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ORIGINAL ARTICLE

Intraoperative Fluorescence Guidance for Breast Cancer Lumpectomy Surgery

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Abstract

BACKGROUND Although lumpectomy and mastectomy provide equivalent survival for patients with breast cancer, local recurrence after lumpectomy increases breast cancer mortality. Positive lumpectomy margins, which imply incomplete tumor removal, are the strongest predictor of local recurrence and are identified days after surgery, necessitating a second surgery.

METHODS In this prospective trial, we assessed margin status with or without pegulicianine fluorescence-guided surgery (pFGS) for stages 0 to 3 breast cancers. To prevent surgeons from performing smaller than standard lumpectomies in anticipation of pFGS assistance, patients were randomly assigned 10:1 to pFGS or control groups, thus randomization was not designed to provide a control group for evaluating device performance. In patients undergoing pFGS, additional pFGS-guided cavity margins were excised at sites of pegulicianine signal. We evaluated three coprimary end points: the percentage of patients for whom pFGS-guided margins contained cancer, sensitivity, and specificity.

RESULTS Overall, 406 patients received 1.0 mg/kg intravenous pegulicianine followed by lumpectomy. Among 392 patients randomly assigned, 316 had invasive cancers, and 76 had in situ cancers. In 27 of 357 patients undergoing pFGS, pFGS-guided margins removed tumor left behind after standard lumpectomy, 22 from cavity orientations deemed negative on standard margin evaluation. Second surgeries were avoided by pFGS in 9 of 62 patients with positive margins. On per-margin analysis, pFGS specificity was 85.2%, and sensitivity was 49.3%. Pegulicianine administration was stopped for adverse events in six patients. Two patients had grade 3 serious adverse events related to pegulicianine.

CONCLUSIONS The use of pFGS in breast cancer surgery met prespecified thresholds for removal of residual tumor and specificity but did not meet the prespecified threshold

A complete list of investigators in the INSITE Study Team is provided in the Supplementary Appendix, available at evidence.nejm.org.

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Introduction

Although survival after breast-conserving surgery (i.e., lumpectomy) is equivalent to survival after mastectomy,[1] local recurrence after lumpectomy increases mortality, with one excess breast cancer death observed for every four local recurrences.[2] The risk of local recurrence is directly related to incomplete tumor removal by lumpectomy surgery.[3-8] Currently, the presence of tumor cells at or near lumpectomy specimen margins is used as a measure of residual tumor in the conserved breast. In current practice, pathological margin evaluation is completed days after surgery. Invasive tumor at the margin or ductal carcinoma in situ (DCIS) within 2 mm of the margin increases risk for both local and distant recurrence[9] and necessitates a second surgery to obtain negative margins.

This approach to breast-conserving surgery has two major flaws. First, excised lumpectomy specimens deform immediately after excision, losing specimen surface orientation relative to the lumpectomy cavity where tumor may remain. Handling and sectioning of specimens can expose tumor not actually at the margin but attributed to the margin, creating falsely positive readings.[10,11] Second, false-negative margin readings are common, as significant tumor may remain in the breast, even when margins on the lumpectomy specimen are deemed "negative." Rates of local recurrence after lumpectomy without radiation highlight the proportion of breasts with clinically significant residual tumor after standard lumpectomy surgery. Local recurrence at 20 years in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-06 trial was 39.2% without radiation versus 14.3% with radiation.[1] For DCIS, rates of local recurrence at 15 years were 15.1 to 35.1% without radiation versus 7.1 to 17.5% with radiation.[12,13]

Lumpectomy margins are positive in 20 to 40% of patients, with wide variation in reexcision rates.[14-16] A comparison of comprehensive cavity shave margins versus standard surgery[14] found positive margins after 34% of standard lumpectomies and 19% with additional shaves. Some centers use frozen section to attempt real-time margin assessment, which requires the presence of a skilled and experienced pathologist. A review of 1102 patients found the sensitivity of frozen section to be only 5.3%, with no statistically significant difference in positive margins (14.3 vs. 16.9%) but longer operative times.[17]

If it were possible to evaluate the entire lumpectomy cavity intraoperatively, allowing the surgeon to immediately identify and remove residual tumor, many of the problems noted above could be avoided. We have previously described a novel fluorescence-guided surgery system that achieves many of these goals.[18-20] In this approach, pegulicianine,[21] an activatable fluorescent imaging agent, is injected intravenously before surgery and produces a signal at sites of residual tumor. During surgery, the surgeon illuminates the cavity with a handheld probe, and a tumor detection algorithm analyzes and displays the images to the surgeon in real time.[18-20] We have previously shown, in a multicenter feasibility study, that pegulicianine fluorescence-guided surgery (pFGS) can identify residual tumor in lumpectomy cavities.[22] We report herein the results of a prospective, multicenter trial of pFGS in patients undergoing lumpectomy for breast cancer.

