Journal of Nuclear Medicine, published on October 9, 2020 as doi:10.2967/jnumed.120.252064

Sentinel Lymph Node Biopsy in breast cancer with 99mTc-Tilmanocept:

A novel tracer in the real life. A multicenter study

Sergi Vidal-Sicart1, María Eugenia Rioja2, Andrea Prieto3, Elena Goñi4, Isabel Gómez5, María Dolores Albala6, Luis Lumbreras7, Luisa Fernanda León8, José Ramón Gómez9, Francisco Campos1.

1 Hospital Clínic Barcelona, Nuclear Medicine department, Barcelona, Spain. svidal@clinic.cat

2 Hospital Universitario Ramón y Cajal, Nuclear Medicine department, Madrid, Spain.

3 Hospital Puerta de Hierro, Nuclear Medicine department, Madrid, Spain.

4 Complejo Hospitalario de Navarra, Nuclear Medicine department, Pamplona, Spain.

5 Hospital General Universitario Gregorio Marañón, Nuclear Medicine department, Madrid, Spain.

6 Hospital Universitario Reina Sofía, Nuclear Medicine department, Córdoba, Spain.

7 Hospital Regional Universitario de Málaga, Nuclear Medicine department, Málaga, Spain.

8 Hospital Rey Juan Carlos, Nuclear Medicine department, Madrid, Spain.

9 Hospital Torrecárdenas, Nuclear Medicine department, Almería, Spain.

Conflicts of interest and sources of funding: none declared.

Correspondence (and First author) to:

Dr. Sergi Vidal-Sicart. Hospital Clinic Barcelona. Nuclear Medicine Department. Villarroel 170. 08036 Barcelona, Spain. Phone +34 932275516. Fax number +34 934518137 E-mail: svidal@clinic.cat; ORCID: 0000-0002-6303-3606

Word count: 5650

Running Title: Multicenter breast cancer and Tilmanocept

ABSTRACT

Purpose: 99mTc-Tilmanocept is a novel radiopharmaceutical for SLN biopsy in breast cancer. The aim was to describe the results with 99mTc-Tilmanocept in a heterogeneous group of breast cancer patients scheduled for SLN biopsy. Methods: Radiotracer preparation followed the manufacturer's indications. Local protocols for SLN detection within 9 participant centers were not changed for the entire duration of the study. Three hundred and forty-four patients with T1-T4, N0-N2 breast cancer (352 lesions) were included. Superficial (intradermal-periareolar) or deep (peritumoral-intratumoral) injections were performed. The doses were adjusted depending on the scheduled time for surgery. Results: Lymphoscintigraphy was able to depict at least one SLN in 339 out of 352 breast lesions (96.3%); and intraoperative SLN detection rate reached 97.2%. On univariable analysis, SLN detection rates did not differ by age, clinical T or N stage, tumor location, histologic subtype or prior neoadjuvant therapy. Lymphoscintigraphy showed higher SLN detection in patients with normal weight (BMI<25) than in those with Overweight/Obesity (BMI ≥ 25); 99.2% *vs.* 94.6%, respectively ($p = 0.031$). The proportion of patients with preoperative lymphoscintigraphic detection and/or excised SLN was higher with superficial than deep injections. Reinjected cases were significantly lower when superficial injection was firstly chosen ($p<0.001$). Site of injection and tumor markers Her2 and RE demonstrated impact over preoperative SLN visualization and intraoperative localization. In 80 cases SLN resulted in a positive lymph node. After a mean follow-up of 19 months, no axillary recurrences have been observed. Conclusion: 99mTc-Tilmanocept showed good results whatever protocol was used in a heterogeneous breast cancer population, although the best results were achieved when a superficial injection was done.

Key words: Breast cancer, Sentinel node, Tilmanocept, lymphoscintigraphy, axillary staging

INTRODUCTION

Sentinel lymph node (SLN) biopsy is the standard of care in regional staging of clinically node negative breast cancer and other types of malignancies. This technique minimizes the extent of the surgery and therefore the associated morbidity(*1*). Several different confirmed methods for SLN mapping exist. One of them relies on a visual identification of lymph nodes after the administration of vital dyes or fluorescent tracers and the most widespread used is a molecular imaging visualization through preoperative lymphoscintigraphy using a radiotracer (lymphatic mapping). Those methods reported high success rates (>95%, when a radiotracer is used). However, sometimes the SLN technique can fail despite correct application and imaging techniques due to an unequal pathologic examination of the SLN or complete metastatic involvement of de SLN causing the injected tracer to bypass the infiltrated node(*1*).

99mTc-Tilmanocept, also known as Lymphoseek®, is a novel radiopharmaceutical specifically designed for lymphoscintigraphy and intraoperative SLN detection. It consists of a macromolecule of multiple units of diethyenetriaminepentaacetic acid and mannose, each attached synthetically to a dextran backbone. It accumulates in lymphatic tissue by avidly binding to mannose receptors (CD206) expressed on macrophages and dendritic precursor cells within lymph nodes(*2*). It was approved in USA by Food and Drug Administration for lymphatic mapping in 2013 and by the European Agency of Medicament (EMA) in 2014 (*3,4*). The small molecular size (7 nm diameter) of 99mTc-Tilmanocept permits rapid injection site clearance and its specific targeting to CD206 mannose-binding receptors allows avid, stable binding within target nodes. Given that 99mTc-Tilmanocept uptake in lymph nodes does not depend of the particle size might offer benefits over the others used radiocolloids (5,6) and exhibits other advantageous properties such as rapid clearance from injection site and low distal node accumulation and detection of sentinel nodes in close proximity to the injection site and high SLN uptake with low leakage to higher echelon nodes(5,7).

