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3 **Cerenkov Luminescence Imaging and Flexible Autoradiography for specimen margin**
4 **assessment during breast-conserving cancer surgery**
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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 breast-conserving surgery (BCS) and to determine the diagnostic performance of intraoperative CLI-FAR imaging with postoperative histopathology as the reference standard.

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%), positive predictive value (PPV) of 83.3% (10/12, 95% CI: 76.2%, 90.5%) and negative predictive value (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 AutoRadiography,

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.

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.

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 ductal carcinoma *in situ* (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 increase 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 S1. 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, ^{18}F -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 Positron Emission Tomography (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.

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

14 **Clinical Trial Setup**

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

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36 Patients were identified during the Breast Multi-Disciplinary Meeting. Following written,
37 informed consent, patients were recruited between November 2022 and December 2023
38 (Figure S1 and S2). The study checklist (figure S3) was completed before the patient proceeded
39 to the nuclear medicine department on the day of surgery (Figure S4).
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46 Patients over the age of 18 with a diagnosis of invasive breast cancer undergoing BCS were
47 included. Patients who have had surgery or radiotherapy to the ipsilateral breast in the past 12
48 months, known hypersensitivity to 18F-FDG, or who were pregnant, or lactating were
49 excluded.
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54 ***In vitro* studies to minimise diathermy artifact:**

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56 Diathermy artifact in CLI has been categorized as chemiluminescence (18); this is a
57 phenomenon of heat energy. Pre-clinical *in vitro* studies were conducted to distinguish between
58 chemiluminescence and radioactivity to minimise the false positive rate of signals obtained.
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3 That the brightness of chemiluminescence reduced with lower diathermy energy (W, watts)
4 levels and time after exposure (figure S5) was noted. Therefore, we chose to use a maximum
5 diathermy level of 20W.
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10 **Radio-tracer administration**

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

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

51 After anesthetic induction, the ^{99m}Tc activity in the axilla was assessed using a gamma probe
52 with a collimator to detect the sentinel node. In conditions with a weak signal of ^{99m}Tc or a
53 generalized high activity level in the axilla, patients were injected with a peri-areolar
54 subcutaneous injection of Patent Blue V (Guerbert, France). Five surgical consultants were
55 involved in the study each performing 150 breast operations on average per annum. The breast
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3 surgery was performed first before any axillary procedure. Surgeons used a scalpel or
4 diathermy (BOWA ARC 303, BOWA Medical, United Kingdom) at a reduced energy level
5 during excision. The excised specimen was orientated for histopathology as follows: one suture
6 and clip for anterior, two sutures and clips for superior and three sutures and clips in the
7 direction of the nipple.
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13 The excised specimen was initially imaged using the 3 Dimensional (3D) X-Ray imaging
14 system (Kubtec Mozart® System, KUB Technologies®, Stratford, Connecticut, USA). If a
15 margin was suspected to be close to the edge of the excised specimen clinically or on intra-
16 operative X-ray, it was imaged using CLI-FAR. If no margins were deemed to be
17 close/involved, the surgeon chose two margins that appeared to be the closest and these were
18 assessed.
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26 On reviewing the images produced by CLI-FAR, the surgeon made a clinical decision whether
27 to intervene surgically by immediately taking further tissue from the residual cavity (cavity
28 shavings). If a specimen was too large for the scintillator, only a CLI image was taken and
29 analysed using one method. The surgeon's interpretation was documented and compared to the
30 final histopathology.
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36 **Specimen analysis**

37 Both imaging techniques were obtained using the LightPath® system (Lightpoint Medical Ltd.,
38 U.K.), an *in vitro* diagnostic device that detects the location and distribution of positron-
39 emitting radionuclides within excised surgical specimens (Figure 1). The system is a bespoke
40 device with an ultrasensitive camera that detects emitted activity between 550 and 850nm. The
41 Lightpath® imaging system is not currently commercially available.
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48 When examining tissue samples after surgery, CLI is a technique that uses non-invasive
49 imaging to view tissues marked with a radiotracer (Figure 1). The LightPath® system's ultra-
50 sensitive camera detects the emitted light and creates an image of the tissue. Each margin must
51 be separately imaged, as the camera can only capture a 2D image at a time.
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56 When performing FAR, a 12-µm-thin scintillator was wrapped on the WLE specimen
57 following BCS, and a 3-µm mylar sheet was placed between the specimen and scintillator to
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3 prevent contaminating the scintillator (figure 1) (18). To detect activity within a specific
4 wavelength range of 550nm +/- 10%, a band path filter is used as the scintillator film produces
5 scintillations in a limited wavelength range. The scintillator is white and completely opaque to
6 chemiluminescence signatures.
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12 It would take 30-60 seconds between images to wrap the scintillator and/or orientate the
13 specimen. Both images had acquisition times of 300s each and 8x8 pixel binning (total pixel
14 resolution 938µm).
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18 **Histopathology**

