ABSTRACT

Interval nodes (IN) are defined as lymph nodes that lie along the course of lymphatic collecting vessels between a primary tumor site and a draining node field. Sometimes INs contain metastases and a consensus on their surgical management is needed. Therefore, to optimize the surgical management of melanoma patients with metastatic lymphatic involvement, especially when the sentinel lymph node biopsy identifies an unusual drainage field, we identified patients treated at the Department of Plastic and Reconstruction Surgery of Bari between July 1994 and December 2012 identified with a primary-cutaneous melanoma who underwent lymphoscintigraphy and subsequent positive-IN the lymphadenectomy to evaluate the impact of this procedure on overall survival and disease-free-period. 51 patients presented INs, and lymphadenectomy (LA) of the subsequent lymphatic field was performed in 13 subjects with positive-IN. In 4 cases additional lymphatic metastases were detected in the usual basin beyond the IN+. Recurrence-free period and survival rate at 5 years were higher in patients with positive-IN who underwent LA than in subjects who underwent LA due to positive lymph nodes in the usual field. Immediate lymphadenectomy of the subsequent lymphatic field in patients with positive-INs may afford patients earlier stage treatment of their disease and improved prognosis.

Keywords: melanoma, interval node, lymphatic mapping, sentinel node biopsy, in-transit lymph node, lymphoscintigraphy, prognosis

Melanoma frequently metastasizes to regional lymphatic pathways and the first lymph node receiving direct lymphatic drainage from a primary tumor site is called the sentinel lymph node (SN) (1). Lymphoscintigraphy (LS) is an indispensable tool to identify dispersion into sequential lymph node basins (2,3); sentinel lymph node biopsy (SNB) allows better staging of disease by identifying patients eligible for loco-regional lymphadenectomy. Lymphatic mapping is useful to identify both conventional basins (axillary, inguinal/pelvic, and cervical) and “unusual” sentinel node fields. Variously termed interval, aberrant, in-transit, ectopic, or intercalated nodes (4-11), these lymph nodes located outside of the conventional nodal basins sometimes contain metastatic disease. The interval nodes (IN) are defined as lymph nodes that lie along the course of lymphatic collecting vessels between a...
primary tumor site and a draining node field (4). They can occur anywhere along the course of the vessels and are usually found in subcutaneous fat. In the literature, there is not a consensus on interval classification although the frequency of these lymph nodes is different, representing 2% in some studies and rising to 22% in others (4,5,12-14). These differences in prevalence are related to difficulty in their detection and to the absence of a consensus in their classification. However, although the presence of occult metastases in IN indicates a worse prognosis (15), there are no guidelines for surgical treatment when interval nodes contain metastases (10,16,17).

**MATERIAL AND METHODS**

**Study Population**

All patients with a primary cutaneous melanoma treated in the Department of Plastic and Reconstructive Surgery of Bari are included in an electronic clinical medical registry after giving informed consent. Patients with another occult primary carcinoma were excluded from the study. The 555 patients (M 53.5%, mean age 54.2 ± 16.5 years) who underwent a lymphoscintigram from 01/07/1994 to 31/12/2012 were enrolled. The clinical-pathological features and the location of the primary cutaneous melanoma are reported in Table 1. Within the 90th day of excision of the primary lesion, patients with a primary melanoma ≥ 0.75 mm in thickness or with Clark level IV or V invasion, or with a thinner tumor associated with adverse prognostic features (regression, ulceration, high mitosis rate) underwent lymphoscintigraphy to identify SN draining fields. The follow-up period is defined as the time between the melanoma diagnosis date until the last visit occurred by