99mTc-Tilmanocept is indicated for imaging and intraoperative detection of SLNs draining a primary tumor in adult patients with breast cancer, melanoma or localized squamous cell carcinoma of the oral cavity(*8*). In breast cancer, 99mTc-Tilmanocept established use is for patients with N0 disease undergoing primary surgery. The recommended dose to administer is 50 microg 99mTc-Tilmanocept with a total tracer activity 18.5 MBq for same day surgery or 74 MBq for next day surgery (doses should not be adjusted for body weight)(*8*).

The aim of this study is to describe and analyze the outcomes in a real clinical practice of SLN biopsy in breast cancer in different centers using 99mTc-Tilmanocept as radiotracer.

MATERIALS AND METHODS

99mTc-Tilmanocept radiopharmaceutical preparation

The vial components of the kit are sterile, non-pyrogenic, and contains 50 micrograms of Tilmanocept. For radiotracer preparation it is recommended to use only eluate from a technetium generator which was previously eluted within 8 hours. In order to obtain the highest radiochemical purity, it must be reconstituted with freshly eluted 99mTc. The Tilmanocept powder contained into the vial must not be vented to or during radiolabelling.

The recommended dose of 99mTc for labelling will depend on the intended use in one or two-day approach and will range, according with the Radiopharmacy Unit in Hospital Clinic Barcelona, from 30 to 140 MBq in 0.65 mL volume (for 0.5 mL dose). At this point, adhesion on the walls of vial, decay correction and dead volume was considered to compensate activity loss. After adding sodium pertechnetate 99mTc and sterile sodium chloride 9 mg/mL (0.9%) solution for injection to the radiolabelled product in the tilmanocept powder vial to bring the volume to the reconstituted vial volume of 0.65 mL solution, this preparation must be let stand at room temperature for at least 15 minutes. Radiochemichal purity of the radiolabelled product should be determined following the manufacturer's guide with Instant Thin Layer Chromatography (ITLC).

99mTc-Tilmanocept solution for injection should be used within 6 hours after reconstitution. Individual injection volumes should not exceed 0.5 mL or be less than 0.1 mL. Total injection volume should

be no greater than 1.0 mL and no less than 0.1 mL. Dilution of the product in volumes greater than 1.0 mL could affect the in vivo disposition of the product. For a complete dose retrieval from the vial it was advisable to use a spinal needle of 90 mm length in order to get the bottom edge of the vial, minimizing the dead volume. No clinical consequences were observed at dose levels of 3.7 times the recommended dose of Lymphoseek in humans (*4*).

SLN biopsy procedure

Local protocols for nine participant centers were not changed for the entire duration of the study. All patients underwent SLN detection technique with 99mTc-Tilmanocept using preoperative lymphoscintigraphy and intraoperative gamma probe and/or portable gamma camera where available.

The way to inject radiotracer depended on each center's protocol. In this study superficial (intradermal (105)/periareolar (80), n=185) or deep (peritumoral (47) /intratumoral (113), n=160) injections were performed. In seven cases, a combination of both (superficial and deep) were done.

The more used procedure was the 99mTc-Tilmanocept injection the day before surgery (two-day protocol) in 314 (89.2%) cases *vs* at the same day of the surgery (one-day protocol) in 38 (10.8%) cases. The doses administered were adjusted depending on the scheduled time for SLN biopsy. The median and interquartile range (IQR) dose used (reinjected patients are excluded) in two-day protocol was 92.5 (74-111) MBq, and in one-day protocol was 55.87 (48.1-63.08) MBq (table 1).

After radiotracer injection most centers performed two sets of images at 30 min and 2-4 hours p.i (early and late images). In some cases, later images (> 6 h) were acquired. A single- or dual-head gamma-camera system with large field -of-view (FOV) detectors was generally used to acquire planar emission (3-5 min each). A 57Co] or 99mTc flood source or a 57Co or 99mTc point source was used for delineation of the patient's body contour during scintigraphy. SPECT/CT acquisition was not mandatory, and it was left to the nuclear medicine physician discretion. When lymphatic drainage was not observed after late images, a second dose was administered (99mTc-Tilmanocept) or other lymphatic mapping tracer (99mTc -nanocolloid).

SLNs were defined as every node with a direct lymphatic channel from the tumor site. In cases where the channel was not visualized, the first lymph node that appeared on scintigraphy was assumed to be an SLN. After images were assessed by the nuclear medicine physician, SLN location was marked on the skin with a small spot of indelible ink.

During surgery, a hand-held gamma probe and/or portable gamma camera was used to check and locate the exact SLN position. Once all SLNs were removed, they were evaluated ex vivo using the probe to demonstrate radioactivity. The wound site was checked for remaining activity. On the other hand, after SLN retrieval guided by gamma probe, the open axilla should be palpated and suspicious lymph nodes harvested, even if these do not present radioactivity.

All SLNs and non-SLN excised specimens were sent for pathological examination. There were, several different protocols, as reported in the literature, and each center used those that fitted better with its capacity. Hence, in several centers SLNs were intraoperatively assessed using imprint cytology, frozen sectioning, or both, and more thoroughly after the operation. Others used molecular method based on a one-step nucleic acid amplification (OSNA).

Study design and data collection

This study was a retrospective multicenter analysis including patients from 9 different Spanish centers in which breast cancer cases were scheduled for SLN biopsy. All clinical scenarios where SLN biopsy is currently clinically applied in breast cancers patients were included (primary breast cancer without clinically node involvement, breast cancer recurrences, after neoadjuvant chemotherapy (with/without node involvement), male patients, after previous surgery…). We want to reflect that performing SLN biopsy with 99mTc-

Tilmanocept in "everyday's routine" does not affect the results obtained. So, every center was free to include the patients scheduled for SLN biopsy in consensus with its own breast committee.

