmed that elevated serum LDH level is a highly significant independent predictor of survival in patients with stage IV melanoma, with 1- and 2-year survival rates of 65% and 45% for stage IV patients with normal LDH level compared with 32% and 18% for those with elevated levels (*P*<.0001).[1,2]

Data in the literature indicate that the number of metastases at distant sites is an important prognostic factor, and a preliminary multivariate analysis confirmed the prognostic significance of this variable for survival outcomes, particularly for 1 site versus either 2 or ≥3 sites (*P*<.0001).[17] However, the number of distant metastases has not yet been incorporated into AJCC staging of melanoma, largely because the various institutions that contributed data to the 2008 database used a wide range of radiologic tests to detect distant metastases without standard methodology.[2]

Staging is more difficult for patients presenting with local, regional, or distant metastases, with an unknown primary tumor site. The 2009 guidelines state that initial presentation of metastases in lymph nodes, or localized to the skin or subcutaneous tissues (in patients with no known primary site) should be classified as having regional disease, and defined as stage III (rather than stage IV) disease, although it is important to differentiate a solitary cutaneous metastasis from a variant of a primary melanoma, including those with a regressed junctional component.[1] Metastases to any other distant site without a known primary melanoma should be categorized as stage IV disease, and subcategorized using site(s) of metastasis and serum LDH level.

Summary

Proper cancer staging is essential for clinicians to determine the best treatment for their patients, to evaluate treatment responses in clinical trials, and to serve as a standard for reporting cancer incidence and outcomes. Clinicians and pathologists are encouraged to review the recently published 7th Edition of the AJCC Staging Manual for Melanoma and become familiar with its contents.

The Role of SLNB in Prior Resected and Recurrent Melanoma

By Robert H. I. Andtbacka, MD, CM, FRCS(C)

Sentinel lymph node biopsy (SLNB) is a widely used technique to assess the existence of microscopic metastatic cells in patients with clinically node-negative melanoma. Information obtained from SLNB is useful for accurate staging and for determination of prognosis, and hence helps guide subsequent treatment decisions. However, while SLNB is considered routine practice for most patients with stage IB or II melanoma, its utility in certain other populations is less clear. This article examines evidence that helps to clarify the role of SLNB in melanoma patients with prior wide local excision (WLE) or in those with locally or regionally recurrent melanoma.

SLNB and its prognostic implications

SLN status has been demonstrated in multiple studies to be the most important prognostic indicator for survival in melanoma patients without clinical evidence of nodal metastases. A multivariate Cox regression analysis of 4750 melanoma patients identified nodal status as the most powerful independent predictor of survival in these patients (relative risk [RR], 2.2; *P*<.0001), followed by Breslow thickness and ulceration.[4] Another analysis showed 5-year survival rates were significantly lower for SLN+ versus SLN– melanoma patients for all T stages except T4b.[3] Similarly, a 1999 study by Gershenwald and associates of 580 patients with stage I/II melanoma reported SLN status was the most significant prognostic factor for disease-free (DFS) and disease-specific survival (DSS).[18] Both the 3-year DFS and DSS rates were more than 25% lower in patients with SLN+ versus SLN– disease (DFS: 55.8% vs 88.5%, *P*<.0001; DSS: 69.9% vs 96.8%, *P*<.0001).

While SLNB provides valuable information about the presence of micrometastases in the SLN, the SLNB technique may not have a direct impact on survival in patients with clinically node-negative stage I or II melanoma. The first Multicenter Selective Lymphadenectomy Trial (MSLT-I) randomized patients with clinically node-negative melanoma to WLE and nodal observation or WLE and SLNB (and immediate completion lymphadenectomy [CLND] for SLN+ status). There was no statistical difference in the estimated 5-year melanoma DSS rates (86.6% vs 87.1%, respectively).[8] However, the 5-year survival rate in SLN+ patients who underwent immediate CLND was nearly 20% higher than in patients randomized to nodal observation and a delayed therapeutic CLND if they developed clinically palpable disease (72.3% vs 52.4%; HR 0.51; *P*=.004).[8]

Hence, the SLNB technique identifies patients with nodal metastases whose survival can be improved with an immediate CLND. In addition, information about SLN status is helpful to clinicians and patients when considering subsequent treatment, including the possibility of adjuvant or other therapies that may improve outcomes.

As discussed earlier, SLNB is recommended for all stage I/II patients whose primary melanomas are ≥1 mm, and for those whose primary melanomas are <1 mm but possess other negative prognostic features.[1,10] Other patient or disease characteristics that may increase the risk of SLN+ include young age, Clark's level IV/V, and (although more controversial) tumor regression.[10] Patients should be individually evaluated, and SLNB should be discussed with all patients with invasive melanoma.

Some evidence indicates that SLNB may be useful in patients with thin melanomas (≤1 mm) and without other adverse prognostic features. In a study of 1375 patients in the 2001 AJCC database who underwent SLNB, the incidence of SLN+ was only 2% for patients with stage IA melanoma.[19] Andtbacka and Gershenwald recently reviewed the results from a number of studies examining SLN status in melanoma patients with melanoma either <0.75 mm or 0.75 to 1.0 mm thick.[6] In many of these studies, patients with thin melanomas did not have poor prognostic features, or the information was incomplete. As can be seen in **Table 2**, a greater argument can be made for offering SLNB as an option for patients with tumors 0.75 to 1.0 mm thick than for those with tumors <0.75 mm thick, regardless of the presence of other prognostic factors.[6] Since the average risk of SLN metastasis in patients with primary melanomas 0.75 to 1.0 mm in Breslow thickness is >5%, we now routinely offer SLNB to these patients.

Should SLNB be performed in patients with prior WLE?

Questions have been raised as to whether a delayed SLNB should be performed in stage I/II melanoma patients who have had a prior WLE without a concurrent SLNB. The principle concern is that prior surgery may have disrupted lymphatic drainage pathways, and that skin grafts and flap closures may further distort these pathways. If the lymphatic channels are disrupted, then the reliability of subsequent lymphoscintigraphy and the results of SLNB may be compromised. In fact, any disruption of lymphatic drainage is a general contraindication for SLNB. In addition, there is a concern that use of vital blue dye as part of the SLN procedure may "tattoo" the skin if a second WLE is not performed. And there are questions as to where the lymphatic mapping tracer should be injected to optimize results.

