The controversy about the surgical management of the regional lymph nodes in early-stage melanoma began more than 100 years ago. In 1892, Herbert Snow advocated wide excision and elective lymph node dissection as a method to control the lymphatic permeation of metastases. His studies suggested a direct connection between the primary and the regional lymph nodes, indicating that treatment of melanoma should routinely include excision of these nodes. Although multiple retrospective studies suggest a survival benefit for patients undergoing elective lymph node dissection in addition to excision of the primary melanoma, none of the randomized trials supported a survival benefit for elective lymph node dissection (1). However, each of these studies supports a potential survival advantage for early dissection of microscopically tumor-positive regional lymph nodes compared with delayed (therapeutic) dissection of clinically apparent nodal metastases.

In 1992, Morton and associates described their initial experience with detection of occult regional lymph node metastases by intraoperative lymphatic mapping and sentinel node biopsy. This technique, devised as an alternative to elective lymph node dissection, enables the surgeon to map the direct route of lymphatic spread from a primary melanoma to the regional drainage basin and then selectively excise the first (sentinel) draining lymph node(s) in this basin. Because the sentinel node is the most likely site of tumor cells in the regional lymph node basin, focused pathologic examination of the lymphatic mapping and sentinel node biopsy specimen by H&E and immunohistochemistry is a useful method of ultrastaging the regional nodes. Complete lymph node dissection is reserved for patients who are most likely to achieve a survival benefit from the procedure (i.e., those with metastasis to the sentinel node). Patients without regional lymph node metastases have such a favorable prognosis they could avoid the cost and morbidity of complete lymph node dissection.

Development of Lymphatic Mapping and Sentinel Node Biopsy

In their initial report of lymphatic mapping and sentinel node biopsy, Morton et al. (2) at the John Wayne Cancer Institute described the feasibility of lymphatic mapping and sentinel node biopsy in 223 patients. All patients underwent lymphatic mapping and sentinel node biopsy using blue dye alone, followed immediately by complete lymph node dissection to verify the accuracy of lymphatic mapping and sentinel node biopsy. The sentinel nodes were localized in 194 of 237 (82%) lymph node basins. Nodal metastases were identified by H&E and immunohistochemical staining in 40 of the 194 (21%) specimens. In only 2 of 194 (1%) lymphadenectomy specimens was the tumor status of the sentinel node not

**Abstract**

Minimally invasive intraoperative lymphatic mapping and sentinel node biopsy has become the standard approach for staging the regional lymph nodes for early-stage melanoma. The procedure requires close collaboration of surgeon, pathologist, and nuclear medicine physician. The strength of lymphatic mapping and sentinel node biopsy is its accuracy of detecting occult lymph node metastases. Reverse transcriptase-PCR (RT-PCR) analyses of either fresh-frozen or paraffin-embedded sections of the sentinel lymph nodes have been found to be more sensitive than H&E staining or immunohistochemistry techniques, but lack of specificity and limits in the availability of tissue specimens make this technique impractical for routine use. Three randomized clinical trials are examining the therapeutic value of lymphatic mapping and sentinel node biopsy for melanoma. Preliminary results of the Multicenter Lymphadenectomy Trial I show the high level of accuracy and low morbidity of lymphatic mapping and sentinel node biopsy done through an international working group. The therapeutic value of lymphatic mapping and sentinel node biopsy is still unclear. Multicenter Lymphadenectomy Trial II will test the clinical significance of lymph nodes evaluated by RT-PCR and the value of completion lymph node dissection for patients found to have tumor-positive sentinel lymph nodes by H&E, immunohistochemistry, or RT-PCR. The Sunbelt Melanoma Trial examines the therapeutic value of completion dissection and benefits of Intron A. The ability to detect occult nodal metastases and evaluate the interaction of primary tumor with the regional lymph nodes may provide for better understanding of the metastatic process in patients with melanoma and help to determine the function of the regional lymph nodes as markers of metastases or incubators of tumor cells in the metastatic cascade.
indicative of the tumor status of the rest of the regional basin. The success rate of lymphatic mapping and sentinel node biopsy was 82%, unacceptable by current standards but impressive for a feasibility study.