Data concerning patient and tumor characteristics as well as the approach used were collected. A total of 344 patients (99.1% women, n= 341) with T1-T4, N0-N2 breast cancer were enrolled from 3/2018 to 10/2018. Of the 344 patients, 45 women had received previous neoadjuvant chemotherapy. Also, eight patients presented bilateral tumors, meaning that the total number of breast lesions assessed were 352 (Fig.1). The Institutional Review Board approved this study and all patients provided a signed informed consent.

It was anticipated that a few patients would not have a SLN in the lymphoscintigraphy and/or biopsy detection, therefore we want to examine the factors impacting failure to identify a SLN in both preoperative (lymphatic mapping findings) and intraoperative times (biopsy findings). In order to quantify the success of the technique, the following concepts were considered:

The image or preoperative Lymphoscintigraphy Detection Rate (LDR) and the intraoperative Biopsy Detection Rate (BDR) were referred to the proportion of patients with at least 1 SLN identified by lymphoscintigraphy or intraoperatively harvested, respectively, among all the breast lesions included. Sensitivity of SLN procedure was defined as the percentage of patients with positive SLN divided by all patients with positive lymph nodes(*9*). False Negative Rate (FNR) was defined as the percentage of a negative SLN result when the patient had positive lymph nodes in the regional axillary lymphadenectomy or in the follow-up period of the study(*10*). Given that lymphadenectomy was not performed in most patients, FNR was calculated based on the clinical follow-up, taking in consideration that it was short (until March 2020). Finally, we calculated the False Negative Detection Rate defined as the percentage of a positive pathology lymph node which were not radioactive. The statistical analysis was done with SPSS v25.0. Differences were considered significant when the value was <0.05.

RESULTS

A total of 344 patients (99.1% women, n= 341) with 352 breast lesions were included. The table 1 shows the protocols and reinjections performed with the doses administered in this study. The table 2 shows the patient and tumor characteristics. Two-hundred and forty-seven patients (71.8%) were 50 years old or older (the 3 men are included in this group). The patient group aged less than 50 years old showed a lower BMI (table 2). There were no statistical differences between age groups regarding pathological findings and tumor characteristics, except in Ki67 which was higher in <50 group (p=0.04, Mann–Whitney U test).

Following the 99mTc-Tilmanocept injection, regional lymphatic drainage was not observed in 35 cases and 33 of them were reinjected. After second dose, lymphatic drainage was achieved in 22/33 cases (66.7%) while in 11/33 (33.3%) of the reinjected patients it failed to visualize any drainage. Regarding radiotracer used in the second injection, 13 out of 33 lesions were reinjected with 99mTc-Tilmanocept and the remaining 20 with a nanocolloidal tracer (99mTc- nanocolloid). The number of cases in which the reinjection achieves the lymph node drainage was higher with 99mTc-Tilmanocept than with the nanocolloidal tracer, 76.9% (10/13) *vs* 60% (12/20) respectively. However, the difference was not statistically significant (p=0.314, χ^2). In summary, lymphoscintigraphy was able to depict at least one SLN in 339 out of 352 breast lesions (LDR_{total}: 96.3%; $LDR_{Lymphoseek}$: 92.9%) and BDR reach 97.2% (Fig. 2). On univariable analysis, SLN detection rates did not differ by age, clinical T stage, clinical N stage, tumor location, histologic subtype or prior neoadjuvant therapy. (Table 3)

Extraaxillary SLN (inner mammary chain and intramammary) were depicted in 29 cases (8%) and it was related to the way of injection (superficial 0- 4% LDR vs deep 11-14.2% LDR). The management of these SLNs were different depending on the centers. Five of them did not pursue any extraaxillary node where present. The remaining four tried to perform the biopsy and obtained a SLN in 75% of cases. Four cases depicted inner mammary chain SLNs only (without axillary tracer uptake), and it was possible to retrieve it in 3 cases.

Focusing on the forty-five women included in the study which had received previously neoadjuvant chemotherapy, Chi-square test does not show statistical differences in LDR (p=0.257), BDR (p-value=0.098) and positive pathology results (p=0.082) in comparation to patients without neoadjuvant therapy. The percentage of SLN detection by preoperative lymphoscintigraphy in patients with normal weight (BMI<25) was significantly higher than in those who presented Overweight/Obesity (BMI ≥ 25) (LDR: 99.2% *vs.* 94.6%, respectively; p= 0.031), however BMI did not affect to BDR (p= 0.325).

The variables which showed impact in both SLN detection rates were site of injection and tumor markers Her2 and RE. Regarding tumor Her2 and RE markers, the data showed a relationship between patients with Her2- and/or RE+ and better detection rates. Considering only the cases with Luminal or Her molecular subtype (so excluded basal like tumors) univariable analysis signaled that higher proportion of Luminal cancers get satisfactory results, with significantly better LDR and BDR (Luminal *vs* Her molecular subtype: LDR= 97.1% *vs* 88.2%, p-value=0.012; BDR= 98.5% *vs* 85.3%, p-value<0.001).

Both LDR and BDR resulted in higher values when superficial administration (intradermal or subareolar) vs deep administration (peritumoral or intratumoral) was used (LDR=98.8% vs 93.8%, p-value=0.009; BDR= 99.5% vs 95%, p-value=0.010). On the other hand, the reinjected cases were significantly lower when superficial injection was chosen than deep injections ($p<0.001$, χ^2).

The number of detected SLN by preoperative lymphoscintigraphy was 603 while the number of intraoperative detected SLN was 640 (table 4). The number of SLNs retrieved during surgery was coincident with SLN depicted on lymphoscintigraphy in 239 cases. Lymphoscintigraphy detected, however, a higher number of SLN than those intraoperatively harvested in 41 cases (11.65%), but in 72 cases (20.45%), the number of depicted SLNs on lymphoscintigraphy was lower than those retrieved during surgery. It is interesting to emphasize that in the 75.3% of the patients the number of excised SLN were 1-2 and focusing on breast lesions in which the biopsy was successful 77.5% obtained between 1-2 SLN (Fig. 3).

