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A Phase 2 Study of ^{99m}Tc -Tilmanocept in the Detection of Sentinel Lymph Nodes in Melanoma and Breast Cancer

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Abstract

Background—Several ^{99m}Tc -labeled agents that are not approved by the U.S. Food and Drug Administration are used for lymphatic mapping. A new low-molecular-weight mannose receptor-based, reticuloendothelial cell-directed, ^{99m}Tc -labeled lymphatic imaging agent, ^{99m}Tc -tilmanocept, was used for lymphatic mapping of sentinel lymph nodes (SLNs) from patients with primary breast cancer or melanoma malignancies. This novel molecular species provides the basis for potentially enhanced SLN mapping reliability.

Methods—In a prospectively planned, open-label phase 2 clinical study, ^{99m}Tc -tilmanocept was injected into breast cancer and cutaneous melanoma patients before intraoperative lymphatic mapping. Injection technique, preoperative lymphoscintigraphy (LS), and intraoperative lymphatic mapping with a handheld gamma detection probe were performed by investigators per standard practice.

Results—Seventy-eight patients underwent ^{99m}Tc -tilmanocept injection and were evaluated (47 melanoma, 31 breast cancer). For those whom LS was performed (55 patients, 70.5%), a ^{99m}Tc -tilmanocept hot spot was identified in 94.5% of LS patients before surgery. Intraoperatively, ^{99m}Tc -tilmanocept identified at least one regional SLN in 75 (96.2%) of 78 patients: 46 (97.9%) of 47 in melanoma and 29 (93.5%) of 31 in breast cancer cases. Tissue specificity of ^{99m}Tc -tilmanocept for lymph nodes was 100%, displaying 95.1% mapping sensitivity by localizing in 173 of 182 nodes removed during surgery. The overall proportion of ^{99m}Tc -tilmanocept-identified nodes that contained metastatic disease was 13.7%. Five procedure-related serious adverse events occurred, none related to ^{99m}Tc -tilmanocept.

Conclusions—Our results demonstrate the safety and efficacy of ^{99m}Tc -tilmanocept for use in intraoperative lymphatic mapping. The high intraoperative localization and lymph node specificity

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CONFLICTS OF INTEREST None.

of ^{99m}Tc -tilmanocept and the identification of metastatic disease within the nodes suggest SLNs are effectively identified by this novel mannose receptor–targeted molecule.

Sentinel lymph node (SLN) mapping is a widely adopted intraoperative procedure for the identification of lymph nodes (LNs) draining the pathway from a primary tumor. Two types of agents are used for mapping lymphatic structures: colorimetric agents, e.g., vital blue dyes (VBDs), and radiopharmaceuticals, e.g., ^{99m}Tc -labeled sulfur colloid (TcSC). Despite widespread use, TcSC is not approved by the U.S. Food and Drug Administration (FDA) for intraoperative lymphatic mapping (ILM), and no agents, including VBDs, are FDA approved specifically for SLN identification. Moreover, none of these agents has any inherent biotargeting structure that provides biospecificity. VBDs, including isosulfan blue (Lymphazurin), are currently considered standard agents for ILM, and some are approved for this general indication. VBDs are contrast agents that depend on line-of-sight visualization of only transiently blue-stained tissue. VBDs also have potential to cause clinically important allergic adverse reactions.^{1–6}

Colloid-based radiopharmaceuticals are also used for SLN mapping, often in conjunction with a VBD. These agents vary greatly in particle size and availability. Several of the most common ^{99m}Tc -labeled agents are TcSC (diameter: filtered TcSC <220 nm, unfiltered TcSC ~650 nm), antimony sulfide (3–30 nm), stannous fluoride (50–600 nm), and microcolloidal albumin (200–1000 nm).⁷ None has regulatory approval for ILM. Radiocolloids are not biotargeted, and all passively transport through the lymphatics. Importantly, these large-molecular-weight species are slowly cleared from the injection site, resulting in delayed migration to the lymphatics. Such injection-site retention, typically proximal to the tumor bed, may mask SLNs that exist regionally. Moreover, current colloid formulations are associated with notable injection site pain and are often injected with local anesthetics that may reduce lymphatic flow, further affecting SLN identification.^{8,9}

