Title: Use of $^{99m}$Tc Labeled-Tilmanocept versus $^{99m}$Tc Labeled-Sulfur Colloid in Lymphoscintigraphy: Sentinel Lymph Node Identification and Patient Reported Pain

Authors:

1,2 Brittany L Murphy MD MS  
3 Allison R. Woodwick CNMT  
3 Katie M Murphy CNMT  
3 Kimberly J Chandler CNMT  
3 Geoffrey B Johnson MD PhD  
3 Christopher H Hunt MD  
3 Patrick Peller MD  
1 James W Jakub MD  
3 Andrew C Homb MD

Author Affiliation: Departments of 1 Surgery, 2 The Robert D. and Patricia E. Kern Center for the Science of Health Care Delivery, 3 Diagnostic Radiology, Mayo Clinic Rochester MN, USA

Address correspondence to:

Andrew C. Homb MD  
Mayo Clinic  
200 First Street Southwest  
Rochester, MN, 55905  
Phone: +001-(507)774-1728  
Homb.Andrew@mayo.edu

First author information:

Brittany L. Murphy MD MS
General Surgery Resident
200 First Street Southwest
Rochester, MN, 55905
Phone: +001-(507)255-5123

**Type of article:** Original Article

**Short Title:** Tilmanocept vs Sulfur Colloid

**Funding/Support:**

The Mayo Clinic Robert D. and Patricia E. Kern Center for the Science of Health Care Delivery provides salary support for Dr. Murphy. No external funding was used.

**Conflicts of Interest:** All authors disclose no conflicts.

**Word Count:** 3,632 (limit 6000), 215 Abstract (limit 350)
Abstract

Rationale
Lymphoscintigraphy plays a vital role in sentinel lymph node (SLN) identification in oncologic breast surgery. The effectiveness of SLN localization and patient pain between filtered $^{99m}$Tc labeled-Sulfur Colloid (Tc-SC) and $^{99m}$Tc labeled-Tilmanocept (Tc-T) were compared.

Methods
A retrospective review of patients undergoing lymphoscintigraphy for breast cancer using Tc-SC (6/1/2010-12/31/2011) or Tc-T (6/1/2013-1/31/2014) was performed. SLN appearance time and uptake, SLN pathology, proportion of positive SLNs removed, and comparative pain scores were compared for each radiopharmaceutical using chi-squared, fisher’s exact, and unequal variance t-tests, as appropriate.

Results
A total of 76 patients, with 86 evaluated axillae, underwent lymphoscintigraphy: 29 Tc-SC and 47 Tc-T. Mean SLN appearance time was 11.0 minutes for Tc-SC and 19.3 minutes for Tc-T, p=0.003. There was no difference in the mean transit uptake percentage: 2.2% Tc-SC and 1.9% Tc-T, p=0.55. Tc-T identified a greater proportion of intraoperative blue nodes than Tc-SC, p=0.03. There was no significant difference between Tc-SC and Tc-T in the number of SLNs removed, number of patients with positive SLNs, or comparative pain score.

Conclusion
Filtered $^{99m}$Tc labeled-Sulfur Colloid use in lymphoscintigraphy is an acceptable alternative to $^{99m}$Tc labeled-Tilmanocept for SLN detection in breast cancer, based on filtered $^{99m}$Tc labeled-Sulfur Colloid’s similar intraoperative SLN identification and comparative pain scores.

Key words: Tilmanocept, Lymphoseek®, sulfur colloid, lymphoscintigraphy
Introduction

Sentinel lymph node (SLN) surgery continues to play a vital role in staging of breast cancer. When identifying SLNs, a detection method must have adequate sensitivity to detect nodal metastases, while maintaining a specificity that will minimize removal of benign lymph nodes. SLNs may be identified via injection of blue dye and/or lymphoscintigraphy. Two popular radiopharmaceuticals used for lymphoscintigraphy are filtered $^{99m}$Tc labeled Sulfur Colloid (Tc-SC) and $^{99m}$Tc labeled-Tilmanocept (Tc-T). Tc-T is composed of a synthetic macromolecule called tilmanocept that specifically targets and binds to CD-206 receptors of macrophages found within lymphatic vessels, theoretically targeting SLNs and not migrating to non-sentinel lymph nodes. (1) Tc-SC is a radiocolloid particle with an average size of 0.3 to 1.0 µm, which is then filtered to a size of >0.22 µm prior to injection to improve lymphatic absorption. The smaller more uniform particle size is translocated from the injection site into the lymphatic channels, eventually reaching the sentinel lymph node(s) draining the injection site; however, unlike Tc-T, the filtered Tc-SC remains unbound and can migrate beyond the sentinel node(s) over time. (2, 3)