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

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34 The sample size for this study was defined based on the assumption that this is a feasibility
35 study. The primary endpoint of the study was to report the sensitivity and specificity of CLI
36 and FAR LightPath® imaging for tumour detection compared to positive tumour detection
37 using standard-of-care histopathology methodology (positive margin of a WLE sample). Using
38 an estimate of the incidence of positive excision margins on histopathology as 20%, with 95%-
39 confidence (alpha = 0.05 two-sided) and with 10% precision, a sample size of 54 patients or
40 tumour specimens was estimated to provide sufficient power to detect sensitivity of 95% and
41 specificity of 90% (25).
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49 Patient demographics and tumour characteristics as well as radiotracer administration and
50 timing were reported with descriptive statistics. To assess the performance of CLI-FAR, we
51 calculated sensitivity, specificity, positive and negative predictive value whilst considering
52 histopathological assessment as the golden standard. All statistical analyses were conducted in
53 Stata (version 18.0, StataCorp LLC, College Station, TX).
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3 The per protocol population is defined as all patients who completed BCS and study procedures
4 as per protocol description. The primary endpoint was analysed based on the per protocol
5 population.
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10 **Results**

11 Overall, 54 specimens were imaged in 52 patients with a total of 104 margins were reviewed
12 using CLI-FAR (Figure 2). No adverse events were reported.
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16 **Patient Demographics and Tumour Characteristics**

17 Demographic data for the patient and tumour characteristics are shown in Table 1 and Table
18 S2. Of the 52 patients recruited with 54 tumours, 20 patients (38.5%) underwent NACT with
19 11 (20.4%) tumours achieving a complete radiological response on Magnetic Resonance
20 Imaging (MRI) before surgical resection. The remaining 43 tumours ranged in size (measured
21 on pre-operative MRI, ultrasound or mammogram) from 4mm to 56mm. The mean tumour size
22 was 20.6mm excluding those with complete radiological response.
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30 **Radiotracer administration and timing**

31 Table 2 shows the results and details of the patients administered with Tc99m and 18-FDG.
32 The mean dose of 18-FDG injected was 250.5MBq (SD 14.2) and the maximum dose was
33 273MBq.
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39 The dose of 18F-FDG injected was standardised and checked by the nuclear medicine
40 department. The protocol and pathway created for patients flowed well and allowed them to
41 undergo surgery without any delays to the theatre list. The target dose of 250MBq (+/-10%)
42 18F-FDG was given to 50 of the 52 patients; the remaining 2 received 213.3MBq and
43 223.7MBq, respectively. The reason for lower doses being administered are likely due to the
44 pre-injection decay which were due to timing of delivery. The aim was for the 18F-FDG to be
45 administered 145mins before imaging.
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53 On average, the first CLI-FAR image was taken 175.1 minutes after the 18F-FDG injection;
54 this ranged from 82 to 362 minutes. There was a minimum of five minutes between each CLI-
55 FAR image, and most patients had four images taken in total.
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Diagnostic performance of CLI-FAR

Of the 54 specimens, 50 had two margins assessed with CLI-FAR, results shown in table S1. 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

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3 showed proof of principle using CLI on 10 excised specimens observing radioactivity in
4 tumour cells and followed this with assessment of 15 margins in 12 patients (16) in which all
5 margins were negative for invasive cancer on imaging and histopathology. Jurrius et al.
6 investigated 385 margins on BCS specimens in 66 patients using FAR in a multi-centre trial in
7 Poland, and showed 46.2% sensitivity, 81.7% specificity, 8.1% positive predictive value
8 (PPV), 97.7% negative predictive value (NPV) and overall accuracy of 80.5%, detecting both
9 invasive carcinoma and DCIS (17). These studies have previously shown that radiation
10 exposure is low and safe for staff members(16,24); this has also been reflected in other 18F-
11 FDG breast assessment studies (26,27).
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20 A total of five surgical consultants were involved in the study, from recruitment to assessing
21 the CLI-FAR images. Thus, different interpreters were able to use the technology.
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25 **Future work:**

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

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

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

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

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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 ECMCQR-2022/10005 new award for ECMC since March 2023 to March 2028.

Data Availability

Data underlying this article will be shared on reasonable request to the corresponding author.

