Cerenkov Luminescence Imaging and Flexible Autoradiography for specimen margin assessment during breast-conserving cancer surgery

Abstract

Background

Among women with breast cancer who undergo breast-conserving surgery (BCS), 20-25% require further surgery due to close or involved margins. Improved techniques are needed to assess resection margins.

Purpose

The study aims were to assess the feasibility of the combined techniques of Cerenkov Luminescence Imaging - Flexible AutoRadiography (CLI-FAR) to assess excision specimen margins in women undergoing BCS and to determine the diagnostic performance of intraoperative CLI-FAR imaging with postoperative histopathology as the reference standard.

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Materials and Methods

Women undergoing BCS were recruited prospectively at a single centre over thirteen months. Patients were injected with 250MBq +/- 10MBq of 18F-fluorodeoxyglucose (18F-FDG), 145 minutes before surgery and the excised specimens were imaged intraoperatively. The surgically excised tumour was initially imaged using conventional x-ray, and margins suspected to be involved by tumor were then imaged using CLI-FAR. CLI-FAR imaging was performed using the LightPath system® (Lightpoint®), an *in vitro* diagnostic device designed to identify and locate positron-emitting radionuclides. Any suspicious margin underwent an immediate re-excision in the form of cavity shavings. Sensitivity, specificity, positive and negative predictive value whilst considering histopathological assessment as the golden standard were used to assess the performance of CLI-FAR.

Results

In all, 54 specimens were imaged in 52 patients with a total of 104 margins reviewed using CLI-FAR. The results showed a specificity of 97.8% (89/91, 95% CI: 95.0%, 100.6%), sensitivity of 76.9% (10/13, 95% CI: 68.3%, 85.0%), PPV of 83.3% (10/12, 95% CI: 76.2%, 90.5%) and NPV of 96.7% (89/92, 95% CI: 93.3%, 100.2%). In all, 8 patients had 10 positive

margins on CLI-FAR imaging and were treated accordingly. CLI-FAR imaging reduced the re-excision rate by (17.3/25) 69%.

Conclusion

CLI-FAR imaging is a promising technique for intraoperative margin assessment in women undergoing BCS for invasive breast cancer.

Key Words

Breast Cancer, Breast-Conserving Surgery, Intra-operative novel imaging, Cerenkov Luminescence, Flexible AutoRadiograpy,

Key Results

- Intraoperative Cerenkov Luminescence Imaging - Flexible AutoRadiography (CLI-FAR) can decrease the re-excision rate in breast-conserving surgery for invasive cancer by up to (17.3/25) 69%.
- Intraoperative margin assessment using CLI-FAR during breast-conserving surgery for invasive cancer showed a specificity of 97.8% (89/91) and sensitivity of 76.9% (10/13).
- Mean delay between surgical excision and CLI-FAR images was 6 minutes indicating CLI-FAR is feasible for use in hospitals without disrupting standard practice or causing significant delays in theatre.
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Summary Statement

Cerenkov Luminescence Imaging - Flexible AutoRadiography (CLI-FAR) is a novel technique that shows promise for reducing the re-excision rate by assessing intraoperative margins during breast-conserving surgery for invasive breast cancer.

Abbreviations

^{18}F-FDG	18F-fluorodeoxyglucose
3D	Three Dimentional
^{99m}Tc	^{99m}Tc-albumin-nanocolloid
BCS	Breast Conserving Surgery
CLI	Cerenkov Luminescence Imaging
DCIS	Ductal Carcinoma in Situ
FAR	Flexible AutoRadiography
MRI	Magnetic Resonance Imaging
NACT	NeoAdjuvant ChemoTherapy
PET	Positron Emission Tomography
RCB	Residual Cancer Burden
SLNB	Sentinel Lymph Node Biopsy
UK	United Kingdom
WLE	Wide Local Excision

Introduction

Women diagnosed with breast cancer undergo breast-conserving surgery (BCS) or mastectomy (+/-reconstruction) for the primary tumour. Alternatively, patients receive neoadjuvant chemotherapy (NACT), followed by BCS (1). Approximately 70% of women undergo BCS. However, around 20-25% of patients who undergo BCS require further excision due to positive margins (2–5). In the United Kingdom, the institutions follow the Association of Breast Surgeon Guidelines (9), with a positive margin indicated if the tumor is found in the inked edge of the specimen, within 1 mm for invasive or DCIS associated with invasive disease, or within 2 mm for pure DCIS, while other institutions call a positive margin as tumor on ink. Despite variations worldwide in the definition of positive margin (5-7), overall, positive margins increases risk of local recurrence with potential increased risk of distant recurrence and death (6-8). As such re-excision is recommended.

