Near Infrared Fluorescent Lymph Node Mapping with Indocyanine Green in Breast Cancer Patients: A Prospective Trial

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BACKGROUND: Near infrared (NIR) fluorescence imaging is an emerging modality that can enable real-time image-guided procedures. Indocyanine green (ICG) is an FDA-approved, inexpensive, and widely available NIR dye. We hypothesized that axillary lymphatic mapping with ICG is equivalent to lymphatic mapping with technetium 99m (^{99m}Tc) in breast cancer patients.

STUDY DESIGN: Breast cancer patients (cT1-2, N0) were prospectively enrolled. Patients underwent lymphatic mapping with ^{99m}Tc preoperatively and ICG mapping intraoperatively (0.8 mL). Sentinel lymph node (SLN) biopsy was guided by NIR camera and gamma probe. Rate of failed mapping, number of SLNs identified, and rate of identifying pathologically positive SLNs were compared between the 2 techniques ($p < 0.05$ was considered statistically significant).

RESULTS: Ninety-two female patients were enrolled (median age 59 years). Mean transit time from ICG injection in the breast to localization in the axilla was 5 minutes (range 2 to 29 minutes). No adverse reactions to ICG were noted. Mean number of SLNs identified with ICG and ^{99m}Tc was 2.4 (SD 1.42) and 2.2 (SD 1.23), respectively ($p = 0.34$). Pathologically positive SLNs were identified in 18 (19.8%) patients. A total of 24 pathologically positive SLNs in 18 patients were identified by ICG in 24 of 24 (100%) patients and by ^{99m}Tc in 23 of 24 (96%) patients ($p = 0.99$).

CONCLUSIONS: Indocyanine green with NIR fluorescence imaging can be safely and efficiently used for real-time intraoperative lymphatic mapping in breast cancer patients. Indocyanine green performs similarly to ^{99m}Tc with regard to the number of SLNs identified, rate of failed mapping, and identification of pathologically positive SLNs. (J Am Coll Surg 2019;228:672−678. © 2018 by the American College of Surgeons. Published by Elsevier Inc. All rights reserved.)

Lymphatic mapping and axillary SLNB are the standard procedures for the surgical staging of patients with early-stage breast cancer. Sentinel lymph node (SLN) biopsy has been shown to be safe, accurate, and limits the need for traditional axillary dissection in most patients.[1] Conventionally, SLN lymphatic mapping for breast cancer has involved the use of colloid radiotracer labeled with technetium 99 (^{99m}Tc),[2] visible blue dye (methylene blue or isosulfan blue),[3] or a combination of both.[4] Best mapping results are achieved with periareolar, subdermal injection of the tracer.[5,6] Sentinel nodes are defined as axillary lymph nodes in which there is the accumulation of 1 or more of these tracers or dyes higher than defined thresholds.[7]

The use of ^{99m}Tc-labeled radiotracer is one of the more commonly used techniques of SLN mapping. In large studies, the ^{99m}Tc SLN detection rate is 97.5% accurate,[8] however, it has significant limitations, including the requirement for nuclear licensing; the need to prepare, handle, and dispose of nuclear material; and patient inconvenience with painful injections typically occurring before operation. Although there are reports of surgeons

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Abbreviations and Acronyms
ICG = indocyanine green
NIR = near infrared
SLN = sentinel lymph node
^{99m}Tc = technetium 99

performing ^{99m}Tc injections in the operating suite, this still requires regulatory resources and is not common.[9] Blue-dye injections can be performed during the operation, but risk skin necrosis or anaphylaxis[10] and, when used alone, have a lower sensitivity yield of 91%.[11] In large studies involving experienced surgeons, the SLN detection rate using dual mapping with ^{99m}Tc and a visible dye is approximately 99%, therefore, use of both methods combined is recommended by some to achieve the highest accuracy of SLNB and to reduce the false-negative rate.[1,12]

Indocyanine green (ICG) dye is a fluorescent dye with moderate optical properties that can be detected using near infrared (NIR) cameras. Indocyanine green has been used in humans for more than 50 years and has an excellent safety profile.[13] There are many uses for ICG during image-guided procedures. After IV injection, it can be used for NIR angiography of blood vessels, skin flap perfusion for mastectomies, identification of the extrahepatic bile ducts, and identification of oncologic tumor metastases.[14] Indocyanine green has also been reported by numerous authors to be safe for subcutaneous injection and SLN mapping in gastrointestinal,[15] melanoma,[16] and breast cancer.[17-20] Use of ICG for lymphatic mapping and SLN identification offers many potential advantages over the use of other lymphatic mapping techniques, including the ability to inject the material while the patient is under anesthesia in the operating room; the ability to use NIR to directly, instantly visualize lymphatic anatomy and flow; no special material handling is required; and low cost.[19,21]

