The efficacy of $^{99m}$Tc-rituximab as a tracer for sentinel lymph node biopsy in cutaneous melanoma patients

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**Background:** The sentinel lymph node (SLN) status is a vital prognostic factor for malignant melanoma (MM) patients. There is increasing evidence that a radioactive agent, rather than its combination with blue dye, is sufficient for a SLN biopsy (SLNB). Thus, we discussed the efficacy of $^{99m}$Tc-rituximab as a tracer in MM patients.

**Methods:** A total of 502 consecutive patients with MM who underwent SLNB were enrolled in this study. All participants were peritumorally injected with $^{99m}$Tc-rituximab before imaging, and scanned with single-photon emission computed tomography-computed tomography (SPECT-CT) to detect the number and location of the SLN. A gamma detection probe was employed to detect radioactive SLNs in operation. Follow up was conducted to observe whether nodal or distant recurrence occurred.

**Results:** The SLNs were successfully imaged via SPECT-CT and harvested from all 502 participants. No drainage tube was indwelled and 32 (6.3%) participants experienced the following complications: seroma (n=26, 5.2%), wound infections or lymphangitis (n=6, 1.2%), sensory nerve injuries (n=4, 0.8%). There were 380 patients who were diagnosed as SLN-negative and 122 (24.2%) were SLN-positive. A total of 85 SLN-positive patients received complete lymph node dissection, and 28 (32.9%) had additional positive lymph nodes. During a median follow-up of 24 months, 28 participants were found to have a false negative (FN) SLN. The FN rate was 18.7%. A higher T stage was a predictive factor for FN [odds ratio (OR) 1.77; P<0.05]. There was no significant difference in the positive or FN rate between the acral and cutaneous groups.

**Conclusions:** The radiopharmaceutical $^{99m}$Tc-rituximab could be employed as a simple and safe tracer in acral and cutaneous melanoma SLN biopsies.

**Keywords:** Melanoma; sentinel node biopsy; $^{99m}$Tc-rituximab; false negative; radioactive tracer

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Introduction

A sentinel lymph node biopsy (SLNB) is a vital procedure in melanoma patients as recommended by the National Comprehensive Cancer Network (NCCN) guideline (1). It is a key staging procedure for patients with clinical stage I-II melanoma according to the presence or absence of nodal metastasis. The available evidence strongly supports the sentinel lymph node (SLN) status as the most powerful independent factor for predicting survival (2-4). Accurate staging is generally accepted as the basis for counseling, therapeutic decision-making, and prognostication in melanoma. Depending on a variety of factors, 5–40% patients will be detected as SLN-positive (1,2,5-8). The commonly acknowledged risk factors of a positive SLN include the Clark level, mitotic rate, ulceration, lymphovascular invasion, anatomic site, tumor infiltrating lymphocytes, and primary tumor thickness (1,3,9-14). Compared to Caucasian patients, Chinese melanoma patients tend to be associated with a longer delay to diagnosis and a higher proportion of T-stage III–IV status (15,16). Therefore, an accurate SLNB procedure is particularly essential for clinical work.

The NCCN-recommended technique for SLNB consists of preoperative dynamic lymphoscintigraphy, intraoperative identification using isosulfan blue or methylene blue dye, and a gamma probe to detect radiolabeled lymph nodes (1). However, recent studies have increasingly indicated that the use of blue dye is associated with a negative outcome. Ranson et al. performed SLNB in 537 patients using both a $^{99}$mTc radiocolloid and blue dye, and reported that if the blue dye was excluded and only hot nodes were harvested, it would only result in a 5% reduction of dissected nodes without any compromise in the sensitivity of the test (17). van der Ploeg et al. retrospectively assessed 681 patients, and discovered that a blue, nonradioactive sentinel node was removed in only 0.9% of patients (18). There was a minor reduction in the collected nodes; however, there are other negative impacts of using blue dye, including anaphylaxis [ranging from 0.1% to 2.7% (19-21)], prolonged cutaneous staining (22), and its contraindication in pregnant women. In 2013, the US Food and Drug Administration approved Lymphoseek ($^{99}$mTc-tilmanocept) as a radiotracer for detecting SLN in breast and melanoma patients (23). Inspired by this invention, we developed $^{99}$mTc-labeled rituximab as a novel radiotracer. The accuracy and specificity of this tracer has been tested and verified in breast cancer patients in our institution, with a sensitivity 97.4% and specificity of 100% (24,25). But its efficacy in melanoma has not been proved.

