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Sentinel Lymph Node Mapping of Breast Cancer via Intradermal Administration of *Lymphoseek*

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Abstract

Lymphoseek™ is a molecular imaging agent specifically designed for sentinel lymph node (SLN) mapping. We conducted a Phase I trial which measured the injection site clearance and sentinel lymph node accumulation after a single intra-dermal injection of Lymphoseek or [^{99m}Tc]sulfur colloid protocol. Ten patients with breast cancer participated in this study. Five patients received an intradermal administration of 1.0 nmole of ^{99m}Tc-labeled Lymphoseek and five patients received an intra-dermal administration of filtered [^{99m}Tc]sulfur colloid (fTcSC). Lymphoseek, exhibited a significantly ($P < .001$) faster injection site clearance than fTcSC. The mean Lymphoseek clearance half-time was 2.61 ± 0.72 hr compared to 24.1 ± 17.7 hr for fTcSC. The mean sentinel lymph node uptake of Lymphoseek (1.1 ± .5%), and TcSC (2.5 ± 4.9%) were statistically equivalent ($P = 0.28$). When an intra-dermal injection was employed, Lymphoseek demonstrated faster injection site clearance than filtered [^{99m}Tc]sulfur colloid.

Keywords

sentinel lymph node biopsy; radiopharmaceutical - [^{99m}Tc]DTPA-mannosyl-dextran; Lymphoseek; molecular imaging; breast cancer

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1. Introduction

Lymphoseek™ is a molecular imaging agent specifically designed for sentinel lymph node mapping. The technetium-99m-labeled radiopharmaceutical attains rapid clearance from the injection site by virtue of it's small molecular diameter [10]. In rabbits Lymphoseek exhibited significantly faster injection site clearance technetium-99m sulfur colloid [10]. Submucosal administration into pig colon and stomach resulted in lymph node accumulation within 10 minutes [2, 6, 11]. Rapid sentinel lymph node uptake was also observed after direct injection into the porcine prostate gland [7]. The molecular structure of Lymphoseek contains the carbohydrate, mannose, with provides the radiopharmaceutical with a high affinity [10] for a receptor, mannose binding protein [9], which is specific to lymphoid tissue. This molecular feature provides Lymphoseek with sustained sentinel lymph node uptake without distal lymph node accumulation, a property demonstrated in rabbit [10] and pig [2] studies.

Clinical trials of Lymphoseek also demonstrated rapid injection site clearance compared with filtered technetium-99m-labeled sulfur colloid and sustained sentinel lymph node uptake. In women with breast cancer, Lymphoseek demonstrated significantly faster clearance from the injection site and equivalent sentinel lymph node accumulation [13]. No adverse events or clinically significant changes in clinical and laboratory values were observed. These findings were also demonstrated at the 5-nmole dose level [1] and by patients with melanoma [12].

We present the injection site clearance and sentinel lymph node accumulation after a single intradermal injection of Lymphoseek or filtered [^{99m}Tc]sulfur colloid which employed a protocol for sentinel lymph node (SLN) mapping of breast cancer. Our previous studies employed paratumoral/intradermal technique where the injection of the radiopharmaceutical was initiated paratumorally and finished with the needle in the intradermal position.

2. Materials and Methods

2.1. Patient Enrollment

Ten female patients with breast cancer who would normally be offered sentinel lymph node biopsy as per University of California, San Diego guidelines participated in this study. We entered women that presented a challenge to successful SLN mapping and lymphoscintigraphy; at least one of the following criteria was required: 1) over 60 years old, 2) having a nonpalable lesion, or 3) having an upper-outer quadrant lesion. The need to have follow-through node dissection was determined by pathologic outcome of the sentinel node and did not affect this study. Consenting subjects were randomly into one of two groups: Lymphoseek, or filtered [^{99m}Tc]sulfur colloid. Pregnant and lactating females, patients with known metastatic disease, and patients currently enrolled in another protocol were excluded from this study. The subjects ranged in age from 46 to 83 years (Table 1). Lesion size ranged from 0.8 to 2.1 cm. Two sentinel lymph nodes from 2 subjects were positive on frozen section. A third sentinel lymph node from a third subject was positive on immunohistochemistry.

