J of Nuclear Medicine Technology, first published online July 13, 2017 as doi:10.2967/jnmt.117.194415

Use of Tc-99m tilmanocept as a single agent for sentinel lymph node identification in breast cancer: A retrospective pilot study

Jonathan T. Unkart MD[1] and Anne M. Wallace MD[1]

[1]Department of Surgery, Moores Cancer Center, University of California, San Diego, La Jolla, CA

Corresponding Author:
Jonathan T. Unkart, MD
Department of Surgery
200 W. Arbor Drive MC #8220
San Diego, CA 92103
Phone: 858-822-6193
Fax: 858-822-6194
Email: junkart@ucsd.edu

Short title: Tilmanocept single agent use

Keywords: tilmanocept, sentinel node biopsy, lymphatic mapping, breast cancer

Conflict of Interest: The authors have no conflicts of interest to report.

Abstract

Introduction: Tc-99m tilmanocept (TcTM) received recent FDA-approval for lymphatic mapping. However, no prior studies have evaluated the use of TcTM as a single agent in sentinel lymph node (SLN) biopsy in breast cancer.

Methods: We executed this retrospective, pilot study to assess the ability of TcTM to identify sentinel nodes as a single agent in clinically node-negative breast cancer patients. Patients received a single, intradermal injection overlying the tumor of either 0.5 mCi TcTM the day-of surgery or 2.0 mCi TcTM the day-prior to surgery by a radiologist. Immediate, three-view lymphoscintigraphy was performed. Intraoperatively, SLNs were identified with the use of a portable gamma probe. A node was classified as "hot" if counts per second of the node > 3 times that of background counts. Descriptive statistics are reported.

Results: 19 patients underwent SLN biopsy with single-agent TcTM. Immediate lymphoscintigraphy identified ≥ 1 sentinel node in 13/17 (76.5%) patients. Intraoperatively, ≥ 1 (mean 1.7 ± 0.8, range 1-3) "hot" node was identified in all (100%) patients. Three (15.8%) patients had one disease-positive SLN.

Conclusion: In this small retrospective pilot study, TcTM performed well as a single agent for intraoperative sentinel node identification in breast cancer. A larger, randomized clinical trial is warranted to compare Tc-99m tilmanocept as a single agent to other radiopharmaceuticals for sentinel node identification in breast cancer.

Introduction

Sentinel lymph node (SLN) biopsy is the standard of care for axillary staging in early-stage clinically node-negative breast cancer patients. Numerous lymphatic mapping agents exist on the market available to surgeons with various strengths and limitations. In addition to the numerous dyes available (i.e., isosulfan blue dye, methylene blue, and indocyanine green (ICG)),Tc-99m sulfur colloid was the radiopharmaceutical used in the United States to validate the technique of SLN biopsy in breast cancer (*1*). However, a newer agent Tc-99m tilmanocept (TcTM) recently received Food and Drug Administration (FDA) approval for lymphatic mapping in breast cancer in 2013.

TcTM is a small, receptor-targeted lymphatic mapping agent with demonstrated technical success in numerous tumor types (*2-5*). Tilmanocept accumulates in SLNs by binding to the CD206 receptor residing on the surface of lymph node macrophages and dendritic cells (*6*). TcTM consists of multiple units of DTPA and mannose, each covalently linked to a dextran backbone (*7,8*). Mannose binds the CD206 receptor, and the DTPA moieties serve as the binding site for Tc-99m (*6-8*). It is a macromolecule with an average diameter of 7 nm (*7,8*). This size differs significantly from the other radiopharmaceutical used for lymphatic mapping in the United States, Tc-99m sulfur colloid, with filtered particles varying in size between 100 and 220 nm (*9*). For radiopharmaceuticals used outside the United States, Tc-99m nanocolloid human serum albumin ranges in size from 5 to 100 nm and Tc-99m antimony trisulfide particles range from 3 to 30 nm (*9*).

While axillary SLN biopsy is performed typically with two agents (radiopharmaceutical + blue dye) (*10*) to increase SLN identification, numerous studies report on the success of SLN biopsy with radiotracer alone and question the utility of blue dye (*10-13*). No prior studies have reported on the use of TcTM as a single agent for SLN biopsy in breast cancer. Thus, we have executed this retrospective pilot study to evaluate the technical outcomes of TcTM when used as a single agent in breast cancer.