Recent studies have shown the ability of Tc-T to identify SLNs in breast cancer was superior to Tc-SC, with less pain on injection. (4-17) Two clinical trials performed at the University of California San Diego showed that Tc-T exhibited faster injection site clearance times with lower mean number of SLNs identified, with a higher concordance than Tc-SC, while Tc-T and Tc-SC had equivalent SLN uptake. (14, 16) Similarly, a retrospective study also from the University of California San Diego showed that Tc-T patients had fewer nodes removed, while having a greater proportion of positive nodes removed among node-positive patients. This study also found that injection with Tc-SC independently predicted a removal of greater than 3
nodes, when adjusted for tumor characteristics. (4) Finally, it has been shown that significantly more pain was associated with the Tc-SC injection than Tc-T. (17)

Tc-SC has been standard at Mayo Clinic Rochester for SLN biopsy; however, Tc-T was trialed in a prospective cohort of patients for SLN detection in breast surgery patients. Both radiopharmaceuticals were evaluated in their ability to detect SLNs based on localization time, transit uptake, ability to intraoperatively localize SLNs, and pain associated with injection. The aim of the study was to determine if the Mayo Clinic Rochester experience was similar to previously published reports. (4-17)

Materials and Methods

After the study was approved by the Institutional Review Board, and the requirement to obtain informed consent was waived, a retrospective review of patients undergoing lymphoscintigraphy for breast surgery utilizing either Tc-SC or Tc-T was performed. Patient data for the Tc-SC cohort was retrospectively collected for consecutive patients from June 2010 to December 2011. For the Tc-T cohort, patient data was collected from June 2013 to January 2014. Tc-T was trialed at the Mayo Clinic Rochester between June 2013 and January 2014 for use of lymphoscintigraphy for all breast cancer patients after previous standard use of Tc-SC. The 18 month separation between data collection was to allow for a transition between Tc-SC use and Tc-T. A total of 76 patients were included in the study, with 86 axillae evaluated. Each axilla was evaluated independently. Patients who were pregnant or breast-feeding, received prior radiation therapy, ipsilateral recurrence, or previous surgery involving the ipsilateral breast tissue were excluded from the study. Ten patients underwent bilateral lymphoscintigraphy for bilateral breast surgery.

Sentinel Lymph Node Identification
An institution specific standard SLN injection technique was used on all patients. Tc-SC patients received four intradermal, periareolar injections of filtered $^{99m}$Tc labeled-Sulfur Colloid (0.2 micron filter) in the quadrant of the primary breast tumor. Each syringe contained 3.7–14.8 MBq (0.1-0.4 mCi) of activity with a total volume of less than or equal to 0.1 mL per saline solution. Tc-T patients received two intradermal, periareolar injections, in the quadrant of the breast tumor, of $^{99m}$Tc labeled-Tilmanocept as manufactured from Navidea Biopharmaceuticals. Each syringe was calibrated to contain 18.5-37 MBq (0.5-1.0 mCi) of activity with a total volume of less than 0.4 mL per injection. Immediately following injection, which occurred in the same room as the gamma camera, patients were imaged for SLN appearance in both groups.

Dynamic and static imaging was performed with a gamma camera immediately post injection. With the patient positioned supine and arms above their head, anterior oblique views of the injection site were required for all patients until sentinel node visualization. Any additional imaging was acquired as needed. If patients received bilateral injections, static anterior views were acquired in addition to anterior oblique images required for each side. A Co-57 sheet source was utilized for a transmission source.

