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Imaging of Lymph Flow in Breast Cancer Patients after Microdose Administration of a Near-Infrared Fluorophore: Feasibility Study¹

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Abstract

Purpose—To prospectively demonstrate the feasibility of using indocyanine green, a near-infrared (NIR) fluorophore at the minimum dose needed for noninvasive optical imaging of lymph nodes (LNs) in breast cancer patients undergoing sentinel lymph node mapping (SLNM).

Materials and Methods—Informed consent was obtained from 24 women (age range, 30–85 years) who received intradermal subcutaneous injections of 0.31–100 µg indocyanine green in the breast in this IRB-approved, HIPAA-compliant, dose escalation study to find the minimum microdose for imaging. The breast, axilla, and sternum were illuminated with NIR light and the fluorescence generated in the tissue was collected with an NIR-sensitive intensified charged-coupled device. Lymphoscintigraphy was also performed. Resected LNs were evaluated for the presence of radioactivity, blue dye accumulation, and fluorescence. The associations between the resected LNs that were fluorescent and (a) the time elapsed between NIR fluorophore administration and resection and (b) the dosage of NIR fluorophores were tested with the Spearman rank and Pearson product moment correlation tests, respectively.

Results—Lymph imaging consistently failed with indocyanine green microdosages between 0.31 and 0.77 µg. When indocyanine green dosages were 10 µg or higher, lymph drainage pathways from the injection site to LNs were imaged in eight of nine women; lymph propulsion was observed in seven of those eight. When propulsion in the breast and axilla regions was present, the mean apparent velocities ranged from 0.08 to 0.32 cm/sec, the time elapsed between “packets” of propelled fluid varied from 14 to 92 seconds. In patients who received 10 µg of indocyanine green or more, a weak negative correlation between the fluorescence status of resected LNs and the time

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See Materials and Methods for pertinent disclosures.

between NIR fluorophore administration and LN resection was found. No statistical association was found between the fluorescence status of resected LNs and the dose of NIR fluorophore.

Conclusion—NIR fluorescence imaging of lymph function and LNs is feasible in humans at microdoses that would be needed for future molecular imaging of cancer-positive LNs.

Currently, standard-of-care staging of breast cancer requires surgical resection of the first tumor-draining, or sentinel, lymph node (SLN) for subsequent pathologic examination (1). If the SLN is cancerous, then additional lymph nodes (LNs) in the axillary basin are subsequently removed for accurate staging. Recently, the accuracy of nodal staging has been challenged by (i) the observed heterogeneity of outcomes for node-negative breast cancer (2–5), and (ii) the demonstration of false-negative rates of conventional cytologic evaluation when compared with molecularly specific immunohistochemical analysis (2). Unfortunately, immunohistochemical analysis is time-consuming and often impractical for use in pathologic surgery where immediate evaluation is required to decide whether additional LNs in the axilla are to be resected.

Noninvasive molecular imaging modalities such as nuclear and/or optical techniques could reduce the morbidity of nodal dissection and improve the accuracy of nodal staging by providing the molecular specificity inherent in immunohistochemical analysis. Optical imaging with molecularly specific near-infrared (NIR) fluorophores may offer the added opportunity to (a) intraoperatively guide resection, (b) postsurgically evaluate LNs more accurately by using fluorescence microscopy, and (c) allow sufficient time for clearance from normal noncancerous LNs without concern of physical tracer half-life. In addition, owing to the lack of autofluorescence in the NIR wavelength range, optical imaging has low background noise levels (6).

However, to date there has been no demonstration of noninvasive optical imaging in humans by using microdose administration of NIR fluorescent contrast agents to justify the use of optical molecular imaging agents in humans. The Food and Drug Administration defines “microdose” as 1/100 of the dose that yields a pharmacologic dose for imaging agents, the microdosing limits of 100 µg or less of labeled protein or 30 nmol or less of labeled biologic agent (7).

