THE ROLE OF SENTINEL LYMPH NODE BIOPSY IN THE MANAGEMENT OF MELANOMA

Farin Amersi, MD and Donald L. Morton, MD
From the Department of Surgical Oncology and the Roy E. Coats Research Laboratories of the John Wayne Cancer Institute at Saint John’s Health Center. Supported by grant CA29605 from the National Cancer Institute and by funding from the Amyx Foundation, Inc. (Boise, ID), Mrs. Alice Johnson McKinney, the Harold J. McAlister Charitable Foundation (Los Angeles, CA), and Nancy and Carroll O’Connor (Los Angeles, CA).

INTRODUCTION

Cutaneous melanoma has become an increasingly growing problem, with a rapid rise in incidence rates in the United States over the last several decades. Melanoma now accounts for 5% of all cancers diagnosed. According to the American Cancer Society an estimated 62,190 new cases of melanoma were diagnosed in 2006, and approximately 7,910 patients will die of this disease.¹ Surgical management with wide excision remains the most important treatment modality; it reliably affects outcome of patients whose disease is diagnosed early in its course. Thus the average rate of 5-year survival is about 85% for patients with early-stage melanoma corresponding to American Joint Committee on Cancer (AJCC) stage I/II disease. However, 5-year survival rate drops to less than 40% for patients who present with stage III regional nodal disease, and to 10% for patients with distant metastases (stage IV).², ³

The disease status of the tumor-draining regional lymph nodes remains the most important predictor of survival and recurrence in patients with melanoma.⁴, ⁵ Predictors of nodal metastases include the patient’s age and the tumor’s thickness, ulceration, mitotic index, drainage pattern (number of nodal basins), and Clark level.⁶–⁸ However, these predictors of nodal status have not replaced surgical resection and histopathologic examination of the lymph nodes. Imaging modalities such as ultrasonography, magnetic resonance imaging (MRI) and position emission tomography (PET) have facilitated detection of distant metastases and can identify suspicious regional nodes, but they cannot accurately identify small metastases within these nodes. Therefore, removal and histologic examination of lymph nodes is considered the most accurate method for assessing spread of disease to the lymph nodes.

In patients with clinically involved lymph nodes, lymphadenectomy is the only effective therapeutic option for local control and potential cure. Because the number of tumor-positive lymph nodes is the single most important prognostic factor in AJCC stage III melanoma, complete lymphadenectomy (CLND) also allows accurate assessment of the regional extent of disease. Complete lymphadenectomy has been widely performed as an elective procedure in patients with clinically normal regional lymph nodes. However, routine elective lymphadenectomy (ELND) entails significant morbidity, including wound complications such
as seromas, paresthesias, and extremity lymphedema. In addition, more than 80% of all patients with clinically normal regional lymph nodes will not have histopathologic evidence of nodal metastases.\textsuperscript{9,10} In these patients, ELND is associated with significant short-term and long-term morbidity and no therapeutic benefit. A far preferable alternative to ELND would be a surgical staging procedure that is less invasive and potentially more accurate.

This alternative in fact had its origins in the 1970s. In 1977, Fee and colleagues\textsuperscript{11} introduced cutaneous lymphoscintigraphy to determine the route of lymphatic drainage from a primary cutaneous melanoma. Their lymphoscintigraphic mapping studies and subsequent immunohistochemical investigations with S100 antibodies in the 1980s showed that early-stage regional metastasis targets one or two nodes proximal to the primary melanoma.\textsuperscript{12} In 1990, Morton et al\textsuperscript{13} introduced the lymphatic mapping (LM) of the first tumor-draining lymph node, the sentinel lymph node (SLN), at the annual meeting of the Society of Surgical Oncology. Morton hypothesized that mapping and biopsy of the SLN is based on the assumption that the afferent lymphatic channel from a primary tumor drains first to one or more SLNs in the regional lymphatic basin.\textsuperscript{14} Because the SLN is the first nodal drainage site, its tumor status can be used to predict the tumor status of all nodes in the regional basin (Figure 1).\textsuperscript{15} Although the SLN is usually proximal to the primary tumor, the SLN concept is based on function, not anatomy; thus the location of the SLN is unique to each patient and depends on the drainage pattern from that patient’s primary lesion. This makes intraoperative LM and SLN biopsy extremely useful for truncal melanomas located in the midline or near the umbilicus, which have the potential to drain to two or more lymphatic basins.\textsuperscript{16} In these cases, biopsy and analysis of a fixed anatomic node, such as Cloquet’s node, cannot accommodate variations in lymphatic drainage.\textsuperscript{17}

