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Research Article

A Prospective Double-Blinded Randomized Controlled Trial Comparing the Intraoperative Injection of Technetium Tc 99m Tilmanocept with Technetium Tc 99m Sulfur Colloid in Breast Cancer Lymphatic Mapping

Anyul Ferez-Pinzon,[1] **Samuel L. Corey**,[2] **Haresh Rochani**,[3] **Elena A. Rehl**,[4] **and William E. Burak Jr**[5]

[1]*Department of Surgery, Memorial Health University Medical Center, Savannah, GA, USA*
[2]*Department of Surgery, Division of Plastic Surgery, University of Louisville, Louisville, KY, USA*
[3]*Department of Biostatistics, Epidemiology, and Environmental Health Sciences, Georgia Southern University, Statesboro, GA, USA*
[4]*Department of Surgery, Good Samaritan Medical Center, West Palm Beach, FL, USA*
[5]*Department of Surgery, Mercer University College of Medicine, Memorial Health University Medical Center, Savannah, GA, USA*

Correspondence should be addressed to William E. Burak Jr; william.burak@hcahealthcare.com

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Introduction. Technetium-labeled sulfur colloid (TSC) is a radiolabeled mapping agent commonly used for sentinel lymph node biopsy (SLNBx). Tilmanocept, a CD206 receptor-targeted mapping agent, has gained recent popularity due to potential advantages of rapid and quick uptake to the SLNs. The objectives of this study were to assess (1) the difference in the number of SLNs harvested using tilmanocept versus TSC and (2) the difference in time to transcutaneous localization when using an intraoperative injection approach. *Methods*. Patients undergoing breast conservation and SLNBx were consented and randomized to receive either 0.5 mCi of filtered TSC or 0.5 mCi of tilmanocept injected intradermally immediately after induction of anesthesia. Axillary transcutaneous gamma detector probe counts were taken at 1-minute intervals until a hot spot was identified. SLNs were then identified and excised. Additional nodes were excised if their counts per second (cps) were greater than 10% of the cps of the hottest SLN. The number of SLNs was based on both number of nodes collected intraoperatively and the number recorded in the final pathology report. *Results*. The study population consisted of 86 patients, 48 randomized to tilmanocept and 38 to TSC. There were no significant differences in patient or tumor characteristics between the two groups. Localization rates were 100% for both cohorts. The mean number of SLNs identified and removed was not significantly different ($p = 0.34$, intraoperatively; $p = 0.57$, pathology reported). Time to transcutaneous localization was 3.3 ± 2.0 minutes for tilmanocept and 3.9 ± 2.3 minutes for TSC ($p = 0.19$). The average cps for the hottest node was $2{,}180.0 \pm 2{,}460.5$ in the tilmanocept group compared to $2{,}679.3 \pm 2{,}687.5$ in the TSC group ($p = 0.94$). *Conclusion*. There was no significant difference in the number of SLNs harvested or in the time to transcutaneous localization when using tilmanocept versus TSC as the radiolabeled mapping agents for intraoperative injection and mapping. Either agent can be used without any significant difference in performance.

1. Introduction

The goal of advancement in the care of breast cancer has focused on improving outcomes while reducing the side effects of treatment. An example of this is the use of intraoperative lymphatic mapping (ILM) and SLNBx for clinically node negative patients [1]. This will spare most patients from an axillary node dissection and its associated comorbidities [2]. Lymphatic mapping agents most commonly consist of injected radiotracers with or without blue dye. Filtered TSC is approved in the US for ILM in breast cancer [3]. These colloidal agents migrate to the draining axillary SLN and are subsequently detected by a hand-held gamma detector probe during the operation. They can be injected into the affected breast in various manners prior to the operation [3]. Most experience has been with a preoperative injection (2–24 hours prior to surgery), although recent studies have shown that intraoperative injection has comparable localization and accuracy rates [4, 5]. Blue dye is often also injected intraoperatively since it has rapid uptake and can be visualized in the lymphatic channels and nodes, aiding in the identification of the SLN.

A low-molecular weight mannose receptor-based, reticuloendothelial cell-directed, ^{99m}Tc-labeled lymphatic imaging agent named ^{99m}Tc tilmanocept (tilmanocept) was developed as an alternative to TSC [6]. This compound is directed towards reticuloendothelial cells located in the SLNs, with studies showing superior performance compared to TSC and labeled albumin [7]. It is a small synthetic molecule that binds to mannose receptors (CD206) expressed on reticuloendothelial cells within lymph nodes leading to its accumulation in lymphatic tissue [7]. Tilmanocept has been shown to have superior localization rates compared to labeled albumin and has been shown to have nearly 100% tissue specificity [7, 8]. In addition, when used as a preoperatively injected mapping agent for early-stage breast cancers, tilmanocept has been shown to result in a decrease in the number of SLNs removed when compared to TSC [9].