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Figures Captions

Figure	
1	<i>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.</i>
2	<i>Figure 2—Patient and specimen flow. n—number of patients, s—number of samples, m—number of margins.</i>
3	<i>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.</i>
4	<i>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.</i>

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 (Mbcq)	
0	6 (11.5%)
20	39 (75.0%)
40	7 (13.5%)
Dose of ¹⁸F-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)[†]	
< 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

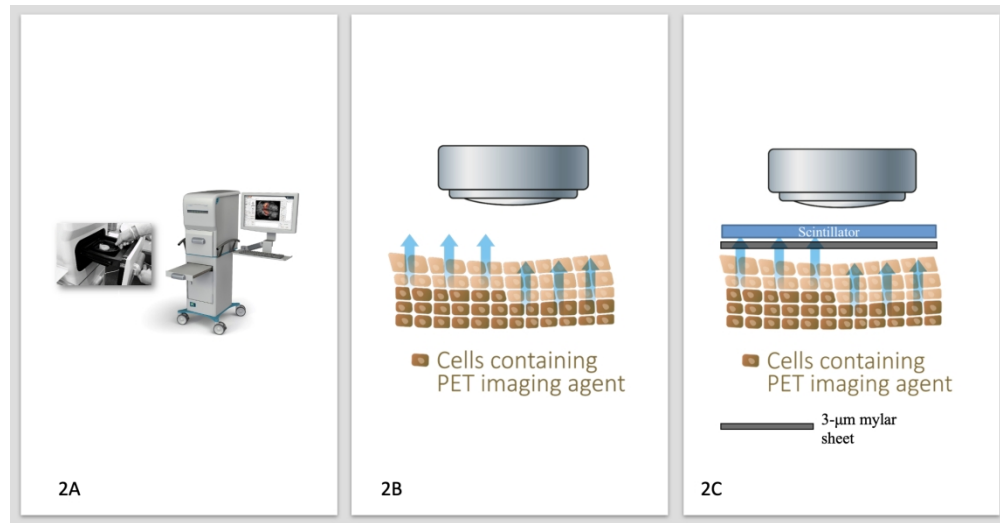


Figure 1 – This figure shows the LightPath[®] System, with how each technology works and how the specimen releasing beta particles would be detected using an ultrasensitive camera for each imaging modality (CLI and FAR). 1A- The LightPath[®] System, 1B - Cerenkov Luminescence Imaging (CLI), 1C - Flexible Autoradiography (FAR) Imaging, 3-µm mylar sheet placed between scintillator and specimen.

373x194mm (144 x 144 DPI)

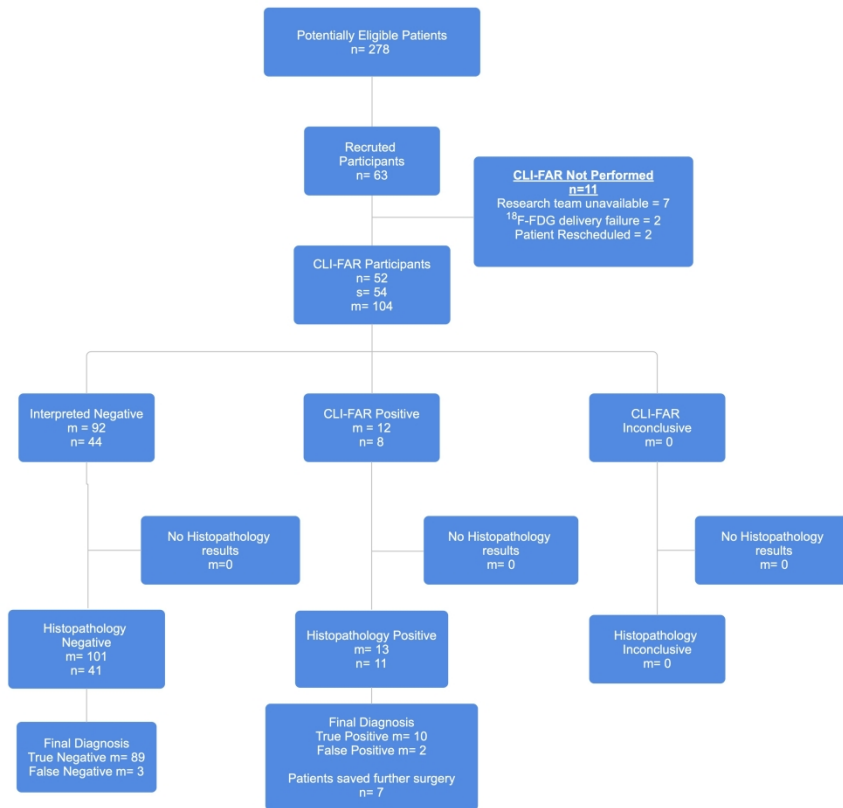


Figure 2— As per STARD guidelines this diagram depicts the transparency in the study design, showing the number of patients that were eligible, included, excluded and the dropout rate. Patient/specimen flow diagram per STARD, including all numbers from eligibility till close of study. Abbreviations are as follows: n— number of patients, s—number of samples, m—number of margins.