A review of 6 studies examining the utility of delayed lymphatic mapping and SLNB (LM/SLNB) after prior WLE in melanoma demonstrated a 99% to 100% success rate for accurately and reliably identifying the SLN and a 2% to 9% SLN basin false-negative rate.[20-25] In 2006, Gannon and colleagues evaluated the feasibility and accuracy of LM/SLNB after WLE and determined that delayed SLNB after prior WLE (median follow-up, 51 months) does not adversely affect the ability to detect lymphatic metastases.[21] Examination of this and the other studies reviewed also suggests that primary or skin graft closures do not negatively impact SLNB rates.[15-20] However, the studies did indicate

Table 2. SLNB May be Indicated for Some Patients With Thin Melanomas

Breslow Thickness	Risk of SLN Metastasis	Average % SLN Metastasis
<0.75 mm	0%–4.3%	2.7%
0.75–1.0 mm	3.9%–18.1%	6.2%

Source: Andtbacka RH, Gershenwald JE. *J Natl Compr Canc Netw.* 2009;7:308-317.[6]

that rotational flap or complex flap closures resulted in a greater likelihood of LM error. In addition, axial lesions may require more surgery since they may drain to more than 1 lymphatic node after a prior WLE.[15-20] **Table 3** summarizes general suggestions regarding LM/SLNB following WLE.

Table 3. Suggestions for LM/SLNB following WLE

- LM and SLNB should be offered to patients with prior WLE if they are candidates for SLNB based on the primary tumor factors
- Whenever possible, WLE and SLNB should be performed concomitantly
- The LM tracer should be injected intradermally in multiple locations around the WLE to ensure proper drainage

Should SLNB be performed in patients with satellite/in-transit recurrence?

Whether SLNB should be performed in patients with recurrent satellite or in-transit melanoma has been controversial. Satellite and in-transit recurrence occurs in 2% to 11% of patients after resection of the primary melanoma, and SLN+ patients are more likely to develop satellite or in-transit recurrence (11%-24% vs 4%-6%).[26-30] Satellite/in-transit recurrence as a first manifestation of recurrence is a harbinger of poor prognosis, with 5-year survival rates of 20% to 55%.[26-28,30-32] In addition, 28% of patients with satellite recurrence as the first event subsequently develop in-transit or lymph node metastasis, and 5-year survival is lower in stage III patients with intralymphatic metastases plus nodal involvement than in those with intralymphatic metastases but no nodal involvement (46% vs 69%).[1]

A limited number of studies have evaluated the use of LM/SLNB in patients with satellite/in-transit recurrence of malignant melanoma. The largest study to date examining this issue involved a review of 1600 patients who had undergone LM/SLNB.[33] Of these 1600, 30 were identified who had undergone LM/SLNB for recurrent melanoma. Fourteen (47%) of the patients with satellite recurrence had at least 1 positive SLN, and 11 of these underwent CLND. All 14 patients were followed for a median of 20 months after LM/SLNB. Median DFS after LM/SLNB for satellite recurrence was significantly shorter for those with SLN+ versus SLN– status (16 vs 36 months, *P*=.03). The authors concluded that LM/SLNB can accurately identify SLNs draining a recurrent melanoma, and that LM/SLNB should be routinely considered for patients with isolated recurrent local/in-transit melanoma, given the high rate of metastases and poor prognosis in SLN+ patients.[33]

Similarly, a study of 12 patients with locally recurrent melanoma determined that LM/SLNB was successful in the vast majority (92%) of patients.[34] Four (33%) patients with recurrence had at least 1 positive SLN after a median follow-up of 23 months post-LM/SLNB for recurrence. All 12 patients underwent LM/SLNB for satellite/in-transit metastasis within 5 cm of prior WLE, and none had a prior LM/SLNB or CLND. LM tracer (radiotracer and vital blue dye) was injected peritumorally (not intradermally) around recurrence.[34] In another study, LM/SLNB was successful in all 5 melanoma patients who underwent the procedure for recurrent in-transit metastases.[35] The median time to in-transit recurrence was 5 years, and none of the patients had prior LM/SLNB or CLND. Four (80%) patients had SLN metastases. LM tracer was injected intradermally around recurrence in 1 patient, and at the primary tumor WLE site in the other 4.[35]

Given the limited number of patients evaluated thus far, firm recommendations cannot be made at this time. The studies do suggest LM/SLNB can accurately detect nodal metastases in patients with an isolated recurrent satellite/in-transit melanoma lesion. Furthermore, LM/SLNB findings may guide decisions to perform CLND in these patients, which might improve their prognosis. Remaining controversies include location of the injection (intradermally, peritumorally, site of WLE) and use of LM/SLNB in patients with multiple in-transit metastases.

The Management of In-transit Melanoma

By Robert H. I. Andtbacka, MD, CM, FRCS(C)

Melanoma consisting of in-transit but no distant metastases is staged as stage IIIB or IIIC. The 5-year survival rate is 69% if the in-transit metastasis occurs in the absence of regional lymph node metastasis (IIIB), and 46% if the in-transit metastasis is combined with regional lymph node metastasis (IIIC).[1]

The presence of in-transit melanoma presents a treatment dilemma. There is no current standard of care for these patients,[10,36] and there are no or few randomized controlled trials comparing different modalities to guide decision-making,[36] although a number of studies have reviewed management of in-transit melanoma.[36-39] A number of options are available **(Table 4)**, depending on whether the in-transit metastases are amenable to surgical resection, the extent of the disease and number of in-transit metastases, and other patient and disease characteristics.[37] Thorough patient evaluation with a complete physical examination and radiographic staging work-up to determine the presence of absence of distant metastases should be done before embarking on a treatment plan.

Local treatment

Local therapies for in-transit melanoma include surgical excision, laser ablation, radiation therapy, and intralesional therapy.

Surgical excision. The 2010 NCCN guidelines recommend complete surgical excision with histologically negative margins as the preferred option for patients with only 1 or a small number of in-transit metastases clustered in a circumscribed area, if feasible.[10] Surgical resection is not appropriate for patients with extensive disease, and limb amputation is rarely (if ever) indicated. Locoregional recurrence is high in patients undergoing curative surgical resection for 1 or few reasonably circumscribed in-transit metastases (82% recurrence within 5 years).[40] Because of the high possibility of occult nodal involvement, the NCCN guidelines recommend consideration of SLNB for these patients.[10]

Laser ablation. Many melanoma patients do not present with in-transit disease that is suitable for complete surgical resection. Carbon dioxide (CO_2) laser ablation is an option for some of these patients, primarily those with visible and superficial subcutaneous lesions of low volume that are small (≤1.5 cm) and not too extensive.[36,38] CO_2 laser ablation is performed in the outpatient setting under local anesthesia, is typically used for palliative effect, and can be employed multiple times. In a 1996 study by Hill and Thomas, 34 of 53 (64%) patients with stage III in-transit melanoma had the disease controlled with 4 or fewer CO_2 laser ablation treatments.[41] Overall survival rates are relatively low, with only 17% of patients still alive at 5 years.[41]

The main drawback of this technique is that it can only be used for visibly apparent, low-volume, subcutaneous lesions.[36] As such, it is unsuitable for patients with microscopic disease or higher volume or deep subcutaneous lesions.