Additional surgery may result in poorer cosmesis, increased psychological morbidity and costs for the patient and healthcare system (5)(10). Techniques identified for intraoperative margin assessment are shown in table A1. However, these are not used widely. Due to their low sensitivity, specificity, and high cost (2,11), only intraoperative radiography in BCS has become the standard of care internationally (12-14). Therefore, innovative techniques are required.

Cerenkov Luminescence Imaging (CLI) and Flexible AutoRadiography (FAR) is a novel dual-modality imaging method for detecting cancer cell radioactivity using optical and molecular imaging. For molecular imaging, 18F-fluorodeoxyglucose (^{18}F-FDG) is used (15). These modalities have been individually investigated in BCS margin assessment (16,17). CLI detects Cerenkov luminescence directly. This luminescence is generated as a faint blue light when a charged particle, like positrons, moves through a medium at a speed greater than light (15). The imaging modality utilizes real-time imaging, which includes advantages from optical white light and PET imaging. FAR indirectly detects scintillations caused by charged particles like positrons exciting a thin scintillating film. The advantage of using a scintillator is that it ensures that only charged particles can produce scintillations, eliminating any diathermy artifact in FAR.

The primary aims of this study were to assess the feasibility of CLI-FAR to assess excision margins in women undergoing BCS and to determine the diagnostic performance of intraoperative CLI-FAR imaging with postoperative histopathology as the reference standard. The secondary objectives were to compare the margin status of specimens obtained during BCS using CLI-FAR and routine specimen x-rays, re-operation rates, and assess additional surgical time.

Material and Methods

Clinical Trial Setup

A single-arm interventional first-in-human study was designed to evaluate the diagnostic accuracy of CLI-FAR in conjunction with 18F-FDG to assess tumour margins in BCS. The study was approved by a UK independent ethics committee (REC15/LO/0029), the Administration of Radioactive Substances Advisory Committee (ARSAC), and the Health Research Authority (IRAS314460) (ClinicalTrials.gov identifier: NCT05496101). All the documents submitted to these regulatory bodies detailed the intraoperative intervention in patients with a positive margin on CLI-FAR, and approvals were granted based on this information.

Recruitment:

Patients were identified during the Breast Multi-Disciplinary Meeting. Following written, informed consent, patients were recruited between November 2022 and December 2023 (Figure A1 and A2). The study checklist (figure A3) was completed before the patient proceeded to the nuclear medicine department on the day of surgery (Figure A4).

Patients over the age of 18 with a diagnosis of invasive breast cancer undergoing BCS were included. Patients who have had surgery or radiotherapy to the ipsilateral breast in the past 12 months, known hypersensitivity to 18F-FDG, or who were pregnant or lactating were excluded.

In vitro studies to minimise diathermy artifact:

Diathermy artifact in CLI has been categorized as chemiluminescence (18); this is a phenomenon of heat energy. Pre-clinical *in vitro* studies were conducted to distinguish between chemiluminescence and radioactivity to minimise the false positive rate of signals obtained. That the brightness of chemiluminescence reduced with lower diathermy energy (W, watts)

levels and time after exposure (figure A5) was noted. Therefore, we chose to use a maximum diathermy level of 20W.

Radio-tracer administration

Patients were injected intravenously with 250Mbq$^{+/-}$10% of 18F-FDG approximately 145 minutes before the expected time of imaging the excised specimen. This dose was based on the previous study by Grootendorst et al (16). Patients undergoing axillary lymph node dissection (ALND) only received the 18F-FDG injection intravenously, whereas patients undergoing an SLNB received intravenous 18F-FDG, as part of the study and received up to 40Mbq of ^{99m}Tc-albumin-nanocolloid (^{99m}Tc) injected intradermally at the peri-areolar region on the ipsilateral breast, as the standard of care.

Radiation safety

Previous studies with 18F-FDG in similar settings, with patients undergoing BCS and SLNB, have shown minimal exposure to radioactivity (16,17). Surgeons received a mean dose of 34 and 61.8 mSv, whereas anesthetists received a mean dose of 11 and 26.4mSv over the two previous studies(16,20). UK legislation regarding the use of ionizing radiation was fully complied with (21–23). Staff members were provided personal body radiation dosimeters (MYDOSE mini, ALOKA, Mure, Mitaka-shi, Tokyo, Japan), and surgeons and anesthetists were provided with thermos-stimulated luminescent rings (Saturn TLD Rings, Landauer®, Illinois, USA) to ensure radiation doses were monitored. All cases had dosimeters (41/44A, series 300 mini-monitor; Thermoscientific) measuring the activity of the room, equipment, staff, and waste. No additional measures were required for the 18F-FDG as its half-life (110m) is shorter than of ^{99m}Tc (362m) (24).