The protocol received the consent of the Division of Medical Imaging and Radiopharmaceutical Drug Products of the U. S. Food and Drug Administration as a Physician-Sponsored Investigational New Drug. The protocol and the informed consent form were approved by the University of California, San Diego, Office for Human Research Protection, the Moores UCSD Cancer Center Protocol Review Monitoring Committee, and the UCSD Human Exposure Review Committee.

2.2. Agent Preparation

Lymphoseek was synthesized [10] and radiolabeled [3] as previously described. This study used the same Lymphoseek preparation as our Phase I Breast cancer [1, 13] and melanoma trials [12]. The mean molecular diameter was 7.0 nanometers and the average molecular weight was 28,200 grams per mole. The average DTPA and mannose densities were 2.1 and 42 moles per mole of dextran, respectively. The mean Lymphoseek radiochemical purity was 97% (range: 96 – 98). Technetium-99m-labeled filtered sulfur colloid (CIS-US, Bedford, MA) and was prepared by a commercial radiopharmacy. All radiopharmaceutical preparations were administered within two hours of preparation.

2.3. Nuclear Imaging

Each patient received a single injection (0.1 ml) of either Lymphoseek (1 nmole) or filtered [^{99m}Tc]sulfur colloid above the lesion using an intradermal technique [4, 5]. The administration site was massaged for several minutes. Each subject was monitored for any sign of an allergic reaction such as the occurrence of a rash, hives, edema, or other cutaneous manifestations.

Nuclear imaging of the Lymphoseek or filtered [^{99m}Tc]sulfur colloid employed the same imaging protocol. Images of the injection site were acquired immediately after the injection and at 15-minute intervals for two hours. An imaging standard of a known dilution and volume of injectate was placed within the field-of-view. All images were acquired (256x256x16) for 3 minutes and stored on an image processing computer. The injection site clearance rate constant k_c and half-life T_c of Lymphoseek and filtered [^{99m}Tc]sulfur colloid were calculated using decay-corrected counts obtained from the nuclear images of the injection site.[13]

2.4. Sentinel Lymph Node Detection and Measurement

Sentinel lymph node biopsy was performed utilizing standard technique. At the start of the surgical procedure, isosulfan blue (Lymphazurin 1%, U. S. Surgical Corp., Norwalk, CT) was injected at the 3-, 6-, 9-, and 12-O'clock positions (1 ml per position) surrounding the lesion. The administration site was massaged for several minutes. Also during this time a hand-held gamma probe (Neoprobe 2000, Neoprobe Corp., Dublin, OH) was used to localize the sentinel lymph node. Scinitigrams were used to guide the search. After marking the skin at the highest count rate, the patient was prepped and an incision was made at the marked location. With the aid of the gamma probe the dissection was carried out to the hot lymph node and/or to the lymphatic with blue dye accumulation. The lymph node was isolated, removed, and placed on the tip of the gamma probe for radioactive counting. A background measurement was made by placing the tip of the gamma probe perpendicular to

the skin surface at lease 20 cm from the administration site. Finally, the gamma probe was placed back within the nodal basin to ensure no significant residual radioactivity remained. Verification that the node contained radioactivity at least ten times background was performed before sending it to pathology. Frozen section analysis was then performed to identify metastases. Sentinel lymph nodes were defined by having a node-to-background ratio of at least 10-to-1. If the sentinel node was histologically positive, the lymph node dissection was completed.

All lymph nodes and injection standards were assayed for radioactivity using a dose calibrator located adjacent to the operating room. The percent-of-injected dose *%ID* in each lymph node was calculated as previously described [13].

2.5. Subject Monitoring

Vital signs and EKGs were obtained before Lymphoseek and filtered TcSC administration and at 15, 30, 45, 60, and 120 minutes post-administration. Samples for urinalysis, CBC with differential and platelet counts, and a blood chemistry panel were acquired at the pre-op anesthesia appointment (baseline) and again just prior to surgery.