Radiation therapy. Contrary to earlier beliefs, radiation is effective against melanoma cells in select patients. Radiation therapy is primarily used in a palliative setting for unresectable in-transit melanoma, particularly when lesions are small and in a circumscribed area. Two early studies reported overall response rates (ORRs) ranging from 60% to 79% when radiation therapy was used for palliation in patients with various stages of melanoma.[42,43]

A more recent study by Olivier and colleagues of 84 patients with metastatic melanoma suggested a higher radiotherapy dose than has typically been used might be associated with higher and more durable response rates.[44] In this study, patients were treated with a median dose of 30 grays (Gy), and the ORR was 84%, although most responses were partial (75% partial response [PR] vs 9% complete response [CR]). Moreover, patients treated with >30 Gy versus ≤30 Gy had significantly longer freedom from disease progression (FFP) (P=.01) and significantly longer median survival (8 vs 2 months, P<.0001), as did those treated with >39 Gy versus ≤39 Gy (FFP: P=.03; median survival: 8 vs 2 months, P<.0001).[44]

Intralesional therapies. These treatments include Bacille Calmette-Guérin (BCG), interleukin-2 (IL-2), interferon-alpha 2b (IFN-α), electroporation, TNFerade, and others, as discussed below.

• BCG. A number of different compounds have been examined for effect when administered as direct injections into the in-transit melanoma lesion site. The first of these was BCG, a vaccine prepared from the bacillus *Mycobacterium bovis*, introduced in the late 1960s and early 1970s. In an initial report by Morton and associates, 91% of directly injected intracutaneous metastatic lesions demonstrated regression, and there was no evidence of disease in 11 (31%) of the 36 patients following treatment.[45] Furthermore, uninjected lesions regressed in 6 (17%) patients. Controversy currently exists as to whether intralesional BCG therapy is associated with a survival benefit in at least certain subsets of patients.[46,47]

The major disadvantage of BCG as intralesional therapy is that it is associated with a high number of often severe local complications around the injection site, as well as sometimes systemic allergic reactions.[36,38,39] Because of this morbidity, and its questionable survival benefit, intralesional BCG therapy is seldom used today as treatment for in-transit melanoma.

• IL-2 and IFN-α. Systemic IL-2 is an approved therapy for patients with advanced stage IV melanoma, and has been associated with durable responses in a small subset of these patients,[48,49] but its role as intralesional treatment for in-transit disease is less well characterized. Promising results were obtained in a 2003 study by Radney and coworkers of salvage intralesional IL-2 therapy in 24 melanoma patients with stage III/IV disease and soft-tissue or in-transit melanoma metastases after failure of surgery, limb perfusion, radiotherapy, or chemotherapy.[50] Intralesional IL-2 therapy produced a CR of treated metastases in 15 (63%) patients and a PR in another 3 (21%). In addition, the treatment was generally well tolerated, with mostly grade I/II adverse events. The time-intensive nature of intralesional IL-2 therapy is a drawback.[37]

IFN-α is currently approved for adjuvant therapy of resected stage IIB/III melanoma, primarily based on its consistently demonstrated ability to increase the RFS of patients in all trials conducted to date, and its ability to increase OS in some studies (high-dose only) but not others.[49] However, there is only very limited experience with IFN-α as intralesional treatment for in-transit melanoma. An early study by von Wussow and associates involving 51 melanoma patients with at least 1 skin metastasis who received intralesional IFN-α therapy demonstrated 24 (47%) CRs or PRs.[51] As with IL-2, larger clinical trials are needed before the effectiveness of IFN-α as intralesional therapy can be more fully evaluated.

• Electroporation with bleomycin or cisplatin. Electroporation, also known as electrochemotherapy (ECT), involves the use of short, high-intensity electric pulses that generate small defects or pores in the membrane of melanoma cells.[37,39] This increases the permeability of these cells, which would otherwise be relatively impermeable to cytotoxic chemotherapy agents. ECT has been shown to increase the intracellular activities of bleomycin and cisplatin by 1000-fold and 100-fold, respectively.[52] ECT with intralesional bleomycin has been reported to produce a CR rate of 77% in patients with subcutaneous melanoma metastases, compared with 32% with intralesional bleomycin omitting concomitant ECT, and 45% when bleomycin is given intravenously.[53,54] Cisplatin produces similar CR rates when used concomitantly with ECT (67% when used with ECT and 48% when administered intravenously).[53] Adverse effects are very minimal, usually involving minor irritation at the injection site or a transient electric shock sensation from the pulse current.[37,39]

ECT plus bleomycin or cisplatin has been suggested for in-transit melanoma that is unsuitable for surgical excision due to the number or location of the tumor(s).[36,39] It can be performed on an outpatient basis without the use of local, regional, or general anesthesia, and can be used to treat previously irradiated areas. The ultimate role of ECT has yet to be established in larger multicenter trials, however.

• TNFerade. This relatively new intralesional treatment for in-transit melanoma uses a special delivery complex to take advantage

Table 4. Treatment Options for In-Transit Melanoma

Local treatment
- Surgical excision
- Laser ablation*
- Radiation therapy
- Intralesional therapy

Regional treatment
- Hyperthermic isolated limb perfusion
- Isolated limb infusion

Systemic treatment
- Chemotherapy
- Immunotherapy
- Gene therapy
- Targeted therapy

*Cryosurgery was once a more frequent option for local treatment of in-transit melanoma but has largely fallen out of favor since the introduction of laser ablation.

of the antitumor effects of tumor necrosis factor-alpha (TNF-α), while avoiding the troublesome toxicity associated with systemic TNF-α. More specifically, TNFerade is a complex formed by cloning the *TNF-α* gene downstream from a radiation- and chemotherapy-induced promoter (Egr-1) in a nonreplicating adenovirus.[39,55,56] The TNF-α component of TNFerade becomes activated when irradiated, and locally injected TNFerade and external radiation have been shown to interact in a synergistic fashion for antitumor activity in patients with in-transit melanoma, without inducing damage to normal tissue.

So far, only small phase I trials have been performed with TNFerade, in which melanoma was only one of many solid tumors examined. In a study by McLoughlin and associates, 3 patients with melanoma metastases of the axillary or axillary node received intralesional TNFerade and experienced CRs lasting for the 24-month follow-up.[57] In another phase I trial, 3 patients with melanoma metastases (2 axilla, 1 groin) similarly demonstrated a CR to intralesional TNFerade.[58] These early results support the use of TNFerade for in-transit melanoma, but additional larger trials are required to more fully evaluate its effectiveness in this setting.