Technetium-99m-labeled tilmanocept (Fig. 1) is a novel molecular species that accumulates in lymphatic tissue by binding mannose receptors residing on the surface of lymphatic-resident reticuloendothelial cells.^{10,11} ^{99m}Tc -tilmanocept is a synthetic macromolecule consisting of multiple units of mannose and diethylene triamine penta acetic acid (DTPA), each attached to a 10-kDa dextran backbone. The mannose units act as ligands for the receptor and DTPA units serve to chelate ^{99m}Tc . As such, ^{99m}Tc -tilmanocept is a low molecular weight substance (~19,000 Da) with a diameter of ~7 nm—substantially smaller than other ^{99m}Tc -labeled radiopharmaceuticals. ^{99m}Tc -tilmanocept is injected in close proximity to a primary tumor and used with an intraoperative gamma detector to localize LNs in the lymphatic pathway draining the tumor site. ^{99m}Tc -tilmanocept's advantages to both VBDs and radiocolloids are inherent in its structure: it is biotargeted to lymphatic tissue for rapid uptake and retention in SLNs; the small diameter of the molecule permits rapid injection site clearance, thus allowing identification of regional SLNs for either same-day or next-day mapping procedures; it permits stable ^{99m}Tc radiolabeling but with low radiation absorption; and it has a remarkable safety profile.³

In phase 1 clinical studies in breast cancer patients, where injection site clearance of ^{99m}Tc -tilmanocept was directly compared to ^{99m}Tc -sulfur colloid, ^{99m}Tc -tilmanocept cleared the injection site statistically significantly faster than filtered TcSC, and its uptake into the primary SLN increased dose dependently ($P = 0.009$).^{12–15} Phase 1 studies have also shown that ^{99m}Tc -tilmanocept use is safe, and its performance has excellent correlation with isosulfan blue in the identification of SLNs.¹² In phase 1 studies for both breast cancer ($n = 24$) and melanoma ($n = 24$), no clinically important alterations were observed in serum chemistry, hematology, or urinalysis parameters; no alterations attributed to ^{99m}Tc -

tilmanocept administration were observed in electrocardiograms or vital signs.^{12,16} No serious adverse events were reported in any of the phase 1 trials.

The purpose of this phase 2 study was to determine the safety and efficacy of ^{99m}Tc-tilmanocept for lymphatic mapping in patients with primary breast cancer and cutaneous melanoma.

MATERIALS AND METHODS

Study Agent

The formulation of tilmanocept (DTPA mannosyl dextran; Lymphoseek, Neoprobe Corporation, Dublin, OH) (Fig. 1) was previously described.^{10,11} Good manufacturing practice (GMP)-grade tilmanocept was provided by Neoprobe and was radiolabeled with ^{99m}Tc at each study site's nuclear medicine department or a commercial radiopharmacy; radiolabeling yield of >97% was required.

Clinical Study Sites and Study Design

This phase 2, single-arm, open-label, single-dose study was undertaken in five U.S. sites. Before study initiation, the study protocol, informed consent forms, and other documentation were approved by institutional review boards of each site. The study was conducted in accordance with regulatory and ethical standards of U.S. IND regulations (21 CFR 56) and the most recent guidelines of the Declaration of Helsinki.

The primary objective of the study was to determine the preoperative and intraoperative lymphoscintigraphic (LS) localization of LNs in lymphatic pathways draining the primary site of melanoma or breast cancer with ^{99m}Tc-tilmanocept used as a radiotracer. Patients who met all of the inclusion and none of the exclusion criteria were enrolled sequentially. All patients received 50 µg (2.6 nmol) of ^{99m}Tc-tilmanocept, radiolabeled with either 0.5 mCi for same-day surgery or 1.0 mCi for next-day surgery, injected in close proximity to the primary tumor, followed by ILM. The specific route and volume of injection varied according to specific tumor type and location, and the interval between injection and ILM ranged from 15 minutes to 24 hours, depending on the scheduling of the surgery. Preoperative evaluation of the patient followed normative practice and could include LS. VBD injection at surgery was optional as an ILM aid.