Rituximab is an antibody that specifically targets the CD20 molecules expressed in B lymphocytes. Theoretically, $^{99}$mTc-rituximab can combine with B lymphocytes in the first lymph node on the lymph circulating route, which is the precise definition of “sentinel lymph node”, and avoids imaging of second-tier lymph nodes. Because this radio tracer can be detected within at least 6–8 h post-injection, surgeons are guaranteed to have sufficient time during the operation. Besides, the side effects of blue dye, such as anaphylaxis and skin staining, can be avoided. In this study, we aimed to report the feasibility, effectiveness, and safety of using $^{99}$mTc-rituximab for the clinical testing of SLN in melanoma.

We present the following article in accordance with the STROBE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-21-6890/rc).

Methods

Patients

From February 2009 to March 2019, a total of 502 consecutive patients with melanoma underwent a biopsy at the Department of Bone and Soft Tissue Sarcoma of Peking University Cancer Hospital. The indications for SLNB included: (I) primary melanoma, Breslow thickness ≥1 mm, or Breslow thickness <1 mm and with other adverse features (e.g., ulceration, high mitotic rate, and lymphovascular invasion); (II) clinically negative lymph nodal basin (examined by palpation and ultrasound); (III) absence of distant metastasis [confirmed by routine physical examination, chest and abdominal computed tomography (CT) scan, or positron emission tomography (PET)-CT]; and (IV) feasibility for anesthesia. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics committee of Peking University Cancer Hospital and informed consent was taken from all the patients.

SLNB

The radiotracer was prepared as described in a previous study (24). The radiotracer solution was approximately 74 MBq/mL with saline. All participants were administered with peritumoral intradermal injections of $^{99}$mTc-rituximab.
(10.0 MBq, 0.2 mL) on the same day or the day prior to the operation. Planar lymphoscintigraphy was then acquired by a single-photon emission computed tomography-computed tomography (SPECT-CT) system (E. Cam; Siemens, Erlangen, Germany) to determine the location and number of SLNs (Figure 1A). During the operation, the precise position of the SLNs was located using a handled gamma detection probe (Neoprobe, Dublin, OH, USA or Crystal Photonics, Berlin, Germany) before the surgical incision, after which a minimal incision (2–4 cm) was performed (Figure 1B). All radioactive nodes with a counting rate ≥10% of the hottest node were removed.

Pathology

During routine pathologic examination, SLNs were cut along the major axis, formalin-fixed, embedded in paraffin, and stained with hematoxylin and eosin (H&E). Intraoperative frozen section investigations were not performed. Paraffin-embedded specimens were examined by light microscopy at magnifications of ×40 and ×200. Additional immunohistochemical (IHC) staining (S100, HMB-45 and Melan-A) was used for suspicious cases identified with H&E. All negative SLNs with local nodal recurrence were retrospectively reviewed by an experienced pathologist (Lai). Additional serial sectioning (at a 2-mm interval) was performed on all of the reviewed SLN biopsy specimens, and doubtful cases were stained for IHC.

Statistical analysis

For the statistical analysis of the data, contingency tables were analyzed using a χ² test. For the survival analysis, a Kaplan-Meier estimator was used with generalized Wilcoxon and log-rank tests for bivariate comparisons. In the analysis of risk factors, binary logistic regression was deployed. The disease-free survival (DFS) time was calculated from the time of the primary diagnosis to the time point of disease recurrence or death due to any reason. The overall survival (OS) time was calculated from the time of primary diagnosis to the time point of death for any reason. All statistical analyses were performed using SPSS 25.0 (SPSS Inc., Chicago, IL, USA). The differences between groups were considered to be significant for P values <0.05.

Results

A total of 502 patients were enrolled in this study, among whom 239 were men and 263 were women. Acral malignant melanoma (MM) and T3–4 stage patients comprised the majority, accounting for 74.5% (374 patients) and 59.4% (298 patients), respectively (Table 1). The SPECT-CT scanning was omitted in 3 participants due to patient rejection or an operation being pressed for time. We detected SLNs in the remaining 499 participants (100%). The imaging time ranged from 0.33 to 16 h (median: 1 h).
The number of imaged SLNs ranged from 1–8 (median 1). During the operation, SLNs were successfully harvested from all participants, including the 3 patients without SPECT-CT results. The average number of harvested SLN was 2.75.