Recent studies from Europe and Japan have demonstrated high concordance between the number of breast cancer SLNs identified between ICG and ^{99m}Tc , suggesting a benefit of NIR-guided SNLB for standard use.[20,22-25] There has been very limited work on the use of ICG for breast cancer axillary lymph node mapping in the US.[19,25] The aim of this study was to prospectively compare the equivalency of ICG to ^{99m}Tc for axillary SLN lymphatic mapping in patients with early-stage breast cancer in terms of the numbers of nodes identified and the identification of pathologically positive nodes.

METHODS

With IRB approval, between 2016 and 2018, patients with stage T1 or T2 clinically node-negative breast cancer undergoing either a lumpectomy or mastectomy were prospectively consented and enrolled to receive ICG- and ^{99m}Tc-guided SLNB. All patients with breast cancer underwent preoperative axillary ultrasound to confirm clinical node-negative status before operation. Patients received standard preoperative periareolar ^{99m}Tc sulfur colloid injection in accordance with routine clinical practice before operation. The procedure was performed by 4 surgeons trained in ICG SLN mapping and ^{99m}Tc SLN mapping.

At the time of operation, after the induction of anesthesia, 0.8 to 1 mL 0.5% ICG solution (a 25-mg vial of indocyanine green [trial medication] was prepared with 5 mL sterile water) was injected subdermally into the breast subareolar region. Indocyanine green movement into the periareolar lymphatics was facilitated by manual massage and immediately observed with NIR fluorescence imaging. Indocyanine green lymphatic drainage was detected and monitored in real time by a handheld photodynamic eye camera (Mitaka USA). Once the ICG dye reached the axillary basin, transcutaneous imaging was facilitated by use of a translucent "image enhancer" (Hamamatsu Photonics), which was used to compress the axillary subcutaneous tissue and allowed for visualization of the ICG avid lymph nodes (Fig. 1). This precise spot of fluorescence in the axilla was marked for incision placement for the SLNB procedure. With assistance of the photodynamic eye camera, the fluorescent lymphatic channels were then dissected and followed to the first ICG avid lymph node. The lymphatic channels were meticulously clipped and ligated so that ICG leakage into the dissection field was avoided.

Fluorescent lymph nodes (ICG-positive) were sequentially identified and removed. The node in which the lymphatic flow first entered was excised as the first SLN. The following subsequent fluorescent nodes were identified and removed in a similar manner. Sentinel lymph node removal continued until no residual fluorescence was visible in the axilla. Excised nodes were then tested for ^{99m}Tc radioactivity using a standard gamma-detecting probe and the counts per 10 seconds were recorded. Finally, the axillary region was inspected with the gamma probe to determine whether there were any residual radioactive nodes.[26] With regard to radioactivity, SLN were defined as any node with at least 10% of the hottest nodal counts.[7] All SLNs were sent for pathologic analysis as per standard surgical practice. Failed mapping