The objectives of this study were (1) to assess the performance of tilmanocept versus TSC as measured by the difference in the number of SLNs harvested and (2) to assess the difference in time to transcutaneous localization when using an intraoperative injection approach for both agents.

2. Methods

2.1. Patient Selection. This IRB-approved study (clinical study registration number NCT03199560) was a double-blinded, randomized controlled trial comparing tilmanocept to TSC as intraoperative radiolabeled mapping agents in female patients with early-stage breast cancer undergoing breast conserving surgery with SLNBx. Patients were screened, consented, and randomized to either tilmanocept or TSC. Patients were eligible if they were older than 18 years of age with biopsy proven invasive breast cancer, were clinically node negative (cN0) by physical exam, and scheduled to undergo partial mastectomy with SLNBx. Mastectomy patients were not included because one of the goals was to identify SLNs immediately after injection of radiotracer to determine time to first node excision. Including total mastectomy patients would have necessitated a separate axillary incision just for study purposes as it is the surgeons' practice to perform the SLNBx through the mastectomy incision. Axillary ultrasound and selective core needle biopsy were used routinely to exclude patients with suspicious nodes. All individuals involved in the study were blinded to the treatment arm of each patient.

2.2. Intraoperative Injection and Surgery. Injection of the study agents and the subsequent operation were both performed by one of two breast surgeons at a single institution. Both radiotracers were injected intraoperatively using the same delivery device and volumes. Tilmanocept was prepared in two syringes, 0.250 mCi at 0.1 mL each, totaling 0.500 mCi; needle size was 28 g. TSC was prepared in two syringes, 0.250 mCi at 0.1 mL each, totaling 0.500 mCi; needle size was 28 g.

Following induction of general anesthesia, each patient received two intradermal injections to the lateral and inferior edges of the areola [10, 11]. After patients were injected with the radiotracer (Time 0), transcutaneous probing of the axilla using a gamma detector probe (Neoprobe GDS^{TM}), measuring counts per second, was performed at 1-minute intervals continuing until a "hot spot" was detected. A "hot spot" was defined as an area of increased radioactivity in the axilla with a fall-off in radioactive counts in adjacent tissues. If a "hot spot" was detected by the gamma detector probe within the first 5 minutes, patients were injected with methylene blue (5 ml–10 ml) in the subareolar space at the time of detection, the breast was massaged, patients were immediately prepped and draped and the SLNBx was performed. If a "hot spot" was not detected within the first 5 minutes, patients were injected with methylene blue in the subareolar space immediately after the 5-minute count was obtained, massaged, and then prepped and draped. Transcutaneous probing and counting continued at 1-minute intervals until a "hot spot" was identified for a maximum of 20 minutes.

Once the axillary incision was made, the axilla was explored using the hand-held gamma detector. Once a radioactive nodes were detected, it was removed, labeled, and sent to pathology for H&E staining at 2 mm intervals. Immunohistochemistry was employed only if the pathologist requested it to confirm diagnosis of metastatic cells. Radioactive nodes were defined as nodes with counts per second (cps) greater than 10% cps of the hottest SLN. An "ex vivo" count was then taken of the SLNs and recorded. If the background radioactivity of the axilla was less than 10% of the cps of the hottest harvested SLN, the search for SLN was deemed complete. Additional nodes that were blue but not radioactive, or those deemed clinically suspicious per the operating surgeon were also excised. Once the SLNBx was completed, the partial mastectomy was performed.

2.3. Statistical Analysis. A power analysis was performed to determine the sample size. The sample size was determined based on the data presented in a previous study comparing

the two agents in a preoperative injection setting [9]. A standard deviation of 2.8 was used, a power of 0.90, and ? = 0.05 to determine the sample size. The minimum total sample size was estimated to be 86. A randomization table with a 150-patient sample size was generated using Microsoft Excel, and each patient was randomized to either treatment arm independently, which resulted in unbalanced groupings.

Descriptive statistics were used to compare patient demographics and pathologic characteristics between the two treatment arms. Distributions of demographic and clinical characteristics were compared for both groups using independent *t*-test, chi-square test, or Fisher's exact test. Categorical variables were summarized using frequencies and percentages, while statistics for continuous variables included mean, standard deviation, and minimum and maximum values.

The number of SLNs identified was compared using independent *t*-test based on the number of lymph nodes that were identified intraoperatively and the number of nodes identified based on the final pathology report. Time to localization was analyzed and compared based on time to transcutaneous identification, time to first node excised, and the time to the last node excised.

3. Results

The study population consisted of 86 women. There were 48 patients who were randomized to tilmanocept and 38 to TSC. Patient and tumor characteristics of both groups are shown in Table 1. There were no significant differences between any of the tumor or patient characteristics listed.