SLN BIOPSY VS ELND

The success of LM and SLN biopsy for staging of regional lymph nodes in melanoma is particularly appealing. Several studies have shown that the status of regional lymph nodes is the most important prognostic indicator of survival and the risk of recurrence.\textsuperscript{18–20} Balch et al\textsuperscript{21} used data from 17,600 patients in the AJCC melanoma database to evaluate the prognostic significance of the patient’s age and sex; the primary lesion’s ulceration, thickness, Clark’s level and site; and the number of tumor-positive regional nodes. Cox multivariate analysis demonstrated that number of metastatic nodes (P < .0001), tumor status of the SLN (microscopic versus macroscopic involvement) (P < .0001), and ulceration of the primary melanoma (P < .0001) were the most significant predictors of outcome. The number of positive SLNs was the most important predictor of survival in patients with stage III disease; 5-year survival rates were 59–69% for patients with a non-ulcerating lesion and one positive node, as compared with 13% for patients with an ulcerated lesion and three positive nodes. Other studies have confirmed an inverse relationship between survival and the number of positive lymph nodes.\textsuperscript{22–25}

Removal of all lymph nodes in a clinically normal drainage basin is hard to justify in patients who are unlikely to derive any survival benefit from this procedure. In addition, the accuracy of ELND has been questioned as the technique may not target the correct lymphatic basin, negating any potential survival benefit.\textsuperscript{26–28} Moreover, detection of micrometastasis in the large number of lymph nodes removed during the procedure becomes increasingly difficult and time-consuming.

The attraction of a minimally invasive procedure such as LM/SLN biopsy for patients and physicians includes accurate staging, without the potential morbidity of lymphedema and nerve injury; this is important because only 20% of patients with intermediate-thickness primaries are expected to have metastasis to regional lymph nodes. Furthermore, detailed

\textit{Adv Surg.} Author manuscript; available in PMC 2008 January 2.
histopathological and molecular analysis of the SLN provides far more accurate staging information than the standard pathological assessment of the numerous nodes randomly removed during ELND. Immunohistochemical assessment can detect occult SLN metastases that have prognostic significance, and identifies a subset of patients who might benefit from a completion lymphadenectomy.

**PATIENT SELECTION FOR SLN BIOPSY**

The eligibility criteria for SLN biopsy include clinically negative nodes and primary lesions between 1–4 mm in thickness. These patients with intermediate-thickness melanomas, can derive substantial therapeutic benefit from LM/SLN biopsy. Lymphatic mapping may be considered in patients who have thinner lesions (0.76–1 mm) associated with histological ulceration or Clark IV invasion, as the incidence of occult nodal metastases is 5%. In our experience, these patients usually choose SLN biopsy instead of nodal observation. In patients with thick lesions (>4 mm), and clinically node-negative disease, the incidence of nodal metastases may be as high as 60–70%, and the pathological status of the SLN is an independent predictor of recurrence and survival. 29–30

The type of biopsy previously performed, excisional or shave, as well as the histological subtype does not affect the accuracy of the procedure. However, wide excision of a primary lesion prior to LM/SLN biopsy compromises the accuracy of SLN biopsy due to aberrant lymphatic drainage patterns caused by disruption of lymphatic channels during the initial surgical procedure.

ELND remains the standard of care in patients with clinically palpable nodal disease. In this group of patients lymphadenectomy is potentially therapeutic, and is important for local control of disease. Patients who develop nodal metastases after SLN biopsy should undergo completion lymph node dissection. Many clinicians believe lymph node involvement precedes systemic metastases, and removal of nodal metastases may prevent systemic metastases.