2.6. Statistical Methods

For the measures k_c, T_c, $\%ID_{IS}$, and $\%ID_{SN}$, statistical significance was evaluated by comparisons of the Lymphoseek group to the filtered [^{99m}Tc]sulfur colloid group using the nonpaired Students–*t* -test (JMP software, SAS Institute, Cary NC).

3. Results

Table 1 lists the subject number and age. The table also lists the tumor diagnosis, size, stage and location. Also, listed are the agent and the amount of radioactivity administered. Table 2 lists the subject number, radiopharmaceutical, the injection site clearance rate constant and half-life, the time at which the sentinel lymph node was excised relative to the time injected. Also, listed is the probe count rate after excision of each sentinel lymph node as defined by the radiotracer or blue dye. Entries for subjects 1 and 5 contain zeros, indicating a sentinel lymph node detected only via blue dye. Subjects 3 and 10 exhibited blue sentinel lymph nodes that did not attain probe counts that exceeded 10-times background.

Lymphoseek, exhibited a significantly ($P < 0.001$) faster injection site clearance than filtered or unfiltered TcSC. The mean Lymphoseek clearance half-time (Table 3) was 2.62 ± 0.55 hr (n = 5) compared to 24.1 ± 17.7 hr for filtered TcSC (n = 5). The mean Lymphoseek sentinel lymph node uptake of 1.1 ± 0.5 % and percent-of-injected dose of 2.5 ± 4.9 % for filtered TcSC, which were statistically equivalent ($P = 0.28$). Figure 1 demonstrates the **c**learance of radioactivity from the injection site of two sentinel node cases: Lymphoseek (diamonds, Subject #8) exhibited a half-life of 2.15 ± 0.16 hours, filtered [^{99m}Tc]sulfur colloid (triangles, Subject #8) exhibited a half-life of 36.7 ± 6.5 hours.

None of the adverse events that occurred during this study were attributed to either radiopharmaceutical. No grade 3 or 4 hematologic adverse events occurred on this study, and one non-hematologic adverse event occurred. This subject (#8), who was a member of

the filtered TcSC group, experienced a mild rash on her left breast from the time of surgery. Approximately one month after the operation, some fullness and erythema was noted toward the incision. This was aspirated and found to be a hematoma.

Both radiopharmaceuticals exhibited the same mean number of sentinel lymph nodes per study. The five subjects in the Lymphoseek group yielded a total of eleven sentinel lymph node for an average of 2.2 nodes per study. The five filtered TcSC subjects yielded nine sentinel lymph nodes; one study failed to produce a hot lymph node. To calculate the average we used four as the denominator to calculate 2.3 nodes per study.

4. Discussion

This study demonstrated significantly faster injection site clearance by Lymphoseek than filtered [^{99m}Tc]sulfur colloid. Contrary to our expectation, the intradermal administration did not exhibit a faster clearance half-life (2.62 +/− 0.55 hr) than Phase I study group that utilized a periturmoral/intradermal injection [13], which exhibited a clearance half-life of 2.72 +/− 1.57 hr. This was also true for our melanoma study [12], where the measured clearance half-life was 2.05 +/− 0.89 hr. One noticeable difference between the three studies was the wide dispersion of clearance half-lives exhibited by the periturmoral/intradermal injections; this was denoted by relative standard deviation of 0.58, which is almost three-fold greater than the relative standard deviation of the intradermal injection (0.21).

The sentinel lymph node accumulation of the Lymphoseek group was higher compared to the accumulation of Lymphoseek after intradermal and intradermal/peritumoral administration to melanoma or breast cancer patients. The mean sentinel lymph node accumulation after a 1-nmole dose of Lymphoseek using a standard mapping protocol for SLN of melanoma [12] was 0.49 ± 0.59 percent of injected dose. A breast cancer mapping protocol using an intradermal/periturmoral administration of Lymphoseek [13] yielded mean SLN uptakes of 0.55 ± 0.43 %. These values are lower than the means 1.1 ± 0.5 % exhibited by the Lymphoseek group of this study; the differences are not, however, significant.