The development of NIR optical imaging agents for molecularly based nodal staging in humans requires the demonstration of an imaging device capable of collecting images following microdose (ie, ≤100 µg) administration of NIR fluorescent agents in humans. We hypothesized that the tissue penetration of NIR light, the ability to repeatedly excite a nonspecific NIR fluorescent indocyanine green dye (IC-Green; Akorn, Buffalo Grove, Ill) in vivo, and the use of an integrating NIR-sensitive camera would enable dynamic imaging of lymph flow from the site of injection to sentinel and axillary LNs in humans.

The purpose of our study was to prospectively demonstrate the feasibility of using an NIR fluorophore at the minimum dose needed for noninvasive optical imaging of LNs in breast cancer patients undergoing standard-of-care SLN mapping (SLNM).

Materials and Methods

The protocols used for this feasibility study were conducted under investigative new drug applications 73 275 and 74 975 for off-label use of indocyanine green. The Health Insurance Portability and Accountability Act–compliant studies were approved by the Institutional Review Boards of Baylor College of Medicine (Houston, Tex) and its affiliated clinical sites (Saint Luke’s Episcopal Hospital, Ben Taub General Hospital, and the Methodist Hospital, Houston, Tex). Informed consent was obtained. Patient studies which included the use of

microdelivery devices were financially supported in part by Becton-Dickinson Technologies (Research Park, NC). However, the authors had control of the data and information submitted for publication.

Patients

We enrolled 24 women (age range, 30–85 years; mean age, 57.7 years; body mass index range, 20.5–61.6; mean body mass index, 30.1) who had breast cancer histologically confirmed from biopsy of lesions smaller than 3 cm and who were scheduled to undergo standard-of-care SLNM between March 20, 2006, and February 15, 2007, and prior to lumpectomy or mastectomy. Additional inclusion criteria were a negative serum or urine pregnancy test 24 hours prior to the study, if the woman was of childbearing potential, and she had to agree to a medically accepted method of contraception for 1 month following the study. Pregnant women or women who were breast feeding were excluded because of the unknown effects of indocyanine green on the fetus or infants. Since indocyanine green potentially causes anaphylaxis related to iodine allergy, patients with a history of allergy to iodine or shellfish were excluded from the study. Patients with a preexisting skin condition at the site of indocyanine green injection were also excluded.

Imaging Agent

Indocyanine green was diluted in saline to achieve the desired dose (Table). In the first eight women, the indocyanine green solution was added to the radiocolloid to constitute a 1 $\mu\text{mol/L}$ solution for intradermal and intraparenchymal injections with a 27-gauge needle. Although the combination of the indocyanine green solution with the radiocolloid was required by the Food and Drug Administration and Institutional Review Board to reduce the number of injections that patients would experience, the acidity of the radiocolloid solution reduced the fluorescent yield of indocyanine green as much as 10-fold (Fig E1, [<http://radiology.rsnajnl.org/cgi/content/full/2463070962/DC1>]) and yielded poor imaging results. Consequently, after approved protocol amendments, injections of indocyanine green were conducted separately and prior to injection of the radiocolloid in the remaining 16 women. The indocyanine green was administered either intradermally in 0.1–0.3-mL volumes by using 0.5-, 0.7-, or 1-mm-long microneedle devices (Research Catheter Set, 34-gauge; Becton-Dickinson Technologies, Research Park, NC) or 1.5-mm-long needles (Needle with Limiter, 34-gauge; Becton-Dickinson Technologies) and conventional 27-gauge needles, or subcutaneously in 1- to 3-mL volumes with conventional 27-gauge needles. The total indocyanine green mass administered to the patients escalated from 0.31 to 100 μg . After injection of indocyanine green and radiocolloid, the breast, supraclavicular, and axilla regions were gently massaged for 1–7 minutes.

In this feasibility study, the locations of contrast agent administration were in the breast quadrant in which the tumor resided (subjects 1–8), periareolar (subjects 9–20); and periareolar with one injection in the quadrant in which the tumor resided (subjects 21–24). Optical imaging began immediately afterward, followed by nuclear imaging. Vital signs were monitored at 15-minute intervals for two hours following agent administration and again at 4 hours. Follow-up monitoring to assess the appearance of skin rash or fever as indication of allergic response occurred at 24 and 48 hours after NIR fluorophore administration.