**TECHNICAL ASPECTS OF SLN BIOPSY**

Cutaneous lymphoscintigraphy is performed with technetium-99m (99mTc)-labeled sulfur colloid (SC). Unfiltered sulfur colloid has a slower transit time and is optimal for prior-day injection, whereas filtered radiocolloid migrates faster and is used for same-day procedures. The procedure is performed with intradermal injection of up to 18mBq (0.5mCi) of the radiopharmaceutical in four quadrants of the primary lesion.31 A scintillation camera is used to document the drainage patterns from the lesion, through the dermal lymphatics into the regional lymph nodes. Dynamic images are performed to differentiate true SLNs from non-SLNs. Sentinel nodes can be identified 1–30 min after injection; after 4 hours, SLNs cannot be differentiated from non-SLNs. 32

Intraoperative localization of the SLN is by intradermal injection of 1–2 ml of isosulfan blue dye injected at the site of the primary lesion. If the lesion has been excised, the dye is injected on either side of the scar. The dye enters the subdermal lymphatics and passes through lymphatic channels to the regional nodal basin that drains the particular area of skin. The SLN(s) is located by visual identification of its blue stain and by gamma-probe measurement of a high level of radioactivity. Each SLN is selectively excised embedded in paraffin, and cut into multiple sections for histopathologic analysis with immunohistochemistry (IHC) as well as standard hematoxylin and eosin (H&E) staining.33,34 If metastatic melanoma is identified, a CLND is performed. The initial study by Morton et al.14 defined the technical aspects and inclusion criteria for SLN biopsy in 223 patients with stage I melanoma. The authors used 1% isosulfan blue dye to identify an SLN in 194 of 237 lymphatic basins (82%). This SLN
accurately predicted the tumor status of the lymph node basin; of the 194 basins, only two (1%) contained evidence of metastases when the SLN was tumor-free.

Several other investigators subsequently verified the accuracy of SLN biopsy (Table 1). Krag et al. used unfiltered technetium and blue dye to identify the SLN in 118 of 121 (98%) patients, with only one regional nodal recurrence in 220 days of follow-up. Leong et al. used technetium Tc 99m-labelled sulfur colloid and blue dye to identify an SLN in 160 of 163 (98%) patients. Of 30 patients with microscopic metastases in the SLN, 22 underwent CLND; only 4 (18%) patients had further evidence of metastatic disease.

Validation of the SLN concept in multiple single-institution studies led to design of the first Multicenter Selective Lymphadenectomy Trial (MSLT-I). In MSLT-I, 2001 patients whose primary cutaneous melanoma had a Breslow thickness \( \geq 1 \) mm with a Clark level \( \geq III \), or a thickness <1 mm with a Clark level \( \geq IV \) were randomly assigned to wide excision followed by observation, or to wide excision plus SLN biopsy followed by CLND if the SLN contained tumor. The primary goal of this phase III, randomized trial is to compare survival in the two treatment arms. Each of MSLT-I’s 18 participating melanoma centers mastered a multidisciplinary SLN protocol that combined surgery, nuclear medicine, and pathology. The SLN was identified in 98% of the cases, with an average of 1.65 nodes excised per procedure. The incidence of lymph node metastases correlated with younger age (p = 0.003), thicker lesions (p = 0.001), higher Clark level (p = 0.001), and truncal location (p = 0.001). This group reported a statistically significant difference (p< 0.014) in the rate of identification of the SLN with blue dye alone (94%) versus blue dye plus radiocolloid (98%).

In a study of 612 patients, Gershenwald et al. reported results of SLN mapping with a combination of radioactive colloid and blue dye. At least one SLN was identified in 580 patients (95%), with a mean of 1.51 SLNs per basin. At least one SLN was identified in 634 (93%) of the 683 basins that were mapped; the rate of SLN identification was highest (97%) for nodal basins in the inguinal region.

The findings of these studies have revolutionized the staging and management of patients with melanoma because they demonstrated the accuracy of SLN biopsy for prediction of the histopathologic status of the lymphatic basin. Results indicate that identification and extensive histopathological analysis of the SLN is less time-consuming, less morbid, and more cost-effective than routine ELND. SLN biopsy is accepted by many centers worldwide as the standard of care in the staging and management of clinically node-negative melanoma patients with primary lesions between 1–4 mm (intermediate thickness). Despite these advances, the management of patients with melanoma and the role of SLN biopsy remain a significant challenge.