Concerning T-stage the SLN detection rate for lymphoscintigraphy was similar within T1-T3 groups. SLN was successfully depicted between 81 and 100% in T1-T3 and 50% in T4 tumors. Concerning intraoperative harvesting, SLN was excised in 96.7-100% in T1-T3 and 50% of cases in T4 patients. Regarding N-stage, 96.4% of N0 cases were successfully depicted with lymphoscintigraphy (LDR) and during surgery (BDR) 97.5% of cases were harvested. In N1 patients, LDR was 97.2% of N1-N2 cases with equal intraoperative detection rate (97.2). These differences were not statistically significative.

In 80 cases histopathologic SLN result resulted in a positive lymph node (table 2). In those patients the analysis showed 41 micrometastasis and 39 macrometastasis. Interestingly, only one metastatic lymph node was identified in a non SLN. Therefore, the sensitivity was 98.76% while the False Negative Detection rate was 2.3%. The percentage of positive SLN was moderately superior in patients below 50 years old (27%) versus those with more than 50 years (21%) but without statistically significance. The overall FNR, after a mean follow-up of 19 months (range 16-25) was 0% (no axillary recurrences).

DISCUSSION

Lymphatic mapping and SLN biopsy have demonstrated their accuracy for correctly staging those patients with clinically negative regional nodes. Lymphoscintigraphy is the only modality ascertaining the SLN location and lymphatic drainage. The main aim of lymphoscintigraphy is to provide a visual roadmap of nodal basins at risk before surgery showing the orderly progression of lymphatic flow reaching lymph node (*11*). Lymphoscintigraphy for SLN mapping must be reproducible and this issue has been properly assessed in different malignancies (*12-14*). There is a large radiotracer geographical variability and 99mTc-nanocolloids ranging from 15 to 100 nm are most widely used in Europe, while filtered and unfiltered 99mTc-sulfur colloid (particle size ranging from 20 nm to 1000 nm) is usually managed in the USA (*15*).

The evolving oncologic multidisciplinary approach has generated new indications for SLN (especially in breast cancer) that result in controversial issues and protocols between different researchers (*16*). Against this background a new radiotracer, designed specifically for the identification of SLNs, 99mTc-Tilmanocept, has become available in USA and Europe. This tracer presents distinct properties for SLN identification and lymphatic mapping with the potential to overcome the shortcomings described for the conventional radiotracers used until now for SLN biopsy (*17*). Some studies showed its superiority in comparison to filtered sulfur colloid and vital dyes probably based on specific properties like quick clearance from injection site and high uptake in first-tier lymph nodes during the first hour leading to adequate number of excised SLN per patient.

A comparison between 99mTc -filtered sulfur colloid and 99mTc-Tilmanocept has been performed in some studies. Baker et al. reported a similar SLN detection rate accompanied by the excision of fewer SLNs in patients injected with 99mTc-Tilmanocept (mean 1.85 SLNs) in comparison to those patients where 99mTc-filtered sulfur colloid was used (mean 3.24 SLNs)(*18*). These results are concordant with those obtained in the present study where the mean harvested SLNs were 1.82.

Wallace et al. reported results from a trial of 13 different centers that included 148 patients(*19*). Each patient received 99mTc-Tilmanocept and a vital blue dye. The primary endpoint was the proportion of SLNs detected by blue dye and radiotracer. During surgery, 207 out of 209 SLNs located by blue dye were also detected by 99mTc-Tilmanocept (concordance rate 99%). However, the radiotracer showed intra-operatively 320 SLNs and identified at least 1 SLN in 146 patients and blue dye identified SLNs in 131 patients. 99mTc-Tilmanocept accurately detected 31 out of 33 positive SLNs whereas blue dye detected only 25 of those 33 nodes(*19*). In our protocol blue dyes were not specifically used, but 99mTc-Tilmanocept accurately identified 80 out of 81 positive SLNs (similar to Wallace's et al study).

Unkart et al. reported that performance results in 617 patients, after a single intradermal injection of 99mTc-Tilmanocept, did not differ significantly between one-day and two-day injection protocols(*20*). The prolonged time provided by the two-day protocol showed that SLN accumulation of 99mTc-Tilmanocept can persist for at least 24 h after administration(*20*). In our study, 314 (89.2%) of cases were performed in a two-day approach, but only in 3 patients a SLN could not be intraoperatively found after a well-depicted lymph node in the preoperative lymphoscintigraphy.

On the other hand, we found statistically significant differences in LRD and BRD between superficial versus deep injections of radiotracer. This fact was previously reported by Hellingman et al. in a large study (2050 patients) using a deep intratumoral injection of nanocolloids (a way that results in a higher percentage of visualization of extra-axillary drainage). Preoperatively, the SLN(s) were visualized on lymphoscintigraphy in 86.7% of the procedures (93.8% in our group with the same injection way) and lymphatic drainage nonvisualization occurred in 13.3% (273/2050) of the procedures. These authors recommended to use a superficial periareolar injection in those patients with limited prognostic and therapeutic relevance of inner mammary chain SLN identification(*21*). As Hellingman et al, the present study showed a higher extraaxillary drainage, ranging from 11 to 14.2% when deep tracer injection was performed.

Tokin et al. retrospectively compared 99mTc-Tilmanocept with 99mTc-nanocolloid (the most frequently used radiotracer in Europe)(*22*). Six studies were included in a meta-analysis and five of them, involving 6,134 patients, were used to calculate the 99mTc-nanocolloid SLN localization rate (mean 95.9%). 99mTc-Tilmanocept were used in 148 patients, and pooled analysis revealed a mean 99.9% localization showing its superiority in this endpoint(*22*). The comparison between both radiotracers is out of the scope of this study but the BDR in our study was 97.2% (342/352), a little bit higher than the results of this meta-analysis with 99mTc-nanocolloid. There are, however, some concerns to be addressed. A direct comparison head-to-head with the standard radiotracer used in Europe has not been done. This issue must be documented to ascertain none or minimal variations through the time with this new tracer.