Surgery

After anesthetic induction, the ^{99m}Tc activity in the axilla was assessed using a gamma probe with a collimator to detect the sentinel node. In conditions with a weak signal of ^{99m}Tc or a generalized high activity level in the axilla, patients were injected with a peri-areolar subcutaneous injection of Patent Blue V (Guerbert, France). Five surgical consultants were involved in the study each performing 150 breast operations on average per annum. The breast surgery was performed first before any axillary procedure. Surgeons used a scalpel or

diathermy (BOWA ARC 303, BOWA Medical, United Kingdom) at a reduced energy level during excision. The excised specimen was orientated for histopathology as follows: one suture and clip for anterior, two sutures and clips for superior and three sutures and clips in the direction of the nipple.

The excised specimen was initially imaged using the 3D X-Ray imaging system (Kubtec Mozart® System, KUB Technologies®, Stratford, Connecticut, USA). If a margin was suspected to be close to the edge of the excised specimen clinically or on intra-operative X-ray, it was imaged using CLI-FAR. If no margins were deemed to be close/involved, the surgeon chose two margins that appeared to be the closest and these were assessed.

On reviewing the images produced by CLI-FAR, the surgeon made a clinical decision whether to intervene surgically by immediately taking further tissue from the residual cavity (cavity shavings). If a specimen was too large for the scintillator, only a CLI image was taken and analysed using one method. The surgeon's interpretation was documented and compared to the final histopathology.

Specimen analysis

Both imaging techniques were obtained using the LightPath® system (Lightpoint Medical Ltd., U.K.), an *in vitro* diagnostic device that detects the location and distribution of positron-emitting radionuclides within excised surgical specimens (Figure 1). The system is a bespoke device with an ultrasensitive camera that detects emitted activity between 550 and 850nm. The Lightpath® imaging system is not currently commercially available.

When examining tissue samples after surgery, CLI is a technique that uses non-invasive imaging to view tissues marked with a radiotracer (Figure 1). The LightPath® system's ultra-sensitive camera detects the emitted light and creates an image of the tissue. Each margin must be separately imaged, as the camera can only capture a 2D image at a time.

When performing FAR, a 12-μm-thin scintillator was wrapped on the WLE specimen following BCS, and a 3-μm mylar sheet was placed between the specimen and scintillator to prevent contaminating the scintillator (figure 1) (18). To detect activity within a specific wavelength range of 550nm +/- 10%, a band path filter is used as the scintillator film produces

scintillations in a limited wavelength range. The scintillator is white and completely opaque to chemiluminescence signatures.

It would take 30-60 seconds between images to wrap the scintillator and/or orientate the specimen. Both images had acquisition times of 300s each and 8x8 pixel binning (total pixel resolution 938μm).

Histopathology

Three histopathologists were blinded to the results of the CLI-FAR and X-ray imaging. All excised tissue specimens were examined for the presence of invasive or *in-situ* disease, its size, and distance from all six margins, also reviewing the tumour type, grade, receptor status, presence or absence of vascular invasion, lymph node status, any additional molecular characteristics as requested by the multidisciplinary team to aid patient management, and the Residual Cancer Burden (RCB) if the patient had undergone NACT.

Statistics

The sample size for this study was defined based on the assumption that this is a feasibility study. The primary endpoint of the study was to report the sensitivity and specificity of CLI and FAR LightPath® imaging for tumour detection compared to positive tumour detection using standard-of-care histopathology methodology (positive margin of a WLE sample). Using an estimate of the incidence of positive excision margins on histopathology as 20%, with 95%-confidence (alpha = 0.05 two-sided) and with 10% precision, a sample size of 54 patients or tumour specimens was estimated to provide sufficient power to detect sensitivity of 95% and specificity of 90% (25).

Patient demographics and tumour characteristics as well as radiotracer administration and timing were reported with descriptive statistics. To assess the performance of CLI-FAR, we calculated sensitivity, specificity, positive and negative predictive value whilst considering histopathological assessment as the golden standard. All statistical analyses were conducted in Stata (version 18.0, StataCorp LLC, College Station, TX).