The main efficacy parameter was the localization of ^{99m}Tc-tilmanocept in regional SLNs. During surgery, a handheld gamma detection probe was used to detect ^{99m}Tc-tilmanocept. Radioactivity counts were recorded in vivo and ex vivo. A set of three 2-s counts was recorded in situ, and a separate set of three 2-s counts was recorded on the hand contralateral to the side of injection for establishing background gamma counts. A positive finding (localization) was defined as a mean LN gamma count greater than the quantity of three square roots of the mean background count (i.e., standard deviation) added to the mean background count (referred to as the 3σ rule or the 68–95–99.7 empirical rule).^{17–19} Any LN gamma count not meeting the 3σ rule was considered a nonlocalized finding. The mean count of the ex vivo LNs was then compared to the mean room background counts, and the same rule was used to determine a positive finding (gamma counts exceeding the threshold) for the ex vivo specimens. Probing of the resection bed was considered complete when all gamma counts were negative by the 3σ rule.

After completion of probing, the surgeon continued with visualization and palpation according to local practice to ensure that no grossly disease-positive LNs remained at the site of resection. The hypothesis that the localization rate (P) of ^{99m}Tc-tilmanocept is >80% for the lower confidence interval was used because recent clinical evidence suggests that the

detection rate of tumor-draining LNs approaches 90% when TcSC and/or VBD are used in patients with melanoma or breast cancer. Therefore, this study was designed to determine whether ^{99m}Tc -tilmanocept was at least as effective as imaging agents currently used in ILM.

Secondary objectives of the study included histopathological assessment of the resected LNs to confirm histology and determine the presence or absence of tumor metastases, and the evaluation of safety (adverse event signals and patterns; AEs). Patients were assessed for safety before surgery and at 24 hours, 14 days, and 30 days after ^{99m}Tc -tilmanocept injection. Safety was assessed through the observation of AEs, routine clinical laboratory tests, electrocardiograms, vital signs, and physical examination findings.

Statistical Analysis

All analyses of efficacy were based on the evaluable patient population ($n = 78$); this population included patients who received an injection of ^{99m}Tc -tilmanocept and completed intraoperative gamma probe survey. Patients with major violations per protocol, e.g., patients with missing primary data, were also excluded. The primary efficacy parameter was the in vivo localization rate (i.e., detection rate) of ^{99m}Tc -tilmanocept in tumor-draining LNs as defined by the 3σ rule. The localization rate was calculated on a per-patient basis and was defined as the number of patients with at least one ^{99m}Tc -tilmanocept-identified LN (as confirmed by histology) divided by the total number of evaluable patients. Tissue samples identified during surgery as tumor-draining LNs that were either gamma-count negative or were identified as non-LNs were considered a negative finding. For the overall localization rate per patient, a 95% exact binomial confidence interval was constructed for the hypothesis, $H_0: P \leq 0.80$ vs. $H_a: P > 0.80$.

Additional exploratory analyses were undertaken to examine the localization rate of ^{99m}Tc -tilmanocept by tumor type. Secondary measures of efficacy were based on the proportion of patients for whom the excised LNs were both ^{99m}Tc -tilmanocept-positive and positive for tumor metastases in the LNs as confirmed by histopathologic evaluation. This contrast was based on the evaluable patient population. Measures of mapping sensitivity were calculated on a per-tissue and per-patient basis to assess the performance of ^{99m}Tc -tilmanocept in detecting LNs with occult tumor.

Safety analyses included all patients injected with ^{99m}Tc -tilmanocept, regardless of post- ^{99m}Tc -tilmanocept injection surgical status.

RESULTS

A total of 84 patients with breast cancer or melanoma were enrolled onto the study from August 2006 to August 2007, of whom 80 were administered ^{99m}Tc -tilmanocept. These 80 patients comprised the safety population (for AE compilation) and included 49 patients with primary melanoma and 31 patients with breast cancer (Tables 1, 2). Of the safety population patients, 78 were evaluated for efficacy (evaluable patient population); demographics and clinical staging are listed in Table 3.