Optical Imaging, Gamma Scintigraphy, and Evaluation

Optical imaging of the fluorescent NIR fluorophores was performed (R.S., J.C.R., K.E.A., J.P.H., and E.M.S.) with an intensified charged-coupled device (CCD) (8). Briefly, the device has three principal components: (i) an NIR-sensitive image intensifier; (ii) a 16-bit dynamic-range frame-transfer CCD camera; and (iii) an 80-mW, 785-nm laser diode to

provide the excitation light to activate the NIR fluorophore. A technical failure was deemed to occur when less than $130 \mu\text{W}/\text{cm}^2$ of power was measured at 30 cm away from the laser diode.

The laser diode beam was expanded by using a plano-convex lens and a holographic optical diffuser such that approximately 0.02 m^2 of the breast and axilla surfaces were illuminated with a surface fluence of less than $1.9 \text{ mW}/\text{cm}^2$. A 785-nm holographic notch band rejection filter (HNPF-785.0–2.0; Kaiser Optical Systems, Ann Arbor, Mich) and an 830-nm image quality bandpass filter (830.0–2.0; Andover, Salem, NH) were placed before the 28-mm lens (Nikkor; Nikon, Japan) to selectively reject the excitation light and allow the emitted 830-nm fluorescence to pass. A collection of 50–400 images with 512×512 resolution and 200- to 800-msec exposure time were acquired, enabling near-real-time visualization of NIR fluorophore trafficking, quantitative analysis (R.S., see below), and evaluation (J.A.W. and E.M.S.). For image registration, white-light images were acquired with a neutral density filter as the camera captured focused images of the skin surface.

After 30–60 minutes of optical imaging, gamma scintigraphy images were acquired. A gamma camera with a low-energy, high-resolution collimator was used to acquire 256×256 -pixel, 140-keV peak energy images. Anterior, oblique, and lateral images of the chest were obtained with a cobalt 57 sheet source behind the patient to provide a silhouette of the body (to aid in anatomic localization of LNs). Both optical images and scintigrams, when available, were evaluated by nuclear medicine physicians (J.A.W., R.E.F., and S.B.C., with 15, 12, and 3 years clinical experience, respectively) immediately after each study to determine whether the isotope deposition seen at scintigraphy corresponded to that imaged by using fluorescence. In patients 4, 12, and 13, scintigraphy was not performed owing to the fact that the referring surgeon did not request it and the lack of sufficient time between optical imaging and surgery to enable scintigraphy.

Surgical Resection

Intraoperative localization and extraction of SLNs were performed by surgeons (H.Q.P., E.B., and D.K.B., each with at least 3 years experience) as part of standard-of-care. In the surgical suite, isosulfan blue was injected around the areola and the breast tissue was massaged. Typically, the blue dye is taken up through the lymphatic system and used to identify lymph vessels and nodes intraoperatively. Guided by the gamma probe, the SLN was initially localized and an incision made in the axillary region. The blue dye was then used as an intraoperative guide for lymphatic drainage, enabling identification of the lymphatic channel or LNs. Identifiable LNs that were radioactive, blue, and/or palpable were resected. After resection, the intact excised LNs were imaged (J.C.R.) for fluorescence in a light-tight dark box with a commercial imaging system (EC3; UVP Bioimaging Systems, Upland, Calif) with an overhead-mounted intensified CCD camera and a laser diode for excitation similar to that used with human subjects. A technical failure was deemed to occur when less than $130 \mu\text{W}/\text{cm}^2$ of power was measured at 30 cm away from the laser diode. The fluorescence status, blue color, and radioactivity of each resected LN were recorded and the LNs were then sent for pathologic analysis for touch preparation cytologic classification.