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients without IN</th>
<th>Patients with IN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total, n (%)</strong></td>
<td>504</td>
<td>51 (9.19)</td>
</tr>
<tr>
<td>Gender (M), n (%)</td>
<td>271 (53.8)</td>
<td>26 (51.0)</td>
</tr>
<tr>
<td><strong>Age (y)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median Range</td>
<td>53.6</td>
<td>56.5</td>
</tr>
<tr>
<td><strong>Breslow thickness, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2 mm</td>
<td>352 (69.8)</td>
<td>28 (54.9)</td>
</tr>
<tr>
<td>2-4 mm</td>
<td>91 (18.0)</td>
<td>12 (23.5)</td>
</tr>
<tr>
<td>≥4 mm</td>
<td>61 (12.1)</td>
<td>11 (21.6)</td>
</tr>
<tr>
<td><strong>Ulceration, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>121 (24.0)</td>
<td>17 (33.3)</td>
</tr>
<tr>
<td><strong>Primary site, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior Trunk</td>
<td>165 (32.7)</td>
<td>21 (41.2)</td>
</tr>
<tr>
<td>Anterior Trunk</td>
<td>90 (17.9)</td>
<td>9 (17.6)</td>
</tr>
<tr>
<td>Head and Neck</td>
<td>48 (9.5)</td>
<td>10 (19.6)*</td>
</tr>
<tr>
<td>Upper limb</td>
<td>55 (10.9)</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Lower limb</td>
<td>146 (29.0)</td>
<td>10 (19.6)*</td>
</tr>
<tr>
<td><strong>Histological subtype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial Spreading</td>
<td>251 (49.8)</td>
<td>27 (52.9)</td>
</tr>
<tr>
<td>Nodular</td>
<td>103 (20.4)</td>
<td>13 (25.5)</td>
</tr>
<tr>
<td>Others</td>
<td>150 (29.8)</td>
<td>11 (21.6)</td>
</tr>
</tbody>
</table>

*p<0.05
the end of June 2013, so that the hypothetical
shorter-term follow-up is fixed at 6 months.

Mapping, Surgical, and Histological
Techniques Used for Lymphatic Metastasis
Diagnosis

Lymphoscintigraphy was performed
using technetium 99m nanocolloid HSA
(human serum albumin) (18-20) with a dosage
of 18-37 MBq injected in 4 sites closely
around the scar of the primary lesion biopsy
(18,19). Ultrahigh resolution collimators
were used to ensure that all the territory
between the primary melanoma site and the
recognized draining node field or fields was
imaged and to reduce artifacts. Dynamic and
planar images (anterior, posterior and lateral)
were acquired using a large field of view
dual-headed digital gamma camera both
immediately after the radio-labelled colloid
injection and then after every lymph node
visualization to ascertain all drainage basins
and the total node number. A 25-min dynamic
image at 1 frame/min in 64x64 matrix in
word mode was used to determine where the
lymphatic collectors were headed. Further
5-10 min static images in word mode 128x128
were acquired over the node field to identify
the collectors as they reach the actual SNs.
Static images were performed to ensure that
all SNs were marked. A static imaging node
that appears in a separate field only in a
subsequent image was considered as a
different SN, unless it was on the same path
in the dynamic scans. A handheld gamma
probe was used during surgery to guide SN
detection. Multiple sections of each SN were
examined by conventional hematoxylin and
eosin (H&E) and by immunohistochemical
stains at both S100 and HMB-45 (21). A
positive SN was defined as a lymph node
containing melanoma cells detected by either
H&E or immunohistochemistry. All histo-
pathologic slides of the interval SNs
containing metastatic disease were reevalu-
ated for this study to document the deposit
size, the tumor penetrative depth, and the
intranodal tumor volume. The histological
presence of metastasis was categorized into
micrometastasis (with deposits ≤2mm) and
macrometastasis (with deposits >2mm)
affecting the peripheral sinus of a lymph
node (20,22). Complete lymph node
dissection was performed in all cases with
a diagnosis of lymph node metastasis. All
patients underwent a clinical and imaging
follow-up every six months for the first five
years and yearly thereafter.

Sentinel Lymph Node Assessment

Axillary, inguinal, and cervical (levels
I-V) regions are considered as usual (or major)
lymph node drainage fields. Supraclavicular,
preauricular/parotid, and chin lymph nodes
were included in the cervical basin; iliac and
pelvic nodes in the inguinal field; all the
lymph nodes between the anterior and
posterior axillary line and the 6th intercostal
space were considered to be in the axillary
drainage basin. In some studies, popliteal
and epitrochlear nodes are classified as IN
(4,5,14), while – in accordance with other
authors (24,25) – we considered these sites as
a functional extension respectively of the
inguinal and axillary fields. The lymph nodes
that lie along the course of lymphatic
collecting vessels between a primary tumor
site and a draining node field (4), recognized
as being outside anatomical lymph node
basins (5,14), are defined IN.