Methods

STUDY DESIGN AND PATIENT SELECTION

We conducted a prospective, multicenter trial enrolling patients at 14 U.S. sites. The research protocol was approved by a central institutional review board and by institutional review boards at participating sites. Most surgeons had previously received supervised training in three to five cases using the pFGS system[22] before enrolling patients. All others underwent required training and had their initial pFGS procedures supervised by an experienced pFGS surgeon.

All study participants provided written informed consent. Eligible individuals included female patients 18 years of age or older undergoing lumpectomy for stages 1 to 3 invasive breast cancer and/or DCIS. Patients receiving neoadjuvant therapy or undergoing margin reexcision following prior lumpectomy were excluded. Sentinel node mapping used isotope-only guidance as blue dyes fluoresce in the same wavelengths as pegulicianine. Patients received standard preoperative isotope injections, with gamma-probe scanning before surgery confirming sentinel node identification by isotope. Surgeons could use blue dye and withdraw the patient from the trial if the isotope signal was insufficient for sentinel node identification. Isotope-guided sentinel node biopsy could be performed before or after lumpectomy.

STUDY PROCEDURES

All patients received 1.0 mg/kg pegulicianine as a 3-minute intravenous infusion 2 to 6 hours before surgery. Surgeons completed their standard lumpectomy, excising the malignant lesion with a rim of normal-appearing tissue guided by palpation or marker localization (wires or seeds) for nonpalpable lesions, including the removal of selective additional shaves for grossly close margins or comprehensive shaves by surgeons for whom that was standard practice. To optimize orientation, all specimens were oriented with sutures and/or six-color inking in the operating room.

To prevent surgeons from performing a smaller than standard lumpectomy in anticipation of pFGS assistance, patients were randomly assigned in a 10:1 ratio to the pFGS group or a control group without the use of pFGS, with group allocation revealed only after the surgeon declared that the standard lumpectomy was completed (Fig. 1). For this reason randomization was not designed to provide a control group for analysis of device performance. In this study design, each patient undergoing pFGS served as her own control, with analysis on the basis of paired data points of final margin pathology after standard lumpectomy and final margin pathology after standard lumpectomy plus additional pFGS-guided cavity margins. Patients in the control group were included only in the safety analysis. In the pFGS group, the lumpectomy cavity was imaged using a 2.6-cm-diameter (5.3 cm^2) field-of-view optical head after the pFGS software determined the normal tissue fluorescence baseline for each patient.[19,20,22] When the pFGS software indicated a positive pFGS signal, the surgeon removed additional lumpectomy cavity tissue, termed pFGS margins, from that orientation and then, reimaged that cavity orientation. A maximum of two pFGS margins were taken from any single-cavity orientation. All tissue removed underwent standard histopathologic margin assessment. Positive margins were defined as invasive tumor