Evaluation of Lymph Function

Fluorescent images were processed by using software (Matlab, version 7.1.0.183, Mathworks, Natick, Mass; ImageJ, National Institutes of Health, Washington, DC; and V++, Digital Optics, Auckland, New Zealand). To reveal the frequency of the pulsatile lymph flow, the mean fluorescence intensity from a region of interest located at a lymph channel was selected and plotted as a function of imaging time. Lymph velocity was computed by

tracking the position of a “packet” of indocyanine green moving along the apparent length of a lymph channel as a function of time.

Statistical Analysis

The Spearman rank correlation test was used to test the association between the resected LNs that were fluorescent and the time elapsed between contrast agent injection and node resection. The Pearson product-moment correlation test (9) was performed to analyze associations between resected LNs that were fluorescent and the administered dose of NIR fluorophore. A *P* value of less than .05 indicated a significant difference. The mean and standard deviation of the velocity of NIR fluorophore packets and mean period of time between packets were determined for the number of specified packets transiting the lymphatic vessels. All calculations were performed by using statistical software (Matlab Toolbox, version 7.1.0.183; Mathworks).

Results

Regardless of administration route or use of delivery device, noninvasive NIR imaging of lymph channels failed in all 15 patients who received less than 10 µg indocyanine green (Table). A technical failure prevented noninvasive imaging of one woman, and two technical failures prevented the imaging of two surgical specimens. There were no adverse events associated with the optical imaging or the administration of indocyanine green.

Dynamic Lymph Trafficking

Of nine women who received 10 µg NIR fluorophores or more, lymph architecture was noninvasively imaged in eight. Of those eight, dynamic lymph trafficking was seen in seven. In one woman who was administered a total of 20 µg in four periareolar intradermal injections, only the epidermal “depot” of indocyanine green was fluorescent, and no apparent lymph uptake or trafficking was observed. In the remaining seven patients, the propulsive lymph flow velocities ranged from 0.08 cm/sec ± 0.03 to 0.32 cm/sec ± 0.08 (Table; Fig 1), similar to that found in swine (10). The time between consecutive NIR fluorophore packets ranged from 14.3 seconds ± 2.5 to 94 seconds ± 16. As the lymph vessels drain into deeper tissues, the fluorescent intensities diminished and became less resolved owing to the scatter experienced by propagating NIR light. Movies that illustrate the dynamic propulsion and results from two additional example studies are provided in the online supplemental material (Figs E2, E3; Movies E1–E3, [<http://radiology.rsna.org/cgi/content/full/2463070962/DC1>]). Not all women exhibited lymph propulsion (Fig 2). In patient 5 (Table), who was injected peritumorally and subcutaneously in the inner lower quadrant, two distinct mediastinal fluorescence signals were observed. However, lymphoscintigraphy showed no corresponding drainage of the radiocolloid. Since internal mammary nodes are not typically resected for pathologic evaluation, we could not confirm whether lymph nodes associated with the region were also fluorescent.

Lymph Nodes

Of the 56 resected LNs (Table), we had complete information (ie, no technical failures) for 49 LNs. Of these 49, 43 were radioactive, 30 were blue, and 27 were fluorescent. Eighteen nodes were radioactive, blue, and fluorescent; 11 were radioactive and blue; nine were radioactive and fluorescent; five were radioactive; one was blue; and five were not radioactive, blue, or fluorescent. Of the 22 resected nodes in patients who received 10 µg or more of indocyanine green, 15 were fluorescent (Fig 3). Owing to the small sample size in this feasibility study, we were unable to determine whether there were statistical differences in whether the resected LNs were marked by the radiocolloid, NIR fluorophore, or blue dye.

We did not find a significant association between the fluorescent status of the resected node and the amount of NIR fluorophores administered (Pearson correlation, $r = 0.51$; $P > .05$). However, when indocyanine green doses were 10 μg or more, we found a weak negative trend between the fluorescent status of resected nodes and the time between NIR fluorophore administration and resection, although the correlation was not significant (Spearman rank correlation, $r = -0.42$, $.5 > P > .2$).