The primary objective of this phase 2 clinical study was to determine the pre- and intraoperative tilmanocept localization of ^{99m}Tc -tilmanocept in LNs in the lymphatic pathway or pathways draining the primary site of melanoma and breast cancer. Whole-body scans after injection of ^{99m}Tc -tilmanocept and before surgery were performed for 55 of the 78 evaluable patients (Fig. 2; Table 4). Of those patients for whom LS was performed, a ^{99m}Tc -tilmanocept hot spot was located on the scans of 52 patients (94.5%); in 3 patients, a hot spot did not localize in a preoperative scan.

Intraoperatively, ^{99m}Tc -tilmanocept showed a per-patient localization rate (P), identifying at least one tissue sample in 75 (96.2%) of 78 patients (95% exact binomial confidence interval, 89.2–99.2; $P < 0.0001$) (Tables 4, 5). The rates were similar across the two diseases: ^{99m}Tc -tilmanocept localized in 46 (97.9%) of 47 (95% exact binomial confidence interval, 88.7–99.9; $P < 0.0004$) melanoma patients and 29 (93.5%) of 31 (95% exact binomial confidence interval, 78.6–99.2; $P < 0.0374$) breast cancer patients. Of those patients with ^{99m}Tc -tilmanocept localization, 64.2% had 1–2 gamma-count-positive LNs; 32% of the remaining patients had ≥ 3 LNs identified by ^{99m}Tc -tilmanocept. The average number of ^{99m}Tc -tilmanocept-identified LNs per patient over both malignancies was 2.22 (2.30 LNs per melanoma patient; 2.10 LNs per breast cancer patient).

In addition to high per-patient localization rates, ^{99m}Tc -tilmanocept also exhibited high mapping sensitivity (resected tissues) and tissue specificity (LNs). Of the 182 resected tissues surveyed by histopathology, 173 were ^{99m}Tc -tilmanocept identified in vivo (95.1% sensitivity, total LNs removed), and all were subsequently determined to be LNs (100% tissue specificity) (Table 5). The secondary efficacy objective was the assessment of the resected LNs to confirm the presence or absence of tumor metastases. Of the 110 tissue specimens received and assessed for disease in tissues from melanoma patients, 18 (16.4%) contained tumor, and specimens were confirmed to be LNs. Of 72 tissue specimens received and assessed for disease in tissues from breast cancer patients, 9 (12.5%) contained tumor, and all specimens were confirmed to be LNs. The overall proportion of ^{99m}Tc -tilmanocept-positive LNs containing metastatic disease was 13.7%: 15.5% from melanoma patients and 11.1% from breast cancer patients. One melanoma patient with a disease-positive LN did not have radioactive counts recorded in vivo; therefore, that LN is not included in the ^{99m}Tc -tilmanocept-identified LN gamma count where such LNs may be positive for disease. In a single breast cancer patient, one disease-positive LN was not identified by ^{99m}Tc -tilmanocept; this patient additionally had two tumor-negative LNs removed that were also not localized by ^{99m}Tc -tilmanocept. However, of the 26 disease-positive tissues with intraoperative gamma counts recorded, 25 (96.2%) were effectively identified by ^{99m}Tc -tilmanocept (Table 5). The remaining 7 of 182 tissue specimens that were removed but not identified by ^{99m}Tc -tilmanocept were all negative for metastases.

An exploratory data analysis was conducted to evaluate the effect that time between injection and surgery had on ^{99m}Tc -tilmanocept localization. ^{99m}Tc -tilmanocept had a 96.8% localization rate (60 of 62) in same-day surgery patients, versus a 93.8% localization rate (15 of 16) in next-day surgery patients (Table 4; $P > 0.05$, not significant). Additional gamma-count-positive specimens were identified for same-day surgery (135 of 141, 95.7%) than next-day surgery (38 of 41, 92.7%) (Table 5). In melanoma patients, the injection-to-surgery times had no effect on the performance of ^{99m}Tc -tilmanocept, as there was 97.7% (85 of 87) localization rate seen in the same-day group and 100% (23 of 23) in the next-day group (by positive specimens). In breast cancer patients, those who had surgery on the same day had a 92.6% (50 of 54) localization rate; however, those who had surgery the next day showed an 83.3% (15 of 18) localization rate. The rate difference in the breast cancer patients was attributed to the small number of patients in the next-day group (9 of 31).