In our study, results showed that the patient age does not affect neither pathology results nor tumor characteristics except for Ki67 marker. Patients older than 50 years had higher values of BMI and BMI >25

affected to the LDR, therefore patients with overweight or obesity could offer difficult lymphoscintigraphy results. However, the BMI does not affect the BDR, so obese patients are good candidates for SLN biopsy.

SPECT/CT is usually performed when SLN cancer patients showing non-visualization on lymphoscintigraphy (especially with the new indications concerning recurrent surgery, previous radiotherapy, or neoadjuvant chemotherapy) or in difficult-to evaluate nodes (e.g. in obese patients with faint SLN uptake). It is well-known that SPECT/CT improves the SLN detection in several tumors and, especially, in obese patients(*23,24*) . Pouw et al. showed that SPECT/CT was able to clearly depict a SLN in 23% of patients without SLN visualization in planar scintigraphy (*25*). SPECT/CT was not mandatory in the present study patients and those cases that SPECT/CT was performed were due to clinical judgement by Nuclear Medicine physician. So, the use of SPECT/CT could increase the positive detection results in our study but we decided to perform the basic approach (planar images) in all centers.

In the same study, Pouw et al. showed that reinjection of the tracer achieved 62% of SLN visualization in those cases with persistent negative SPECT/CT visualization. Following this approach of reinjection in those cases with negative SLN on planar images, 22 out of 33 cases succeeded on SLN visualization in our study. In those cases, 99mTc-Tilmanocept resulted in a higher percentage of visualization than those cases reinjected with 99mTc-Nanocolloid (76.9% vs 60%).

While 99mTc-Tilmanocept established use is for patients with N0 disease undergoing primary surgery, in this study different patient profiles were included and the data pointed that there were not statistical differences in LDR, BDR and pathology results between different N and/or T stage and even in patients with neoadjuvant chemotherapy. Due to the therapeutic interest of patients with neoadjuvant therapy, it is remarkable to note that, despite the small number of patients in this group, the results indicate that these patients are as good candidates for the technique as patients without prior neoadjuvant, since they are not they found differences in the detection rates between the different groups.

Further, the recommended dose is 50 microgr of 99mTc-Tilmanocept in 18.5 MBq for same day surgery or 74 MBq for next day surgery. In the present study, higher doses did not show adverse effects. Additionally, reinjection resolved the no drainage situation most of the times. Therefore, 99mTc-Tilmanocept and the availability to use higher dose without adverse events could be an advantage in real clinical practice. Some patients received 100 microg 99mTc-Tilmanocept, but no side-effects were recorded.

99mTc-Tilmanocept offer good results regardless of the type of patient or the characteristics of the tumor, the factors that affect to the SLN biopsy results were the injection site and the molecular subtype. In this respect, intradermal o subareolar injection site resulted in better LDR and BDR and showed the best results in those patients with luminal tumors. There is a tendency to use superficial tracer injection in patients considered at low risk for lymph node metastases, whereas in high-risk patients, with large or multifocal tumors or lesions located deep or medio-caudally in the breast, deep tumor-related injections are recommended in order to stage as accurately as possible both lymph nodes in the axilla and outside the axilla. Another alternative is to combine deep with superficial injections at the same time (*26*). Based on the data obtained in this study, there were found statistically significant differences in SLN visualization/excision rates between superficial and deep injections of radiotracer. This issue could warrant a trend to use superficial injections (or combined with deep ones) but a more specific recommendation will require other study well-designed for this end.

In today's every-day practice of medicine, costs are important. As far as we know, the cost of one vial of Tilmanocept is higher than the cost of the most used tracer for lymphatic mapping. The reported advantages of 99mTc-tilmanocept versus the radiocolloid tracers include faster clearance from the site and higher retention in the SLN (avoiding extensive node dissection and morbidity). Other potential benefits could be the reduced number of SNs to be assessed by pathologist (time reduction) with similar staging results. However, a head-to head comparison of 99mTc-tilmanocept with standard SLN radiotracers in larger series of patients is necessary.

Finally, 99mTc-Tilmanocept was able to demonstrate metastatic involvement (macro or micrometastasis) in 22.7% of patients. When a positive result in a SLN was obtained, the next therapeutic step

was left to local breast committee decision. Most of the centers used the criteria derived from the study ACOSOG Z0011 when conservative breast surgery was used (avoiding lymphadenectomy in most of cases even with positive SLN). In cases where a positive SLN was observed after neoadjuvant chemotherapy (macro or micrometastasis), a complete axillary lymphadenectomy was performed. On the other hand, after surgery, all decisions regarding systemic adjuvant treatment and radiotherapy were based on international guidelines (ESMO, ESTRO, NCCN). In the present study, after a mean follow-up of 19 months, the FNR was 0%. The results are similar to those obtained in previous studies (*11,18,19*) with the same radiotracer, confirming its consistency throughout different approaches and clinical situations.

CONCLUSION

99mTc-Tilmanocept is a new radiotracer for lymphatic mapping and SLN biopsy that shows good results in breast cancer SLN biopsy whatever protocol is used in a heterogeneous breast cancer population, although the best results are achieved when a superficial radiotracer injection is performed.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design.
Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by Elena Goñi and Sergi Vidal-Sicart and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that no one of them have conflict of interest.
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.
Informed consent was obtained from all individual participants included in the study.

ACKNOWLEDGEMENTS:

The authors wish to thank Mrs. Ana Villarejo López for her help in statistical analysis as well as for her dedication and collaboration during the whole process of this work.