The per protocol population is defined as all patients who completed BCS and study procedures as per protocol description. The primary endpoint was analysed based on the per protocol population.

Results

Overall, 54 specimens were imaged in 52 patients with a total of 104 margins were reviewed using CLI-FAR (Figure 2). No adverse events were reported.

Patient Demographics and Tumour Characteristics

Demographic data for the patient and tumour characteristics are shown in Table 1 and Table A2. Of the 52 patients recruited with 54 tumours, 20 patients (38.5%) underwent NACT with 11 (20.4%) tumours achieving a complete radiological response on MRI before surgical resection. The remaining 43 tumours ranged in size (measured on pre-operative MRI, ultrasound or mammogram) from 4mm to 56mm. The mean tumour size was 20.6mm excluding those with complete radiological response.

Radiotracer administration and timing

Table 2 shows the results and details of the patients administered with Tc99m and 18-FDG. The mean dose of 18-FDG injected was 250.5MBq (SD 14.2) and the maximum dose was 273MBq.

The dose of 18F-FDG injected was standardised and checked by the nuclear medicine department. The protocol and pathway created for patients flowed well and allowed them to undergo surgery without any delays to the theatre list. The target dose of 250MBq (+/-10%) 18F-FDG was given to 50 of the 52 patients; the remaining 2 received 213.3MBq and 223.7MBq, respectively. The reason for lower doses being administered are likely due to the pre-injection decay which were due to timing of delivery. The aim was for the 18F-FDG to be administered 145mins before imaging.

On average, the first CLI-FAR image was taken 175.1 minutes after the 18F-FDG injection; this ranged from 82 to 362 minutes. There was a minimum of five minutes between each CLI-FAR image, and most patients had four images taken in total.

Diagnostic performance of CLI-FAR

Of the 54 specimens, 50 had two margins assessed with CLI-FAR, results shown in table A1. The remaining four had only one margin assessed due to technical errors, leading to a total of 104 margins being assessed with CLI-FAR. Most specimens were assessed using CLI (figure

3) and FAR imaging (figure 4), however 8 specimens were too large for the scintillator, so these were assessed only with CLI imaging, and there was a technical error of system software failure on one CLI image, therefore only one margin was assessed using FAR.

In total 13 margins were positive, and 91 margins were negative when assessed by final histopathology. CLI-FAR margin assessment was compared with final histopathological assessment (table 3). This showed a margin specificity of 97.8% (89/91), sensitivity of 76.9% (10/13), positive predictive value of 83.3% (10/12) and negative predictive value of 96.7% (89/92).

The diathermy setting used for one specimen only imaged with CLI was relatively high at 30W and the false positive result can be attributed to chemiluminescence. Furthermore, the time between 18F-FDG injection and CLI imaging for two false positive interpretations were 97 and 99 minutes, significantly lower than the planned 145 minutes, which could have contributed to this result.

Re-excision rate

In all, 10 margins in 8 patients were correctly identified as positive on CLI-FAR imaging, which were acted upon intraoperatively. In these patients, all initial margins were also positive on histopathology, but cavity shavings were benign on 7, and therefore, these patients avoided a second operation. One patient with a positively identified margin on CLI-FAR underwent an intra-operative cavity shave, the excised cavity shave on histopathology showed further disease at the new margin. The histopathology report showed three false negative interpretations of margins on CLI-FAR. One specimen had a positive margin for DCIS. The other two specimens had invasive cancer at the margin. The re-excision rate using CLI-FAR was 7.7% (4/52). The overall re-excision rate was decreased by (17.3/25) 69%.

Out of 52 patients, 3 patients' margins assessed as negative on CLI-FAR required further surgery due to invasive cancer or associated DCIS within 1mm of the margin. Of the three specimens that were incorrectly considered to be negative, two were too large to be assessed with FAR and were only assessed using CLI imaging.

Comparison with conventional X-ray

CLI-FAR identified positive and negative margins more frequently than intraoperative X-ray. Intra-operative X-ray correctly identified 82 of the 91 negative margins on histopathology, but incorrectly identified 9 as positive, leading to more healthy tissue being excised in the form of cavity shavings. Intraoperative X-ray correctly identified 2 out of the 13 positive margins on histopathology, whereas CLI-FAR identified 10 margins correctly.