Methods

We queried our prospectively maintained, Institutional Review Board (IRB)-approved sentinel lymph node database to identity patients undergoing single agent lymphatic mapping and SLN biopsy with Tc-99m tilmanocept. The IRB granted a waiver of consent for this retrospective review. After FDA approval in March 2013, TcTM was added to our institutional radiopharmacy formulary in May 2013 and became the routinely ordered radiopharmaceutical for sentinel node imaging in breast cancer. Patients with previous axillary surgery, known positive-node disease or undergoing lymphatic mapping with two or more agents were excluded from this analysis.

Radiopharmaceutical preparation

Tc-99m tilmanocept was prepared and quality control performed by a local central radiopharmacy (Cardinal Health, San Diego, CA) per manufacturer (Navidea Biopharmaceuticals, Inc. Dublin, OH) package insert. Radiolabeling was performed offsite at Cardinal Health and the agent was delivered during one of three delivery time-windows based on scheduled injection. The agent was delivered in a single, 27-gauge tuberculin syringe to the hospital's Nuclear Medicine Department. Upon arrival and immediately prior to injection, the agent was routinely surveyed for radioactivity by trained nuclear medicine technicians using a Capintec (Florham Park, NJ) CRC-25R dose calibrator.

Tc-99m tilmanocept injection

Injections were performed or supervised by one of three licensed radiologists. Per protocol, after confirming the correct patient and side for injection, and placing the patient supine on the table, the radiologist uses an alcohol wipe to clean off the patient's skin above the tumor. The radiologist uses the prior biopsy site scar and confirmation with the patient regarding reported location of the tumor. For "one-day" injections, patients received a single intradermal 0.1mL 0.5 mCi (18.5MBq) (actual 0.53 +/- 0.02 mCi (19.6 +/- 0.74 MBq)) Tc-99m tilmanocept injection approximately 2-3 hours prior to surgery. Patients in the "two-day" protocol received a single intradermal 0.1mL 2.0 mCi (74.0 MBq) (actual 2.06

+/- 0.08 mCi (76.2 +/- 2.96 MBq)) Tc-99m tilmanocept injection approximately 15-20 hours prior to surgery on the day before surgery. A skin “wheal” confirms successful injection. Residual radioactivity within the syringe is not routinely surveyed.

Lymphoscintigraphy

Images were obtained with a Philips (ADAC) Forte Gamma Camera (Amsterdam, Netherlands), with 20% energy window and low energy general purpose collimator. Image acquisition was 180 seconds and three views were typically obtained (anterior, oblique and lateral). Acquisition was typically performed five minutes post-injection. However, if a patient was summoned immediately to the operative area, lymphoscintigraphy was not performed.

Surgery

Operations were performed by one experienced surgical oncologist with >15 years of experience performing SLN biopsies. Intraoperatively, background radioactivity levels were measured using a handheld intraoperative probe (Neoprobe) (programmed to read out in counts per second). To qualify as a hot node, the intraoperative counts in the node had to exceed the background count (using either one 10-second count or the average of three 2-second counts, with background measured directly on the patient ~20 cm from the primary site) plus 3 times the standard deviation of the background.

Removed lymph nodes were submitted to pathology for either a frozen section or permanent histopathologic staining. Frozen sections are typically performed within one hour of lymph node removal and a positive-result may warrant a patient to immediately undergo axillary lymph node dissection depending on clinical scenario. Permanent section results are typically finalized in 5-7 days postoperatively.

Statistical Methods

We report on baseline patient and technical characteristics. Descriptive statistics were calculated using R (https://www.r-project.org, v. 3.1.2).

Results

During May 2013- July 2016, a total of 19 patients underwent SLN biopsy after single agent injection with TcTM. Patient characteristics are summarized in Table 1. In brief, most patients had breast conserving surgery (84.2%), had T1/T2 sized tumors (78.9%) and hormone-receptor positive tumors (89.5%). Additionally, the majority (78.9%) of patients underwent injection with the two-day protocol. Of the patients who underwent immediate imaging with lymphoscintigraphy, 76.5% of patients had at least one visualized node. Two patients did not undergo lymphoscintigraphy as they were summoned to the operative area before imaging could be completed.

At least one intraoperative SLN was identified in 100% of patients. A total of 32 radioactive nodes were detected and removed (mean 1.7 ± 0.75, range 1-3). Three-patients (15.8%) had one identified node that was positive for disease. Of the three patients with disease positive sentinel nodes, two of the patients underwent axillary lymph node dissection as part of their treatment pathway. No other disease was found in the completion axillary lymph node dissection specimens of the two patients (Table 2).