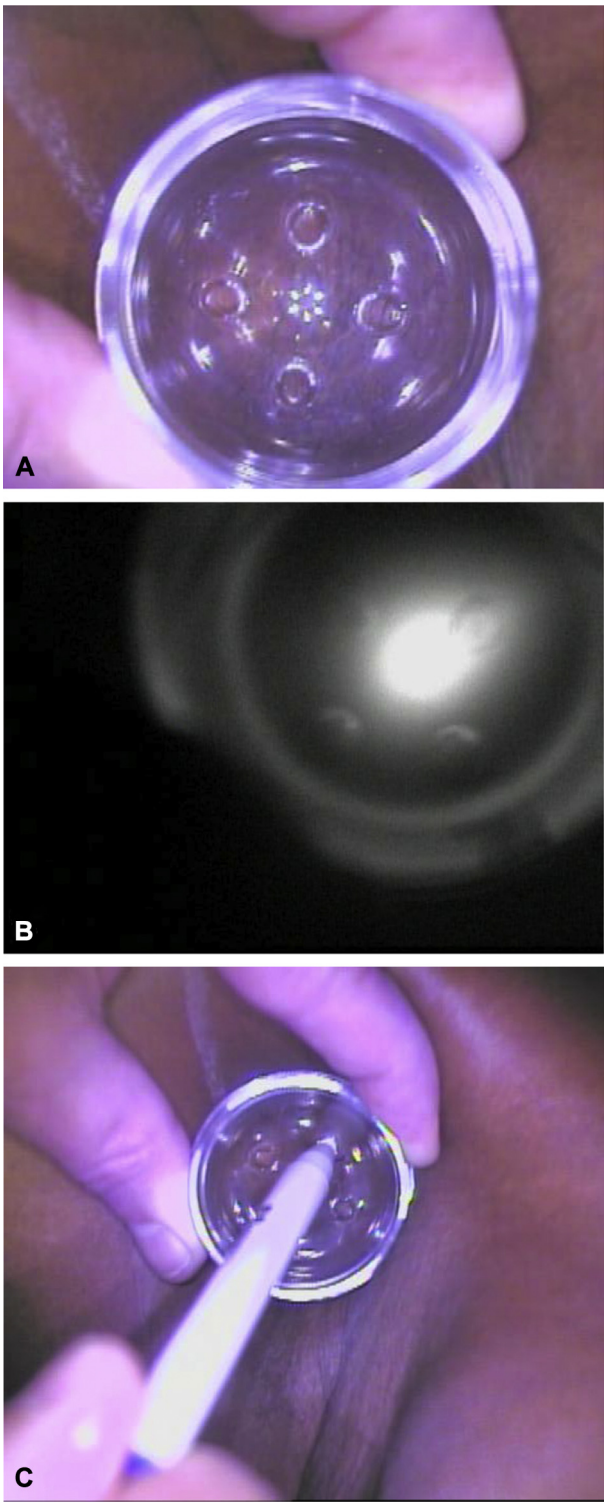

Figure 1. Use of the "image enhancer" to help identify indocyanine green (ICG) sentinel lymph nodes in the axilla. (A) Image enhancer is used to visualize lymphatic drainage and ICG avid lymph nodes in the axilla using the near infrared camera. (B) Site of node is marked using the image enhancer. (C) Fluorescent node in the axillary nodal basin.

was defined as no uptake of the ICG dye and/or ^{99m}Tc into an axillary SLN. For the purposes of this study, SLNs were defined as "sentinel" if they were fluorescent and/or met threshold for radioactive positivity. Clinically palpable nodes that did not map, but were removed based on the judgment of the operating surgeon, were not included in this analysis.

Patient and tumor data collected included patient age, BMI, tumor size, and tumor histology. Indocyanine green transit time (time from injection to the time fluorescence reached the axilla) for each procedure was documented. Sentinel lymph nodes were compared to identify concordance among those identified as fluorescent, radioactive, and both. The total number of SLNs identified by ICG, ^{99m}Tc, or both, was then recorded and analyzed.

Statistical methods

Data were described using medians and ranges for continuous variables and counts and percentages for categorical variables. The null hypothesis for equivalence of the 2 SLN mapping techniques (ICG and ^{99m}Tc) is that the difference between the proportion of SLNs detected by each method lies outside the margin (-5%, 5%). Tests for equivalence were based on the methods for analyzing clustered paired binary data and results presented by a 2-sided 95% CI compared with 5% equivalence region.[22] For pathologically positive axillary SLNs, McNemar test was used to compare the difference in detection rate between the 2 methods. R software was used for all analyses.

RESULTS

A total of 92 women underwent SLNB using both ^{99m}Tc and ICG. There were no adverse reactions to the injection of ICG. One patient in this series (1 of 92 [1%]), with a history of a breast reduction, had a failed SLN mapping to both ICG and ^{99m}Tc, leaving 91 patients for SLN analysis. One patient had 1 SLN identified with ICG but not with ^{99m}Tc. In total, the failed mapping rate for the 2 techniques was 1% (1 of 92) for ICG and 2% (2 of 92) for ^{99m}Tc.

Table 1 summarizes the patient and tumor characteristics. Patients had a median age of 59 years (range 35 to 81 years) and a median BMI of 27.5 kg/m^2 (range 17 to 51 kg/m^2). Median transit time from intraoperative ICG injection to NIR fluorescence localization in the axilla was 5 minutes (range 2 to 29 minutes). Longer ICG transit time was found to be positively correlated with higher BMI ($p = 0.025$).