TECHNICAL ADVANCES IN SLND

SLN biopsy can pose a technical challenge depending on the experience of the surgeon, pathologist and the nuclear medicine radiologist. Based on their expertise, the rate of accurate identification and histopathological evaluation of SLN can range from 75% to 100% in patients with clinically node-negative disease. Use of the blue dye technique alone identified the SLN in only 85% of patients. The subsequent addition of pre-operative lymphoscintigraphy and 99Tc-labeled sulfur colloid enabled surgeons to identify multiple draining lymphatic nodal basins, as well as drainage to areas away from standard nodal basins. Use of the hand-held gamma probe for intraoperative lymphatic mapping has increased the accuracy of SLN identification to greater than 96%. A recent study on the crucial need of a learning period for lymphatic mapping and SLN biopsy was reported by Morton and his colleagues. Their findings based on the MSLT-I trial indicates that the learning phase of
30 cases is not sufficient to master the technique of LM/SLN biopsy; a minimum of 55 cases is needed to identify the SLN with 95% accuracy.

The blue dye remains the gold standard for identifying SLNs. The radiopharmaceutical serves as a useful adjunct but is impractical as the sole mapping agent because of its tendency to migrate up the nodal chain beyond the SLN. The node identified as sentinel by the surgeon and nuclear medicine physician must be confirmed and analyzed by the pathologist. Confirming the identity of the SLN is not straightforward; the blue dye that is injected intraoperatively may be visible on a fresh lymph node and partially visible on frozen section, but it is usually not visible by H&E or IHC. In addition, the levels of radioactive isotope rapidly decline, and most pathology laboratories lack the ability to measure radioactivity in tissue specimens.

Carbon particles injected with the blue dye have been successfully used as an inert marker in identifying micrometastases in the SLN. The particles enter lymphatic tissue through afferent lymphatics, in the same manner that tumor cells travel through lymphatic channels from the primary tumor, and are deposited in the subcapsular sinus, which is the preferential site of nodal metastases (Figure 2). The use of carbon as an alternative approach to identify the SLN is presently not being used by most centers world-wide. A study by Morton et al. reported that LM with a suspension of carbon particles and isosulfan blue dye enabled histopathologic confirmation of a SLN as a true SLN; moreover, the pattern of carbon deposition within the node indicated the intranodal site of micrometastatic tumor cells. Their findings provide the rationale for the use of carbon dye to enable the pathologist to identify specific areas of the node that should be examined for micrometastases.

PATHOLOGICAL ANALYSIS OF THE SLN

Intra-operative frozen section of the SLN

When the SLN technique was initially developed, intra-operative frozen section analysis was performed so that patients could undergo CLND during the same operation if findings were positive for nodal disease. As more experience was gained in the histopathological evaluation of SLNs, investigators found that permanent sections yielded more reliable results than frozen sections. In SLNs, tumor cells are usually found peripherally in the subcapsular sinus. Numerous studies have demonstrated that frozen sections fail to identify micrometastatic tumor deposits < 2mm in diameter. A whole cross section would be required for an accurate diagnosis, decreasing the amount of tissue available for permanent sections. In addition, frozen section preparation varies considerably, leading to problems in comparison of histopathology results from different institutions.

Technique/Processing of the SLN for pathological analysis

Standard histopathologic analysis of the SLN draining a primary melanoma involves H&E staining and IHC with S100, HMB45, or MART-1 antibodies. The success of histopathologic or molecular analysis depends in part on correct processing of the specimen after the SLN has been fixed briefly in 10% formalin. Since most tumor cells are found along the midplane and capsular sinus of the SLN, the node is bisected through its length, and representative cross sections are submitted for IHC. The remaining nodal tissue is then placed into formalin-fixed cassettes for 48 hours, paraffin-embedded, and serially sectioned into at least 2–4 µm sections for H&E and IHC (Figure 3). Despite this technique, routine pathological analysis of the SLN can miss micrometastases due to sampling error. In a study by Gershenwald et al., patients with AJCC stage I or II melanoma underwent SLN biopsy. Of these patients, 270 had histologically negative SLNs, and recurrence patterns were analyzed over a study period of 35 months. Ten patients (4%) developed recurrences in the dissected lymph node basin; 8 of the

Adv Surg. Author manuscript; available in PMC 2008 January 2.
10 patients had micrometastases that were retrospectively identified by additional serial sections and IHC.