Discussion

In this retrospective pilot study Tc-99m tilmanocept performed well as a single agent for sentinel node biopsy in early breast cancer. Intraoperatively, at least one SLN was identified in all patients. TcTM identified three nodes with pathology proven disease in three patients. For the two patients who had completion axillary lymph node dissections, no further disease was found. Furthermore, patients who did not have a node identified on immediate lymphoscintigraphy still had a "hot" sentinel node detected intraoperatively with a gamma probe. Additionally, TcTM offered patient scheduling flexibility as successful SLN biopsy was performed in both patients receiving the injection the day-of surgery or the day-prior to surgery.

TcTM recently received initial FDA-approval for lymphatic mapping in March 2013 in breast cancer and melanoma. No prior trial or study exists evaluating the performance of TcTM as a single agent for intraoperative lymphatic mapping in breast cancer. The initial clinical trials validating the use of TcTM in breast cancer and melanoma involved comparisons and measurements of concordance with blue dye (*3,5,14*). While data showed consistent performance and reliability of TcTM, the use of blue dye may have assisted with identification of the hot lymph nodes. In a separate disease process, TcTM identified at least one SLN in 97.6% of patients when used as a single agent in oral squamous cell carcinoma of the head and neck (*4*). Preliminary results from our pilot study demonstrate excellent performance for intraoperative SLN identification in breast cancer when TcTM is used as the lone injected agent.

TcTM exhibits properties of an ideal radiopharmaceutical for SLN procedures. Ideal lymph node mapping agents exhibit rapid injection site clearance, rapid and sustained uptake within the sentinel node and low uptake by distal lymph nodes (*15*). In prior work, TcTM exhibited faster injection site clearance than Tc-99m sulfur colloid (*16*). When used as a single agent, this trait is highly desirable as some surgeons prefer to inject their radiopharmaceutical intraoperatively and rapid injection site clearance is desirable (*12*). At our institution, patients are required to receive their radiopharmaceutical injection while awake in the Nuclear Medicine Department by a licensed individual. In these instances, we have found

that using TcTM caused significantly less injection site pain compared to filtered Tc-99m sulfur colloid (*17*).

This study is not without a few limitations. First, the sample size of this study, while sufficient to make a conclusion regarding a small subset of patients at this institution, would be bolstered by a larger sample size. A larger, randomized controlled study could provide more conclusive results and data about the use of TcTM as a single agent in breast cancer. Furthermore, a true false negative rate could not be determined as axillary lymph node dissection is no longer the accepted standard of care in all breast cancer patients. However, in the two patients who underwent completion axillary dissection, TcTM identified the only disease positive lymph nodes. Lastly, as is the case for all retrospective studies, there is a possibility that the results could be biased due to unknown confounding variables.

Conclusion

In this small, retrospective study, Tc-99m tilmanocept performed well as a single agent in sentinel node identification in breast cancer. A larger, randomized clinical trial is warranted to compare Tc-99m tilmanocept as a single agent to other radiopharmaceuticals for sentinel node identification in breast cancer.

References

1. Krag DN, Weaver DL, Alex JC, Fairbank JT. Surgical resection and radiolocalization of the sentinel lymph node in breast cancer using a gamma probe. *Surg Oncol.* 1993;2:335-339; discussion 340.

2. Surasi DS, O'Malley J, Bhambhvani P. 99mTc-Tilmanocept: A novel molecular agent for lymphatic mapping and sentinel lymph node localization. *J Nucl Med Technol.* 2015;43:87-91.

3. Wallace AM, Han LK, Povoski SP, et al. Comparative evaluation of [(99m)tc]tilmanocept for sentinel lymph node mapping in breast cancer patients: results of two phase 3 trials. *Ann Surg Oncol.* 2013;20:2590-2599.

4. Agrawal A, Civantos FJ, Brumund KT, et al. [(99m)Tc]Tilmanocept accurately detects sentinel lymph nodes and predicts node pathology status in patients with oral squamous cell carcinoma of the head and neck: results of a phase III multi-institutional trial. *Ann Surg Oncol.* 2015;22:3708-3715.

5. Sondak VK, King DW, Zager JS, et al. Combined analysis of phase III trials evaluating [(99)mTc]tilmanocept and vital blue dye for identification of sentinel lymph nodes in clinically node-negative cutaneous melanoma. *Ann Surg Oncol.* 2013;20:680-688.