Since the original seminal study, the accuracy of the lymphatic mapping and sentinel node biopsy has been improved with the addition of preoperative lymphoscintigraphy in all cases and intraoperative use of a hand-held \( \gamma \) probe to assist with identifying sentinel nodes (3). The method of pathologic analysis has evolved to include relatively standard serial sectioning techniques and HMB-45 and Melan-A as immunostains (4). The technique of lymphatic mapping and sentinel node biopsy has been shown by a number of investigators to be a reliable indicator of the tumor status of the regional lymph nodes, likely upstaging ~15% of cases compared with elective lymph node dissection (5). With more than 10 years of experience, we and other groups have confirmed the low nodal recurrence rate and minimal morbidity from lymphatic mapping and sentinel node biopsy (ref. 6; Table 1). Based on these studies, lymphatic mapping and sentinel node biopsy has replaced elective lymph node dissection and has become almost standard procedure for staging the regional lymph nodes.

Current controversy remains regarding whether lymphatic mapping and sentinel node biopsy is a diagnostic procedure or a therapeutic intervention. Three major studies are examining the therapeutic utility of lymphatic mapping and sentinel node biopsy. In 1994, we initiated an international multicenter randomized prospective trial comparing wide excision and lymphatic mapping and sentinel node biopsy to wide excision alone in patients with American Joint Committee on Cancer stage I and II melanoma (Multicenter Selective Lymphadenectomy Trial I). Eligible patients were those with intermediate-thickness (>1.5-cm margins), skin grafting, or other procedures that would alter the lymphatic drainage. Complete lymph node dissection was done only in lymphatic drainage basins that contained tumor-positive sentinel nodes. The primary aim of this study is to determine the therapeutic benefit of lymphatic mapping and sentinel node biopsy and the true accuracy of the technique from a multicenter basis (7). As of March 2002, all 2001 patients had entered the study. Each of the centers completed a 30- case training phase showing at least an 83% accuracy rate of sentinel node identification before entering patients into the randomized trial. Preliminary results from the third interim analysis have validated the accuracy of lymphatic mapping and sentinel node biopsy as a staging procedure (8).

Initial sentinel node identification rate was 95.3% overall; 99% for the groin, 95% for the axilla, 84% for the neck, and 87% for the popliteal and other ectopic sites. The accuracy rates remained consistent through the length of the study, showing the importance of preoperative lymphoscintigraphy, blue dye, and \( \gamma \) probe-directed sentinel node localization. A total of 19% of patients had a tumor-positive sentinel node; the majority underwent completion dissection. A total of 59 of the 944 (6.3%) patients with tumor-negative sentinel node recurred in the regional basin after a median follow-up of 54 months. A total of 48 (81%) of the nodal recurrences were in a dissected basin. Eight patients experienced disease recurrence in the intransit skin or s.c. tissue before experiencing disease recurrence in the regional lymph node basin. The rate of false-negative sentinel node, as measured by nodal recurrence in the dissected basin, decreased with increasing experience of each center: 10.3% for the first 25 cases versus 5.2% after 25 cases. These data suggest that although a training phase of 30 cases is essential for learning lymphatic mapping and sentinel node biopsy, the learning curve does not peak for at least 25 additional cases. The rate of tumor-positive sentinel node essentially equals the incidence of lymph node recurrences (144 of 800; 18.1%) after wide excision alone, suggesting the biological significance of sentinel node metastases. The surgical complication rate related to lymphatic mapping and sentinel node biopsy was 10.1%. There were no operative mortalities.

Preliminary data showed a significantly \( (P = 0.01) \) higher 5-year disease-free survival (78%) for patients undergoing lymphatic mapping and sentinel node biopsy compared with those treated by wide excision alone (73%). There is no difference in overall survival between the two treatment groups, yet tumor status of the sentinel node remained the strongest predictor of outcome in these patients (9).