• Others. Additional intralesional therapies are being evaluated for in-transit melanoma.[59-62] Allovectin-7 is a plasmid formulated with a cationic lipid complex. The plasmid component contains coding sequences for HLA-B7 and β2 microglobulin, which together make up a major histocompatibility complex class I. Initial studies indicate that Allovectin-7 activates the immune system both locally at the injection site and systemically. Phase III trials are ongoing to determine the effect of Allovectin-7 when used as intralesional therapy for in-transit melanoma. OncoVEX^{GM-CSF} is an oncolytic herpes simplex virus encoding GM-CSF that is also being investigated as intralesional therapy in a phase III trial of patients with in-transit melanoma.

Regional treatment

Regional therapies for in-transit melanoma include hyperthermic isolated limb perfusion (HILP) and isolated limb infusion (ILI).

• HILP. Isolated limb perfusion (ILP) is a regional therapy for extremity-localized in-transit melanoma that was introduced in 1958.[63] ILP is performed under general anesthesia and involves exposure and cannulation of the artery and vein supplying the limb of interest, and the use of a tourniquet and ligation of collateral vessels to isolate the affected limb from the systemic circulation.[38,64] Then, with the use of an extracorporeal oxygenated pump connected to the cannulas, the diseased limb is perfused with a chemotherapeutic agent. Mild warming of the perfused limb (38.5-40°C) has been shown to improve the effectiveness of ILP,[65] giving rise to the approach known as HILP (**Figure 1, left**).

Use of HILP enables the achievement of regional drug concentrations 15 to 25 times higher than can be achieved with systemic administration due to dose-limiting toxicities.[36,38] Perfusion is usually carried out over a period of 1 to 1.5 hours, followed by a washout period. Although systemic toxicities are generally minimal with HILP, regional toxicities are quite common. In addition, systemic toxicity is sometimes observed when there is some leakage of the chemotherapeutic agent or washout is incomplete.[36,39]

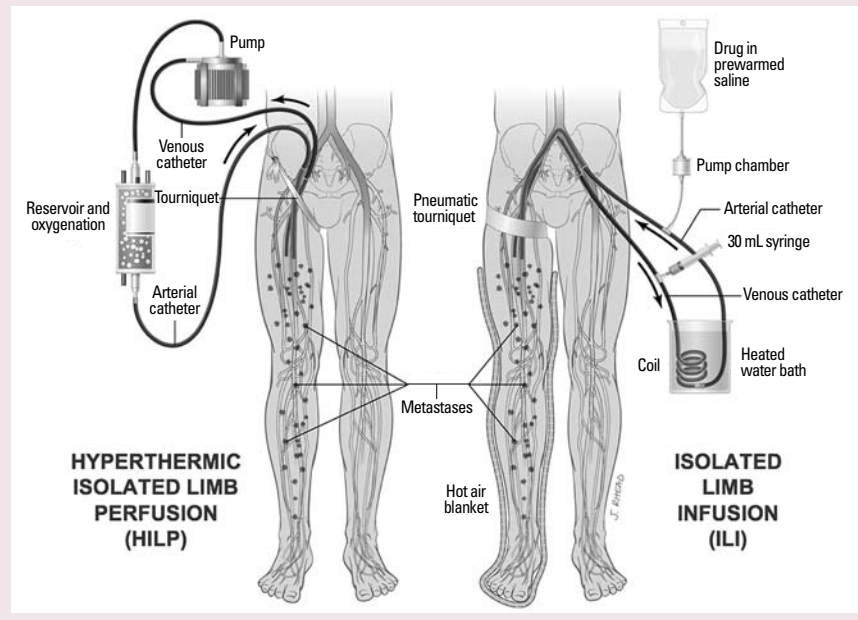
An analysis of various potential chemotherapeutic agents for use with HILP identified melphalan as the most effective, and it is currently used as part of HILP in both the United States and Europe.[36,38,39] Some studies subsequently reported a combination of TNF-α plus melphalan was more effective than melphalan alone (45%-58% CR rate for melphalan alone vs 59% to 78% CR for melphalan plus TNF-α).[66-74] TNF-α is routinely used in Europe in HILP, but is not approved for use in the United States. Toxicities tend to be greater with addition of TNF-α in HILP. Patients who have a CR with HILP experience recurrence 46% to 54% with follow-up.[36,39,72,75]

HILP is indicated for the regional treatment of multiple in-transit melanoma localized to a leg or arm that is not amenable to surgical excision.[76] The lesions must be contained within what would be the perfused area to be suitable for HILP. HILP may also be appropriate for palliative therapy in some patients with distant metastases who also have soft tissue metastases within a limb.

• ILI. This technique, developed in the mid-1990s, was introduced as a simpler alternative to HILP with similar efficacy and less toxicity.[77] ILI is less invasive than HILP and uses the common femoral artery and vein in the contralateral groin to percutaneously insert small catheters to deliver melphalan into the artery and vein of the affected limb (**Figure 1, right**).[36,38] This part of the procedure can be done under local anesthesia, before transfer to the operating room for application of a pneumatic tourniquet to isolate the limb from the systemic circulation for subsequent treatment.

ILI further differs from HILP in that the infusion occurs at a much slower rate than HILP perfusion and for a duration of only 30 minutes.[38,78] Also, the extremity is hypoxic during ILI, leading to an acidic milieu that appears to augment the effects of melphalan.[36,38,78] By contrast, the pump oxygenator used during HILP maintains normal oxygenated and acid/base status.

Figure 1. Schematics of HILP and ILI for In-Transit Melanoma of the Leg



Furthermore, ILI is amenable to repeat usage (whereas repeat use of HILP is difficult because of surgical scarring[77,78]), and does not require a perfusionist.

While ILI has not been evaluated as long as HILP, findings suggest somewhat lower efficacy with ILI, but better tolerability. For example, a recent study by Beasley and colleagues comparing ILI and HILP in patients with in-transit melanoma reported a higher ORR and CR with HILP than ILI (88% and 57% vs 44% and 30%), but more HILP-treated patients experienced grade III or higher toxicities (32% vs 18%, *P*=.037).[79] Another recent study reported similar regional toxicities with HILP and ILI, but lower systemic toxicity with ILI.[80]

Systemic chemotherapy

This treatment is as disappointing for in-transit melanoma as it is for systemic metastatic disease, and is generally considered after the failure of local and/or regional therapy or in patients with high-volume disease or other disease characteristics that render them unsuitable for local or regional therapy options. Dacarbazine (DTIC) is typically considered the standard for systemic chemotherapy of melanoma in the United States, but response rates are low (20% ORR) and of short duration (4-6 months).[36,38,39] Combining DTIC or other chemotherapeutic agents with biochemotherapy does not appear to produce consistent benefits versus chemotherapy alone, while increasing toxicity. Temozolomide may be considered as an alternative to DTIC for patients considering systemic chemotherapy.[81,82]

Targeted therapy

Targeted therapies with small molecule inhibitors of *BRAF*, and *C-Kit* that have recently passed phase I-II trials for widely metastatic disease have yet to be systematically examined in the therapy of in-transit metastatic disease.