A total of 235 SLNs containing either 1 or both of the tracers were identified. Median number of SLNs removed with ICG was 2 (range 1 to 7) and with ^{99m}Tc was 2

Table 1. Patient and Tumor Characteristics

Characteristic	Data
Patient	
n	92
Age, y, median (range)	59 (35–81)
BMI, n (%)	
<25 kg/m^2	36 (39)
25.1 to 30 kg/m^2	30 (32.6)
30.1 to 35 kg/m^2	16 (17.4)
>35.1 kg/m^2	10 (11)
Tumor	
Type, n (%)	
Invasive ductal	68 (74)
Invasive lobular	9 (10)
Mixed	12 (13)
Ductal carcinoma in situ	3 (3)
Size (T stage), n (%)	
Tis	3 (3)
T1a	5 (5)
T1b	32 (35)
T1c	32 (35)
T2	20 (22)
Receptor status, n (%)	
Estrogen receptor positive	81 (88)
HER2 amplified	5 (5.4)
Triple negative	10 (11)

HER2, human epidermal growth factor receptor 2.

(range 0 to 5). Dual tracer was found in 191 (81%) SLNs, ICG alone identified 33 (14%) additional SLNs, and ^{99m}Tc alone was found in 11 (5%) SLNs (Table 2). The 2-sided 95% CI for the difference in proportions of SLNs detected by the 2 methods was 0.036 to 0.151, which shows the ICG SLN mapping method is equivalent to ^{99m}Tc in the ability to identify SLNs.

Pathologically positive axillary SLNs were found in 18 of 91 (19.7%) patients. Among these 18 patients, there were 24 pathologically positive SLNs, which represented 10.2% of all 235 of the SLNs removed. Indocyanine green identified 24 of 24 (100%) positive SLNs and ^{99m}Tc identified 23 of 24 (96%) positive SLNs ($p > 0.99$). The 24 SLNs with metastatic disease included 19 with macrometastatic disease, 1 with micrometastatic disease, and 4 with isolated tumor cells.

DISCUSSION

Although SLNB has become widely accepted for breast cancer staging, several studies have suggested a variation in the techniques and procedural proficiency among surgeons.[27,28] Surgeons who perform fewer SLNBs have been shown to demonstrate less proficiency with the procedure.[29] A recent report also has demonstrated that surgeon-associated variations exist in both the number of SLNs removed and likelihood of finding a positive SLN.[30] To maximize success rates and accuracy of SLN identification, use of dual mapping, which consists of the injection ^{99m}Tc and a blue dye, has been recommended.[1] However, there remains heterogeneity in the conduct of SLN mapping in breast cancer patients.[28] A recent expert panel suggested that there is a need to "improve the technical performance and success rate of SN biopsy."[28]

Table 2. Number of Sentinel Lymph Nodes Identified with Indocyanine Green and Technetium 99m Methods

	Data (n = 235)	
Mapping	n	%
ICG mapped; ^{99m}Tc mapped	191	81
ICG mapped; ^{99m}Tc not mapped	33	14
ICG not mapped; ^{99m}Tc mapped	11	5

ICG, indocyanine green; ^{99m}Tc, technetium 99m.

The current study supports the goal discussed earlier by showing that, in breast cancer patients, ICG with NIR imaging is a safe and precise method for SLN mapping with accurate SLN detection. The ICG technique in this study, when directly compared with ^{99m}Tc SLN mapping, identified both similar numbers of SLN and a similar rate of finding pathologically positive SLNs.

The excellent results obtained with ICG SLN mapping are supported by other studies comparing ICG against either ^{99m}Tc , blue dye, or both.[17,18,20,22,23,25,31-33] Recently, Sugie and colleagues[20] compared ICG with ^{99m}Tc and found individual SLN mapping rates to be identical at 97%, and when used in combination, the rate improved to 99.8%. In our current study, although not statistically significant, ICG performed slightly better than ^{99m}Tc in appropriate identification of positive SLNs (100% vs 96% identification rate, respectively).

A concern raised in some earlier studies is the identification of an excessive number of SLNs using the ICG technique. Removal of excessive nodes during the SLN procedure is not desirable, as upper extremity morbidity risk increases with increasing removal of SLN.[34] Sugie and colleagues[32,35] reported the mean number of SLNs identified with ICG was significantly higher than blue dye or ^{99m}Tc. Samorani and colleagues[36] prospectively evaluated ICG vs ^{99m}Tc for SLN identification and found the median SLNs removed in each group was 2, but range was 1 to 5 SLNs with ICG and 0 to 5 with ^{99m}Tc , which was statistically different. The current study shows no difference in the number of SLNs removed comparing ICG and ^{99m}Tc. It has been suggested that the concentration of ICG injected is associated with the number of SLNs

identified.[25] In the current study, we used a higher concentration of ICG, 0.5%, than used in many other studies. Ballardini and colleagues[22] proposed the reason ICG dye identifies more SLNs than ^{99m}Tc is that the ICG molecule is smaller than the ^{99m}Tc bound to albumin and therefore travels faster.[22] Their group recommended starting the SLN procedure immediately after the ICG is seen reaching the axilla.