Surgical time

The addition of CLI-FAR for imaging did not significantly prolong surgical time (table 4). There was significant variation in the time between administration of 18F-FDG and the start of surgery, and the duration of surgery, leading to a wide range of time between 18F-FDG injection and procuring CLI-FAR images. These time delays are summarised in table 3. For most patients, the delay between surgical resection and CLI-FAR imaging was minimal (a mean of six minutes).

Discussion

This first-in-human study evaluated BCS specimen margin assessment using 18-FDG for CLI-FAR imaging. In all, 104 margins were assessed in 54 specimens, 46 specimens underwent both imaging modalities of CLI and FAR. Overall, 8 specimens were too large for the scintillator to cover the specimen hence FAR was not undertaken. Margin correlation was good between CLI-FAR imaging and final histopathology with 7 of 8 patients avoiding a second operation due to positive margins as detected by CLI-FAR. CLI-FAR shows a margin specificity of 97.8% (89/91) and a sensitivity of 76.9% (10/13). The re-excision rate using CLI-FAR was 7.7% (4/52), which is lower than the current reported 20-25% for intra-operative x-ray (2–5).

CLI-FAR's dual high-resolution imaging technique utilises imaging equipment that can be used in the operating room. CLI-FAR is particularly useful for image-guided surgery with an acquisition time of 10 minutes per margin and instant image analysis. Grootendorst et. al showed proof of principle using CLI on 10 excised specimens observing radioactivity in tumour cells and followed this with assessment of 15 margins in 12 patients (16) in which all margins were negative for invasive cancer on imaging and histopathology. Jurrius et Al. investigated 385 margins on BCS specimens in 66 patients using FAR in a multi-centre trial in

Poland, and showed 46.2% sensitivity, 81.7% specificity, 8.1% PPV, 97.7% NPV and overall accuracy of 80.5%, detecting both invasive carcinoma and DCIS (17). These studies have previously shown that radiation exposure is low and safe for staff members(16,24); this has also been reflected in other 18F-FDG breast assessment studies (26,27).

A total of five surgical consultants were involved in the study, from recruitment to assessing the CLI-FAR images. Thus different interpreters were able to use the technology

Future work:

The current study has already identified that DCIS associated with invasive cancer is detected on CLI-FAR imaging assessment. However, it is unknown whether pure DCIS can be identified using CLI-FAR imaging. Therefore, the CLI-FAR study is to be extended to include a further cohort of patients with pure DCIS requiring BCS.

Limitations

Both CLI and FAR have limitations in the assessment of positive margins. Chemiluminescence continues to be a limiting factor in CLI, which requires teaching and experience to successfully differentiate it from radioactivity. In this study, a standard scintillator that could only accommodate specimens up to 4cm in size was used. However, given the growing prevalence of oncoplastic surgical procedures, it is becoming more frequent for specimens to exceed 4cm in diameter. For such specimens, larger scintillators are now readily available. As this is a feasibility study representing a relatively small series of patients, a larger validation series may be required for confirmation in the future.

Conclusion

CLI-FAR assessment of margins in BCS can be feasibly integrated into standard clinical care. It enables surgeons to accurately assess margin status specimens intraoperatively when compared with gold-standard histopathologic examination.

Disclosure

The authors have no relevant financial or non-financial interests to disclose. The authors have no conflicts of interest to declare.

Acknowledgement

This study was funded by the Guy's and St Thomas Charity and the ECMC (CRUK and NIHR). The UK sites receive support from Cancer Research UK and Department of Health as Experimental Cancer Medicine Centres. Cancer Research UK Award ECMCQQR-2022/10005 new award for ECMC since March 2023 to March 2028.

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Table 1: Subject demographics and tumour features

Patient demographics (n=52)	
Age (years)	
20-44	9 (17.3%)
45-54	18 (34.6%)
55-64	14 (26.9%)
65+	11 (21.2%)
Mean (SD)	55.0 (12.6)
Range	22, 84
Treatment (n=52)	
Primary Surgery	32 (61.5%)
Post-Neoadjuvant chemotherapy	20 (38.5%)
Residual Tumor burden (n=20)	
0	15 (75.0%)
2	3 (15.0%)
Not assessed/not reported	2 (10.0%)
Tumour features (n=54)	
Tumour size (mm)	
0 (rCR)	11 (20.4%)
0-20	27 (50.0%)
21-50	15 (27.8%)
51+	1 (1.8%)
Mean (SD)	16.4 (13.3)
Mean (SD) excluding rCR	20.6 (11.7)
Type	
No Special Type	47 (87.0%)
Lobular	3 (5.5%)
Spindle Cell	1 (1.9%)
Micropapillary	1 (1.9%)
Mucinous	1 (1.9%)
Mixed No Special Type and mucinous	1 (1.9%)
Grade	
1	4 (7.4%)
2	21 (38.9%)
3	29 (53.7%)
ER (Allred score)	
Positive	37 (68.5%)
Negative	17 (31.5%)
PR (Allred score)	
Positive	39 (72.2%)
Negative	15 (27.8%)
Her2	
Positive	4 (7.4%)
Negative	50 (92.6%)