Current Status of Adjuvant IFN-α Therapy in Melanoma

By Ahmad A. Tarhini, MD, MSc

Patients with AJCC stage IIB-III melanoma have a 5-year postsurgical relapse rate of >40%-50% and are therefore candidates for postsurgical adjuvant therapy. Adjuvant immunotherapy with high-dose IFN-α2b has demonstrated relapse-free survival (RFS) and overall survival (OS) benefits in this patient population, and is currently the only FDA-approved adjuvant therapy for these patients in the United States. This article reviews the current status of adjuvant IFN-α therapy in melanoma and the latest on pegylated IFN-α testing in this setting. It also examines the latest on potential biomarkers of predictive or prognostic value in the adjuvant setting.

Impact of adjuvant standard IFN-α on RFS and OS

Various IFN-α regimens that may be categorized as high-, intermediate-, or low-dose have been evaluated as adjuvant therapy for intermediate- to high-risk melanoma. The only regimen consistently shown to produce durable RFS benefits in randomized controlled trials is the high-dose regimen originally tested in the E1684 trial (20 MU/m^2/day intravenously, 5 days a week, during a 4-week induction period, followed by 10 MU/m^2 subcutaneously, 3 times a week, during a 48-week maintenance period).[83]

This regimen has been associated with significantly improved RFS compared with observation or vaccine control in the 3 Eastern Cooperative Oncology Group (ECOG) and Intergroup trials (E1684, E1690, E1694) that explored this modality as adjuvant therapy for high-risk melanoma.[83-85] This regimen was also associated with a significant improvement in OS in E1684 (vs observation) and E1694 (vs the GMK ganglioside vaccine).[83,85] Furthermore, a recent individual patient data (IPD) meta-analysis by Wheatley and colleagues of 13 randomized controlled trials of various adjuvant IFN-α regimens confirmed the event-free survival (EFS) benefit of IFN-α, while also demonstrating a significant benefit of IFN-α for OS (odds ratio [OR] 0.9, *P*=.008).[86] In this meta-analysis evaluating high-, intermediate-, and low-dose regimens without clarifying the optimal dose level, the impact of IFN-α upon OS was relatively small, translating into an absolute benefit of about 3% (95% confidence interval [CI], 1%-5%) at 5 years.[86]

Taken together, the data to date indicate that adjuvant therapy with high-dose IFN-α2b is associated with a significant improvement in RFS and OS. Recently, investigators have been exploring whether outcomes might be improved by: 1) using a different regimen and formulation of IFN-α (pegylated IFN) given for a longer period of time, or 2) by using biomarker analysis to identify those subgroups of patients most likely to benefit from adjuvant IFN-α therapy.

Evaluation of more prolonged treatment (up to 5 years) with pegylated IFN-α2b

The EORTC 18991 trial hypothesized that prolonged treatment is needed to obtain a maximal antiangiogenic benefit and thus has compared observation with an intended 5 years of maximally tolerable doses of pegylated IFN-α2b for patients with resected, stage III melanoma (Tx,N1-2,M0).[87] In this phase III trial, pegylated IFN-α2b was given at 6 µg/kg per week for 8 weeks as induction therapy, then 3 µg/kg per week during the maintenance period.

The trial enrolled 1256 patients and was powered to detect a 9.75% absolute difference in distant-metastasis-free survival (DMFS) at 4 years.[87] This trial reported data based on a median follow-up of 3.8 years. The primary endpoint was DMFS, which showed a nonsignificant benefit in favor of pegylated IFN-α2b (hazard ratio [HR] 0.90, *P*=.20). OS was not significantly different in the 2 groups; however, IFN-treated patients showed a significant improvement in RFS (HR 0.84, *P*=.02).

Subgroup analysis showed a benefit of pegylated IFN-α2b only in patients with N1 disease, (29% reduction in recurrence risk, *P*=.016; 30% improvement in DMFS, *P*=.034). These patients also showed an 18% reduction in mortality, although this was not statistically significant (*P*=.43). Patients with palpable tumor in regional lymph nodes de-

rived no benefit from pegylated IFN-α2b.[87]

In the end, the EORTC trial 18991 testing pegylated IFN-α has shown neither statistically significantly improved OS nor DMFS overall, although RFS benefits that were statistically significant overall have been shown on analysis for regulatory review. These benefits appear to be most pronounced in the subset of patients without gross nodal disease (SLN+). In this subset of patients with the lowest tumor burden (n=382), interferon significantly improved RFS and DMFS, but not OS, compared with observation alone.[87] As initially published at a median follow-up of 3.8 years, this trial is relatively early to allow a firm conclusion regarding the durability of the observed benefits in the subset of patients with microscopic nodal disease, where the disease course of 5 to 10 years is required to assess the durability of benefits for intermediate-risk disease. This trial was designed to deliver 5 years of therapy, but has ultimately shown treatment median duration of little over 1 year, so the question of whether longer therapy with this regimen achieves more significant antitumor effects cannot be answered at this time.

Biomarkers in adjuvant therapy

Identification of biomarkers predictive of adjuvant IFN-α therapeutic benefits would enable the targeting of patients most likely to benefit from therapy, while sparing those least likely to benefit from the significant toxicities associated with treatment. While well-established predictors have not been identified at this time, some progress has been made. In addition, disease prognostic biomarkers may also enable the identification of patients most likely to relapse, who may derive the most benefit from adjuvant therapy.

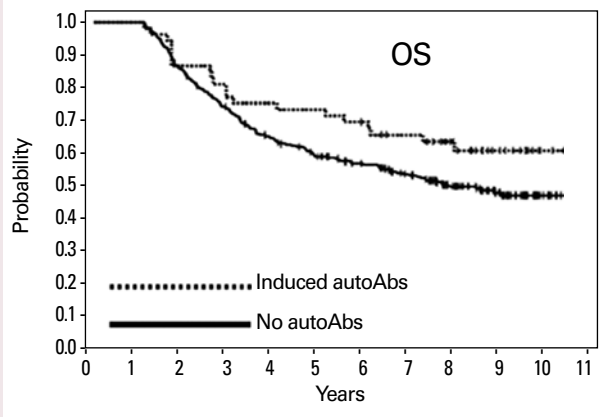
Autoimmunity. Induction of clinical or serologic manifestations of autoimmunity following the initiation of therapy is a potential biomarker for therapeutic response to immunotherapies such as IFN-α, interleukin-2 (IL-2), and anticytotoxic T-lymphocyte antigen 4 (anti-CTLA-4) blockade. Early studies of IL-2 for advanced melanoma suggested that the induction of autoimmune phenomena like thyroiditis, hypophysitis, enteritis, hepatitis, and dermatitis correlated with antitumor effects.[88-95] More recently, studies evaluating the use of CTLA-4-blocking monoclonal antibodies for advanced melanoma pointed to the presence of autoimmune-related adverse events as a possible correlate of therapeutic response.[96-102]

