METHODS

Standard practice requires the use of ^{99m}Tc–labeled radiopharmaceuticals for SLNB. The presence of ^{18}F-FDG in study subjects results in greater than normal background signal being detected by the handheld gamma probe used for SLNB, due to the down-scatter of 511keV Fluorine-18 (^{18}F) gamma photons into the ^{99m}Tc energy window. This cross-talk background could potentially hinder detection of low-activity SLNs. To assess the ^{18}F cross-talk and to determine the activity of ^{99m}Tc-nanocolloid required to successfully perform standard of care SLNB in patients undergoing ^{18}F-FDG CLI-guided surgery, two lead-in studies were conducted prior to commencing the CLI study.

Lead-in study I: SLNB simulations using the GAPS simulator

To determine the effects of the cross-talk background on the detectability of radioactive lymph nodes a simulation study was performed using the computerized gamma probe simulator (GAPS) (*1*). The GAPS system has previously been used in the UK national breast SLNB training program 'NEW START', and provides simulations of the radioactivity distribution and gamma probe response that allows accurate objective assessment of the surgeon's ability to localize SLNs on the surface of a manikin of the female breast and axilla.

The aim of the study was to measure the accuracy of gamma probe guided localization of nodes with varying levels of radioactivity and varying levels of cross-talk background.

The measurements were performed by two breast surgeons (AP and AK), with extensive experience of performing SLNB procedures. After one test case to familiarize the surgeons with the GAPS system, each surgeon was presented with 3 simulated SLNB cases individually. In each case the injection site, two SLNs and a spatially uniform ^{18}F background signal with Poisson distributed noise were simulated. The simulated SLN count rates ranged between 56 and 373 counts per second, and were based on a 150MBq ^{99m}Tc injection, a SLN tracer uptake ranging from 0.06% to 0.4% of the administered dose, an average SLN depth (30mm from the axillary skin surface) (*2*), and a time between tracer injection and SLN detection of 3 hours, which is common for 1-day SLNB protocols. The dose of 150MBq ^{99m}Tc is markedly higher than the standard dose of 20MBq used for 1-day SLNB procedures at Guy's Hospital. This increased dose was chosen to ensure a significant rise in SLN count rate, thus facilitating SLN detection in a ^{18}F background, whilst keeping within a dose range that is well established (*3*). The range in SLN % tracer uptake covers the lower spectrum of tracer uptake

reported in literature (*4*), thus assessing the surgeon's performance in the clinically most challenging situation of identifying SLNs with a low count rate. The ^{18}F background signal was set to a mean of 560 cps, corresponding to an ^{18}F-FDG dose of 5MBq/kg, which is the dose CLI study patients received. Calibration measurements of count rates from ^{99m}Tc and ^{18}F sources with the same gamma probe system and high-energy collimator as in the CLI study, the Europrobe 3 (Eurorad S.A., France), were used to set the count rates simulated by the GAPS system. The signal-to-noise ratio, defined as the ratio of the node count rate to the background count rate, varied from 0.67 and 0.10 in the simulations.

The surgeons performed surface scans with the GAPS gamma probe to localize as many nodes as they could, and to indicate when they thought they had localized a SLN. SLN detection was only considered successful if the surgeon indicated that they had located a node and if the position pointed at by the probe was within 10mm of the virtual node.

Lead-in study II: Gamma probe measurements in 18F-FDG PET patients

Following on from the SLNB simulations, gamma probe measurements of the axilla were performed in patients scheduled for a diagnostic ^{18}F-FDG PET scan to confirm that the simulated ^{18}F background signal corresponded to the gamma probe cross-talk found *in vivo*.

After research ethics committee approval and written informed consent was obtained, a total of 20 female patients were included at the PET Centre at St Thomas's Hospital (ISRCTN29552671). Approximately 60 minutes after receiving an intravenous injection of ^{18}F-FDG, but prior to PET imaging, the Europrobe 3 gamma probe with a high energy collimator was used to perform 10 second measurements of the lowest and highest count rates in the left and right axilla, respectively. The measurements of both axillae were performed shortly after each other (within 5 minutes) so that effects of radioactive decay between measurements were negligible. The background count rate was also measured, and the 10s count rates were averaged to give cps. The gamma probe system and configurations used to perform the ^{18}F-FDG axillary cross-talk measurements were the same as in the CLI study. The axilla was defined as the triangle between the pectoralis major, the latissimus dorsi and the edge of the breast. By placing the probe perpendicular to the skin, the entire axilla was scanned. Patient and injection characteristics such as height, weight and injected activity were recorded. An

independent samples t-test was performed to compare the highest signal in the right axilla and left axilla respectively, and a p-value of <0.05 was considered statistically significant.