SLN localization rates were 100% for both cohorts. The mean number of SLNs identified and removed was not significantly different between the two groups, including when taking into consideration the number of nodes identified intraoperative versus based on final pathology results, as shown in Table 2. The mean number of SLNs identified intraoperatively with tilmanocept was 2.0 compared with 1.8 with TSC ($p = 0.34$). When looking at SLNs identified based on final pathology, mean number was 3.0 for tilmanocept compared to 2.8 with TSC ($p = 0.57$).

Time points were compared between treatment groups based on time to localization transcutaneously, time to excision of first SLN, and time to excision of last SLN. There was no significant difference in the time to localization of SLNs transcutaneously, in the time to first node excised, or in the time to last node excised (Table 3). Time to transcutaneous localization was 3.3 ± 2.0 minutes for tilmanocept and 3.9 ± 2.3 minutes for TSC ($p = 0.19$) (Table 3). Time to first SLN excision was 23.4 ± 4.8 minutes with tilmanocept and 23.7 ± 5 minutes for TSC ($p = 0.79$). Time to last SLN excision was 27.8 ± 7 minutes with tilmanocept and 27.2 ± 6 minutes with TSC ($p = 0.68$).

Although comparison between the average counts per second between radiotracers was not one of the study's objectives, the average counts were compared between both groups as part of the data analysis. There was no significant difference between the two study groups. The average cps for the hottest node was $2{,}180.0 \pm 2{,}460.5$ in the tilmanocept group compared to $2{,}679.3 \pm 2{,}687.5$ in the TSC group ($p = 0.94$).

4. Discussion

Lymph node status is one of the most significant prognostic factors for future systemic disease in patients with breast cancer [4, 7, 12, 13]. The use of SLNBx has become the standard method for axillary staging, however, this method still has a published false negative rate up to 10% and variation in the number of nodes removed based on technical factors that can sometimes lead to the removal of more nodes than necessary for accurate staging [9, 13]. An ideal mapping agent should have a high degree of accuracy for identifying the correct SLNs, minimally pass through non-SLNs, and have quick nodal uptake, so injections can be performed in the operating room to avoid the patient discomfort and logistical issues associated with a preoperative injection. There have been several studies that have reported high localization rates using intraoperative injection of TSC [5, 14]. This has led many institutions to adopt this protocol in an effort to address these issues. Tilmanocept to our knowledge has not been used in this setting.

Tilmanocept was developed in an effort to create an ideal lymph node imaging agent that could achieve superior targeting given its mechanism of action [15]. Developmental goals included rapid injection site clearance, which would not interfere with SLN identification, and binding properties that would limit drainage to more distal nodes. The molecule's size allows rapid lymphatic uptake and cell-specific binding to mannose receptors expressed on the surface of macrophage cells [11].

In this study, there was no significant difference in the number of SLNs either identified intraoperatively or based on final pathology between tilmanocept and TSC when used intraoperatively with transcutaneous probing. These results differ with the findings of other studies that did not use intraoperative injections, where the average SLNs removed when mapped with tilmanocept has been shown to be significantly lower compared to when using TSC as the radiotracer (3 or fewer nodes with tilmanocept compared to about 20% of patients having more than 4 nodes removed with TSC) [9]. We hypothesize that previous studies compared the agents using a preoperative injection where the increased time interval between injection and surgery led to more of a "pass-through effect" in the TSC patient population. This is evidenced by the observation that the mean number of TSC SLNs removed in the current study was 1.8 compared to 3.2 in the preoperative injection study [9].

In addition, there was no significant difference in the time to transcutaneous localization of SLNs in this study, in the time to excision of the first SLN, or in the time to excision of the last SLN when comparing it to TSC. There were very few patients with metastatic SLNs in this study, likely due to the use of pre-enrollment axillary ultrasound. Interestingly, the SLN uptake was rapid in both groups, with all patients in both groups localizing in less than 10 minutes. Therefore, even when using TSC as the radiotracer, which lacks tissue-

Table 1: Demographic and clinical data.