6. Azad AK, Rajaram MV, Metz WL, et al. gamma-tilmanocept, a new radiopharmaceutical tracer for cancer sentinel lymph nodes, binds to the mannose receptor (CD206). *J Immunol.* 2015;195:2019-2029.

7. Vera DR, Wallace AM, Hoh CK. [(99m)Tc]MAG(3)-mannosyl-dextran: a receptor-binding radiopharmaceutical for sentinel node detection. *Nucl Med Biol.* 2001;28:493-498.

8. Vera DR, Wallace AM, Hoh CK, Mattrey RF. A synthetic macromolecule for sentinel node detection: (99m)Tc-DTPA-mannosyl-dextran. *J Nucl Med.* 2001;42:951-959.

9. Giammarile F, Alazraki N, Aarsvold JN, et al. The EANM and SNMMI practice guideline for lymphoscintigraphy and sentinel node localization in breast cancer. *Eur J Nucl Med Mol Imaging.* 2013;40:1932-1947.

10. He PS, Li F, Li GH, Guo C, Chen TJ. The combination of blue dye and radioisotope versus radioisotope alone during sentinel lymph node biopsy for breast cancer: a systematic review. *BMC Cancer.* 2016;16:107.

11. O'Reilly EA, Prichard RS, Al Azawi D, et al. The value of isosulfan blue dye in addition to isotope scanning in the identification of the sentinel lymph node in breast cancer patients with a positive lymphoscintigraphy: a randomized controlled trial (ISRCTN98849733). *Ann Surg.* 2015;262:243-248.

12. Johnson CB, Boneti C, Korourian S, Adkins L, Klimberg VS. Intraoperative injection of subareolar or dermal radioisotope results in predictable identification of sentinel lymph nodes in breast cancer. *Ann Surg.* 2011;254:612-618.

13. Kang T, Yi M, Hunt KK, et al. Does blue dye contribute to success of sentinel node mapping for breast cancer? *Ann Surg Oncol.* 2010;17 Suppl 3:280-285.

14. Leong SP, Kim J, Ross M, et al. A phase 2 study of (99m)Tc-tilmanocept in the detection of sentinel lymph nodes in melanoma and breast cancer. *Ann Surg Oncol.* 2011;18:961-969.

15. Wallace AM, Hoh CK, Vera DR, Darrah DD, Schulteis G. Lymphoseek: a molecular radiopharmaceutical for sentinel node detection. *Ann Surg Oncol.* 2003;10:531-538.

16. Wallace AM, Hoh CK, Ellner SJ, Darrah DD, Schulteis G, Vera DR. Lymphoseek: a molecular imaging agent for melanoma sentinel lymph node mapping. *Ann Surg Oncol.* 2007;14:913-921.

17. Unkart JT, Baker JL, Hosseini A, et al. Comparison of post-injection site pain between technetium sulfur colloid and technetium tilmanocept in breast cancer patients undergoing sentinel lymph node biopsy. *Ann Surg Oncol.* 2015;22 Suppl 3:S559-565.

Tables

Table 1: Patient and Performance Characteristics

Variable (n = 19)	Value
Age (years)	57.5 ± 7.4
BMI (kg/m^2)	27.2 ± 3.3
Injection Protocol	
One-Day	4 (21.1%)
Two-Day	15 (78.9%)
Surgery	
Lumpectomy	16 (84.2%)
Mastectomy	3 (15.8%)
Tumor (T stage)	
Tis	1 (5.3%)
T1	7 (36.8%)
T2	8 (42.1%)
T3	3 (15.8%)
Estrogen-Receptor	
Positive	17 (89.5%)
Negative	2 (10.5%)
Progesterone-Receptor	
Positive	17 (89.5%)
Negative	2 (10.5%)
Her2-Receptor	
Positive	1 (5.3%)
Negative	18 (94.7%)
Lymphoscintigraphy	
Performed	17 (89.5%)
Nodal Uptake	13 (76.5%)
Not Performed	2 (10.5%)

Legend: Values are reported as means ± standard deviation or as numbers (percentages).

Table 2: Patients with a Positive Sentinel Lymph Node

Patient	SLNB Pathology	ALND Performed?	ALND Pathology*
A	1/3	Yes	0/15
B	1/2	Yes	0/19
C	1/2	No	N/A

Legend: SLNB: Sentinel lymph node biopsy, ALND: Axillary lymph node dissection, *Pathology for the axillary lymph nodes removed in addition to those removed during the sentinel lymph node biopsy reported in first column, N/A: Not applicable