Localization time was defined by the elapsed time from radiotracer administration to sentinel node visualization as indicated on patient images by the imaging technologist. Confirmation of a sentinel node was verified by the nuclear medicine reading physician or radiologist and annotated in final patient imaging. Manual regions of interests (ROI) were drawn around the injection site and sentinel node(s), as identified by physician, on anterior oblique images to yield count information. These values were then used for mathematical manipulation to determine the transit uptake percentage using the equation below:

\[
\text{Transit uptake} \% = \frac{\text{SLN ROI}}{\text{Injection ROI} + \text{SLN ROI}} \times 100
\]
During surgery, SLNs were identified using radionuclide activity via gamma probe plus or minus the addition of methylene blue. Excised nodes were submitted for pathological examination. Pathology reports were reviewed for report of blue nodes, number of SLNs removed, and positive SLNs. Patients who did not undergo SLN surgery, as they were undergoing breast surgery for risk reduction or atypia, were excluded from intraoperative SLN identification analyses. This included 1 axilla (from 1 patient) in the Tc-SC group and 18 axillae (from 15 patients) in the Tc-T group.

Pain Associated with Intradermal Injection

Only patients who received topical EMLA (Eutectic Mixture of Local Anesthetic) cream prior to injection were included in the pain analysis, which consisted of 22 women in the Tc-SC group and 47 women Tc-T group. For all patients, EMLA cream was applied to the skin and covered with an adhesive patch around the areola in the quadrant of the tumor 30 minutes prior to the injections. Intradermal periareolar injections were performed with a 25-gauge needle by the nuclear medicine radiologist using sterile technique. Filtered $^{99m}$Tc labeled-Sulfur Colloid was given with four injections per breast each containing 3.7–14.8 MBq (0.1–0.4 mCi) in less than or equal to 0.1 mL of saline solution volume. $^{99m}$Tc labeled-Tilmanocept was given with two injections per breast each containing 18.5-37 MBq (0.5-1.0mCi) in less than 0.4mL of volume. Patients were asked to give a pain score immediately after injections using a linear comparative pain scale (CPS) from 0 to 10 (0=no pain, 10=unbearable pain).

Statistical Analysis

Data between the Tc-SC and Tc-T groups were compared using chi-square, fisher exact, and unequal variance t-tests as appropriate. All analyses were completed using JMP 10.0 statistical software. The alpha level was set at 0.05 for statistical significance.
Results

Sentinel Lymph Node Identification

A total of 76 patients, with 86 evaluated axillae, underwent lymphoscintigraphy: 29 Tc-SC patients (29 axillae) and 47 Tc-T patients (57 axillae). Average patient age was 57.0 in the Tc-SC group and 59.5 in the Tc-T group, p=0.22 (Table 1). In the Tc-SC group, more patients underwent lumpectomy than mastectomy (18/29 (62.1%) versus 11/29 (37.9%)), whereas more patients underwent mastectomy than lumpectomy in the Tc-T group (22/47 (46.8%) versus 25/47 (53.2%)); these differences were not statistically significant, p=0.24 (Table 1). One patient in the Tc-T group did not undergo concurrent breast surgery, as no breast lesion was seen on preoperative imaging and patient declined a breast operation.

Localization Time

The average localization time for the Tc-SC patients was 11.0 minutes ± 7.4, versus 19.3 min ± 18.1 for the Tc-T group, p=0.003. Of the Tc-SC patients, 25/29 (86.2%) had visible SLN within 12 minutes from the time of injection, 1/29 (3.5%) had a localization time of 18 minutes and 3/29 (10.3%) took 30 minutes. For the patients in the Tc-T cohort, 37/57 (64.9%) patients had verified SLN appearance within 12 minutes, and an additional 9 (total 46/57 (80.7%) were included when the time was extended to 18 minutes. 5/57 (8.8%) had a localization time of 30 minutes and 6/57 (10.5%) patients had localization times greater than 30 minutes. Total node visualization was 31 (average of 1.1 per patient) for the Tc-SC group and 77 (average of 1.3 per patient) for the Tc-T group, p=0.02.

The average transit uptake for patients in the Tc-SC data pool was 2.2% ± 2.4 and 1.9% ± 2.7 for the Tc-T group, p=0.55.

Pain Associated with Intradermal Injection
Both groups had CPS scores ranging between 0 and 10 with the Tc-SC having a higher mean pain scale at 4.2±2.3 versus 3.3±2.6; however, this was not statistically significant, \( p = 0.16 \) (Figure 1). Additionally, 44.4\% (4/9) of the Tc-SC patients attested to a CPS of greater than or equal to five while only 20.4\% (10/49) of Tc-T patients attested to pain greater than five.