A follow-up study (Multicenter Lymphadenectomy Trial II) began in the winter of 2005. Patients with tumor-positive sentinel nodes identified by H&E staining, immunohistochemical techniques, or reverse transcriptase-PCR (RT-PCR) evaluation of paraffin sections are randomized to observation of the lymph node basin with serial clinical examination and ultrasound or immediate completion dissection (10). The study is designed to determine if a therapeutic benefit exists for completion lymph node dissection, although in a majority of cases metastases are limited to the sentinel node alone. Patients are randomized based on the technique in which the sentinel node metastasis was identified (H&E/immunohistochemistry versus RT-PCR) and thickness of the primary tumor. Patients with tumor-positive sentinel node shown by RT-PCR analyses of paraffin sections are included in this study as their survival estimates fall midway between patients found to have H&E tumor-positive sentinel node and those with tumor-negative sentinel node (Fig. 1). Several clinicopathologic factors have been shown to be suggestive, but not absolutely predictive, of metastases to nonsentinel node (ref. 11; Table 2). Patients can enter this trial through Multicenter Lymphadenectomy Trial I centers or other participating sites.

The Sunbelt Melanoma Trial is a prospective randomized study that examines the therapeutic value of completion lymphadenectomy and Intron A (IFN-\( \alpha \), Schering-Plough, Kenilworth, NJ) for patients whose sentinel nodes contain tumor cells identified by conventional H&E staining, immunohistochemical techniques, and/or RT-PCR evaluation of fresh

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**Table 1. Estimated risk of recurrence following tumor-negative sentinel lymph node dissection**

<table>
<thead>
<tr>
<th>Investigator, year</th>
<th>No. sentinel node dissections</th>
<th>Lymph node recurrence</th>
<th>Median follow-up (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gershenwald, 1998</td>
<td>243</td>
<td>10 (4)</td>
<td>35</td>
</tr>
<tr>
<td>Gadd, 1999</td>
<td>89</td>
<td>7 (8)</td>
<td>23</td>
</tr>
<tr>
<td>Jansen, 2000</td>
<td>151</td>
<td>6 (4)</td>
<td>32</td>
</tr>
<tr>
<td>Clary, 2001</td>
<td>252</td>
<td>11 (4)</td>
<td>24</td>
</tr>
<tr>
<td>Zogakis, 2005</td>
<td>773</td>
<td>13 (2)</td>
<td>37</td>
</tr>
</tbody>
</table>
specimens. The organizers of this study anticipate that this trial should provide further insight to the therapeutic value of lymphatic mapping and sentinel node biopsy and subsequent treatment with Intron A (12).

**Biological Significance of Sentinel Node Metastases**

When melanoma metastases, it is usually detected first in the regional lymph nodes and later at distant sites. Two alternative hypotheses have been created to explain the metastatic process: the incubator hypothesis suggests metastases develop in the regional lymph nodes allowing for expansion of metastatic clones with subsequent spread to distant sites. If this hypothesis is correct, then surgical removal of the regional lymph nodes early in the biology of melanoma should reduce the incidence of distant metastases and should improve survival as compared with those patients treated by delayed lymph node dissection. The marker hypothesis suggests that metastases to the regional lymph nodes are only indicative of a metastatic phenotype and surgical removal of the lymph nodes is diagnostic of occult distant metastases. Surgical excision of the regional lymph nodes may be palliative but provides no meaningful therapeutic benefit. The clinical trials of lymphatic mapping and sentinel node biopsy should help to answer the metastatic process in melanoma.

It is believed that regardless of which of the two hypotheses are correct, when melanoma metastases, it is generally first to the regional lymph nodes or sentinel node. Because the sentinel node is the first site of metastases, evaluation of sentinel node provides a unique opportunity to study the early phases of tumor-lymph node immune interaction. Recent evidence suggests that the regional lymph nodes (sentinel node) are immunosuppressed when compared with adjacent nonsentinel node (13, 14). Lymphocytes from lymph nodes closest to the primary melanoma show diminished ability to proliferate in response to lectins or interleukin (IL)-2. These lymph nodes also generate migration inhibitory lymphokines and increased number of suppressor cells. Melanoma sentinel nodes have been found to have significant reduction in number and density of interdigitating dendritic cells that are essential for antigen processing and T-cell activation (13). These changes in the sentinel node are evident even in the absence of lymph node metastases, suggesting that the primary tumor interacts with the sentinel node to create an immunosuppressive microenvironment that favors melanoma growth and metastases (ref. 15; Fig. 2).