**CLINICAL SIGNIFICANCE OF OCCULT METASTASES IN SLNs**

Occult metastatic melanoma in the SLN may represent the earliest stage of metastatic disease. The AJCC divides lymph nodes metastases into those that measure > 2 mm or < 2 mm. Micrometastases or tumor deposits < 2 mm are usually visualized only through IHC or molecular techniques such as RT-PCR. The prognostic significance of solitary micrometastases is unclear, and it has been suggested that SLN biopsy is adequate surgery in these cases. However, the detection of occult tumor cells in the SLN is limited by technique, as well as sampling error during pathological analysis. A small number of patients with tumor-free SLNs develop recurrence, possibly due to the presence of undetected subclinical or occult disease. Clinically, nodal micrometastases affect further treatment decisions regarding CLND and adjuvant therapy. Occult metastases in an SLN remain a topic of debate, and the impact on disease progression is under investigation. Morton and colleagues are presently conducting a multicenter worldwide trial (MSLT-II) that evaluates outcomes of patients with SLN metastases, including occult metastases detected only by RT-PCR, and the need for CLND when nodal metastasis is limited to submicroscopic deposits in the SLN. For now, it is recommended that regardless of the tumor burden, patients with a positive SLN should undergo CLND dissection.

**SLN BIOPSY AND THE CONTROL OF REGIONAL DISEASE**

Lymphadenectomy has clearly enhanced control of regional disease in patients with melanoma. SLN biopsy allows control of regional nodal disease by identifying patients with microscopic disease who would benefit from an early CLND. The small SLN specimen can be comprehensively analyzed for evidence of tumor. SLN evidence of microscopic disease is a useful criterion for CLND because it targets high-risk patients and spares node-negative patients the potential morbidity associated with further nodal dissection. CLND performed for microscopic disease provides the potential for improved regional control, emphasizing the importance of SLN biopsy as an important therapeutic modality in achieving regional nodal control and potentially cure. In addition, a study by Gershenwald et al demonstrated that less than 10% of patients who underwent LM/SLN biopsy, and were found to be tumor-free had regional nodal recurrences, of which none were in the previously mapped nodal basin. This recurrence rate is comparable to the recurrence rates seen in patients who underwent ELND.

**Molecular Analysis of SLN**

RNA and DNA markers for melanoma offer clinical opportunities for diagnosing and detecting occult disease in the SLN, for selecting candidates for adjuvant therapy, for monitoring response to therapy, and for predicting disease outcome and detecting early recurrence (Table 2). These markers include tumor suppressor genes, oncogenes, tumor-associated antigens, transcription factors, and cellular mediators of apoptosis. Recently reverse transcriptase polymerase chain reaction (RT-PCR) techniques have been developed to detect cancer cells in tissue, lymph nodes, bone marrow or blood of patients. Although RT-PCR detection of tumor marker mRNA expression in histopathologically negative SLNs has upstaged a significant number of melanomas, RT-PCR analysis of the SLN remains very controversial, especially since the clinical significance of IHC-identified micrometastases is still subject to debate. Moreover, use of molecular analysis is limited by the specificity and sensitivity of markers, and by the lack of standardization. Which molecular markers are predictors of disease outcome and which merely detect tumor cells? Considerable work is needed to develop RT-PCR markers specific for malignant melanocytes. Most laboratories that have reported
molecular staging of lymph nodes in melanoma focus on the same markers used for the last 10 years, and have failed to identify more informative markers.