The study design did not prospectively dictate or exclude the use of VBD, and its use was discretionary. As such, 57 patients (73.1%) were also evaluated with VBD (Table 4). An additional exploratory review of available operative and pathology reports was conducted for the concordance (coidentification of a LN by two agents) of ^{99m}Tc -tilmanocept hot LNs and VBD blue LNs in these patients. This analysis indicated a concordance rate of 94.4% for ^{99m}Tc -tilmanocept hot LNs that also contained blue dye. Conversely, the rate for concordance of VBD with ^{99m}Tc -tilmanocept was 86.7%.

As part of the drug safety analysis, AEs were monitored in all patients who received an injection of ^{99m}Tc -tilmanocept ($n = 80$). Overall, 37 patients (46.3%) experienced one or more AEs during the study. No patients experienced AEs that were considered probably or definitely related to study drug. Only 3 patients (3.8%) experienced AEs possibly related to study drug. Adverse events were generally mild to moderate (grade 1–2) in severity. Overall, 5 patients (6.3%) experienced AEs that were considered serious, but all were resolved and were unrelated to the study drug. These procedure-related serious AEs (with cause) included: fever (infection), bladder perforation (surgical, melanoma wide excision), vascular injury (surgical), pneumothorax (surgery reconstruction, infusion port placement), and wound hematoma (surgical). There were no deaths during the study, and no patients were withdrawn from the study as a result of an AE.

DISCUSSION

The results from this study indicate that ^{99m}Tc -tilmanocept accurately identifies tumor-draining LNs in melanoma and breast cancer patients (100% tissue specificity). The statistical assessment of the performance of ^{99m}Tc -tilmanocept exceeded the prospectively established assessments for both malignancies where these thresholds reflected the current state of SLN practice during the conduct of this trial. Moreover, the performance numbers, even by current standards that assume that the practice of SLN biopsy is improved, meets or exceeds the level of this surgical standard. ^{99m}Tc -tilmanocept identified at least one LN in 96.2% of the patients, greatly exceeding the prospective statistical end point ($P < 0.0001$). In addition, the localization rates between melanoma (97.9%) and breast cancer (93.5%) were not statistically significantly different, which suggests that ^{99m}Tc -tilmanocept's ability to identify LNs is not disease specific. Of the resected tissues surveyed and confirmed to be LNs, 95.1% were identified by ^{99m}Tc -tilmanocept. These results demonstrate that ^{99m}Tc -tilmanocept is highly effective in identifying LNs that have the highest potential for containing metastases (i.e., SLNs) in melanoma and breast cancer patients. Secondary analyses in this study addressed the presence or absence of tumor metastases in ^{99m}Tc -tilmanocept-identified LNs. The overall proportion of ^{99m}Tc -tilmanocept-positive nodes containing metastatic disease (13.7%) was in alignment with previous studies and was not statistically significantly different between the two malignancies.^{20–22} Additionally, 96.2% of the intraoperatively counted, disease-positive tissues (95% of those patients with nodal metastases) in this study were identified by ^{99m}Tc -tilmanocept.