IL-10 is a potent regulator of immunosuppression and may play a significant role in melanoma progression. IL-10 can interfere with T-cell activation by inducing immunosuppressive dendritic cells. The recent discovery of indolamine 2,3-dioxygenase–expressing dendritic cells in melanoma patients provides insight into the relationship of sentinel node immunosuppression and the local cytokine environment. We obtained fresh sentinel node and nonsentinel node tissue specimens from 21 patients undergoing lymphatic mapping and sentinel node biopsy (16). In the 13 patients with sentinel node metastases or evidence of residual melanoma at the primary site (post-residual melanoma), quantitative RT-PCR analyses showed significantly higher levels of IL-10, IFN-γ, and indolamine 2,3-dioxygenase than those sentinel node without post-residual melanoma (Fig. 3). The pattern of up-regulation in cytokine gene expression is indicative of a immunosuppressive phenotype. We also examined sentinel node and nonsentinel node for expression of a variety of markers of dendritic cell and T-cell activation. In all cases, activation signals were expressed at significantly higher levels from nonsentinel node whereas IL-10 expression was significantly higher in sentinel node and seemed to correlate with tumor thickness.

A pilot study was done to evaluate the reversibility of sentinel node immune dysfunction. Recombinant human granulocyte macrophage-colony stimulating factor (GM-CSF; Leukine, Berlex Corp, Seattle, WA) was chosen for its function as a monocyte differentiation factor, its in vivo function against melanoma when injected intradermally, and its potential role in reversing the immunosuppressive process.

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**Table 2.** Factors predictive of more than one tumor-positive lymph node [as adapted from Lee et al. (11)]

<table>
<thead>
<tr>
<th>Factor</th>
<th>Relative risk [95% confidence interval]</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor thickness, &gt;3 mm</td>
<td>96 [1.47-5.99]</td>
<td>0.002</td>
</tr>
<tr>
<td>Sentinel node metastasis, 2 mm</td>
<td>0.93 [1.41-6.06]</td>
<td>0.003</td>
</tr>
<tr>
<td>No. tumor-positive sentinel node, 2</td>
<td>2.05 [98-4.27]</td>
<td>0.054</td>
</tr>
<tr>
<td>Age, &gt;60 y</td>
<td>84 [0.92-3.68]</td>
<td>0.082</td>
</tr>
<tr>
<td>High mitotic rate</td>
<td>0.92 [0.87-4.20]</td>
<td>0.100</td>
</tr>
</tbody>
</table>
as adjuvant therapy in patients with stage III and IV melanoma. Thirty-four sentinel node and 9 nonsentinel node were obtained from 15 patients who received preoperative peritumoral injection of recombinant human GM-CSF, and 21 pairs of sentinel node and nonsentinel node were obtained from 21 patients not receiving recombinant human GM-CSF. By using antibodies to S-100 and CD43, immunohistochemistry staining was done to identify intradigitating dendritic cells and T cells. Intradigitating dendritic cell and T-cell areas were significantly (P < 0.001) higher in the sentinel node after peritumoral injection of recombinant human GM-CSF with an appearance similar to nonsentinel node. Vuylsteke et al. (17) from VU University Medical Center found similar results with preoperative peritumoral injections of recombinant human GM-CSF with up-regulation of dendritic cell markers CD1a, CD83, CD86, CD40, S-100, and CD14. Other studies suggest that tumor-specific CTLs may be drawn to the sentinel node by activated dendritic cells. The question remains if the reactivation of sentinel node with recombinant human GM-CSF can lead to a diminished number of metastases in the regional lymph nodes and overall improved patient survival.