Discussion

Previously, Motomura et al (11) injected 25 mg of indocyanine green in 5 mL of diluent in the breast parenchyma peritumorally to identify stained LNs. Later, Kitai et al (12) demonstrated the use of subareolar administration of 25 mg indocyanine green and a light-emitting-diode-charged-coupled device system to collect fluorescence for guiding SLNM in breast cancer patients following intradermal injection. In contrast to these studies, we are *not* attempting to develop new methods for SLNM in breast cancer patients, as current techniques by using radiocolloid and isosulfan dye show excellent SLN identification rates (13). Rather, we seek to show the feasibility of NIR optical imaging following microdose ($\leq 100 \mu\text{g}$) administration of indocyanine green to use comparable fluorophore concentrations in molecularly targeted agents to noninvasively assess nodal status in humans.

We found initially that doses of indocyanine green less than 1.0 μg were insufficient for adequate NIR imaging in 15 of 15 patients. With doses of 10–100 μg however, we were able to successfully image lymphatic drainage pathways and SLNs in eight of nine women. Our results with low doses of indocyanine green suggest the feasibility of future NIR molecular imaging for nodal staging with microdose administration of protein- and peptide-labeled NIR agents in humans. Microdosing is expected to result in a low probability of adverse events, as is achieved with positron emission tomographic imaging agents, and facilitate translation of NIR molecular imaging (7). We believe the opportunity to employ molecularly targeted NIR conjugates for guiding surgical resection of cancer-positive LNs has the potential to improve the accuracy and morbidity of nodal staging, not only in breast, but other cancers as well.

The NIR dye used in this feasibility study was nonspecific and passed through the lymphatic system, as evidenced by the trend of negative correlation between the number of resected LNs that were fluorescent and the time elapsed between NIR fluorophore administration and LN resection. Consequently, we were restricted to early time imaging whereby the high photon count rate from superficial lymphatics and injection sites saturated the CCD and limited the noninvasive detection of deeper LNs. We believe that the use of target-specific NIR-labeled imaging agents that pass from superficial lymphatics, but are retained in cancer-positive LNs, would not create saturation of the camera and would enable noninvasive nodal staging by signals needed for deeper tissue imaging.

Indocyanine green dyes do not have reactive groups for conjugation to targeting moieties, but on conjugating other NIR fluorophores such as cypate (14), Cy7 (15), or IRDye800 CW (16) with targeting peptides or proteins, the microdosing limits of 100 μg or less of labeled protein or 30 nmol or less of labeled biologic agent (7) could be achieved. Indeed, for the minimum detectable signal offered by 10 μg of indocyanine green shown in this study, the minimum molar ratio of NIR dye (with molecular weight and fluorescent yield similar to that of indocyanine green) to antibody would be an easily achievable 0.43, while labeling ratios of dye to antibody that retains binding affinity can be expected to be between 1.0 and 7.0.

Additional improvements to reduce the noise by reducing excitation light leakage with proper filtering and optics (17) can further improve sensitivity. Finally, fluorescence tomography, as recently described and demonstrated by others (18), can be used to more accurately locate diseased LNs in three dimensions.

To our knowledge, ours is the first description of functional NIR lymph imaging in humans. Unexpectedly, we discovered that the lymph trafficking hypothesized to result from lymphangion contraction (19) exists in humans and can be directly imaged. Although angiographic and scintigraphic techniques are current reference standards of lymph imaging, neither offer the ability to dynamically image lymph function with temporal resolutions of milliseconds or less.

Owing to the high photon count rate associated with NIR fluorescence and the ability to perform measurements with millisecond integration times, we discovered that the lymph propulsion characteristic of lymphangions occurs, even if not consistently, within the lymphatics draining the breast tissue. We believe that the use of short integration times of an NIR-sensitive camera and optics enabled imaging of lymph propulsion, and we hypothesized that the ability to monitor lymph trafficking could enable noninvasive diagnostic imaging of lymph function in patients with lymphatic disorders. In our study of women undergoing SLNM, we could not discern the reasons for the variability of lymph propulsion among patients and note that insufficiency in lymph function is not measured or known as a risk factor for breast cancer-related lymphedema following nodal resection.