	Tilmanocept ($n = 48$)	TSC 2 ($n = 38$)	Total ($n = 86$)	p value
		Age (years)		
Mean±SD	66.3 ± 10.1	66.0 ± 10.7	66.2 ± 10.3	0.87
Range	(39.0–82.9)	(38.5–87.1)	(38.5–87.1)	
		BMI (kg/m^2)		
Mean± SD	30.7 ± 7.8	30.9 ± 7.5	30.8 ± 7.6	0.91
Range	(21.0–54.9)	(19.1–50.7)	(19.1–54.9)	
		Tumor size (cm)		
Mean± SD	1.1 ± 1.0	1.2 ± 0.8	1.1 ± 0.9	0.69
Range	(0.1–4.7)	(0.1–3.5)	(0.1–4.7)	
		Preoperative histology		
Ductal	37 (80.4%)	31 (83.8%)	68 (81.6%)	0.91
Lobular	5 (10.9%)	3 (8.1%)	8 (9.6%)	
Mixed	4 (8.7%)	3 (8.1%)	7 (8.4%)	
		Tumor grade		
1	13 (27.7%)	11 (29.7%)	24 (28.6%)	0.63
2	26 (55.3%)	17 (46.0%)	43 (51.2%)	
3	8 (17.0%)	9 (24.3%)	17 (20.2%)	
		LVI		
No	47 (97.9)	37 (97.4)	84 (97.7)	0.81
YES	1 (2.1)	1 (2.6)	2 (2.3)	
		Location		
UOQ	28 (58.3)	26 (26.4)	54 (62.8)	0.40
UIQ	12 (25.0)	7 (18.4)	19 (22.1)	
LOQ	5 (10.4)	1 (2.6)	6 (7.0)	
LIQ	1 (2.1)	3 (7.9)	4 (4.7)	
Subareolar	2 (4.2)	1 (2.60	3 (3.5)	
		T stage		
T0	1 (2.1)	1 (2.8)	2 (2.4)	1.0
T1	40 (85.1)	30 (83.3)	70 (84.3)	
T2	6 (12.8)	5 (13.9)	11 (13.3)	
		N Stage		
SLN neg	46 (95.83)	34 (89.47)	48 (55.81)	0.40
SLN POS	2 (4.17)	4 (10.53)	38 (44.19)	
		ER		
+	45 (93.8)	33 (86.8)	78 (90.7)	0.46
−	3 (6.2)	5 (13.2)	8 (9.3)	
		PR		
+	42 (87.5)	34 (89.5)	76 (88.4)	0.78
−	6 (12.5)	4 (10.5)	10 (11.6)	
		HER2		
+	2 (4.2)	3 (8.1)	5 (5.9)	0.65
−	46 (95.8)	34 (91.9)	80 (94.1)	

SD, standard deviation; BMI, body mass index; LVI, lymphovascular invasion; UOQ, upper outer quadrant; UIQ, upper inner quadrant; LOQ, lower outer quadrant; LIQ, lower inner quadrant; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

Table 2: Number of SLNs identified.

	Tilmanocept ($n = 48$)	TSC 2 ($n = 38$)	p value
	Intraoperative		
Mean± SD	2.0 ± 1.2	1.8 ± 0.9	0.34
Range	(1–6)	(1–4)	
	Based on pathology		
Mean± SD	3.0 ± 2.0	2.8 ± 1.7	0.57
Range	(1–9)	(1–7)	

SD, standard deviation.

specific binding properties, this study shows no compromise in the time to localization of the first SLN compared to tilmanocept.

One observation from the study's data is that the study groups were unbalanced due to the fact that our statical methodology called for the randomization of each patient

Table 3: Time to localization.

	Tilmanocept ($n = 48$)	TSC 2 ($n = 38$)	p value
	Transcutaneously (min)		
Mean± SD	3.3 ± 2.0	3.9 ± 2.3	0.19
Range	(1–9)	(1-9)	
	First node excised (min)		
Mean± SD	23.4 ± 4.8	23.7 ± 5.0	0.79
Range	(17–35)	(14–36)	
	Last node excised (min)		
Mean± SD	27.8 ± 7.0	27.2 ± 6.0	0.68
Range	(17–48)	(16–41)	

SD, standard deviation.

individually. Using that method can result in unequal number of patient's per group, as seen in our study, without compromising the power of the study.

A potential limitation of the study is that it was powered based upon the results of the study by Baker et al. [9], which showed a significant difference between the number of nodes localized after preoperative injection of tilmanocept (mean number of nodes = 1.85) or TSC (mean number of nodes = 3.24). Since the time of this study, more recent data suggest the average number of nodes reported for both agents in the Baker et al.'s [9] study might be somewhat skewed. A prospective study by Unkart et al. evaluated number of nodes localized after preoperative injection of tilmanocept and TSC as a secondary outcome and reported comparable means of 2.16 and 2.26, respectively [16]. Similarly, a retrospective study by Murphy et al., injecting preoperatively, reported comparable means of 2.41 and 2.57 for tilmanocept and TSC, respectively [17]. Even though the mean SLNs localized were close to those in the studies mentioned above, there is a possibility that waiting longer between injection and starting the operation could have led to more SLN localization, which could have affected the accuracy and false negative rate of our data. However, larger studies would be necessary to identify that effect on accuracy and false negative rate.

5. Conclusion

This study presents evidence that when using intraoperative injection protocols, there is no significant difference in the number of SLNs harvested nor the time to localize them when comparing TSC to tilmanocept; however, larger studies are necessary to further evaluate these and other performance metrics to truly determine which is the ideal mapping agent for intraoperative injection protocols.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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