This study's findings provide strong evidence that ^{99m}Tc -tilmanocept identifies the SLN by, first, the overall localization rate of disease-positive LNs is in agreement with published figures, and second, a high concordance between VBD and ^{99m}Tc -tilmanocept (localization of ^{99m}Tc -tilmanocept and VBD to the same SLN). Additionally, analysis of injection-to-surgery time suggests that there is no difference in ^{99m}Tc -tilmanocept's ability to localize LNs between same-day and next-day surgery for either melanoma or breast cancer patients; this feature allows for greater flexibility in hospital scheduling. With regard to safety, previous nonclinical and phase 1 studies of Tilmanocept indicate no safety concerns related to ^{99m}Tc -tilmanocept, and no clinically important drug-related events were identified in this study.^{12–16,23–26}

Last, tilmanocept has the potential to be the first and only FDA-approved biomarker-targeted radiolabeled ILM agent, affording enhanced specificity and sensitivity for more reliable mapping of lymphatic structures. SLN mapping is a procedure used worldwide, and adoption of ^{99m}Tc -tilmanocept has the potential to impact SLN mapping in all international medical venues. In the United States, there were 209,060 new cases of breast cancer in 2010, and 1.38 million women were diagnosed with breast cancer worldwide in 2008.^{27–29} The highest rates were in Europe, with 420,800 new cases in 2008. Additionally, there were

197,400 new cases of malignant melanoma diagnosed worldwide, with 68,130 new cases in the United States and 84,000 in Europe. In the United States and the European Union, 85% to 95% of all breast cancer or melanoma cases are estimated to undergo curative surgeries that use ILM procedures.

Tilmanocept is currently undergoing phase 3 clinical evaluation in breast cancer, melanoma, and head and neck squamous-cell carcinoma patients. Preliminary analysis of combined data of melanoma and breast cancer patients from the single completed phase 3 study data has shown highly statistically significant positive results.^{30,31} The preliminary phase 3 results are consistent with our phase 2 study results and suggest that prospective drug design of an ILM agent based on receptor targeting provides a novel and clinically valuable approach to support the diagnostic paradigm of ILM as an adjunct to melanoma and breast cancer surgery.

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FIG. 1.
Structure of Tilmanocept (DTPA mannosyl dextran)

FIG. 2.

Representative dynamic lymphoscintigraphy (LS) after injection of ^{99m}Tc -tilmanocept. A melanoma patient underwent LS approximately 5 min after injection of 0.8 mCi-labeled ^{99m}Tc -tilmanocept before same-day surgery. One hot spot (right axilla) was located in the dynamic whole-body scan. Intraoperatively, one sentinel lymph node was localized (right axilla) by ^{99m}Tc -tilmanocept in vivo and was determined to be positive for melanoma disease

TABLE 1

Analysis of patient populations

Characteristic	Melanoma	Breast cancer	Overall
No. enrolled	52	32	84
^{99m} Tc-tilmanocept treated, <i>n</i> (%) (safety population) ^a	49 (94.2%)	31 (96.9%)	80 (95.2%)
Evaluable patients, <i>n</i> (%) ^b	47 (90.4%)	31 (96.9%)	78 (92.9%)

^aFour patients who consented for study participation were excluded from the safety population analysis because they did not receive an injection of ^{99m}Tc-tilmanocept

^bTwo patients were excluded from the evaluable patient population. One patient did not receive intraoperative survey of the tumor site, and one patient had only one tissue specimen removed during surgery that was histologically confirmed to be nonlymphoid tissue

TABLE 2**Patient inclusion and exclusion criteria**

<i>Inclusion criteria</i>
• Identifiable primary melanoma or breast cancer, known or suspected   • Candidate for surgical intervention, with lymph node mapping being a part of the surgical plan   • Clinical negative node status at the time of study entry   • Nodal status a key factor in the decision to receive additional therapy   • Eastern Cooperative Oncology Group performance status of grade 0 or 1, or a corresponding Karnofsky ambulatory rating   • Female patients with a negative pregnancy test within 72 hours of administration of ^{99m}Tc-tilmanocept, has been surgically sterilized, or has been postmenopausal for at least 1 year   • At least 18 years of age
<i>Exclusion criteria</i>
• Clinical or radiological evidence of metastatic cancer   • Currently enrolled onto another clinical study or scheduled to receive experimental therapy during active participation in this study   • Pregnant or lactating