Open Discussion

Dr. Sondak: Do you have any data that a Clark level III melanoma at 0.9-mm thickness is any different than a Clark level IV melanoma at 0.9 mm?

Dr. Essner: Clark level is important in a melanoma <1 mm. The incidence of nodal metastases is higher for a Clark level IV than III of the same thickness. Patient prognosis is worse for higher Clark levels.

Dr. Sondak: For predicting whether or not you’ll find something in the regional lymph node?

Dr. Essner: The incidence of regional nodal involvement is higher. There is a clear separation between the incidence of nodal metastases from Clark level III and IV lesions.

Dr. Sondak: All your data are about RT-PCR on the paraffin-embedded nodes suggesting a worse outcome or death from melanoma. Nothing that you’ve shown us suggests that patients are more likely to have disease in their lymph nodes if the lymph nodes are not removed. You’ve told us that if you do immunohistochemical staining and H&E on the lymph nodes and it’s negative, then there is a 2% recurrence rate in those lymph nodes. You’ve also told us that 30% of those people will have positive PCR results in the lymph nodes. The Multicenter Selective Lymphadenectomy Trial II suggests that these patients should have their lymph nodes dissected, despite being immunohistochemistry and H&E negative. What evidence is there that there is any reason to concentrate therapy on the lymph nodes of a patient whose sentinel node is RT-PCR positive?

Dr. Essner: If you do H&E and immunohistochemical analysis of the sentinel nodes, the nodal recurrence rate is ~ 2%. The average number of lymph nodes removed was ~ 1.5 or 2 lymph nodes. It may be that when you remove those 2 lymph nodes, you’ve basically rid the patients of all their disease.

Dr. Sondak: In the Multicenter Lymphadenectomy Trial II trial, we do a sentinel node biopsy, H&E, and immunohistochemistry, and if the results are negative, we tell that patient, “Over the next 10 years, there’s about a 2% chance that you’ll fail in your lymph nodes, but you’ve got this PCR signal for N-acetyl-D-galactosamine in your lymph node, so we want you to have all your lymph nodes removed.” I still have a concern about whether offering additional surgery on the histologically negative regional nodes can possibly help in that setting.

Dr. Essner: Perhaps the risk of nodal recurrences will diminish when lymph nodes are analyzed by RT-PCR. The other issue is that there is a group of patients who experienced distant recurrence after analysis of presumed tumor-negative sentinel nodes. We went back and looked at their nodes and found disease in these nodes, suggesting the metastases were biologically active.

Dr. Sondak: But you didn’t see failure in those other nodes clinically. If these PCR-positive patients had disease in other nodes, they would have shown up as “false negative” sentinel node biopsies and developed nodal recurrence. There are makers of progression and markers of lethality and you’re making a case that PCR is a marker of lethality, yet there’s no evidence that PCR will lead to a diminished number of metastases in the regional lymph nodes.

Fig. 2. Differential expression of IL-10 based on primary thickness. Semiquantitative RT-PCR analyses of sentinel node and nonsentinel node pairs showed decreased relative gene expression of CD80, CD86, CD40, CTLA4, and CD28 from sentinel node as compared with matched nonsentinel node. Gene expression of IL-10 was ~ 3-fold higher in sentinel node (light boxes) than in nonsentinel node (dark boxes). [Adapted from Essner and Kojima (15)].
real reason to think it’s a marker of nodal failure or residual disease remaining in the lymph nodes, which is the only thing that you can treat with a node dissection. You won’t find anything histologically in any of those node dissections because you didn’t find anything histologically in the sentinel lymph node to begin with. So what could a node dissection offer that patient?

Dr. Essner: Despite that 2% recurrence rate after H&E and immunohistochemistry, some people will likely have disease in nonsentinel lymph nodes after RT-PCR identified sentinel lymph node metastases. I understand what you’re asking. Why subject these people to a complete node dissection when we think it may not be relevant? I can’t answer your question because I don’t know how the trial is going to pan out.

Dr. Sondak: Having more data about the regional nodal risk of these patients would be important.