**Sentinel Lymph Node Surgery**

Of the 29 Tc-SC patients, 28 underwent SLN surgery. All had SLNs identified using a gamma probe. The average number of SLNs removed was 2.6 \( \pm \) 1.6 (range 1-9). Six patients had positive nodes, ranging from one to two positive SLNs. Of the 57 breasts injected with Tc-T, 39 underwent SLN surgery. The average number of SLNs removed was 2.4 \( \pm \) 1.6 (range 1-8). Five patients had positive SLNs, all with one positive node. There was no statistical difference between the average number of SLNs removed, number of positive nodes, or proportion of positive nodes excised between each group, \( p = 0.66, 0.89, \) and 0.72, respectively (Table 2).

Of all patients injected with methylene blue who underwent SLN surgery, 15/27 (55.5\%) Tc-SC patients had blue SLNs identified, two of which were positive. In the Tc-T group, 22/30 (73.3\%) patients had blue nodes, 3 of which had a positive blue node. The proportion of blue nodes identified as SLNs was greater for the Tc-T group than the Tc-SC group, \( p = 0.03 \) (Table 2).

**Discussion**

**Sentinel Lymph Node Identification**

The localization time for the Tc-SC group was 11 minutes versus 19 minutes in the Tc-T group, suggesting that Tc-SC lymphatic transit time may be quicker than Tc-T with standard use. Additionally, fewer SLNs were identified with Tc-SC than Tc-T, although the difference was not clinically significant. There was no statistical difference in the other evaluated variables,
including transit uptake, intraoperative SLN identification, or percent positive node identification.

The faster localization time of Tc-SC may be beneficial for institutions where injections are performed intraoperatively and the surgeon must wait for the radiopharmaceutical activity to be present in the axilla before proceeding with SLN surgery. Additionally, one of the perceived benefits of Tc-T over Tc-SC is the large size and macrophage specific receptor binding of Tc-T prevents it from traveling to non-sentinel lymph nodes.\(^{(1)}\) The data presented in this study showed that fewer SLNs were identified with Tc-SC, while the percent of positive SLNs identified in node positive patients remained statistically equivalent. This suggests that the smaller size and absence of a specific binding target of Tc-SC does not limit its ability in intraoperative SLN identification and that Tc-SC remains at least equivalent to Tc-T for this purpose.

Previous studies by Wallace et. al., that evaluated the use of Tc-SC vs Tc-T in SLN identification, showed that SLNs removed from patients in the Tc-T group had a higher concordance with blue dye.\(^{(14, 16)}\) Also, they found that Tc-T had a faster injection site clearance time.\(^{(14, 16)}\) This study did not evaluate clearance time; however, it was determined that the Tc-T group had a greater number of SLNs identified by imaging and an equivalent number identified intraoperatively. This study did agree with previous studies in identifying a greater proportion of blue SLNs in the Tc-T group. Primary SLN uptake in this study was greater than that reported by Wallace et. al. for both groups, but neither study found a statistical difference between the two radiopharmaceuticals.\(^{(16)}\) Reasons for differences in this study and previously published prospective studies may include the larger sample size and the retrospective design of this study.
Baker et. al. also performed a retrospective review of 84 Tc-T and 115 Tc-SC patients.\textsuperscript{4} Their study showed that fewer SLNs were identified in the Tc-T group compared to Tc-SC.\textsuperscript{4} Additionally, they found that both groups had a similar proportion of LN positive patients; however, the Tc-T group identified a greater number of positive nodes among the node positive patients.\textsuperscript{4} Collected data in this study suggest that the two groups identify a similar number of SLNs, with an equal proportion of positive nodes identified in each group. Reasons for the difference in these findings compared to the Baker et al. study likely include the small sample size and small proportion of patients with positive lymph nodes in both groups in the previously published study.