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TABLE 3

Demographic characteristics and clinical staging for all evaluable patients

Demographic variable	Category	Melanoma (<i>n</i> = 47)	Breast cancer (<i>n</i> = 31)	Overall (<i>n</i> = 78)
Age (years)	Mean (SD)	54.8 (14.62)	54.7 (10.86)	54.7 (13.18)
	Median	55.0	52.0	52.5
	Min, Max	22, 84	37, 89	22, 89
Sex, <i>n</i> (%) ^a	Male	28 (59.6%)	0 (0.0%)	28 (35.9%)
	Female	19 (40.4%)	31 (100.0%)	50 (64.1%)
Race, <i>n</i> (%) ^a	White	45 (95.7%)	28 (90.3%)	73 (93.6%)
	Black	1 (2.1%)	3 (9.7%)	4 (5.1%)
	Asian	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Other	1 (2.1%)	0 (0.0%)	1 (1.3%)
Clinical TNM staging, <i>n</i> (%) ^{a,b}	TX	0 (0.0%)	1 (3.2%)	1 (1.3%)
	Tis	0 (0.0%)	2 (6.5%)	2 (2.6%)
	T1	19 (40.4%)	20 (64.5%)	39 (50.0%)
	T2	16 (34.0%)	5 (16.1%)	21 (26.9%)
	T3	4 (8.5%)	1 (3.2%)	5 (6.4%)
	T4	8 (17.0%)	1 (3.2%)	9 (11.5%)
	N0	46 (97.9%)	27 (87.1%)	73 (93.6%)
	N1	1 (2.1%)	2 (6.5%)	3 (3.8%)
	N2	0 (0.0%)	0 (0.0%)	0 (0.0%)
	N3	0 (0.0%)	2 (6.5%)	2 (2.6%)
	N4	0 (0.0%)	0 (0.0%)	0 (0.0%)
	M0	47 (100.0%)	21 (67.7%)	68 (87.2%)
	MX	0 (0.0%)	10 (32.3%)	10 (12.8%)
	ECOG performance status, <i>n</i> (%)			
	0	45 (95.7%)	30 (96.8%)	75 (96.2%)
	1	2 (4.3%)	1 (3.2%)	3 (3.8%)
	2	0 (0.0%)	0 (0.0%)	0 (0.0%)
	3	0 (0.0%)	0 (0.0%)	0 (0.0%)
	4	0 (0.0%)	0 (0.0%)	0 (0.0%)

TNM tumor, node, metastasis system, *ECOG* Eastern Cooperative Oncology Group performance status^aThe denominator for all percentages is the number of evaluable patients^bOne breast cancer patient did not have tumor staging data available

TABLE 4

LS and intraoperative localization of ^{99m}Tc -tilmanocept in all evaluable patients

Characteristic	Patient population, <i>n</i> (%)		
	Melanoma (<i>n</i> = 47)	Breast cancer (<i>n</i> = 31)	Overall (<i>n</i> = 78)
Patients with LS performed ^a			
Yes	46 (97.9%)	9 (29.0%)	55 (70.5%)
No	1 (2.1%)	22 (71.0%)	23 (29.5%)
Patients with hot spot located on scan ^b			
Yes	45 (97.8%)	7 (77.8%)	52 (94.5%)
No	1 (2.2%)	2 (22.2%)	3 (5.5%)
Surgery time disposition ^a			
Patients with same-day surgery	40 (85.1%)	22 (71.0%)	62 (79.5%)
Patients with next-day surgery	7 (14.9%)	9 (29.0%)	16 (20.5%)
^{99m}Tc -tilmanocept localization by patient			
All patients (weighted) ^a	46 (97.9%)	29 (93.5%)	75 (96.2%)
Patients with same-day surgery ^c	39 (97.5%)	21 (95.5%)	60 (96.8%)
Patients with next-day surgery ^c	7 (100.0%)	8 (88.9%)	15 (93.8%)
Patients with no. of ^{99m}Tc -tilmanocept-positive lymph nodes ^{a,d}			
NA	1 (2.1%)	0 (0.0%)	1 (1.3%)
0	0 (0.0%)	2 (6.4%)	2 (2.6%)
1	16 (34.0%)	9 (29.0%)	25 (32.1%)
2	15 (31.9%)	10 (32.3%)	25 (32.1%)
3	7 (14.9%)	4 (12.9%)	11 (14.1%)
> 3	8 (17.0%)	6 (19.4%)	14 (17.9%)
Average no. of ^{99m}Tc -tilmanocept-positive lymph nodes per patient ^e	2.30	2.10	2.22
Patients with use of blue dye ^a			
Yes	34 (72.3%)	23 (74.2%)	57 (73.1%)
No	13 (27.7%)	8 (25.8%)	21 (26.9%)