rCR= complete radiological response; ER = oestrogen receptor; PR = progesterone receptor; NST= No specific type ***SD – Standard Deviation, n – number***

Table 2: Patient preparation and surgery

Patient features (n=52)	
^{99m}Tc injection	
Yes	46 (88.5%)
No	6 (11.5%)
Dose of ^{99m}Tc (Mbq)	
0	6 (11.5%)
20	39 (75.0%)
40	7 (13.5%)
Dose of ^{18}F-FDG	
210-230	3 (5.8%)
230-250	22 (42.3%)
250-270	24 (46.1%)
270+	3 (5.8%)
Mean (SD)	250.5 (14.2)
Range	213.3, 273.0
Specimen features (n=54)	
Diathermy setting (Watts)	
Not used	6 (11.1%)
20	31 (57.4%)
25	4 (7.4%)
30	3 (5.6%)
40	8 (14.8%)
45	2 (3.7%)
Duration of surgery (minutes)[l]	
< 15	13 (24.1%)
15-30	32 (59.3%)
30-60	8 (14.8%)
60+	1 (1.8%)
Mean (SD)	24.4 (19.64)
Range	9, 150
Median (IQR)	19.5 (16, 26)
Time between injection and knife-to-skin (minutes)	
50-90	14 (25.9%)
90-120	6 (11.1%)
120-180	19 (35.2%)
180-240	12 (22.2%)
240+	3 (5.6%)
Mean (SD)	144.8 (62.5)
Range	50, 341
Median (IQR)	138.5 (88, 185)

SD – standard deviation, IQR – interquartile range

Table 3 – Diagnostic performance of each imaging modality for tumour-positive margins.

104 margins on 54 specimens in 52 patients (95% confidence interval in parentheses)				
	X-Ray	CLI	FAR	CLI-FAR
Sensitivity	(2/13) 15.4% (8.5%, 22.3%)	(10/13) 76.9% (68.8%, 85.1%)	(3/9) 33.3% (23.4%, 43.3%)	(10/13) 76.9% (68.3%, 85.0%)
Specificity	(82/91) 90.1% (84.4%, 95.9%)	(86/90) 95.6% (91.6%, 99.5%)	(76/77) 98.7% (96.3%, 101.1%)	(89/91) 97.8% (95.0%, 100.6%)
PPV	(2/11) 18.2% (10.8%, 25.6%)	(10/14) 71.4% (92.47%, 80.2%)	(3/4) 75.0% (65.9%, 84.2%)	(10/12) 83.3% (76.2%, 90.5%)
NPV	(82/93) 88.2% (82.0%, 94.1%)	(86/89) 96.6% (93.1%, 100.1%)	(76/82) 92.7% (65.9%, 84.2%)	(89/92) 96.7% (93.3%, 100.2%)
Number of margins assessed per specimen				
1		4 (7.4%)		
2		50 (92.6%)		
Number of margins with lack of concordance (CLI-FAR vs Histopathology)				
0		49 (90.7%)		
1		5 (9.3%)		

Table 4: Time between key points of the intraoperative specimen analysis procedure

Specimen specific (n=54)	
Time between injection and intra-operative X-ray (minutes)	
60-90	3 (5.5%)
90-120	11 (20.4%)
120-180	19 (35.2%)
180-240	14 (25.9%)
240+	7 (13.0%)
Mean (SD)	169.1 (64.6)
Range	74, 356
Median (IQR)	163 (113, 217)
Time between tracer injection and first CLI-FAR image (minutes)	
60-120	13 (24.1%)
120-180	20 (37.0%)
180-240	13 (24.1%)
240+	8 (14.8%)
Mean (SD)	175.1 (63.2)
Range	82, 362
Median (IQR)	167.5 (131, 221)
Time between intraoperative X-ray and first CLI-FAR image (minutes)	
<3	6 (11.1%)
3-4	16 (29.6%)
5	11 (20.4%)
6-7	13 (24.1%)
>7	8 (14.8%)
Mean (SD)	6.0 (5.6)
Range	0, 40
Median (IQR)	5 (4, 6)
Time between first image and last image (minutes)	
<11	9 (16.7%)
11-15	25 (46.3%)
16-20	9 (16.7%)
>20	11 (20.32%)
Mean (SD)	15.4 (5.6)
Range	6, 31
Median (IQR)	14 (13, 20)

CLI-FAR = Cerenkov Luminescence Imaging - Flexible AutoRadiography

Appendix Tables

Table A1 – Intraoperative margin assessment methods with their corresponding status in clinical practice.