RESULTS

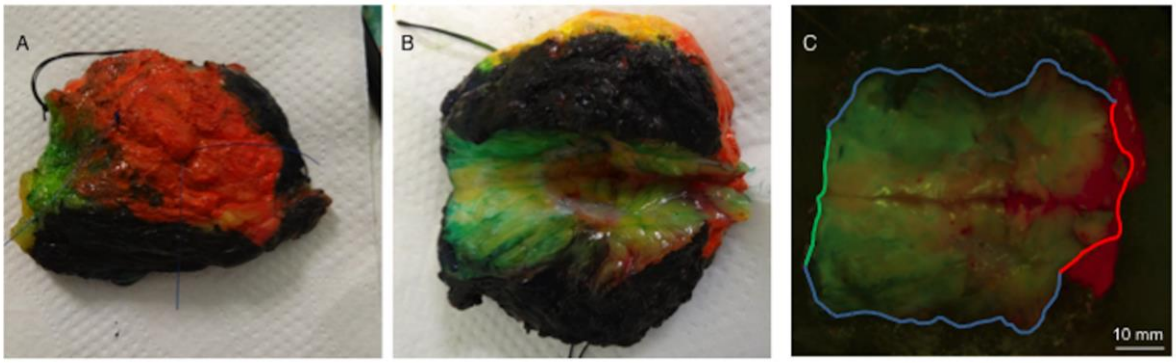
Lead-in study I: SLNB simulations using the GAPS simulator

The SLN detection results per surgeon can be found in Supplemental Table 1. The majority of the SLNs were accurately detected. Nodes with 0.4% (373 cps), 0.3% (280 cps) and 0.1% (93 cps) of injected activity were found by both surgeons, representing successful localization of nodes even with a signal-to-noise ratio as low as 0.17. Both surgeons missed the SLN with the lowest simulated uptake (0.06% uptake, 56 cps). The mean spatial accuracy for detected nodes was 2.6mm and 4.0mm for surgeon 1 and surgeon 2, respectively.

Lead-in study II: Gamma probe measurements in ^{18}F-FDG PET patients

The patient and ^{18}F-FDG injection characteristics can be found in Supplemental Table 2. The mean and standard deviation of the lowest and highest gamma probe signal in the right axilla was 310 ± 77 cps (range 133 – 488) and 372 ± 85 cps (range 233 – 616), respectively. The mean and standard deviation of the lowest and highest gamma probe signal in the left axilla was 299 ± 80 cps (range 161 – 553) and 359 ± 74 cps (range 236 – 582), respectively. These mean values are lower than the 560cps (5MBq/kg) used in the SLNB simulations, which were obtained from gamma probe calibration measurements with ^{18}F distributed in a water volume. The lower values probably reflect the renal excretion and non-uniform uptake *in vivo*. The highest count rate, which is clinically most relevant as it causes the greatest interference when detecting SLNs, did not differ between left and right axilla ($p = 0.596$), thus indicating that the cross-talk is similar in both axillae.

Based on the findings from both lead-in studies the investigators were confident that by using an increased administered activity of 150 MBq ^{99m}Tc-nanocolloid, a gamma probe collimator suitable for 511 keV energy photons and blue dye, SLNB could be performed safely and successfully, and patient recruitment to the CLI study commenced.



Supplemental Figure 1: Intraoperative inking and incising of WLE specimen. (A) Inks and sutures were applied to the WLE specimen to aid anatomical specimen orientation. (B) WLE specimen following initial incision through the posterior (black) margin, exposing the primary tumor and margins of excision. (C) White-light image of incised WLE specimen obtained with the CLI imaging system. Compared to (B) the specimen was further incised and opened to maximize the visibility of the posterior margin (outlined in blue), medial margin (outlined in green) and lateral margin (outlined in red). This image was used to assess tumor margins on CLI (Fig. 4B in manuscript).

Supplemental Table 1: SLN detection results from SLNB simulations using the GAPS simulator.

Surgeon	Case	^{18}F BG[1] (cps)[2]	SLN 1 (cps)	SLN 1 detected?	Localization error SLN 1 (mm)	SLN 2 (cps)	SLN 2 detected?	Localization error SLN 2 (mm)
1	1	560	373	Y	2.50	93	Y	6.40
	2	560	280	Y	1.40	93	Y	2.43
	3	560	280	Y	0.50	56	N	-
2	1	560	373	Y	1.27	93	Y	3.99
	2	560	280	Y	3.00	93	Y	9.70
	3	560	280	Y	2.00	56	N	-

1. *BG = background signal*
2. *CPS = counts per second*

Supplemental Table 2: Patient and ^{18}F-FDG injection characteristics from the gamma probe cross-talk study in PET patients.

Characteristic	**Mean (range)**
Age (years)	61.5 (40-81)
Height (cm)	161 (145-178)
Weight (kg)	69.3 (45-101)
Blood glucose level (mmol/L)	5.7 (4.3-16.4)
Injected Activity (MBq)	343.8 (307.5-387.0)
Time between injection and first measurement (min)	59.7 (49-79)

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