LS lymphoscintigraphy, NA not available

^aThe denominator for percentages calculated is number of evaluable patients^bThe denominator for percentages calculated is number of patients for whom LS was performed^cThe denominator for percentages calculated is number of patients in corresponding time of surgery group^dOne melanoma patient did not have available nodal data (NA)^eDenominator for average nodes is number of evaluable patients

TABLE 5

Histology and pathology results in all evaluable patients

Characteristic	Patient population		
	Melanoma (<i>n</i> = 47)	Breast cancer (<i>n</i> = 31)	Overall (<i>n</i> = 78)
No. of tissue specimens surveyed and assessed by pathology	110	72	182
Specimens confirmed to be lymph nodes by histology, ^a <i>n</i> (%)	110 (100.0%)	72 (100.0%)	182 (100.0%)
^{99m} Tc-tilmanocept-localized lymph nodes (positive by 3 σ gamma count), ^a <i>n</i> (%)	108 (98.2%)	65 (90.3%)	173 (95.1%)
Lymph nodes from same-day surgery patients ^b	85/87 (97.7%)	50/54 (92.6%)	135/141 (95.7%)
Lymph nodes from next-day surgery patients ^b	23/23 (100.0%)	15/18 (83.3%)	38/41 (92.7%)
No. of patients with ^{99m} Tc-tilmanocept-localized lymph nodes	46	29	75
Localization rate ^c	0.979	0.935	0.962
95% exact binomial confidence interval	(0.887–0.999)	(0.786–0.992)	(0.892–0.992)
<i>P</i> value ^d	0.0004	0.0374	\ 0.0001
Lymph nodes with tumor present, ^a <i>n</i> (%)			
Yes	18 (16.4%)	9 (12.5%)	27 (14.8%)
No	92 (83.6%)	63 (87.5%)	155 (85.2%)
^{99m} Tc-tilmanocept localization pathology findings by lymph nodes, ^{a,e} <i>n</i> (%)			
Probe positive, disease positive	17 (15.5%)	8 (11.1%)	25 (13.7%)
Probe positive, disease negative	91 (82.7%)	57 (79.2%)	148 (81.3%)
Probe negative, disease positive	0 (0.0%)	1 (1.4%)	1 (0.6%)
Probe negative, disease negative	1 (0.9%)	6 (8.3%)	7 (3.8%)
Probe (NA), disease positive	1 (0.9%)	0 (0.0%)	1 (0.6%)
Probe (NA), disease negative	0 (0.0%)	0 (0.0%)	0 (0.0%)
^{99m} Tc-tilmanocept localization pathology findings by patient, ^f <i>n</i> (%)			
All nodes probe negative, but with ≥ 1 node probe negative, disease positive	0/12 (0.0%)	1/9 (11.1%)	1/21 (4.8%)
Any nodes probe positive, but also ≥ 1 node probe negative, disease positive	0/12 (0.0%)	0/9 (0.0%)	0/21 (0.0%)

“Palpable masses” are not included in the efficacy assessment as lymph nodes NA not available

^aThe denominator for percentages calculated is the number of tissue specimens

^bThe denominator for percentages calculated is the number of specimens in corresponding time of surgery group

^cThe denominator for rates calculated is the number of evaluable patients

^dThe *P* value represents the localization rate (*P*) hypothesis, $H_0: P \leq 0.80$, $H_a: P > 0.80$ for the lower limit of the confidence interval

^eLocalization with gamma detection probe by 3 σ count

^fThe denominator for percentages calculated is the number of patients with corresponding pathology-positive nodes