**FUTURE DIRECTION: STATUS OF MULTICENTER LYMPHADENECTOMY TRIALS**

Over the last several decades, multiple randomized trials have failed to show a survival benefit for ELND. But what about SLN biopsy – can it affect survival? To answer this question, Morton and colleagues designed MSLT-I, a phase III trial of 1973 stage I melanoma patients randomized to wide excision alone or wide excision plus SLN biopsy (Figure 4). After a median follow-up of 59.5 months, an interim analysis revealed that although there were no significant differences in 5-year overall survival between the two treatment arms, 5-year disease-free survival was significantly higher among patients assigned to SLN biopsy (78.5% vs 73% \(p < 0.0065\)). Among the subgroup of patients with nodal metastases from an intermediate-thickness primary melanoma (Breslow thickness 1.2 – 3.5 mm), immediate lymphadenectomy for clinically occult disease significantly prolonged 5-year survival compared with delayed lymphadenectomy for clinical nodal recurrence during observation (71.1% vs. 53.4 %, \(p < 0.0034\)). These recently reported results of this trial indicate that SLN biopsy, followed immediately by CLND if the SLN contains tumor, clearly prolongs disease-free survival and spares the patient the psychological trauma of anticipating possible nodal recurrence during the traditional “watch and wait” approach. Results also show that failure to identify and remove occult sentinel node metastases may allow development of more extensive regional node disease. These interim results confirm the prognostic significance of the tumor status of the SLN.

To further examine the importance of SLN metastases and the relevance of molecular assessment of the SLN, organizers of MSLT-I designed a second Multicenter Selective Lymphadenectomy Trial (MLST-II). In MLST-II, patients are randomized according to multi-marker RT-PCR analysis of SLN specimens (Figure 5). Patients whose SLNs are negative for tumor by H&E/IHC will undergo multimarker RT-PCR at John Wayne Cancer Institute; those whose molecular assessment is negative will undergo routine follow-up, whereas those whose assessment is positive will be randomized to either observation or to CLND. The study plans to accrue 3,500 patients, and is presently open to enrollment.

**CONCLUSIONS**

Over the last two decades, the development and wide acceptance of SLN biopsy have profoundly affected the management of melanoma. This technique represents a considerable improvement in our ability to evaluate the tumor status of the regional lymph node basin, which is the most important predictor of survival in patients with melanoma. Histopathologic and molecular assessment of the SLN has enhanced detection of clinically occult nodal metastases, thereby distinguishing patients who might benefit from immediate lymphadenectomy from those in whom this procedure is unlikely to be helpful. This technique also identifies patients who would be candidates for clinical trials of adjuvant therapy. Centers can offer SLN biopsy without routine CLND once they reach a level of proficiency that usually corresponds to a learning phase of 55 cases.

The role of molecular technology in identification and analysis of the SLN remains to be established. Although molecular evidence of SLN metastasis has been identified in patients with early-stage melanoma, its clinical relevance cannot be determined until marker selection is improved. The markers presently under study lack sensitivity and specificity. The role of molecular biomarkers can only be validated through large multicenter randomized controlled...
trials such as MSLT-II, a trial that will determine the benefit of multi-marker RT-PCR assay in SLN specimens.

SLN offers a promising future in staging lymph nodes and will improve management of patients with melanoma. Although SLN biopsy has become widely accepted as a minimally invasive technique of staging regional lymph nodes, its use in patients with melanoma continues to be challenged. The future of SLN biopsy holds promise if prospective multicenter trials confirm a survival benefit for SLN biopsy as compared with watch-and-wait observation.

References


Figure 1.
Schematic representation of intraoperative lymphatic mapping shows blue dye injected in a primary forearm melanoma. The dye moves along the lymphatic channel into the drainage basin. The sentinel node is removed during selective lymphadenectomy.
Figure 2.
A. Schematic representation of intraoperative lymphatic mapping showing carbon particles injected intradermally with blue dye. The carbon particles enter the node through the afferent lymphatics and are found in the subcapsular sinus and in the lymphoid tissues by microscopic exam. B. Carbon dye identifies lymphatic drainage within SLN. C. Carbon dye identifies specific site within SLN where melanoma cells are located.
Figure 3.
Technique of processing the SLN for histopathology and molecular studies. A. Bisect SLN, B. Section SLN, C. 8–12 sections examined for IHC, H & E staining, and RT-PCR studies.
In the first Multicenter Selective Lymphadenectomy Trial (MSLT-I), patients with biopsy-proven melanoma were randomly assigned to receive wide excision alone or wide excision and sentinel node biopsy, in a ratio of 6:4. (LM/SLN, lymphatic mapping/selective lymphadenectomy; SLN, sentinel node; CLND, completion lymph node dissection).