Statistical Analysis

Patients' baseline characteristics were
reported as the frequency (percentage) and
mean ± standard deviation (SD) or median
and interquartile ranges. Categorical
variables were assessed by the chi-squared
test to compare the results for specific
subgroups with those of the rest of the patient
population. Recurrence-free survival was
defined as the time between the definitive
surgical treatment of the primary melanoma
and clinical detection of the first recurrence.
Follow-up time was defined as the time between definitive surgical treatment of the primary melanoma and the last contact with patients. Time-to-death analyses were performed using multivariate Cox proportional hazards regression models, and risks were reported as hazard ratios (HRs) along with their 95% confidence interval (CI). Survival curves and probabilities were reported according to the Kaplan-Meier method. All statistical analyses were performed using SAS Software Release 9.2 (SAS Institute, Cary, NC).

RESULTS

Frequency of Interval Lymph Node

Among 555 subjects who underwent lymphoscintigraphy, 51 patients (9.19%) had interval SLNs identified. Some examples of interval nodal drainage on lymphoscintigraphy are shown in Fig. 1. The majority of interval SNs (58.8%) occurred in cutaneous melanomas arising on the trunk, but INs are reported significantly more frequently in patients with melanomas on head and neck. The incidence of interval nodes in upper and lower limbs was significantly lower (p<0.001). No further major differences were found in age and gender or primary tumor characteristics between patients with and without interval SNs. With regard to the location of INs, we classified the following anatomical regions:

- nuchal region, bounded by a horizontal line passing through the occipital prominence and a horizontal line passing through the 7th cervical vertebra;
- mastoid region, laterally to the nuchal region and bounded by auricular-mastoid sulcus;
- anterior thoracic region, bounded at the top by the inferior margin of clavicles, below by the xiphoid process and the lower margin of the 12th rib, and laterally by the anterior axillary lines;
- posterior thoracic region, bounded at the top by a horizontal line passing through the occipital prominence, below by the lower margin of the 12th rib, and laterally by the posterior axillary lines;
- anterior abdominal wall, including
lymph nodes located above the inguinal ligament but not classifiable as iliac-obturator basins, because these lymph nodes are in the thickness of the abdominal wall up to the transversalis fascia;

- lumbar region, bounded at the top by the lower margin of the 12th rib and below by the iliac crest.

In our experience, the lymph nodes were distributed as follows: 17 cases (33.3%) in posterior thoracic region, 14 (27.4%) in anterior abdominal wall, 7 (13.75%) in anterior thoracic region, 6 (11.8%) in nuchal region, 6 (11.8%) in mastoid region, and 1 (2.0%) in lumbar region. No INs have been reported in arm, forearm, and thigh. In 21 patients the interval node is the only sentinel node found, while in 30 cases there is at least another draining lymph node field.

**Frequency of Occult Lymphatic Metastasis**

In the 504 patients without IN who underwent biopsy, a total of 614 lymph node fields were detected. Overall SNs positive for metastases were reported in 138 (27.38%) subjects, 356 patients (70.65%) were negative, and in 10 cases no lymph node was found (Fig. 2). Within the group of 51 patients with IN, 13 subjects (25.49%) had a tumor-positive interval SN biopsy (Table 2). In accordance with the sequential dissemination of lymphatic metastases (25), we proposed a radical dissection of the subsequent lymphatic basin to each patient with a positive IN (12): 1 subject refused any further surgical treatment, while in 12 cases, lymphadenectomy was performed. We divided IN+ patients into 3 groups:

- **Group A:** 3 patients with a positive IN and without any usual lymphatic drainage field;
- **Group B:** 6 patients with a positive IN and with a negative-SLN detected in usual field;
- **Group C:** 3 subjects with both IN and usual basin SLN positive for metastasis.