Indocyanine green offers the advantages of real-time imaging, ease of handling, low cost, and rapid localization to the SLNs. The lymphatic transit time documented in our study shows the median time from ICG injection to start of SLN excision of just 5 minutes. Others identified lymphatic channels at 3 minutes and axillary lymph nodes at 15 minutes, which is consistent with our findings.[17,25] We believe that we found similar rates of SLN removal between tracers because our surgeons standardized 3 important steps of the operation: the ICG dose used (0.8 mL 0.5% ICG)[22,23]; the periareolar, subdermal injection site; and the immediate use of the photodynamic eye camera to detect ICG and perform the SLN portion of the operation first.

In the current study, patients with a higher BMI had a longer transit time of ICG mapping. Grischke and colleagues[37] evaluated ICG SLN mapping and BMI and found that a BMI >40 kg/m^2 was associated with failed mapping.[37] The authors point out that NIR penetrates tissue to a maximum depth of 2 cm. Although the mean BMI in our study was 27.5 kg/m^2, the range extended to 51 kg/m^2, with 11% of patients being obese. These patients had longer ICG transit time, but all SLNs were able to be identified. We used the axillary "image enhancer" device to help compress the axillary tissue and attenuate ICG signals, which is particularly helpful in obese patients, and was also used by Sugie and colleagues,[32] who showed SLN identification was independent of BMI.

The ICG NIR imaging method is an efficient, convenient, and equivalent intraoperative method of SLN detection compared with traditional ^{99m}Tc. The ICG dye is inexpensive but does require an NIR camera, which is becoming more common in operating rooms due to the emergence of NIR imaging for surgical guidance.[21] Near infrared technology continues to advance, is becomingly widely available, and can be used for many other types of operations.[15,16] The ICG SLN detection method is known to be associated with very few side effects, and no patient in our study experienced any reaction, confirming its safety. Technetium 99m has a higher cost and more patient inconvenience due to timing of the procedure and discomfort, as well as radioactive material handling issues.[9] Blue dye has no radiation exposure and is more convenient, but it is less accurate than ^{99m}Tc. The blue dyes are also associated with safety concerns.

In the modern treatment of breast cancer, the importance of SLNB has focused on appropriately staging patients to customize their adjuvant treatment recommendations. With fewer completion axillary lymph node dissections being performed due to increased morbidity and no survival benefit for early-stage breast cancer,[38] the routine use of dual tracer for the now standard SLN procedure in a clinically node-negative breast cancer might be overuse of resources and patient's time and, essentially, overtreatment. The results of this study comparing ICG with ^{99m}Tc shows that the ability of ICG to identify SLNs is similar, although more studies are recommended to strengthen this argument. We propose that ICG SLN identification could be used as a sole mapping technique to appropriately and accurately identify SLNs without the cost of dual mapping, without the use of extra ^{99m}Tc resources, and without the side effects associated with the use of blue dyes. Additionally, newer studies have demonstrated the success of NIR-guided ICG SLN mapping to appropriately and consistently identify the lymphatic pathways and SLNs in the neoadjuvant chemotherapy setting.[39,40]

A limitation to this study is that the false-negative rate is unable to be determined, as completion axillary lymph node dissection after SLN mapping was not performed. The National Surgical Adjuvant Breast and Bowel Project trial B32 results showed a 9.8% false-negative rate with use of dual mapping and is the current accepted standard.[1] Hirche and colleagues[41] did evaluate ICG SLN mapping and subsequent completion dissection and found the SLN detection rate to be 97.7% and sensitivity for SLN positivity to be 94.4% (false-negative rate 5.8%).[41] Additionally, the study meta-analysis by Zhang and colleagues[33] evaluating 19 ICG studies showed SLN sensitivity at 98% with a false-negative rate of 8%.

CONCLUSIONS

Indocyanine green with NIR fluorescence imaging can be safely and efficiently used for real-time intraoperative lymphatic mapping in breast cancer patients. This study demonstrates that ICG safely performs similarly to traditional ^{99m}Tc with regard to the number of SLNs identified, rate of failed mapping, and identification of pathologically positive SLNs.