1410x1232mm (72 x 72 DPI)

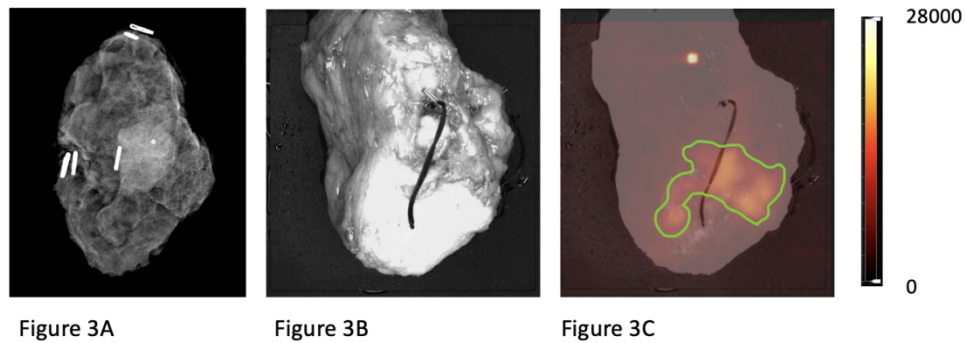


Figure 3 – This figure shows a specimen in which the CLI image was positive, therefore depicting how an intra-operative image would look like. This has a white light image and the intra-operative x-ray image for comparison. The intraoperative X-ray for this specimen was deemed to have negative margins. 3A – Intra-operative X-Ray of Specimen, 3B- the white light image of the specimen, 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.

245x89mm (144 x 144 DPI)

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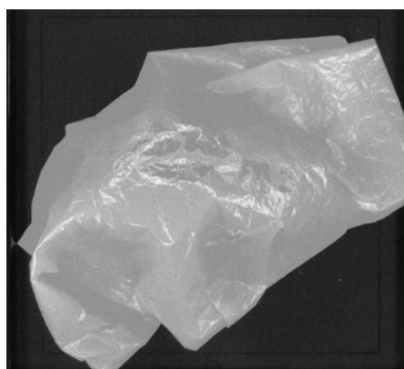


Figure 4A

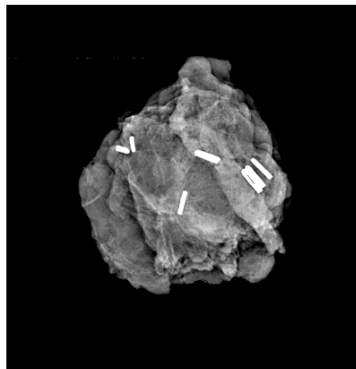


Figure 4B

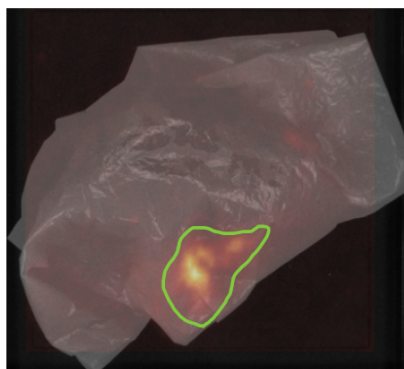


Figure 4C

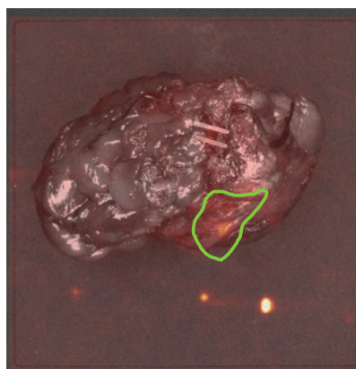


Figure 4D



Figure 4 - This figure shows a specimen in which the FAR image was positive, therefore depicting how an intra-operative image would look like. This has a white light image, the intra-operative x-ray image and a CLI image for comparison. The intraoperative X-ray for this specimen was deemed to have negative margins. 4A - The FAR image with a region of interest drawn in green where activity was seen. 4B - Intraoperative X-Ray of the specimen. 4C - Specimen wrapped in a scintillator, image in black and white. 4D - CLI image of with a region of interest drawn in green where activity was seen.

196x172mm (144 x 144 DPI)