Complete lymphadenectomy of the cervical field was carried out in the 2 patients with mastoid region positive IN. Total axillary
<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Histological subtype</th>
<th>Bone metastasis site</th>
<th>Interval SN</th>
<th>Metastasis deposit type</th>
<th>Subsequent bone biopsy result</th>
<th>Subsequent bone biopsy result</th>
<th>Survival at the end of followup</th>
<th>Distant metastasis</th>
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</thead>
<tbody>
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<td>M</td>
<td>Superficial spreading</td>
<td>Posterior thorax</td>
<td>2.1</td>
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<td>No</td>
<td>No</td>
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<td>78</td>
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<td>Anterior abdominal wall</td>
<td>5.5</td>
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<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
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<td>49</td>
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<td>MICRO</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>48</td>
<td>F</td>
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<td>Thigh</td>
<td>4</td>
<td>ALCRO</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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</tr>
<tr>
<td>42</td>
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<td>Anterior abdominal wall</td>
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<td>MICRO</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>48</td>
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<td>MICRO</td>
<td>No</td>
<td>No</td>
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<tr>
<td>50</td>
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<td>Thigh</td>
<td>1.9</td>
<td>MACRO</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>56</td>
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<td>1.9</td>
<td>MACRO</td>
<td>No</td>
<td>No</td>
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<tr>
<td>32</td>
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<td>Posterior thorax</td>
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<td>MACRO</td>
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<tr>
<td>70</td>
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<td>Head</td>
<td>5</td>
<td>MACRO</td>
<td>No</td>
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<td>44</td>
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<td>Anterior thorax</td>
<td>1.6</td>
<td>MACRO</td>
<td>No</td>
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<tr>
<td>49</td>
<td>M</td>
<td>Superficial spreading</td>
<td>Mastoid region</td>
<td>5</td>
<td>MACRO</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>47</td>
<td>F</td>
<td>Superficial spreading</td>
<td>Mastoid region</td>
<td>5</td>
<td>MACRO</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
lymph node dissection was performed in the 2 cases with posterior thoracic region IN+ and in the 2 cases with anterior thoracic region IN+. A complete lymphadenectomy of the inguinal basin was carried out in the 6 subjects with positive-IN in anterior abdominal wall. Overall metastases were detected in the usual basin subsequent to IN+ site in 4/12 cases: 1 in Group A, 2 in Group B and 1 in Group C. In our experience, there was a higher frequency of metastatic findings in the lymphadenectomy of IN+ patients (33.3%) than in patients with usual side positive-SN (28.2%). Additional nodes were found in all the groups, suggesting the possible presence of lymphatic metastasis beyond the site of INs also in patients in whom the IN is the only positive lymph node or the only draining field. The presence of additional lymphatic metastasis in lymphadenectomy was evident in 3 of 4 patients (75%) with macrometastatic INs, while the frequency of further positive lymph nodes was lower in subjects with micrometastasis in INs (12.5%).

Survival and Recurrence-Free Analysis

Follow-up information was available for 153 of 161 patients with positive or unfound SN; the other 8 patients were lost to follow-up. The mean follow-up period in the population with positive or unfound SN amounted to 56.5 months (median duration 46.4, range 1.2-179.6), while the mean follow-up in the cohort with positive INs was 35.1
months (median 29.8, range 1.8-78.8). Overall, 73 (47.7%) of 153 patients with positive or not found SN developed a local, in-transit, regional, or distant recurrence, after a median follow-up of 28.6 months. Two of 12 patients who underwent lymphadenectomy of the usual field subsequent to positive IN developed distant metastatic disease at a median time of 31.5 months and 1 of them died of melanoma. Recurrence-free period and survival rate at 5 years were analyzed in the cohort with positive INs who underwent lymphadenectomy, in subjects who refused lymphadenectomy, in patients who underwent lymphadenectomy after usual field positive SN, and in the cohort without SN biopsy taken (Figs. 3-4).

Fig. 4. Kaplan-Meier disease-free survival curve for subjects in the 4 groups.

Recurrence-free period and survival rate at 5 years were analyzed in a multivariate Cox proportional hazards regression model corrected for age, gender, Breslow thickness, presence of ulceration, lymphatic metastatic deposit type, and mitotic rate factors (Table 3). There was a trend toward a better prognosis in patients who underwent a lymphadenectomy after a positive IN, when compared to the patients undergoing lymphadenectomy for usual SN+ and with patients without SN taken or who refused lymphadenectomy, but the results did not reach statistical significance.

**DISCUSSION**

In our experience, INs were identified in 51 (9.19%) of 555 patients who underwent
SN biopsy. The reported incidence of INs is similar to that of others recent studies (16-28) but higher than the rate of 2.1-7.2% reported in previous studies (4,5,10,12,14,17-26). This finding can be explained by the better definition of lymphatic drainage provided by the small particle radiocolloid (technetium 99m nanocolloid HSA) combined with the use of ultrahigh resolution collimators currently routinely utilized for lymphoscintigraphy. The majority of INs in our study were collected in patients with primary melanoma on the trunk, and there were significantly more interval SNs in subjects with cutaneous melanomas on head and neck than in those with primary tumors on limbs. In this study, the lower frequency of INs in melanomas located on the upper and lower limbs, reported also by other authors (4-16), can be explained by the exclusion of popliteal and epitrochlear lymph nodes from the fields defined as “interval,” as they are considered to be a functional extension of the inguinal (24) and axillary (24) basins respectively. In our experience, anterior abdominal wall and dorsum are reported as the more frequent location of INs (5), while none were found in limbs. Furthermore, for the first time, an IN is reported in the xiphoid region.