Complications were observed in 32 patients (6.3%): 26 were seroma (5.2%), 6 were wound infections or lymphangitis (1.2%), and 4 were sensory nerve injuries (0.8%). The inguinal region was a high-risk region for SLNB complications compared to the axilla and neck (Table 2). There were 19 (73.1%) participants with seroma that did not require any intervention, and the remaining 7 (26.9%) participants recovered well after suctioning fluid with a syringe. Those with a wound infection or lymphangitis were treated with antibiotics. Participants with sensory nerve injuries were observed and the symptoms were fully or partially relieved within 6 months. No drainage tube needed to be indwelled, and no second operation was performed.

A total of 112 participants were diagnosed as SLN-
positive after a pathological examination, in which 74 had only 1 positive SLN, 27 had 2 positive SLNs, and 11 had 3 or more positive SLNs. The SLN positive rates of the acral and cutaneous groups were not significantly different (23.3% vs. 27.3%; P=0.40). A total of 85 patients received a complete lymph node dissection. Additional positive lymph nodes (non-SLN positive) were discovered in 28 (32.9%) patients. There were 16 (57.1%) participants who had only 1 non-SLN positive lymph node, 5 (17.9%) had 2 non-SLNs positive lymph nodes, and 7 (25.0%) had 3 nodes or more.

A total of 390 patients were initially diagnosed to be SLN-negative. We followed these patients for up to 140 months (3–40 months; median: 24.0 months). There were 44 patients who exhibited local nodal basin recurrence. After a pathological and medical history review, 10 participants were found to be initially misdiagnosed as SLN-negative, and 6 participants had in-transit recurrence before nodal basin metastasis. After these 16 participants had been excluded, 28 participants were confirmed to be false-negative (FN). The FN rate was defined as the proportion of patients with nodal recurrences after negative SLNB in the patients with nodal involvement. The failure rate was defined as the number of FN patients divided by the total number of SLN-negative patients. Based on this definition, the FN rate of our tracer was 18.7%, and the failure rate was 7.2%.

After pathological review, we increased the number of SLN-positive patients from 112 (22.3%) to 122 (24.2%), and the number of SLN-negative patients was amended to 380. We then compared the survival rate between the 2 groups. The mean DFS and OS of the SLN-negative group was 74.4 months [95% confidence interval (CI): 67.0 to 81.9] and 106.1 months (95% CI: 103.2 to 109.0), respectively. The mean DFS and OS of the SLN-positive group was 28.2 months (95% CI: 22.8 to 33.7) and 67.2 (95% CI: 59.2 to 75.1). Statistical differences were identified between the 2 groups regarding both DFS and OS rates (P<0.05) (Figure 2). These results confirmed that the SLN status was a strong predictor of survival.

We further explored the risk factors of FN status. age, gender, T stage, ulceration, Clark stage, mitosis rate, lymphovascular invasion, and primary lesion location (acral or non-acral) were taken into account in the analysis. The FN group was compared with SLN-negative group and SLN-positive group. The results showed that T stage was the only predicting factor for the FN group compared with

### Table 2 Complications after SLNB

<table>
<thead>
<tr>
<th>Complications</th>
<th>N (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients with complications</td>
<td>32/502 (6.3)</td>
<td></td>
</tr>
<tr>
<td>Seroma</td>
<td>26 (5.2)</td>
<td></td>
</tr>
<tr>
<td>Wound infection</td>
<td>6 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Sensory nerve injury</td>
<td>4 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inguina</td>
<td>30/387 (P&lt;0.05)</td>
<td>χ² test</td>
</tr>
<tr>
<td>Axilla</td>
<td>2/112</td>
<td></td>
</tr>
<tr>
<td>Neck</td>
<td>0/3</td>
<td></td>
</tr>
</tbody>
</table>

SLNB, sentinel lymph node biopsy.

**Figure 2** The OS and DFS of SLN-negative and SLN-positive patients. The mean DFS and OS of the SLN-negative group was 74.4 and 106.1 months. The mean DFS and OS of the SLN-positive group was 28.2 and 67.2 months. OS, overall survival; SLN, sentinel lymph node; DFS, disease-free survival.
the SLN-negative group (OR: 1.77; 95% CI: 1.12 to 2.78; P=0.01). Acral or cutaneous subtype was not a risk factor in either comparison (FN vs. SLN-negative, P=0.33; FN vs. SLN-positive, P=0.72). There were no differences between the FN group and SLN-positive group in the listed risk factors (Table 3). The median time to nodal basin recurrence in the FN group was 10.6 months. Although misdiagnosed, the OS of FN group (mean: 94.9 months; 95% CI: 84.8 to 104.9) was not statistically different from the OS of the SLN-positive group (P=0.17) (Figure 3).