Intra-operative Imaging Modality	Current Status
Frozen Section	Clinically used
Ultrasound	Clinically used
Intraoperative X-Ray	Clinically used
Radiofrequency spectroscopy	Clinically used
Enzyme-activable fluoroscopy	Experimental
Protein-targeting Fluoroscopy	Experimental
In-cell based fluorescent Probe	Experimental
Magnetic Resonance Imaging	Experimental
Raman Spectroscopy	Experimental
Ambient Mass Spectrometry	Experimental
Optical Coherence Tomography	Experimental
Diffuse Spectroscopy	Experimental
Confocal Microscopy	Experimental
Molecular Imaging (e.g. Positron Emission Tomography)	Experimental

Table A2: demographics of each patient

Patient	Tumour	Age [years]	Ethnicity	Tumour side	Tumour size [mm]	Tumour type	Tumour grade	ER [Allred]	PR [Allred]	Her-2 [Allred/ FISH]	NACT	RCB
1	1	42	White	Left	14	NST	3	7	8	Negative	No	
2	1	51	White	Left	32	NST	3	4	4	Negative	Yes	2
3	1	50	White	Right	16	NST	2	7	7	Negative	No	
4	1	71	White	Right	45	NST	2	8	8	Negative	No	
5	1	61	Black	Left	41	Spindle Cell	3	0	0	Negative	No	
6	1	47	Black	Right	30	NST	2	7	6	Positive	Yes	2
7	1	47	Other	Right	18	Mixed NST and mucinous	3	7	6	Negative	No	
8	1	51	White	Left	12.5	NST	3	0	4	Positive	Yes	0
9	1	46	Black	Right	19	NST	3	0	0	Negative	Yes	0
9	2	46	Black	Left	10	NST	3	0	0	Negative	Yes	0
10	1	52	White	Right	41	NST	3	8	4	Negative	No	
11	1	65	White	Right	4	NST	1	7	5	Negative	No	
12	1	52	White	Left	21	Lobular	2	8	8	Negative	Yes	Not reported
13	1	46	Black	Right	20	NST	3	0	0	Negative	Yes	0
14	1	62	White	Right	10	NST	3	7	8	Negative	No	
15	1	46	White	Left	0	NST	3	0	0	Negative	Yes	0
16	1	55	White	Right	10	NST	1	7	8	Negative	No	
17	1	58	White	Right	9	NST	3	0	0	Negative	Yes	0
18	1	59	White	Left	7	Lobular	2	8	8	Negative	No	
18	2	59	White	Left	11	NST	2	8	8	Negative	No	
19	1	42	Asian	Right	0	NST	3	7	8	Positive	Yes	0
20	1	58	White	Left	16	NST	2	8	7	Negative	No	
21	1	48	White	Left	20	NST	3	6	8	Negative	Yes	2
22	1	52	White	Right	6.5	NST	2	7	8	Negative	No	
23	1	48	White	Right	56	NST	3	6	6	Negative	No	
24	1	50	White	Left	28	NST	2	8	8	Negative	No	
25	1	62	White	Left	21	NST	2	8	7	Negative	No	
26	1	39	White	Right	0	NST	3	0	0	Negative	Yes	0
27	1	72	Chinese	Left	24	NST	3	8	8	Negative	No	
28	1	49	Black	Left	31	NST	3	8	7	Negative	No	
29	1	56	White	Left	8	Micropapillary	2	8	8	Negative	No	
30	1	63	Black	Left	28	Lobular	2	7	0	Negative	No	
31	1	84	White	Left	25	NST	2	8	8	Negative	No	
32	1	31	Black	Left	0	NST	3	0	2	Negative	Yes	0
33	1	22	Black	Left	0	NST	3	0	0	Negative	Yes	0
34	1	43	White	Right	0	NST	3	0	0	Positive	Yes	0
35	1	67	White	Left	0	NST	2	8	4	Negative	Yes	0
36	1	62	White	Left	13	NST	2	8	7	Negative	No	
37	1	59	Black	Left	0	NST	3	0	0	Negative	Yes	0
38	1	44	White	Right	0	NST	3	0	0	Negative	Yes	0