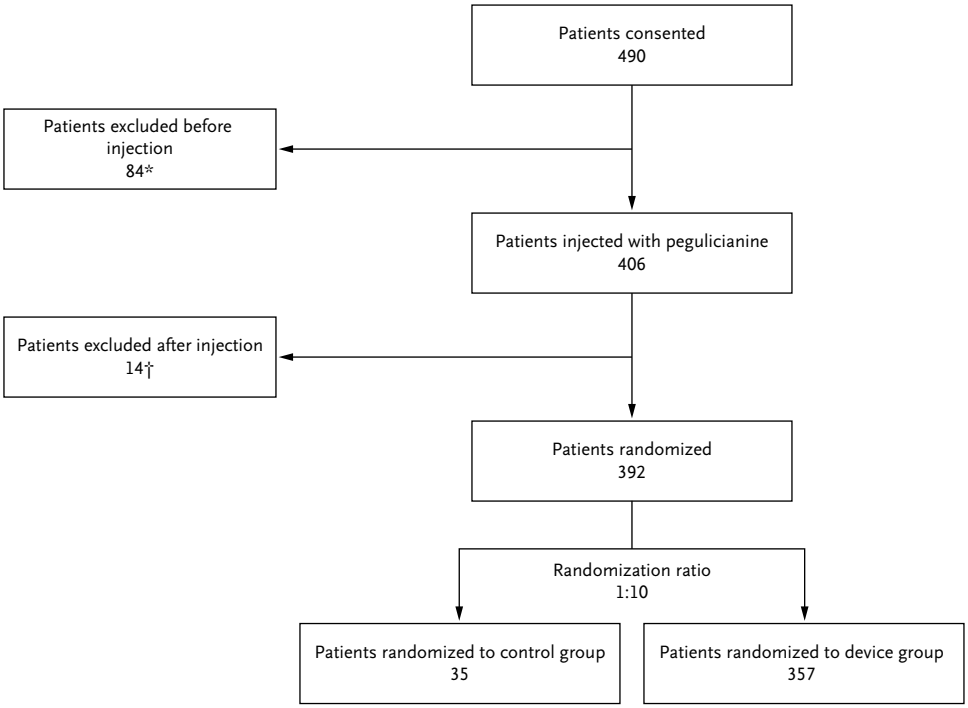

Figure 1. Schema of Pegulicianine Fluorescence-Guided Surgery versus Standard Lumpectomy Surgery for Stages 0 to 3 Breast Cancers.

To prevent surgeons from performing smaller than standard lumpectomies in anticipation of pegulicianine fluorescence-guided surgery (pFGS) assistance, patients were randomly assigned 10:1 to the pFGS or control group. Randomization was not designed to provide a control group for assessing device performance.

* Patients excluded before injection included 58 for not meeting eligibility criteria, 22 for other reasons (withdrawing consent, Covid-19–related impact, consented but study was closed before surgery, and others), and 4 for unknown reasons.

† Patients excluded after injection included seven adverse events, three device issues, three sentinel node blue dye injections prior to imaging, and one prior ipsilateral procedure.

or DCIS at the edge of the specimen[23] and DCIS within 2 mm of the edge of the specimen for patients with a diagnosis of DCIS alone.[24]

All patients were observed for adverse reactions to pegulicianine injection and other adverse events until the occurrence of hospital discharge. Patients had a final safety assessment including complete blood counts and serum chemistry evaluations at the first postoperative visit, with all adverse events followed until the occurrence of resolution. Adverse event data were reviewed by an independent data and safety monitoring board, and severity was categorized per the National Institutes of Health Common Terminology Criteria for Adverse Events scale (version 5.0).[25]

STUDY END POINTS

We evaluated three coprimary end points. The first was the percentage of patients for whom pFGS-guided margins contained cancer left behind after the standard lumpectomy procedure. Success for this end point was defined as a lower bound of the 95% confidence interval (CI) of more than 3%. The second and third coprimary end points evaluated the diagnostic performance of pFGS by measuring the percentage of margins with tumor that were pFGS positive (sensitivity) and the percentage of margins with no tumor that were pFGS negative (specificity). Figure 2 depicts how each pFGS image result was scored relative to histopathology results of the respective lumpectomy specimen margin (additional details are in the statistical analysis section and the Supplementary Appendix). Success for the sensitivity and specificity end points was defined as lower bounds of the 95% CI of more than 40% and more than 60%, respectively, to ensure appropriate cancer detection while sparing normal tissue.

Secondary end points included evaluation of the positive margin rate after removal of pFGS-guided margins and the impact of pFGS margins on the volume of tissue excised.