<table>
<thead>
<tr>
<th></th>
<th>FN</th>
<th>SLN-negative</th>
<th>SLN-positive</th>
<th>P value (FN vs. SLN-negative)</th>
<th>P value (FN vs. SLN-positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient(n)</td>
<td>28</td>
<td>352</td>
<td>122</td>
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<tr>
<td>Male</td>
<td>14</td>
<td>167</td>
<td>58</td>
<td>0.79</td>
<td>0.81</td>
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<tr>
<td>Female</td>
<td>14</td>
<td>185</td>
<td>64</td>
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<tr>
<td>Age [median]</td>
<td>60.0 [27–84]</td>
<td>58.0 [20–86]</td>
<td>60.0 [8–80]</td>
<td>0.91</td>
<td>0.95</td>
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<tr>
<td>Location</td>
<td></td>
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<tr>
<td>Acral MM</td>
<td>19</td>
<td>268</td>
<td>87</td>
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<td>0.72</td>
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<tr>
<td>Cutaneous MM</td>
<td>9</td>
<td>84</td>
<td>35</td>
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<td>4</td>
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<td>97</td>
<td>52</td>
<td></td>
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<td>3</td>
<td>26</td>
<td>9</td>
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<tr>
<td>Ulceration</td>
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<tr>
<td>Positive</td>
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<td>145</td>
<td>58</td>
<td>0.64</td>
<td>0.70</td>
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<tr>
<td>Negative</td>
<td>14</td>
<td>188</td>
<td>53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>1</td>
<td>19</td>
<td>11</td>
<td></td>
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<tr>
<td>Lymphovascular invasion</td>
<td>3</td>
<td>15</td>
<td>9</td>
<td>0.32</td>
<td>0.84</td>
</tr>
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<td>5</td>
<td>83</td>
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<td>V</td>
<td>1</td>
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<td>16</td>
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<tr>
<td>NA</td>
<td>21</td>
<td>164</td>
<td>62</td>
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</tbody>
</table>

FN, false negative; SLN, sentinel lymph node; MM, malignant melanoma; NA, not available.
In our analysis, a higher T stage was a predictive factor for the FN status. These results were in accordance with the findings of a study conducted by Nowecki, who analyzed 1,207 MM patients and found that primary tumor thickness, primary tumor ulceration, Clark stage IV/V, and histological nodular melanoma were strongly associated with FN occurrence (45). The risk factors (e.g., ulceration, Clark stage, and histological type) were not significant in this study. This might have contributed to a relatively small number of FN patients (28 vs. 57; Nowecki study). The OS between the FN group and SLN-positive group was not significant, which indicated that despite an initial misdiagnosis, the survival of FN group patients would not be impaired as long as close follow-up was ensured in each patient (48-50).

The FN rate in our study was 18.7%, which was a reasonable level compared with that of previous studies (7–20%) (5,13,35,36,43-47). In our analysis, a higher T stage was a predictive factor for the FN status. These results were in accordance with the findings of a study conducted by Nowecki, who analyzed 1,207 MM patients and found that primary tumor thickness, primary tumor ulceration, Clark stage IV/V, and histological nodular melanoma were strongly associated with FN occurrence (45). The risk factors (e.g., ulceration, Clark stage, and histological type) were not significant in this study. This might have contributed to a relatively small number of FN patients (28 vs. 57; Nowecki study). The OS between the FN group and SLN-positive group was not significant, which indicated that despite an initial misdiagnosis, the survival of FN group patients would not be impaired as long as close follow-up was ensured in each patient (48-50).
Conclusions

The findings of this study suggested that $^{99m}$Tc-rituximab may be employed as a simple and safe tracer in a melanoma SLN biopsy.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-21-6890/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-21-6890/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-21-6890/coif). All authors report that this study was supported by the Science Foundation of Peking University Cancer Hospital. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics committee of Peking University Cancer Hospital and informed consent was taken from all the patients.

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