39	1	41	Black	Left	0	NST	3	0	4	Negative	Yes	Not assessed
40	1	48	Other	Left	0	NST	2	0	0	Negative	Yes	0
41	1	56	Black - Caribbean	Right	28	NST	2	8	7	Negative	No	
42	1	80	White	Right	32	NST	3	8	5	Negative	No	
43	1	73	White	Left	37	NST	3	8	8	Negative	No	
44	1	49	Not Specified	Left	17	NST	2	8	8	Negative	No	
45	1	62	White	Left	18	NST	2	8	8	Negative	No	
46	1	47	White	Right	10	NST	3	3	4	Negative	No	
47	1	77	Black - Caribbean	Right	17	NST	3	0	0	Negative	No	
48	1	66	Black - Caribbean	Right	8	NST	2	8	8	Negative	No	
49	1	40	Not Specified	Left	10	NST	3	7	8	Negative	Yes	0
50	1	59	White	Left	13	NST	1	8	8	Negative	No	
51	1	78	White	Left	23	Mucinous	1	8	8	Negative	No	
52	1	72	White	Right	25	NST	2	8	8	Negative	No	

Figures Captions

Figure	
1	*Figure 1 –Imaging technology. 1A- The LightPath® System (Lightpoint medical®), 1B - Cerenkov Luminescence Imaging (CLI), 1C - Flexible Autoradiography (FAR) Imaging, 3-µm mylar sheet placed between scintillator and specimen. The specimen releasing beta particles are detected using an ultrasensitive camera for each imaging modality.*
2	*Figure 2—Patient and specimen flow. n—number of patients, s—number of samples, m—number of margins.*
3	*Figure 3 – A specimen in which the margins were deemed positive by CLI (Cerenkov Luminescence Imaging) and negative by intraoperative X-ray. 3A – Intra-operative X-Ray 3B- the white light image, 3C - CLI image as seen at the time of interpretation. The green region of interest shows radioactivity on a positive margin. The illuminated spot on the specimen seen above the radioactivity is either contamination or chemiluminescence.*
4	*Figure 4 – 4A – Specimen wrapped in a scintillator, image in black and white. 4B – Intraoperative X-Ray of the specimen. 4C - The FAR image with a region of interest drawn in green where activity was seen. 4D – CLI (Cerenkov Luminescence Imaging) image of with a region of interest drawn in green where activity was seen.*

Supplemental Figures Captions

Figure	
A1	*This figure shows the pathway that was followed for patients to be informed of the study and them providing their consent to participate in the study. There were multiple steps to this and this has been shown in this figure. CLI-FAR patient consent pathway, Abbreviations: MDM – Multi-disciplinary Meeting, OPA – Outpatient Appointment, EPR – Electronic Patient Records, CTC – Clinical Trial Coordinator, CNS – Cancer Nurse Specialist, SPAR – Surgical Preparation and Recovery.*
A2	*This figure shows the pathway for organising and ensuring all team members were aware of patients that had provided their consent to participate in the study and when their procedure was taking place. There were multiple steps to this which has been shown in this figure. Pathway to ensure that patients consented to CLI-FAR are identified in advance and are placed on the theatre list, ensuring patients not participating are not incorrectly tagged as CLI-FAR Abbreviations: TCI – To Come In, CTC – Clinical Trial Coordinator, CNS – Cancer Nurse Specialist, SPAR - Surgical Preparation and Recovery.*

A3	*This figure shows a checklist that required to be completed for all patients prior to them having an injection of ^{18}F-FDG on the day of surgery.*
A4	*This figure shows the pathway followed on the day of surgery from the point the participating patient attended the hospital for their surgery till they were discharged and what steps were completed.*
A5	*This figure shows that there is a difference in the degree of diathermy artifact when a higher energy level is used. It further shows how the luminescence of the heat energy decreases over time as the area exposed to the diathermy cools down. The image compares three different diathermy settings. A. Diathermy artifact on pre-clinical studies, time shown on the x-axis is in minutes, Diathermy levels are in Watts and the numbers below the images expresses the maximum brightness in luminescence units. B. Diathermy artifact on pre-clinical studies, depicted in a linear-like relationship graph.*