With respect to adjuvant IFN-α for resected stage IIB-III melanoma, a 2006 report by Gogas and colleagues linked the development of de novo manifestations of autoimmunity during adjuvant therapy with improved outcomes.[103] This report included results from a substudy of a larger phase III trial (He 13A/98) conducted by the Hellenic Cooperative Oncology Group, where 200 patients with resected stage IIB-III melanoma were randomized to receive a modified (reduced dosing) IFN-α regimen consisting of induction therapy (15 MU/mm^2/day, 5 times a week for 4 weeks) followed by observation or induction therapy (15 MU/mm^2/day, 5 times a week for 4 weeks) followed by maintenance therapy (10 MU/day, 3 times a week for 48 weeks). The primary efficacy endpoints were RFS and OS. Patients were prospectively consistently examined for vitiligo and other manifestations of autoimmunity, and their blood was evaluated for various autoantibodies.[103]

Clinical manifestations of autoimmunity were observed in 19 (10%) patients, including vitiligo-like depigmentation in 11 (6%), and autoantibodies were detected in 52 (26%).[104] Of particular note, induction of autoimmune phenomena and/or the appearance of autoantibodies in serum was strongly correlated with prolongation of RFS and OS after treatment with this modified adjuvant IFN-α regimen. Furthermore, a Cox multivariate analysis identified the presence of autoimmunity as a significant independent prognostic marker for improved RFS and OS at both 3 months (RFS: HR 0.15, *P*<.001; OS: HR 0.07, *P*<.001) and 12 months (RFS: HR 0.08, *P*<.001; OS: HR 0.02, *P*<.001).[103]

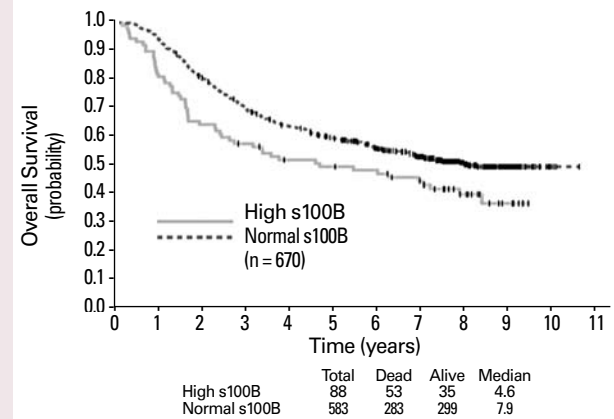
More recently, myself and others have evaluated the phase III E1694 trial comparing high-dose IFN-α and ganglioside vaccine in patients with high-risk melanoma to better understand the prognostic value of treatment-induced autoimmunity.[104] Serum was collected from 691 patients at baseline and up to 3 additional time points and tested by ELISA for development of 5 autoantibodies. To account for lead-time bias, a 1-year landmark analysis was performed. Autoantibodies developed in 19.3% of the high-dose IFN-α group but only 4.4% of the vaccine control group (2*P*<.001). Multivariate analysis demonstrated that IFN-α–induced autoimmunity conferred a survival advantage (**Figure 2**) approaching statistical significance (HR 1.54, *P*=.072).[104]

Figure 2. OS Among IFN-α-Treated Patients With or Without Induced Autoantibodies



Stuckert JJ, Tarhini AA, Lee S, Kirkwood JM. *J Clin Oncol.* 2007;25(18 suppl):8506.[104]

Figure 3. Baseline S100B ≥0.15 µg/L Significantly Correlates With OS (*P*=.010)



Tarhini AA et al. *J Clin Oncol.* 2009;27(1):38-44.[107] Reprinted with permission.

Ulceration. There are now a number of suggestions that ulceration in the primary tumor might be a biomarker for increased responsiveness to adjuvant IFN-α therapy (standard high-dose or pegylated) in patients with high-risk melanoma. The IPD meta-analysis by Wheatley and co-workers of 13 randomized adjuvant IFN-α trials reported that patients with ulcerated tumors experienced greater benefit from IFN-α (EFS: OR 0.76; OS: OR 0.77) than those with no ulceration (EFS: OR 0.94; OS: OR 0.98).[86]

A more recent analysis of adjuvant EORTC trials 18952 and 18991 assessed the predictive value of ulceration in relation to the therapeutic impact of IFN-α in terms of RFS, DMFS and OS, overall and according to stage.[105] Among 2644 patients randomized, 849 had ulcerated primaries and 1336 had nonulcerated primaries, while the ulceration status was unknown for 459. In the group with ulcerated primary melanomas, the impact of IFN was noted to be greater than in the nonulcerated group for RFS (test for interaction: *P*=.02), DMFS (*P*<.001), and OS (*P*<.001). The greatest effects of therapy were noted in patients with ulceration and stages IIB/III-N1.[105]

Based on this analysis, EORTC 18081 trial has been planned to compare the benefit of pegylated IFN-α2b versus observation in patients with ulcerated primaries and Breslow depth >1 mm (node-negative). It is noteworthy that unlike US cooperative groups, the EORTC does not require central pathology review for EORTC melanoma trials.

Proinflammatory cytokine levels. Recently, the detection of serum biomarkers that are either prognostic of clinical outcome or predictive of response to IFN-α was pursued using a multiplex immunobead assay to simultaneously measure the levels of 29 soluble factors associated with tumor immunobiology in the sera of 179 patients with high-risk melanoma from the E1694 study and 378 healthy, age- and gender-matched, controls.[106] Pretreatment levels of proinflammatory cytokines IL-1β, IL-1α, IL-6, and TNF-α, and chemokines MIP-1α and MIP-1β were significantly higher in patients with longer RFS (1-5 or >5 years) than in patients who experienced shorter RFS (<1 year).[106] This suggests that serum levels of particular cytokines prior to adjuvant IFN-α therapy might be prognostic of clinical outcome and serve as potential response predictors in patients with high-risk melanoma.

Serum S100B protein. A recent report from our laboratory highlighted serum levels of S100B as a potential prognostic marker for patients with high-risk melanoma.[107] In this study, sera banked at baseline and 3 additional time points were tested for S100B in 691 patients from the phase III E1694 trial by using chemiluminescence. A univariate analysis (**Figure 3**) showed baseline S100B ≥0.15 µg/L significantly correlated with OS (*P*=.010), and a Cox multivariate analysis identified baseline S100B as a significant independent predictor of OS (*P*=.043) after adjusting for other significant prognostic factors.[107] These results identified another potential prognostic biomarker that may enable more refined patient selection for adjuvant IFN-α.