The study was designed to measure the rates at which Lymphoseek and filtered [^{99m}Tc]sulfur colloid clear the injection site after an intradermal administration to the breast. The study was powered to provide a statistical comparison of the injection site clearance rates exhibited by the Lymphoseek and fTcSC groups. We also measured each subject's sentinel lymph node accumulation. This study was not powered to test for a significant difference in SLN accumulation between Lymphoseek and fTcSC. This is due to the extremely high variability in SLN uptake between subjects [8].

The entrance criteria for this study, over 60 years old, a nonpalable, or upper-outer quadrant lesion, was designed to enroll subjects who's lymphosintigraphic or gamma probe detection is more difficult. The intradermal method [4, 5, 14] is extremely convenient, is less stressful for the patient, generates higher SLN uptake, and produces less scatter from the injection site. Given the propensity for many surgeons to use an intradermal approach, we conducted this study to ensure that Lymphoseek was compatible with this technique. Todate, we have not administered Lymphoseek using a sub-areolar injection.

5. Conclusion

When a single intradermal injection is employed, Lymphoseek demonstrated significantly faster injection site clearance than filtered [^{99m}Tc]sulfur colloid. The injection site clearance rate and sentinel lymph node accumulation were comparable to clearance and accumulation of Lymphoseek after intradermal or intradermal/peritumoral administration to melanoma or breast cancer patients.

Acknowledgments

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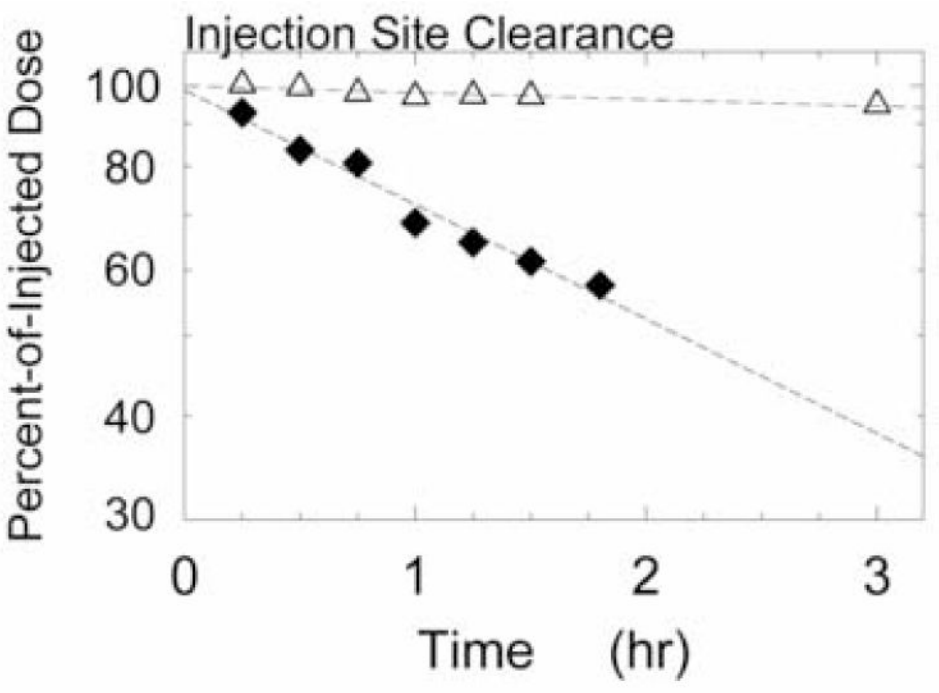

Figure 1.

Clearance of radioactivity from the injection site of two sentinel node cases: Lymphoseek (diamonds, Subject #8) exhibited a half-life of 2.15 ± 0.16 hours, filtered [^{99m}Tc]sulfur colloid (triangles, Subject #6) exhibited a half-life of 36.7 ± 6.5 hours.