KEY POINTS: The aim of this study is to describe and analyze the outcomes in a real clinical practice of SLN biopsy in breast cancer. The study covers different approaches and scenarios and demonstrates that this tracer is able to precisely localize the SLN whatever protocol is used although the best results are achieved when a superficial radiotracer injection is performed. The results foster the reliability of the technique using this novel tracer.

REFERENCES

1. Kircher M, Seifert S, Kircher S, Lapa C. [99mTc]Tilmanocept lymphscintigraphy after inconclusive [99mTc]nanocolloid scan in breast cancer. *Nuklearmedizin.* 2019;58:282-284.

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. Herrera AF, Zain J, Savage KJ, et al. Preliminary Results from a Phase 2 Trial of Brentuximab Vedotin Plus Cyclophosphamide, Doxorubicin, Etoposide, and Prednisone (CHEP-BV) Followed By BV Consolidation in Patients with CD30-Positive Peripheral T-Cell Lymphomas. *Blood.* 2019;134:4023-4023.

4. EMA. Lymphoseek® SmPC. https://www.ema.europa.eu/en/medicines/human/EPAR/lymphoseek#authorisation-details-section. Accessed September 4, 2020.

5. 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.

6. Engering AJ, Cella M, Fluitsma D, et al. The mannose receptor functions as a high capacity and broad specificity antigen receptor in human dendritic cells. *Eur J Immunol.* 1997;27:2417-2425.

7. Vidal-Sicart S, Vera DR, Valdes Olmos RA. Next generation of radiotracers for sentinel lymph node biopsy: What is still necessary to establish new imaging paradigms? *Rev Esp Med Nucl Imagen Mol.* 2018;37:373-379.

8. LYMPHOSEEK® (tilmanocept) 50 mcg kit for radiopharmaceutical preparation, Summary of Product Characteristics. September 16, 2019; https://www.ema.europa.eu/en/documents/product-information/lymphoseek-epar-product-information_en.pdf. Accessed September 4, 2020.

9. Wu Y, Jing J, Wang J, Xu B, Du M, Chen M. Robotic-assisted sentinel lymph node mapping with indocyanine green in pelvic malignancies: A systematic review and meta-analysis. *Front Oncol.* 2019;9:585.

10. Niebling MG, Pleijhuis RG, Bastiaannet E, Brouwers AH, van Dam GM, Hoekstra HJ. A systematic review and meta-analyses of sentinel lymph node identification in breast cancer and melanoma, a plea for tracer mapping. *Eur J Surg Oncol.* 2016;42:466-473.

11. Moncayo VM, Aarsvold JN, Alazraki NP. Lymphoscintigraphy and sentinel nodes. *J Nucl Med.* 2015;56:901-907.

12. Tanis PJ, Valdes Olmos RA, Muller SH, Nieweg OE. Lymphatic mapping in patients with breast carcinoma: reproducibility of lymphoscintigraphic results. *Radiology.* 2003;228:546-551.

13. Vidal M, Vidal-Sicart S, Torrents A, et al. Accuracy and reproducibility of lymphoscintigraphy for sentinel node detection in patients with cutaneous melanoma. *J Nucl Med.* 2012;53:1193-1199.

14. Vitali GC, Trifiro G, Zonta M, et al. Lymphoscintigraphy in clinical routine practice: reproducibility and accuracy in melanoma patients with a long-term follow-up. *Eur J Surg Oncol.* 2014;40:55-60.

15. 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.

16. Ruano Perez R, Rebollo Aguirre AC, Garcia-Talavera San Miguel P, et al. Review of the role of the sentinel node biopsy in neoadjuvant chemotherapy in women with breast cancer and negative or positive axillary node at diagnosis. *Rev Esp Med Nucl Imagen Mol.* 2018;37:63-70.

17. 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.

18. Baker JL, Pu M, Tokin CA, et al. Comparison of [(99m)Tc]tilmanocept and filtered [(99m)Tc]sulfur colloid for identification of SLNs in breast cancer patients. *Ann Surg Oncol.* 2015;22:40-45.

19. 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.

20. Unkart JT, Proudfoot J, Wallace AM. Outcomes of "one-day" vs "two-day" injection protocols using Tc-99m tilmanocept for sentinel lymph node biopsy in breast cancer. *Breast J.* 2018;24:526-530.

21. Hellingman D, Wan OY, de Wit-van der Veen BJ, et al. Predictive risk factors for sentinel lymph node nonvisualization on planar lymphoscintigraphy using an intratumoral injection in patients with primary breast cancer. *Nucl Med Commun.* 2019;40:317-324.

22. Tokin CA, Cope FO, Metz WL, et al. The efficacy of Tilmanocept in sentinel lymph mode mapping and identification in breast cancer patients: a comparative review and meta-analysis of the (9)(9)mTc-labeled nanocolloid human serum albumin standard of care. *Clin Exp Metastasis.* 2012;29:681-686.

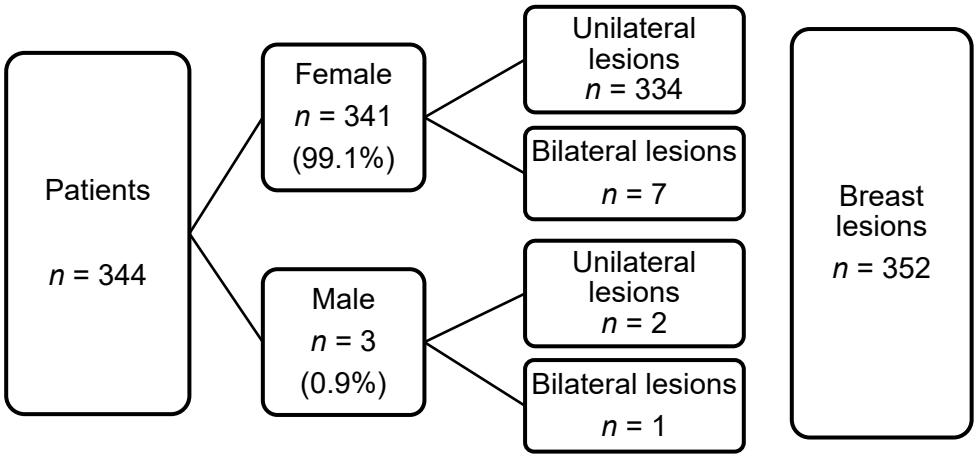
23. Stanzel S, Pernthaler B, Schwarz T, Bjelic-Radisic V, Kerschbaumer S, Aigner RM. Diagnostic and prognostic value of additional SPECT/CT in sentinel lymph node mapping in breast cancer patients. *Nuklearmedizin.* 2018;57:92-99.