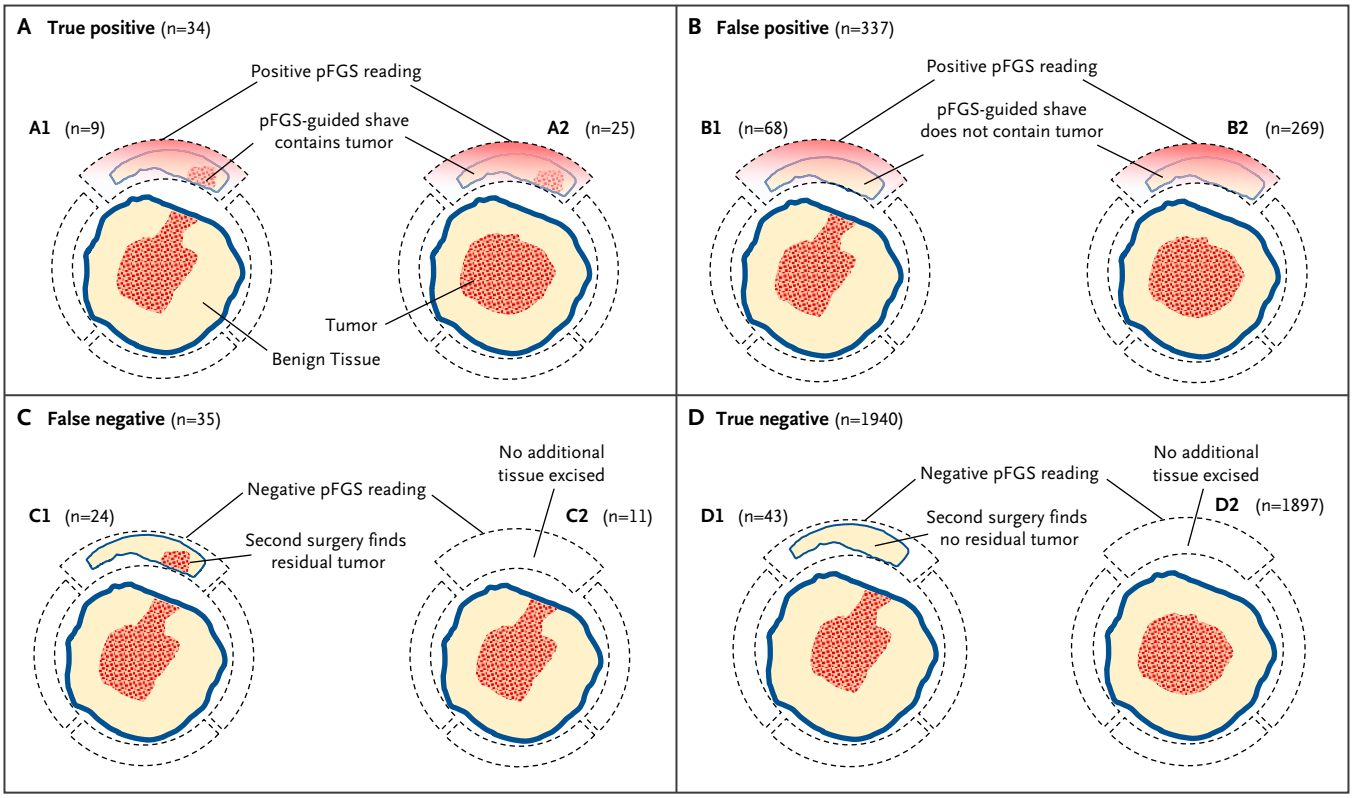

Figure 2. Margin Scoring Schema.

Pegulicianine fluorescence-guided surgery (pFGS) readings (positive or negative) from each lumpectomy cavity orientation were compared with histopathology of the adjacent tissue to classify the pFGS signal as true positive (Panel A), false positive (Panel B), false negative (Panel C), or true negative (Panel D). Positive pFGS readings (Panels A and B) were compared with histopathology of the guided shave whether the prior margin in that orientation was positive (Panels A1 and B1) or not (Panels A2 and B2). Negative pFGS readings (Panels C and D) were compared with histopathology of tissue excised from the imaged orientation at a second surgery (Panels C1 and D1) or with the prior excised lumpectomy margin at that orientation if no additional tissue was excised (Panels C2 and D2).

STATISTICAL ANALYSIS

On the basis of prior results,[22] we estimated that 70 margins with cancer would achieve a power of 90% or greater for all primary end points. We used an event-driven design, and patients were recruited until 70 margins with cancer had occurred. Safety analysis included all patients who received injections of pegulicianine, and efficacy analyses included only patients randomized to the pFGS group.

The three coprimary end points were evaluated using a one-sided alpha of 2.5%, and true proportions were estimated with a two-sided 95% CI. To account for potential intrapatient correlations, the generalized estimating equations[26] method was used for calculation of sensitivity and specificity. Secondary end points were evaluated with a two-sided 95% CI.

Results

STUDY PARTICIPANTS

From November 4, 2019 to September 15, 2021, 406 patients were enrolled in the trial. Patient and tumor characteristics are shown in Table 1. The study population was representative of patients with breast cancer for whom breast-conserving surgery is considered (Table S3 in the Supplementary Appendix). Of the 406 patients enrolled, 14 were withdrawn before randomization: 7 because of adverse events, 2 because of device issues, 3 because sentinel node blue dye injections occurred before imaging (1 for inadequate radioisotope signal and 2 for blue dye mistakenly injected before imaging), 1 because of prior ipsilateral procedure, and 1 because the patient received an incision that was too small to accommodate the imaging device. These patients were followed for safety assessments. For the 392 patients who underwent randomization, median age was 64 years (range, 36 to 83). There were 316 patients (80.6%) with invasive cancer with or without DCIS and 76 patients (19.4%) with DCIS alone. The median largest invasive tumor dimension was 1.5 cm (range, 0.1 to 10.1 cm).