TABLE 1

Subject and Radiopharmaceutical Summary

Subject and Pathology						SN Status[+] (#)	Radiopharmaceutical	
Subject	Age (yrs)	Diagnosis	Size (cm)	Stage	Site		RP	Dose (mCi)
1	59	Inv ductal & Lob	0.8	T1bN0MX	RUOQ	0/2	fTcSC	0.55
2	60	Inv spindle-cell	2.1	T2N0M0	LUOQ	0/2	Lymphoseek	0.48
3	55	Inv ductal	1.0	T1N0MX	RUOQ	0/4	Lymphoseek	0.46
4	65	Multifocal DCIS	1.1	TisN0MX	LUOQ	0/7	Lymphoseek	0.56
5	57	Inv mixed duc & lob	1.5	T1cN1M0	LUOQ	1/2/12	fTcSC	0.49
6	64	Inv mixed duc & lob	2.0	T1cN0MX	LLIQ	0/5	fTcSC	0.47
7	46	Inv ductal	1.2	T1cN0 (I+)MX	RUIQ	$1^{\dagger}/3$	Lymphoseek	0.48
8	51	Inv mixed duc & lob	2.0	T1cN2aMX	RUOQ	4/8/35	Lymphoseek	0.50
9	83	Inv duc & DCIS	1.8	T1bN0MX	LUOQ	0/10	fTcSC	0.43
10	55	Inv duc & DCIS	1.1	T1cN0MX	RUIQ	0/4	fTcSC	0.52

Inv, invasive; DCIS, ductal carcinoma in situ; fTcSC, filtered [^{99m}Tc]sulfur colloid;

[+]positive/SLN/axillary dissection,

[†]Immunopositive

TABLE 2

Injection Site Clearance and Sentinel Lymph Node Uptake

Subject	Radiopharmaceutical	Injection Site Clearance[+]		Sentinel Lymph Node Uptake[‡]				
		Rate Constant k_c (hr^{-1})	Half-Life T_c (hr)	Excision Time (hr)	Probe Count Rate			
					primary (cps)	2nd (cps)	3rd (cps)	4th (cps)
1	fTcSC	0.036 ± 0.044	19.5 ± 24.4	5.1	0[†]			
2	Lymphoseek	0.284 ± 0.026	2.44 ± 0.22	3.4	7,000	1,300		
3	Lymphoseek	0.249 ± 0.022	2.79 ± 0.25	4.3	1,100	650	90[†]	
4	Lymphoseek	0.176 ± 0.034	3.93 ± 0.76	4.4	3,200[*]	450	2,200	1,208[*]
5	fTcSC	0.000		4.4	0[†]	141[*]		
6	fTcSC	0.019 ± 0.003	36.7 ± 6.5	7.3	45	225	109[*]	
7	Lymphoseek	0.294 ± 0.023	2.36 ± 0.19	6.1	2,600	2,136		
8	Lymphoseek	0.322 ± 0.023	2.15 ± 0.16	4.9	5,081			
9	fTcSC	0.032 ± 0.019	21.4 ± 12.7	3.5	6,869	2,169[*]	5,070[*]	
10	fTcSC	0.057 ± 0.018	12.2 ± 3.9	6.1	38,942	2,700	75[†]	

fTcSC, filtered [^{99m}Tc]sulfur colloid;

[+] mean ± SD;

[‡] hot or blue;

[*] not blue,

[†] not hot

TABLE 3

Injection Site Clearance and Sentinel Lymph Node Uptake of Lymphoseek and Filtered [^{99m}Tc]Sulfur Colloid

Group	Radiopharmaceutical	Injection Site Clearance			Sentinel Lymph Node	
		Studies (#)	Rate Constant k_c[+] (hr^{-1})	Half-Life T_c[*] (hr)	Studies (#)	$\%ID_{SN}$[+] (%)
1	Lymphoseek	5	0.265 ± 0.055	2.62 ± 0.55	5	1.1 ± 0.5
2	Filtered [^{99m}Tc]sulfur colloid	5	0.029 ± 0.021	24.1 ± 17.7	5	2.5 ± 4.9
	P-value		< 0.001	< 0.001		0.275

[+] Mean ± SD;

[*] Based on the Rate Constant k_C