24. Jimenez-Heffernan A, Ellmann A, Sado H, et al. Results of a prospective multicenter International Atomic Energy Agency sentinel node trial on the value of SPECT/CT over planar imaging in various malignancies. *J Nucl Med.* 2015;56:1338-1344.

25. Pouw B, Hellingman D, Kieft M, et al. The hidden sentinel node in breast cancer: Reevaluating the role of SPECT/CT and tracer reinjection. *Eur J Surg Oncol.* 2016;42:497-503.

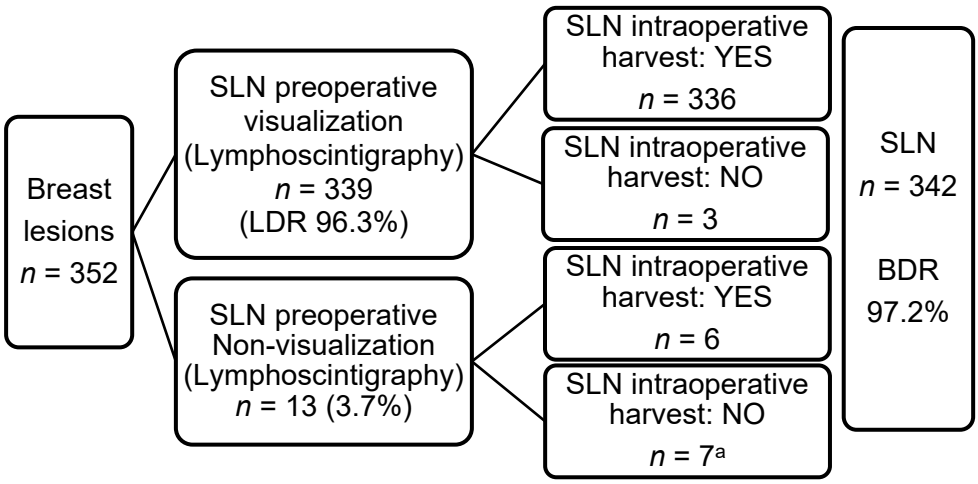
26. Ahmed M, Purushotham AD, Horgan K, Klaase JM, Douek M. Meta-analysis of superficial versus deep injection of radioactive tracer and blue dye for lymphatic mapping and detection of sentinel lymph nodes in breast cancer. *Br J Surg.* 2015;102:169-181.

Figure 1: patients and breast lesions distribution



Total of unilateral lesions: 336 (97.7%); total of bilateral lesions: 8 (2.3%)

Figure 2: Distribution of lymphoscintigraphic and SLN biopsy outcomes



[a] one patient did not undergo biopsy (the patient was chemotherapy)
BDR: biopsy detection rate; LDR: Lymphoscintigraphy detection rate; SLN: Sentinel lymph node.
Of the 352 breast lesions studied, SLN were harvested in 342, SLN not were detected in 9 lesion and a patient did not underwent biopsy (the patient was chemotherapy).

Figure 3. A demonstrative case of breast cancer patient intratumorally injected with 70 MBq of [^{99m}Tc]Tc-Tilmanocept in a two-day protocol. Planar images were performed at 30 min, 2 h and 21 h after injection. Two well-depicted SLN were clearly seen in all sets of images. Three-dimensional volume rendering images, based on SPECT/CT data, accurately reflected the SLM anatomical localization.

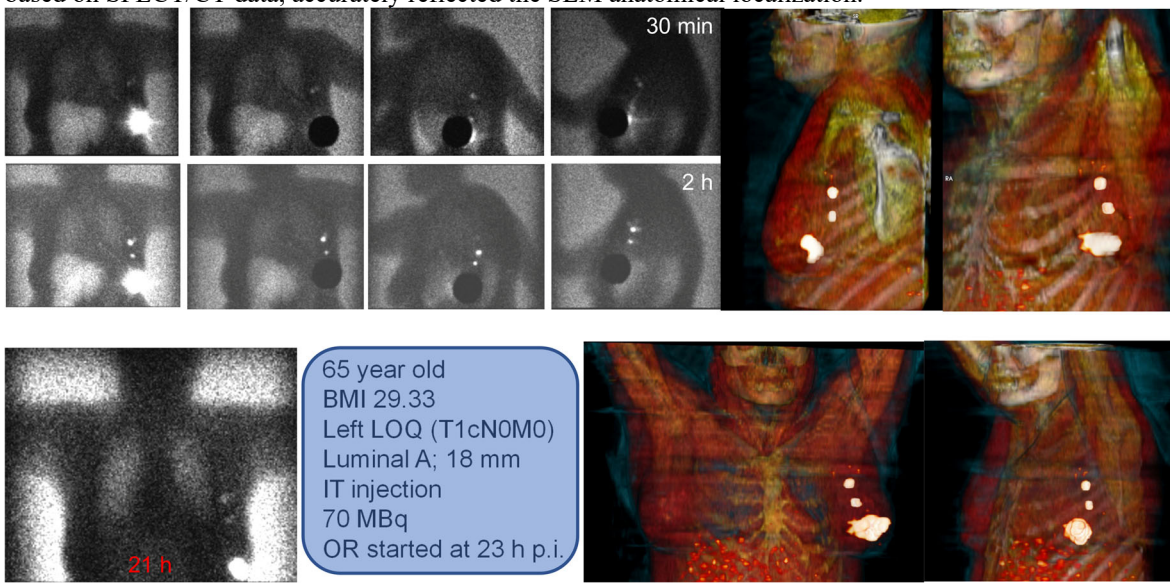


Table 1. SLN procedure: time protocol, doses and volume administered.