The rate of metastatic INs (25.49%) in our study is similar to that shown in other studies (5,14,17,27). Some authors report lower tumor-positive rates (Table 4), but these differences may be explained by the following:

1) Some authors (4-16) reported a low rate of patients with positive INs, but in their studies patients with clinically evident metastases were treated by a direct complete dissection without any preliminary scintigraphy (16) resulting in the exclusion of patients with clinically evident or more infiltrative metastasis in INs, leading to an evident selection bias;

2) Some studies (8,12,27,28) included epitrochlear and popliteal lymph nodes in the
IN group, lowering in this way the frequency of tumor-positive IN (29,30);

3) The frequency (1.98%) of lymph nodes identified in lymphoscintigraphy but surgically not found was low; in fact, the failure to find a SN could result from the failure in searching for an INs.

There are no current guidelines for the management of positive interval SNs. In literature several proposals to manage these patient are described:

1) Some authors recommend only clinical and imaging follow-up of the lymphatic fields (4-14); but some studies demonstrated the simultaneous presence of occult metastases both in INs and in the subsequent lymphatic fields (5-17), suggesting the existence of pathways of drainage. For this reason, if the positive INs were not removed, the lymphatic metastatic spread cannot be properly treated;

2) Sumner et al proposed the completion lymph node dissection of both the unusual site and one regional lymphatic basin upstream from the unusual site for all patients with metastatic INs, but the small patient population and the limited number of events make it premature to draw meaningful conclusions from any analysis of recurrence and survival data (12);

3) Recently Viewer et al recommended lymphadenectomy in patients with both positive IN and positive usual field SN, defining as useless any treatment in patients in whom the only positive node was the interval (16);

4) McMasters et al advised that re-excision of the IN site should be performed when there is evidence of extracapsular nodal extension or contamination of the surgical wound (5).

In our experience, INs were always completely removed during SNB procedure and no patient developed local recurrence or in-transit metastasis. In 1 patient there was extracapsular nodal spread in IN and also in this case no further dissection was necessary.

In accordance with the sequential
diffusion of lymphatic metastasis (25), radical dissection of the subsequent lymphatic basin was proposed for all patients with a positive IN. It is important to note that an additional metastatic lymph node was found in patients with positive INs and positive recognized field SNs (Group C), in subjects with positive IN and negative usual site SN (Group B), and above all in patients with positive IN and without any other drainage field (Group A) (Table 3). The patient with macrometastatic INs showed a higher probability of additional lymphatic metastases in lymphadenectomy, but the involvement of the lymphatic field beyond the micrometastatic IN was reported only in 1 subject. In our experience, the metastatic involvement of the subsequent lymphatic field after positive interval node in melanoma patients confirms the indication for the complete dissection of the subsequent lymphatic basin. Even in the case of positive INs, however, a higher frequency of occurrence of additional lymph nodes is demonstrated in case of detection of macrometastases in INs.

Overall, the 5-year survival rate was 73.6%, in accordance with results of larger multicenter trials (31). Patients with positive IN show a good prognosis; in fact, only 2 patients presented local or distant recurrences. Five year disease-free and overall survival of this cohort is higher than the population who underwent total dissection after usual field positive SN, confirming the utility of immediate lymphadenectomy after a tumor-positive IN. This result suggests that the immediate dissection of the lymphatic field beyond the positive IN could allow patients to be treated in earlier stages of disease before the dissemination of the lymph nodes in the usual basins.

**CONCLUSION**

INs were found in 9.19% of patients and 25.49% of them contained metastatic melanoma. The failure to adequately investigate interval SN sites may result in under-staging of disease, increasing the number of false negatives and not-found sentinel nodes, and increasing the finding of occult metastasis. Furthermore, our data suggest that the complete dissection of the subsequent lymphatic field can avoid metastatic dissemination across the lymphatic pathways, making it possible to treat patients in earlier stages of disease. For this reason, a prospective trial is ongoing with the Italian Melanoma Intergroup in order to define classification, prognosis, and impact of differing surgical management in melanoma patients with interval nodes.

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Eleonora Nacchiero, MD
via A. Einstein
37/1 - 70124- Bari, Italy
Tel: 3392080478
E-mail: eleonora.nacchiero@yahoo.it