Dr. Kirkwood: Within the involved sentinel node, have you seen differences that predict outcome differences that would tell us within the node-involved population what we can potentially attack?

Dr. Essner: We don’t have enough follow-up on these patients to tell the differences to determine these factors.

Dr. Atkins: When you’re looking at indolamine 2,3-dioxygenase or other factors in the sentinel node in patients who had no disease in the sentinel node, were the primaries in place at the time of that procedure or had the primary already been resected?

Dr. Essner: One group had complete biopsy. Another group of patients had incisional biopsies but in the majority of cases the primary tumor was intact.

Dr. Atkins: Were the data you presented from patients who still had their primary in place?

Dr. Essner: Yes, they either had a metastasis in the lymph node or disease at the primary was still intact.

Dr. Atkins: Can you relate anything about the biology of the node to specific anatomic features of the disease in the node, such as parenchymal versus subcapsular tumor location or tumor volume?

Dr. Essner: We didn’t look at them because the numbers of patients were small.

Dr. Gajewski: I have found a way to rule out an effect of the skin injection for lymphoscintigraphy itself on the sentinel lymph node procedure in influencing dendritic cell presence or absence in the draining node?

Dr. Essner: The only thing we’ve been able to get for comparison is fresh human tonsils, which are not even normal. Most tonsils are removed from patients because of abnormal size and presumably function. We’ve looked at a number of variables of time, such as timing of the biopsy, delay to when the sentinel node procedure was done, and when the radioactive agent was injected into the skin. We have found no relationship with timing of the biopsy or sentinel lymph node procedure that predicts the “immune” response of the sentinel lymph nodes. That’s one of the biggest skepticisms of our own work: could this all be a function of the biopsy itself?

Dr. Atkins: My understanding of the Multicenter Lymphadenectomy Trial I data was that the survival was ~86% in one group and 87% in the other group. Therefore, there wasn’t a survival difference related to the actual sentinel node procedure. I thought one of your conclusions was that there was a survival benefit.

Dr. Essner: In 1994, when we created this study, we had a hard time promoting it or just a clinical trial comparing outcomes following positive sentinel nodes versus a wide excision and then subsequent complete node dissection. We now know that 80% of the patients aren’t going to benefit from sentinel node biopsy because they don’t have tumors in the sentinel nodes. So you’ve already diluted your potential benefit to 20% of patients. When the data went through the data monitoring safety board; everybody said it was a negative study, but really there was no chance of ever having an impact on treatment outcome in a sentinel node negative population so the lack of impact on overall survival is not surprising.

Dr. Ross: This is always going to be a tough issue. In a realistic sense, the only way to do the trial is if you’ve got some way to identify a group of patients with positive nodes. Then you decide that you’re going to randomize all those patients to a node dissection now or when they relapse. That trial cannot be done. If you look at retrospective analyses or prospective subsets of elective node dissection trials, the survival advantage of doing node dissections for microscopic disease versus waiting is ~15% versus 20%. Fifteen percent of 20% is 3%, so you’re looking at a survival difference of 3%. Three-percent survival difference is probably not clinically relevant.

Dr. Sondak: I’m not sure 3% isn’t very clinically relevant for a procedure of low morbidity. But proving that 3% is statistically significant, that is another story. Imagine going into a phase 3 clinical trial of any systemic therapy. Imagine saying, “We’re going to enroll 2000 patients, and of those 2000 we’ll randomize 20% to our intervention versus 80% to the placebo.” You wouldn’t be excited about the likelihood that the trial would show a statistically significant increase in overall survival unless the numbers of patients were enormous. But look at what we have learned from Multicenter Lymphadenectomy Trial I. We learned that the outcome for patients who were node positive by the sentinel node was a lot better than that for people who were node positive by the eventual clinical development of nodal recurrence. We can’t prove they’re the exact same groups of patients, but this is melanoma not breast cancer. If you’ve got disease in your nodes, it’s going to show up eventually. Still, there are potential biases in that comparison that could lead you to conclude that a sentinel node–positive patient is going to fare better than a patient who later develops palpable nodes.

References