PATIENT RANDOMIZATION

The trial schema is shown in Figure 1. After the standard lumpectomy procedure was completed, randomization assigned 357 patients to the pFGS group and 35 to the control group. Randomization was not designed to provide a control group for analysis of device performance but rather, to introduce uncertainty as to whether there would be pFGS assistance, thereby encouraging surgeons to perform their best standard lumpectomy before pFGS imaging. Study end points were calculated by comparing pathology margin status before and after pFGS assistance on a per-patient basis only in patients in the pFGS study group.

REMOVAL OF RESIDUAL CANCER BY PFGS

For 27 of 357 patients (7.6%; 95% CI, 5.0 to 10.8%) in the pFGS group, pFGS-guided margins removed tumor left behind after the standard lumpectomy procedure, meeting the success criteria of more than 3% (Table 2). Furthermore, 19 of these 27 patients had all margins negative on standard lumpectomy pathology evaluation, and the residual cancer removed would have remained unrecognized without the pFGS intervention. The remaining eight patients had positive lumpectomy margins on final pathology. Three of these had residual cancer removed in pFGS-guided margins excised from orientations with negative lumpectomy margins by standard pathology; these areas would not have been targeted for reexcision on the basis of standard lumpectomy margin assessment. Thus, in 22 patients, pFGS guidance removed residual cancer from lumpectomy margins negative on standard-of-care evaluation.

Residual tumor deposits removed in pFGS-guided margins included grade 3 histology in 9 of 22 patients with negative lumpectomy margins and tumor deposits greater than 1 mm in 15 of 22 patients (range, 1.5 to 13 mm). These 22 patients included 5 over 70 years old with hormone receptor–positive tumors (Table 3). A total of 139 patients had pFGS shaves removed with no residual cancer found on pathology assessment.

DIAGNOSTIC PERFORMANCE: PFGS MARGIN-LEVEL SENSITIVITY AND SPECIFICITY

Sensitivity and specificity results are shown in Table 2. Margin-level specificity was 85.2% (1940 of 2277 margins; 95% CI, 83.7 to 86.6%), higher than the prespecified lower-bound goal of greater than 60%. Margin-level sensitivity calculated across all orientations (with and without additional tissue margin histopathology for comparison) was 49.3% (34 of 69 margins; 95% CI, 37.0 to 61.6%). We had set a lower-bound goal of greater than 40%, and the trial failed to meet this prespecified end point. A post hoc analysis of margin-level sensitivity calculated using only orientations where histopathology testing was available

Table 1. Patient and Tumor Characteristics.*		
Characteristics	**Patients Dosed with Pegulicianine (N=406)**	**Patients Randomized (N=392)**
Age — yr		
Median — yr (range)	64 (36–83)	64 (36–83)
Race		
American Indian or Alaska Native	1 (0.2%)	1 (0.3%)
Asian	22 (5.4%)	22 (5.6%)
Black or African American	26 (6.4%)	26 (6.6%)
Native Hawaiian or Pacific Islander	1 (0.2%)	1 (0.3%)
White	337 (83.0%)	324 (82.7%)
Other or not reported	19 (4.7%)	18 (4.6%)
Ethnicity		
Hispanic or Latino	12 (3.0%)	12 (3.1%)
Non-Hispanic or Latino	383 (94.3%)	370 (94.4%)
Not reported	11 (2.7%)	10 (2.6%)
BMI		
Median (range)	29.4 (16.8–67.4)	29.4 (16.8–67.4)
Menopausal status		
Post	339 (83.5%)	327 (83.4%)
Pre/peri	67 (16.5%)	65 (16.6%)
Mammographic breast density		
Almost entirely fatty	5 (1.2%)	5 (1.3%)
Scattered areas of fibroglandular density	220 (54.9%)	216 (55.1%)
Heterogeneously dense	163 (40.6%)	153 (39.0%)
Extremely dense	13 (3.2%)	13 (3.3%)
Largest dimension of tumor in main specimen		
Overall median — cm (range)	1.5 (0.1–10.1)	1.5 (0.1–10.1)
Tumor histology		
DCIS only	78 (19.2%)	76 (19.4%)
IDC ± DCIS	284 (70.0%)	274 (69.9%)
ILC ± DCIS	41 (10.1%)	39 (9.9%)
ILC + DCIS	3 (0.7%)	3 (0.8%)
Palpable mass	100 (24.6%)	96 (24.5%)
Receptor status		
ER (+)	378 (93.1%)	365 (93.1%)
PR (+)	311 (76.6%)	300 (76.5%)
HER2 (+)	23 (5.7%)	23 (5.9%)
Triple negative	15 (3.7%)	14 (3.6%)
Node-positive disease (+)	10 (2.5%)	10 (2.6%)