Figure 4.
Figure 5.
In the second Multicenter Selective Lymphadenectomy Trial (MSLT-II), patients with histopathology or RT-PCR evidence of tumor in the sentinel node (SLN) are randomly assigned to receive completion lymph node dissection (CLND) or observation. (LM/SLN, lymphatic mapping/selective lymphadenectomy; H&E, hematoxylin and eosin; IHC, immunohistochemistry.)
<table>
<thead>
<tr>
<th>Study</th>
<th># Pts</th>
<th>#Basins</th>
<th>CLND</th>
<th>SLN Identification Rate (%)</th>
<th>(%) + SLN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morton (14)</td>
<td>223</td>
<td>237</td>
<td>Y</td>
<td>82</td>
<td>22</td>
</tr>
<tr>
<td>Reintgen (71)</td>
<td>42</td>
<td>42</td>
<td>Y</td>
<td>100</td>
<td>19</td>
</tr>
<tr>
<td>Krag (35)</td>
<td>121</td>
<td>121</td>
<td>Y</td>
<td>98</td>
<td>12</td>
</tr>
<tr>
<td>Thompson (44)</td>
<td>118</td>
<td>120</td>
<td>Y</td>
<td>87</td>
<td>21</td>
</tr>
<tr>
<td>Albertini (52)</td>
<td>106</td>
<td>129</td>
<td>N</td>
<td>96</td>
<td>15</td>
</tr>
<tr>
<td>Loong (36)</td>
<td>163</td>
<td>189</td>
<td>N</td>
<td>98</td>
<td>18</td>
</tr>
<tr>
<td>Morton (72)</td>
<td>72</td>
<td>79</td>
<td>Y</td>
<td>90</td>
<td>15</td>
</tr>
<tr>
<td>Bostick (73)</td>
<td>117</td>
<td>120</td>
<td>N</td>
<td>96</td>
<td>12</td>
</tr>
<tr>
<td>Murray (74)</td>
<td>360</td>
<td>360</td>
<td>N</td>
<td>99</td>
<td>17</td>
</tr>
</tbody>
</table>

CLND, completion lymph node dissection

SLN, sentinel lymph node
### Table 2
RNA Molecular Markers in Sentinel Nodes Draining Primary Cutaneous Malignant Melanoma

<table>
<thead>
<tr>
<th>Markers</th>
<th>Descriptions/Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma Antigen Gene A (MAGE-A3)</td>
<td>Encodes for a highly immunogenic protein</td>
</tr>
<tr>
<td>Tyrosinase</td>
<td>Rate limiting enzyme in melanin synthesis</td>
</tr>
<tr>
<td>Melanoma antigen recognized by T cells-1 (MART-1)</td>
<td>Protein recognized by HLA-A2-restricted autologous and allogeneic tumor-infiltrating lymphocytes</td>
</tr>
<tr>
<td>Tyrosinase-related proteins-1 (Trp-1)</td>
<td>Melanosomal membrane glycoproteins recognized by antibodies and T-cells</td>
</tr>
<tr>
<td>Tyrosinase-related proteins-2 (Trp-2)</td>
<td>Melanosomal membrane glycoproteins recognized by T-cells and antibodies</td>
</tr>
<tr>
<td>$\beta_1 \rightarrow 4$ N-acetylgalactosaminyl transferase ($\beta_1$-$4$-GalNAC-T)</td>
<td>Key enzyme in the biosynthetic pathway of the oncofetal glycolipids GM2/GD2 expressed on surface of cancer cells.</td>
</tr>
<tr>
<td>Paired-box homeotic gene transcription (Pax-3)</td>
<td>Regulation of melanin synthesis, migration and anti-apoptosis factor 3</td>
</tr>
</tbody>
</table>