Genomics and Proteomics in Melanoma Biomarker Development

By Sancy A. Leachman, MD, PhD

Cancer biomarkers are disease- or patient-related factors that correlate with, and might be predictive of, an outcome of interest.[108] There is particular interest in biomarkers as potential predictors of disease outcome (prognostic markers) or response to a particular treatment (response markers). If such biomarkers can be identified, they would aid in individualizing treatment and help determine which patient subgroups to treat, and with what particular treatment(s). Since current evidence suggests melanoma is a heterogeneous group of disorders differing in pathogenesis and other characteristics,[109,110] individualization of treatment is an important concept, and identification of biomarkers that could aid in this process would represent an advance in disease management. Some biomarkers may also be important in diagnosis.

This article focuses on the use of genomics and proteomics in melanoma biomarker development. The 3 technologies described here are array comparative genomic hybridization (CGH), cDNA microarray expression profiling, and matrix-assisted laser desorption and ionization time of flight (MALDI-ToF) mass spectrometry (MS).

Array CGH

This molecular tool is used to investigate differences in gene expression in the genomes from healthy individuals and those with tumors, or in comparative tissue samples from these groups.[111] Further characterization of these differentially expressed genes can offer clues important for diagnosis or better understanding of the disease pathogenesis. If genes (or their protein products) are identified as important for disease development or progression (prognostic markers), these or related genes/compounds may serve as potential therapeutic targets.

More specifically, array CGH measures and compares changes in the copy number of genes (losses, gains, or amplifications that might alter function of these genes) between a sample from a cancer patient and benign tissue from a healthy individual.[112] The first step is to extract the DNA from the 2 different sample sets, and then label the extracted DNA from the different samples with 2 different fluorescent dyes (**Figure 4**). Labeled samples are then used to hybridize an array of dots on a glass slide or other display, which represent known regions of DNA. By using appropriate standards and controls, the hybridization intensity of the signal at a given location provides information about the copy number of that location or region of DNA. Comparing the relative intensity of signals produced provides information about differences between the 2 sample populations in copy number for the DNA region. These regions can then be further sequenced to provide additional information about the region.

Curtin and associates recently used array CGH to compare the DNA from 102 different primary melanomas containing 4 melanoma subtypes: 38 from mucosa, 28 from acral skin, 18 from skin with chronic

Figure 4. Array CGH

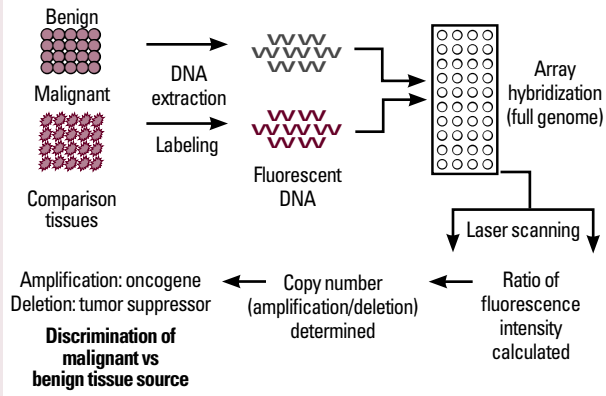
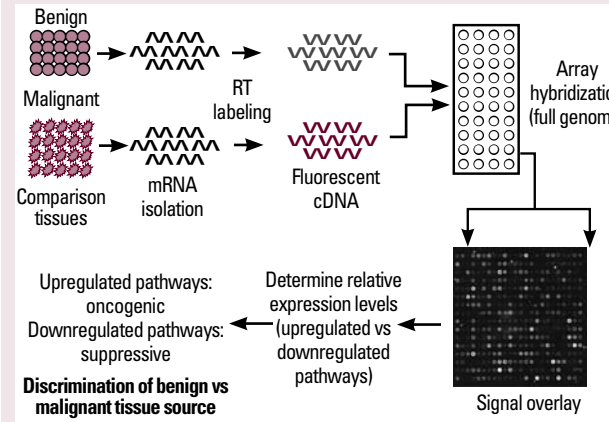


Figure 5. cDNA Microarray Expression Profiling



sun-induced damage (elastosis), and 18 from skin without elastosis.[112] These subtypes were chosen because of their infrequent association with *BRAF* or *NRAS* mutations. Analysis of the data showed increased copy number at chromosome 4q12, a region that contains the genes for *VEGFR*, *PDGFRA*, and *KIT*. Further analyses demonstrated only *KIT* contained oncogenic mutations. *KIT* mutations and/or copy number increases of *KIT* were observed in 39%, 36%, 28%, and 0% of mucosal, acral, skin with elastosis, and skin without elastosis samples, respectively. IHC analyses demonstrated increased KIT protein levels in 79% of tumors with mutations and 53% with multiple copies of KIT.[112]

In this study, array CGH permitted a global analysis of a relatively large number of melanomas of different clinical and histological categories.[112] A previously dismissed melanocyte protein, KIT, was identified as an oncogene. This finding has led to promising ongoing clinical trials testing imatinib, an inhibitor of the KIT receptor, in melanoma.

cDNA Microarray expression profiling

This technique is similar to array CGH, but with a twist (**Figure 5**). For cDNA microarray expression profiling, RNA (instead of DNA) is collected from the test and reference tissues.[113] The RNA is then subjected to reverse transcription, which turns the RNA into a more stable DNA product (cDNA) representative of the original RNA. The samples are then differentially labeled to produce fluorescent cDNA representative of the different samples. The fluorescent cDNA is used to hybridize an array of dots representing the full genome. Differences in signal intensity for the test and reference populations represent relative differences in expression of the gene encoding the RNA originally extracted. Moreover, this technology enables evaluation of patterns of expression, or so-called gene expression profiles or signatures.

cDNA microarray technology was recently employed in a preliminary and follow-up study by the same general group to obtain information important for discriminating benign nevi from melanoma (ie, for aiding in the diagnosis of melanoma).[114,115] The first study used cDNA microarray technology to compare gene expression profiles from 5 sample classes: normal skin, benign nevi, primary melanoma, and 2 types of metastatic melanoma.[114] Multiclass significance analysis of the microarrays identified 2602 transcripts that significantly correlated with sample class (ie, that were able to distinguish nevi from primary melanoma, and primary melanoma from metastatic melanoma, based on molecular events that presumable underlie melanoma pathogenesis).