* The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters. DCIS denotes ductal carcinoma in situ; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; and PR, progesterone receptor.

for comparison showed sensitivity of 58.6% (34 of 58 margins; 95% CI, 44.9 to 71.4%).

The margin-level negative predictive value of pFGS was 98% across all margins (1940 of 1975 margins; 95% CI, 97.7 to 98.8%). Margin-level positive predictive value was 9.2% (34 of 371 margins; 95% CI, 6.4 to 12.6%).

CONVERSION OF POSITIVE MARGINS TO FINAL NEGATIVE MARGINS AND OVERALL PFGS IMPACT

After the lumpectomy procedure and before pFGS imaging, 62 of 357 patients (17.4%) had at least one positive margin. In 9 of 62 patients (14.5%), removing pFGS-guided shaves resulted in conversion of all positive margins to final negative margins during the initial lumpectomy surgery, which

Table 2. Pegulicianine Fluorescence-Guided Surgery Performance on Trial End Points.*		
Coprimary End Points	**Prespecified Performance Goal (Lower Bound of 95% CI; %)**	**Results**
Removal of residual cancer	>3	27/357 (7.6%); 95% CI, 5.0–10.8%
Sensitivity (tissue level)	>40	34/69 (49.3%); 95% CI, 37.0–61.6%
Specificity (tissue level)	>60	1940/2277 (86.2%); 95% CI, 83.7–86.6%

* CI denotes confidence interval.

avoided a second surgery. Among remaining patients with positive margins, 10 patients had cancer found in reexcisions where pFGS indicated negative signals. Overall, 10% (35 of 357) of patients had a favorable impact from pFGS surgery, 27 with residual cancer removed and 9 spared reexcision, including 1 both spared reexcision and with additional tumor removed.

IMPACT ON VOLUME OF EXCISION

Among 357 patients who underwent pFGS, the average tissue volume removed by standard lumpectomy was 74.9 cm^3, with a median of 54.4 cm^3 (interquartile range, 34.7 to 90.7 cm^3) and a range from 5.5 to 963 cm^3. The average tissue volume removed by standard lumpectomy in the control group was 82.0 cm^3, with a median of

Table 3. Characteristics of Residual Cancer Found in Pegulicianine Fluorescence-Guided Surgery–Guided Margins Removed from Negative Margin Orientations (22 Patients).*					
Patient Age	**Primary Tumor Pathology**	**pFGS-Guided Margin Residual Tumor Pathology**	**Largest Tumor Dimension Found in pFGS-Guided Margin (mm)**	**Tumor Grade†**	**Estrogen Receptor (+ or −)**
Detection of residual invasive cancer					
51	IDC + DCIS	IDC	1.5	3	+
77	IDC + DCIS	IDC + DCIS	NR‡	1	+
71	IDC	IDC	NR‡	3	+
52	ILC	ILC	4	2	+
65	ILC	ILC	5	2	+
71	ILC	ILC	6.5	2	+
Detection of residual DCIS					
47	IDC + DCIS	DCIS	1	3	+
60	IDC + DCIS	DCIS	1	2	+
53	IDC + DCIS	DCIS	1.5	1	+
65	IDC + DCIS	DCIS	2	2	+
70	IDC +DCIS	DCIS	2	3	+
36	IDC + DCIS	DCIS	2	3	+
58	IDC + DCIS	DCIS	2	3	+
66	DCIS	DCIS	7	NR‡	+
60	DCIS	DCIS	7	2	+
66	DCIS	DCIS	8	2	+
76	IDC + DCIS	DCIS	11	2	+
58	IDC + DCIS	DCIS	11	3	+
52	IDC + DCIS	DCIS	13	2	+
58	IDC + DCIS	DCIS	NR‡	3	−
42	IDC + DCIS	DCIS	NR‡	3	+
59	IDC + DCIS	DCIS	NR‡	2	+

* There were 22 total patients. DCIS denotes ductal carcinoma in situ; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; NR, not reported; and pFGS, pegulicianine fluorescence-guided surgery.