There were several limitations in our study. First, the small number of patients in the dose escalation trial prevented a direct comparison of whether radioactivity, blue dye, or NIR fluorophores demarked the resected tumor. The inability to control the time between administration of the nonspecific imaging agents and surgical resection also probably contributed to variability of results, especially because indocyanine green drains freely through the lymphatic system. In addition, we believe that excitation light leakage through rejection filters prevented imaging of deep lymphatics when less than 10 μ g of indocyanine green were administered.

Nonetheless, we demonstrated that noninvasive NIR optical imaging by using microdose administration of an NIR fluorophore is feasible in humans. Since indocyanine green cannot be conjugated to target peptides and antibodies, new dyes with reactive groups, greater quantum efficiencies, and excitation and emission spectra similar to indocyanine green must be employed. Future directions include conjugation of NIR fluorophores to target specific markers of cancer-positive LNs, improvement of agent delivery systems for supraclavicular and internal mammary LN detection and study of normal and abnormal lymph function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CCD	charged-coupled device
LN	lymph node

NIR	near-infrared
SLN	sentinel LN
SLNM	SLN mapping

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Advances in Knowledge

- When administered intradermally at total doses of 10 μg or more, soluble near-infrared (NIR) fluorophores can provide imaging of lymph propulsion through the vessels and in draining lymph nodes (LNs) in the breast and axilla of breast cancer patients.
- Lymph propulsion in the breast and axilla region was not always present, but when it was, the mean velocities ranged from 0.08 cm/sec $\pm$ 03 (standard deviation) to 00.32 cm/sec $\pm$ 08 and the time elapsed between consecutive packets of propelled fluid varied from 14 seconds $\pm$ 2.5 to 92 seconds $\pm$ 16.

Implication for Patient Care

- This study demonstrates the feasibility of noninvasive imaging to identify cancer-positive LNs in breast cancer patients by using targeted NIR molecular imaging agents.

Figure 1.

(a) White-light frontal view and NIR fluorescence frontal views (b) at reference time point, (c) after 14.5 and (d) after 20.1 seconds; and (e) frontal scintigram of left breast of patient 21, who received four periareolar intradermal 2.5- μ g NIR fluorophore injections (in 100 μ L solution each). (a–d) Stick diagram shows breast view; yellow circles demark NIR fluorophore packet trafficking. See also Movie E1

(<http://radiology.rsna.org/content/full/2463070962/DC1>). Images a–d correspond to lymphoscintigraphic e. Five cancer-negative LNs were resected 6 hours after NIR fluorophore and radiocolloid administration; one LN was radioactive, blue, and fluorescent; one, radioactive and blue; two, radioactive and fluorescent; and one, radioactive.

Figure 2.

(a) White-light view, (b) NIR fluorescence view, and (c) frontal scintigram of left breast of patient 20, who received four 25- μ g intradermal injections (in 100 μ L solution each) by using needle with limiter. Stick diagram shows breast view in a and b. Two lymphatic channels seen on fluorescence image were also seen on scintigraphic image (c). No lymphatic propulsion was seen in this patient. Scintigram confirms drainage to two LNs in axilla. One radioactive, blue, and fluorescent negative LN was resected 25 hours after agent administration.

Figure 3. White-light (left) and fluorescent (right) images of resected fat containing a sentinel node from patient 23 shows colocalization of blue and NIR fluorescent dyes.