\textit{Pain Associated with Intradermal Injection}

This study found no significant difference between pain associated between Tc-SC and Tc-T. A prior randomized controlled trial found more pain associated with Tc-SC injection than Tc-T, within the first 3 minutes post-injection.\textsuperscript{17} EMLA cream was applied preoperatively to all patients in this study, which may have helped to eliminate differences in the injection associated pain; however, a previous study from the Mayo Clinic Rochester showed that topical anesthetic cream did not help with injection pain.\textsuperscript{18} Today, these findings may be of limited clinical importance as all patients now receive intradermal lidocaine at the injection sites at Mayo Clinic Rochester, which has been shown to improve patient tolerance to the procedure.\textsuperscript{19}

\textit{Limitations}

Limitations to this study include its retrospective design and modest sample size. Additionally, although patients were injected in the same room as the gamma camera, they were not injected directly beneath the camera with immediate dynamic imaging to ensure the most accurate measurement of transit time. Thus, the time to perform the injections and patient
transport time from injection to imaging may have made the times to visualization appear longer than actual transit times. Also, for the pain analysis, patients injected with Tc-SC had four injections, while Tc-T only had two injections. Multiple injection sites were chosen to ensure injection on each side of a tumor or scar to see all possible drainage patterns. Even with the difference of two injections of Tc-T and four injections of Tc-SC, there was only a slightly lower mean CPS for Tc-T and no statistical difference was found. Finally, Tc-SC and Tc-T could not be directly compared in the same patient. In order to do so, one would need to inject a patient with one of the agents, wait for radioactivity to decrease to zero, and inject the patient with the other agent, and then proceed to surgery. Not only is this unreasonable from a patient standpoint, but no pathological comparison data would be available, as only one of the injections would be followed by operative intervention.

**Conclusion**

Comparison of two radiopharmaceuticals, filtered $^{99m}$Tc labeled-Sulfur Colloid and $^{99m}$Tc labeled-Tilmanocept, showed that the usage of filtered $^{99m}$Tc labeled-Sulfur Colloid for lymphoscintigraphy continues to be an acceptable alternative to $^{99m}$Tc labeled-Tilmanocept for use in SLN detection in breast cancer. These findings are based on the similar intraoperative SLN identification and patient perceived pain between the two radiopharmaceuticals.
References


Figure Legend:

Figure 1: Average Localization Time $^{99m}$Tc labeled-Tilmanocept versus filtered $^{99m}$Tc labeled-Sulfur Colloid
<table>
<thead>
<tr>
<th></th>
<th>Filtered $^{99m}$Tc labeled-Sulfur Colloid (n=29)</th>
<th>$^{99m}$Tc labeled-Tilmanocept (n=57)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (± standard deviation)</td>
<td>n=29 Mean 57.0±13.6</td>
<td>n=47 Mean 59.5±12.7</td>
<td>0.22</td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mastectomy</td>
<td>11 (38)</td>
<td>31 (54)</td>
<td>0.24</td>
</tr>
<tr>
<td>Lumpectomy</td>
<td>18 (62)</td>
<td>25 (44)</td>
<td></td>
</tr>
<tr>
<td>No Breast Surgery</td>
<td>0</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Localization Time (minutes)</td>
<td>10.96±7.36</td>
<td>19.31±18.06</td>
<td>0.003</td>
</tr>
<tr>
<td>Transit Uptake (%)</td>
<td>2.20±2.37</td>
<td>1.86±2.71</td>
<td>0.55</td>
</tr>
<tr>
<td>Node Visualization Per Patient</td>
<td>1.07±0.26</td>
<td>1.26±0.48</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Filtered $^{99m}$Tc labeled-Sulfur Colloid (n=28)</td>
<td>$^{99m}$Tc labeled-Tilmanocept (n=39)</td>
<td>P-Value</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------------------------------------------</td>
<td>-------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Number (mean ± standard deviation) of SLNs excised</td>
<td>2.57±1.64</td>
<td>2.41±1.56</td>
<td>0.66</td>
</tr>
<tr>
<td>Number (mean ± standard deviation) of Positive SLNs</td>
<td>0.28±0.60 Range 0-2</td>
<td>0.13±0.34 Range 0-2</td>
<td>0.89</td>
</tr>
<tr>
<td>Proportion (mean ± standard deviation) of Positive SLNs</td>
<td>0.12±0.26</td>
<td>0.08±0.24</td>
<td>0.72</td>
</tr>
<tr>
<td>Number (mean ± standard deviation) of Blue nodes</td>
<td>n=27 0.81±1.00 Range 0-4</td>
<td>n=31 1.45±1.72 Range 1-8</td>
<td>0.04</td>
</tr>
<tr>
<td>Proportion (mean ± standard deviation) of Blue Nodes</td>
<td>n=27 0.32±0.36</td>
<td>n=31 0.52±0.43</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Abbreviations: SLN – sentinel lymph node