Total cases		**Non reinjection cases**	**Reinjections cases**
n=38	**One day protocol**	**37**	**1**
	Mean dose (MBq)	*55.87*	*55.5*
	Mean Volume (mL)	*0.40*	*0.45*
n=314	**Two days protocol**	**282**	**32**
	Mean dose (MBq)	*92.5*	*111*
	Mean Volume (mL)	*0,5*	*0.5*

Table 2: Patient (n=344) and tumor characteristics (breast lesions, n=352)

Characteristics	Value
Sex, n (valid %). [N_{total} 344, missing 0]	
Female	341 (99.1%)
Male	3 (0.9%)
Age in years, median (IQR)	
Total patients, n= 344	59 (49 – 68)
< 50 age group, n= 97	45 (41 – 47)
≥ 50 age group, n= 247	63 (57 – 71)
BMI, median (IQR)*	
Total patients, n= 327 (14 missing)	26.08 (23 – 29.52)
< 50 age group, n=89 (8 missing)	22.6 (20.50 – 26.17)
≥ 50 age group, n=241 (6 missing)	27 (24.22 – 30.15)
Tumor location, n (valid %). [N_{total} 347, missing 5]	
UOQ	115 (33.1%)
UIQ	38 (11%)
LIQ	19 (5.5%)
LOQ	24 (6.9%)
Central regions	151 (43.5%)
Histological Subtype, n (valid %).[N_{total} 351, missing 1]	
IDC	253 (72.1%)
ILC	36 (10.3%)
DCIS	19 (5.4%)
Others	43 (12.3%)
Clinical T stage at diagnosis, n (valid %). [N_{total} 351, missing 1]	
T0	2 (0.6%)
T1	225 (64.1%)
T2	90 (25.6%)
T3	11 (3.1%)
T4	2 (0.6%)
Tis	21 (6.0%)
Clinical N stage at diagnosis, n (valid %). [N_{total} 348, missing 4]	
Nx	2
N0	274
N1	66
N2	6
Ki67, median (IQR) [a]	
Total patients, n= 332 (20 missing)	12.50 (6.25 – 27.25)
< 50 age group, n=95 (3 missing)	20 (8.00 – 36.00)
≥ 50 age group, n=237 (17 missing)	12 (6.00 – 21.50)
Molecular Subtype, n (valid %). [N_{total} 334, missing 18]	
Luminal A	204 (61.1%)
Luminal B	71 (21.3%)
Her2	34 (10.2 %)
Basal like	25 (7.5 %)
Neoadjuvant chemotherapy [b], n (valid %). [N_{total} 344, missing 0]	
Yes	45 (13.1%)
No	299 (86.9%)
SLN pathology results in 352 cases on 344 patients)	
Positive	80 (22.8%)
Negative	262 (74.4%)
n/a (not harvested)	10 (2.8%)

[a] Statistically significant differences. [b] Anyone patient with neadyuvant suffered bilateral lesion. UOQ: upper outer quadrant; UIQ: upper inner quadrant; LIQ: lower inner quadrant; LOQ; lower outer quadrant; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; DCIS: ductal carcinoma *in situ*. IQR: interquartile range

Table 3: Univariable analysis tests results of SLN detection preoperatively and intraoperatively

	SLN in image (n=352)		P statistic (2-sided) (χ^2)	SLN harvested (n=352)		P statistic (2-sided) (χ^2)
	YES	NO		YES	NO	
Age (0 missing)						
<50	96	2	0.307	97	1	0.202
≥50	243	11		245	9	
BMI [a] (17 missing)						
Overweight/obesity	192	11	0.031	196	7	0.325
Normal weight	123	1		122	2	
Tumor location (15 missing)						
UOQ	110	5	0.523	111	4	0.465
Rest of the regions	225	7		227	5	
Molecular Subtype [b]						
Luminal	267	8	0.012	271	4	<0.001
Her	30	4		29	5	
N stage (4 missing)						
N0	267	10	0.744	270	7	0.891
N1-N2	69	2		69	2	
Neoadjuvant therapy (0 missing)						
Yes	42	3	0.257	42	3	0.098
No	297	10		300	7	
Injection area (7 missing)						
Peri/Intratumoral	150	10	0.009	152	8	0.010
Intradermal/Subareolar	183	2		184	1	

[a] there is BMI missing from 14 patients, of which one of them has bilateral lesion. Additionally, 3 patients were excluded in the BMI codification groups because of infraweight ; [b] There are 18 missing dates and Basal like tumors were excluded (n=25).

BMI: Body mass index; NAT: Neoadjuvant therapy; SLN: Sentinel lymph node; UOQ: Upper outer quadrant; χ^2: Chi-Square.

Table 4: Number of SLN detected by lymphoscintigraphy and SLN harvested

Nº SLN	Preoperative -Frequency (%)-	Intraoperative -Frequency (%)-
0	13 (3.7)	10 (2.8)
1	162 (46.0)	151 (42.9)
2	119 (33.8)	114 (32.4)
3	41 (11.6)	59 (16.8)
≥4	17 (4.8)	18 (5.1)
Mean (SD) of SLN detected by lesion	1.71 (±1.027)	1.82 (±1.036)
Total of SLN counted	603	640

SD: Standard deviation; SLN: Sentinel lymph node