† Tumor grades from 1–3, with higher numbers indicating more severe disease.

‡ NR indicates data not reported in the patient's case report form.

Table 4. Adverse Events.					
Patients	All — N (%)	Mild (Grade 1) — N (%)	Moderate (Grade 2) — N (%)	Severe (Grade 3) — N (%)	Life Threatening (Grade 4) — N (%)
Patients with any adverse event	380 (93.6%)	375 (92.4%)	39 (9.6%)	8 (2.0%)	1 (0.2%)*
Patients with chromaturia	368 (90.1%)	367 (90.1%)	1 (0.2%)	0	0

* One patient developed a grade 4 acute respiratory failure and somnolence adverse event after the surgical procedure was completed that was not related to pegulicianine administration. This patient also developed two grade 3 adverse events.

66.8 cm^3 (interquartile range, 40.7 to 99.8 cm^3) and no clear difference from the device group. At least one pFGS-guided shave was removed in 166 of 357 patients. The average number of pFGS margins taken among all 357 patients was 1.0 ± 1.4, with an average total volume of 10.1 ± 17.5 cm^3. The median additional volume of excision was 0 cm^3 (interquartile range, 0 to 14.1 cm^3), as no additional margin excision was performed in 54% of patients who underwent pFGS. In the 166 patients with at least 1 pFGS margin taken, an average of 2.2 ± 1.4 pFGS margins were taken with an average total volume of 21.8 ± 20.1 cm^3 and a median of 16.6 cm^3 (interquartile range, 6.9 to 32.1 cm^3).

SAFETY

The safety analysis included all 406 patients who received pegulicianine (Tables 4 and 5 and Table S1). Blue chromaturia, expected with the blue color of pegulicianine, was documented in 367 patients. Pegulicianine administration was stopped for adverse events in six patients (1.5%). Two patients (0.5%) had grade 3 serious adverse events related to pegulicianine; one had hypersensitivity, and one had an anaphylactic reaction. The other four pegulicianine-related adverse events included allergic reaction, milder hypersensitivity, nausea, and pegulicianine extravasation.

All adverse events resolved, and patients continued with their scheduled standard lumpectomy. The six patients who received only partial pegulicianine doses were not imaged with the pFGS system. There were no deaths reported in this trial.

Discussion

Our results showed that pFGS allowed real-time assessment of breast cancer lumpectomy cavity margins and facilitated removal of tumor left behind after standard lumpectomy surgery. The system met prespecified goals for excision of residual tumor and for specificity, but it fell short of the goal for sensitivity. Although not a primary end point, pFGS had a negative predictive value of 98%, a critical attribute for a margin assessment tool.

Table 5. Summary of Patients with Grade 3 and Grade 4 Adverse Events.*			
Patient	Adverse Event	Severity	Relationship to Pegulicianine Administration†
1‡	Acute respiratory failure and somnolence	Grade 4	Not related
1‡	Acute myocardial infarction and hypotension	Grade 3	Not related
2	Hypersensitivity	Grade 3	Related (SAE)
3	Anaphylactic reaction	Grade 3	Related (SAE)
4§	Allergic reaction	Grade 3	Related (non-SAE)
5	Vascular pseudoaneurysm	Grade 3	Not related
6	Acute kidney injury and breast cellulitis	Grade 3	Not related
7	Back pain	Grade 3	Not related
8	Breast pain	Grade 3	Not related

* SAE denotes serious adverse event.

† The relationship to pegulicianine administration was on the basis of the judgment of the clinical site investigator.

‡ One patient developed a grade 4 acute respiratory failure and somnolence adverse event after the surgical procedure was completed that was not related to pegulicianine administration. This patient also developed two grade 3 adverse events.

§ One patient developed a grade 3 allergic reaction related to the administration of pegulicianine, but it did not meet the criteria for SAE. Thus, two patients had SAEs related to pegulicianine administration.

The positive predictive value was 9.2%, as our detection algorithm was designed to maximize removal of residual tumor and accepted a higher rate of additional margin excision.