Author Contributions

Study conception and design: Valente, Al-Hilli, Radford, Yanda, Tu, Grobmyer

Acquisition of data: Valente, Al-Hilli, Radford, Yanda, Tu, Grobmyer
Analysis and interpretation of data: Valente, Al-Hilli, Radford, Yanda, Tu, Grobmyer
Drafting of manuscript: Valente, Al-Hilli, Radford, Yanda, Tu, Grobmyer
Critical revision: Valente, Al-Hilli, Radford, Yanda, Tu, Grobmyer

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Discussion

DR EDWARD COPELAND (Gainesville, FL): I think that we can all agree that the use of any blue dye is no longer necessary. We all owe a debt of gratitude to individuals like Drs Morton, Giuliano, and Krag for convincing us of the acceptable accuracy of the sentinel lymph node (SN) technique. Dr Philip Efron, of our group, reported, in 2002, one of the initial patients with an anaphylactic reaction to isosulfan blue. Multiple medications and fluids were required to resolve hypotension. The reaction was, indeed, delayed for several minutes after the dye injection. Subsequent to this experience, we abandoned all blue dyes. A hint to all of you who will continue to use blue dye is to ask your patient preoperatively if her eye lids swell after she applies blue mascara to them. The blue color in some preparations contains isosulfan blue.

Our nuclear medicine team used high and low molecular weight colloids as the carriers for the technetium-labeled radiotracer. The small particles identified the SNs rather immediately, and the larger particles remained in the SN to provide visibility the next day in the operating room. This method eliminated the need for injection on the same day. And, yes, we checked the concordance on the 2 days to ensure the same nuclear scan.

Now we move into the discussion of eliminating a radiotracer entirely. Dr Valente has done a good job of convincing me that technetium is no longer necessary. The data with indocyanine green using the patients' lymph nodes as their own controls are quite convincing, and, in the hands of the Cleveland Clinic team, are similar enough to result in discarding a radio label substance. Is this, in fact, correct? Does your group still use a radiotracer and, if so, when? Dr Ramsay Camp has shown that the hottest lymph node with technetium is not necessarily a positive lymph node. Do you grade the fluorescence of the lymph nodes removed and, if so, is there a correlation with the positivity of lymph nodes?

Lastly, you clip all lymphatic channels containing indocyanine green. How arduous is this task and why is it necessary? Dr V Suzanne Klimberg has shown, using the reverse axillary technique, that the afferent lymph nodes would also be needed to be clipped to reduce lymphedema. I expect she will allude to this in her subsequent discussion of this excellent paper.

DR V SUZANNE KLIMBERG (Galveston, TX): I have used indocyanine green (ICG) and found it to be quite messy. It seemed to lighten up the whole background. You mentioned that you clipped the lymphatics so it does not spill. What other practical hints do you have for using ICG? I purposely do not clip the afferent and efferent lymphatics, but reapproximate them to prevent lymphedema. We have shown this in humans as well as in a rat model; if you just reapproximate them, they grow back together.

This is part of the axillary reverse mapping (ARM). Why did you not use ARM in this group of patients? I see on the film you were doing that but did not report it. How many did you do? If you calculate it, just as many women get lymphedema with a negative sentinel lymph node as an axillary node dissection. I know you published an axillary node dissection, but I am a proponent of using ARM with a sentinel lymph node, because that is the real crime. If you have a negative sentinel lymph node, you did not even need the procedure, and so we want to prevent lymphedema in those patients and there are many more patients undergoing sentinel lymph node than an axillary node dissection today.

Did you look at lymphedema in this group of patients? You said ICG was more accurate at finding positive lymph nodes, but 4 of the 24 lymph nodes you reported had only in-transit cells, which is not considered a positive node, so how does removing those 4 negative nodes from your calculation affect the false negative rate between the groups? The average node count was a bit higher in the ICG group, with a range of 1 to 7. How many of the group had 6 or 7 lymph nodes? The cooperative groups would consider those patients to have an axillary node dissection, in fact. Finally, 11% of the patients in your group were obese. In the US, and especially in the South, the rate of obesity is $> 40\%$. What was the false negative rate specifically in your obese patients, as opposed to your regular patients?

DR KEITH DELMAN (Atlanta, GA): I am not a breast surgeon, but from the standpoint of transit time, when you have control of a colloid, you can slow down transit and have it get trapped in the