Data Summary for 24 Patients Imaged Following Microdose Administration of IC-Green

Table

Patient No./Race	Tumor Type	IC-Green (µg)	Technetium 99m Sulfur Colloid (mCi)	Time between Biopsy and Injection (h)	No. of Resected LNs	Resected LN Indications			Packets			
						Radioactive	Blue Dye	Fluorescent	LN Fluorescence Status	Lymph Vessel Imaged	Mean Velocity (cm/s)	No. of Packets
1/White	IDC	0.43*	0.5*	3.7	2	2	2	TF	All nonfluorescent	No	...	...
2/White	DCIS	0.70*	3.3*	19.3	2	2	2	TF	All nonfluorescent	No	...	...
3/White	IDC I	0.70*	3.3*	3.1	2	2	2	2	All nonfluorescent	No	...	...
4/White	ILC	0.70*	1.1*	19.4	1	1	1	0	Nonfluorescent	No	...	...
5/White	IDC	0.77*	1.1*	23.1	3	3	1	1	All nonfluorescent	No	...	...
6/White	IDC III	0.70*	3.3*	18.1	5	3	3	1	All nonfluorescent	No	...	...
7/White	Multifocal ductal carcinoma	0.70*	3.3*	19.1	1	1	1	1	Nonfluorescent	No	...	...
8/White	IDC	0.70*	3.3*	3.1	3	3	2	0	Two fluorescent	No	...	...
9/White-Hispanic	IDC	0.31	0.325	3.6	3	2	2	TF	All nonfluorescent	No	...	...
10/White-Hispanic	IDC	0.31	0.35	6.5	2	2	2	1	All fluorescent	No	...	...
11/White-Hispanic	Residual DCIS	0.31	0.315	2.7	2	2	1	0	All nonfluorescent	No	...	...
12/White-Hispanic	DCIS	0.31	0.35	2.3	2	2	0	2	All nonfluorescent	No	...	...
13/Black-African American	DCIS	0.31	0.25	2.2	4	4	4	3	All nonfluorescent	No	...	...
14/White-Hispanic	DCIS	0.31	0.362	4.5	1	1	1	0	Nonfluorescent	No	...	...
15/White-Hispanic	Infiltrating DCIS	0.31	0.312	3.4	1	1	1	1	Nonfluorescent	No	...	...
16/White-Hispanic	ILC	10	0.37	22.9	2	1	1	1	All fluorescent	Yes	0.32 ± 0.08	66.0 ± 44.0, 54.0 ± 35.0
17/White	IDC	25	1.25	10.0	4	2	3	1	All nonfluorescent	Yes	NA	14.3 ± 2.5

Patient No./Race	Tumor Type	IC-Green (µg)	Technetium 99m Sulfur Colloid (mCi)	Time between Biopsy and Injection (h)	No. of Resected LNs	Resected LN Indications				Lymph Vessel Imaged	LN Fluorescence Status	Packets	
						Radioactive	Blue Dye	Fluorescent				Mean Velocity (cm/s)	No. of Packets
18/White	DCIS	50	0.604	6.2	2	2	2	2		Yes	All nonfluorescent	0.25 ± 0.07	5
19/Black–African American	DCIS	80	0.379	5.0	3	3	0	3		Yes	All nonfluorescent	0.22 ± 0.06	5
20/White-Hispanic	IDC	100	0.355	25.0	1	1	1	1		Yes	Nonfluorescent	...	...
22/White	ILC	10	0.371	3.5	1	1	0	1		Yes	Fluorescent	0.14 ± 0.03	2, 4 [‡]
23/White-Hispanic	IDC	20	0.362	6.3	4	3	2	3		No	All nonfluorescent	...	...
24/White-Hispanic	NA	20	1.2	No surgery	0	0	0	0		Yes	Nonfluorescent	0.14 ± 0.0001	2, 8 [‡]

Note.—DCIS = ductal carcinoma in situ, IDC = infiltrating ductal carcinoma, ILC = infiltrating lobular carcinoma, NA = not applicable, TF = technical failure.

* Technetium 99m sulfur colloid (radiocolloid) and IC-green were combined in one injected solution.

[‡]There were insufficient data along the length of the vessel to compute velocity for the same data where there was sufficient data for the computing period. As a result, we present the period for the same packets that velocity was computed for and separately report the period for studies when more packets were analyzed for period than for velocity.