Our data showed wide variability in lumpectomy size across surgeons with no clear differences in initial lumpectomy size between patients in the pFGS group and the control group, indicating that randomization achieved its goal of blinding surgeons to potential pFGS assistance. The wide range of lumpectomy sizes in part reflects variability in tumor size and geometry, but also, it illustrates the difficult trade-off faced by surgeons and patients — take a larger specimen to avoid a second surgery while accepting more breast deformity or take a smaller specimen to preserve cosmesis, risking a second trip to the operating room. At present, these intraoperative decisions are not evidence based and are often subject to individual operator bias.

We previously described the strengths of the pFGS system for breast cancer margin assessment.[18-20,22] Results are immediately available to the surgeon, requiring approximately 1 minute to scan the entire lumpectomy cavity, with all interventions adding less than 7 minutes to the operative procedure.[20] Unlike standard margin assessment, frozen section, and other available tools,[27] pFGS is a cavity-based tool that identifies residual tumor within 2 to 5 mm from the surface of the lumpectomy cavity, rather than on the surface of the excised lumpectomy specimen. The pFGS cavity-based approach avoids the inherent problem of specimen-based approaches — correlating the location of tumor on an excised deformable specimen surface with the location of residual tumor in the breast cavity. pFGS also allows for repeat imaging of areas of concern during the initial operation to verify the removal of all positive signal areas. We previously found that pFGS performed equally well for DCIS and invasive cancers, in pre- and postmenopausal women, and in dense and fatty breasts.[20] For most patients, the requirement for pegulicianine administration 2 hours before surgery had little impact on the overall length of their hospital stay.

pFGS successfully identified and removed residual tumor remaining after standard lumpectomy in 27 of 357 (7.6%) patients. The residual tumor deposits excised included areas of low- and high-grade tumor, areas of tumor ranging from 1 to 13 mm in size, and tumor deposits in women over 70 years of age who are potentially candidates for omission of radiation on the basis of margin status.[28] Overall, pFGS removed residual tumor and/or avoided second surgeries in 10% of patients in this trial.

Our study failed to meet the prespecified per-margin sensitivity rate, achieving a 49.3% sensitivity with a CI lower bound of 37% rather than the prespecified 40% target. We speculate that our trial design may have affected this outcome. Our trial did not take additional margin specimens from cavity orientations with negative pFGS readings but scored the pFGS cavity reading as falsely negative if the corresponding lumpectomy margin was positive. Ongoing studies (NCT04440982) are designed to obtain additional margin tissue from both pFGS-positive and pFGS-negative cavity margins, which may allow for more precise sensitivity calculation and permit more direct evaluation of the extent of residual tumor after pFGS.

Use of the pFGS system increased the volume of breast tissue excised by an average of 10 cm^3, an 11% volume increase over our average standard lumpectomy. This resulted in less additional tissue removal compared with the 30% volume increase observed with the common practice of taking comprehensive shaved margins.[10,11,14] Patient-reported outcome measures are being collected during follow-up to evaluate cosmetic perceptions before and after pFGS surgery.

Our study population is representative of patients with breast cancer for whom breast-conserving surgery is considered. Eligibility criteria were intentionally broad to include the full spectrum of tumors considered for lumpectomy. At present, 65% of all newly diagnosed cancers present as stage 1.[29] Moreover, approximately 62% of women with stage 1 and 2 disease undergo breast-conserving surgery, making the pFGS approach potentially applicable to a high percentage of patients with breast cancer. We also note that pFGS is agnostic to whether a lumpectomy encompassed a unifocal or multifocal tumor, as it detects residual continuous or discontinuous tumor in lumpectomy cavity walls.

The rate of allergic reactions to pegulicianine was similar to that of other commonly used imaging agents. For example, isosulfan blue, used for sentinel node mapping, has a 1 to 3% allergic reaction rate.[30-32] Pegulicianine injection was integrated into the preoperative workflow and could be administered before or after sentinel node isotope injection or marker localization of nonpalpable tumors.[20] Other optical imaging agents require injection up to a day in advance.[33]

Sentinel node mapping used isotope-only guidance as blue dyes fluoresce in the same wavelengths as pegulicianine. Only one patient was withdrawn from the study for inadequate isotope signal.

Our findings suggest potential benefits of the pFGS approach. In the 17% of patients who had positive margins after standard surgery, 9 of 62 avoided a second surgical procedure as a result of additional margins excised in real time, guided by pFGS signal. This reduces the patient burden of additional surgery and decreases the health care costs associated with a return to the operating room. Perhaps more provocative is the discovery of additional tumor in 27 of 357 patients, where pFGS-guided margins removed tumor left behind by standard lumpectomy. This potential benefit merits evaluation in future trials.

Disclosures

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