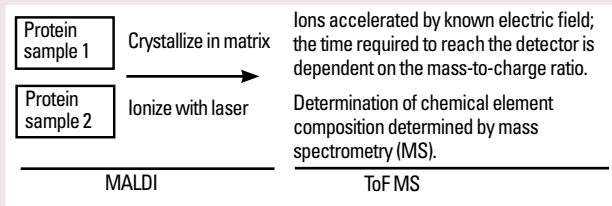
In a subsequent study, investigators selected 5 markers identified in the prior study (ARPC2, FN1, RGS1, SPP1, WNT2) and incorporated them in a multimarker diagnostic assay intended to distinguish nevi from primary melanomas.[115] Initial IHC analyses of the tissue microarray demonstrated the 5 markers were differentially expressed in nevi and primary melanomas, and this information was used to develop a diagnostic algorithm that was subsequently shown to differentiate primary melanomas from nevi with 95% specificity and 91% sensitivity compared with the known histological diagnoses. These results showed cDNA microarray technology was able to identify a number of markers that were differentially expressed in primary melanomas versus benign nevi, and these differentially expressed genes were subsequently incorporated in the multimarker assay. The 5-marker set might permit improved diagnosis of melanoma in difficult cases.

MALDI-ToF MS

This technique utilizes ionization of proteins dissolved within a crystal matrix, together with time-of-flight MS, to detect and characterize proteins from different samples isolated using gel electrophoresis (**Figure 6**). MALDI-ToF MS can be used in a similar fashion to identify proteins that are being differentially expressed in test and reference (eg, malignant versus benign) tissues.[116]

Recently, Findeisen and associates used MALDI-ToF MS to analyze a set of serum samples from patients with stage I or stage IV melanoma, and identified a peak that differentiated between them.[117] Subsequent analyses identified this peak as serum amyloid A (SAA). A second set of serum samples was obtained from patients with either stage I, II, III, or IV melanoma, and immunoassays were used to measure serum concentrations

Figure 6. MALDI-ToF MS



of the candidate prognostic marker SAA and the known biomarkers S100B, LDH, and C-reactive protein (CRP). Univariate analysis identified SAA as a strong prognostic marker in stage I-III (P=.043) and stage IV patients (P=.000083). Multivariate analysis showed SAA, gender, stage, tumor load, S100B, and CRP were all strong independent prognostic factors, with an interaction between SAA and CRP. In patients with stage I/II disease, SAA plus CRP was better than S100B in predicting PFS and OS. In summary, this study used MALDI-ToF MS to evaluate global protein profiles and identify a new candidate biomarker for melanoma prognosis.[117]

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POSTTEST

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

1. Which of the following is NOT true concerning changes in the latest version of the AJCC staging system for melanoma compared with the previous version?
A. Immunohistochemical detection of nodal micrometastases is considered acceptable, whereas previously only routine histology was used
B. There is no lower threshold of staging N+ disease (ie, the size of the isolated tumor cells is no longer used as a determinate of N+ disease)
C. Mitotic rate of the primary melanoma is recognized as an independent prognostic factor and incorporated into the T1b classification
D. LDH level has been dropped from stage IV subclassification, while site of metastases has remained

2. Which of the following accurately describes NCCN recommendations for use of SLNB?
A. SLNB should not be considered standard of care for any subgroup of melanoma patients; patients should be made fully aware of the arguments both for and against the procedure
B. SLNB is recommended for stage IA melanoma that is ≤1.0 mm with no adverse features
C. Discussion of SLNB should be considered for patients with stage IA thin melanomas (≤1.0 mm) if the following features are also present: thickness >0.5 mm, low mitotic rate, and older patient age
D. SLNB is encouraged as a staging tool for patients with stage IB or stage II melanoma (≤1.0 mm thick with ulceration or Clark's level IV, V, or >1.0 mm thick)

3. Which of the following regional treatment options for in-transit melanoma would be most suitable for a patient with multiple, moderately-sized, deep lesions?
A. Surgical excision
B. Laser ablation
C. Radiation therapy
D. Electroporation with bleomycin

4. Which of the following factors has been associated with an improved response to adjuvant IFN-α therapy?
A. An autoimmune response
B. High tumor burden
C. Low baseline IL-1β levels
D. Low baseline S100B values

5. The EORTC 18991 trial found that pegylated-IFN was:
A. Discontinued due to adverse events by ~30% of patients
B. Continued for at least 4 years in the majority of patients
C. Associated with significant improvement in OS in N1 patients
D. Associated with significant improvement in OS in N2 patients

6. A 2006 study by Curtin and associates used array CGH to identify _____ as an important previously unrecognized oncogene in melanoma.
A. *PDGF*
B. *KIT*
C. *RAS*
D. Cyclin D1

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MELANOMA CARE OPTIONS™

MARCH 2010

Update on Advances in Melanoma: Current Progress and Future Promise

EVALUATION FORM

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

Very low Low Moderate High Very High

1. To what degree will you apply the following objectives of the educational activity in your practice and/or professional responsibilities?
   - A. Identify changes in the 7th edition of the American Joint Committee on Cancer (AJCC) melanoma staging system compared with the previous edition ❍ ❍ ❍ ❍ ❍
   - B. Describe the role of sentinel lymph node biopsy in patients with thin (≤1 mm) melanomas, wide local excision of melanoma, or locally or regionally recurrent melanoma ❍ ❍ ❍ ❍ ❍
   - C. Evaluate the role of adjuvant interferon-α therapy and potential markers for treatment response in patients with high-risk melanoma ❍ ❍ ❍ ❍ ❍
   - D. Describe different treatment options for patients with in-transit melanoma ❍ ❍ ❍ ❍ ❍
   - E. Understand the basic principles and uses of comparative genomic hybridization, cDNA microarray expression profiling, and MALDI-ToF mass spectrometry as they to apply to melanoma research ❍ ❍ ❍ ❍ ❍
2. To what extent were you satisfied with the overall quality of the educational activity? ❍ ❍ ❍ ❍ ❍
3. To what extent was the content of the activity relevant to your practice or professional responsibilities? ❍ ❍ ❍ ❍ ❍
4. To what extent did the program enhance your knowledge of the subject area? ❍ ❍ ❍ ❍ ❍
5. To what extent did the program change the way you think about clinical care and/or professional responsibilities? ❍ ❍ ❍ ❍ ❍
6. To what extent will you make a change in your practice and/or professional responsibilities as a result of your participation in this educational activity? ❍ ❍ ❍ ❍ ❍
7. To what extent did the activity present scientifically rigorous, unbiased, and balanced information? ❍ ❍ ❍ ❍ ❍
8. To what extent was the activity free of commercial bias? ❍ ❍ ❍ ❍ ❍

Posttest Answer Sheet

1. ❑ 2. ❑ 3. ❑ 4. ❑ 5. ❑ 6. ❑

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

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