NeuroSAFE frozen section during robot-assisted radical prostatectomy: peri-operative and histopathological outcomes from the NeuroSAFE PROOF feasibility randomized controlled trial

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Objectives
To report on the methods, peri-operative outcomes and histopathological concordance between frozen and final section from the NeuroSAFE PROOF feasibility study (NCT03317990).

Patients and Methods
Between May 2018 and March 2019, 49 patients at two UK centres underwent robot-assisted radical prostatectomy (RARP). Twenty-five patient were randomized to NeuroSAFE RARP (intervention arm) and 24 to standard RARP (control arm). Frozen section was compared to final paraffin section margin assessment in the 25 patients in the NeuroSAFE arm. Operation timings and complications were collected prospectively in both arms.

Results
Fifty neurovascular bundles (NVBs) from 25 patients in the NeuroSAFE arm were analysed. When analysed by each pathological section (n = 250, average five per side), we noted a sensitivity of 100%, a specificity of 99.2%, and an area under the curve (AUC) of 0.994 (95% confidence interval [CI] 0.985 to 1; P ≤ 0.001). On an NVB basis (n = 50), sensitivity was 100%, specificity was 92.7%, and the AUC was 0.963 (95% CI 0.914 to 1; P ≤ 0.001). NeuroSAFE RARP lasted a mean of 3 h 16 min (knife to skin to off table, 95% CI 3 h 2 min–3 h 30 min) compared to 2 h 4 min (95% CI 2 h 2 min–2 h 25 min; P ≤ 0.001) for standard RARP. There was no morbidity associated with the additional length of operating time on in the NeuroSAFE arm.

Conclusion
This feasibility study demonstrates the safety, reproducibility and excellent histopathological concordance of the NeuroSAFE technique in the NeuroSAFE PROOF trial. Although the technique increases the duration of RARP, this does not cause short-term harm. Confirmation of feasibility has led to the opening of the fully powered NeuroSAFE PROOF randomized controlled trial, which is currently under way at four sites in the UK.

Keywords
NeuroSAFE, frozen section, robotic prostatectomy, prostate cancer, margins, nerve-sparing, #PCSM, #ProstateCancer, #uroonc
Patient summary

What is the paper about? When the nerves next to the prostate are removed during prostate cancer surgery men suffer side effects such as impotence and incontinence. Sometimes these nerves do not have to be removed. The NeuroSAFE technique checks the edge of the prostate under the microscope during surgery to see if it is safe to protect the nerves. This paper investigates the feasibility of studying the NeuroSAFE technique in men with prostate cancer.

What does it mean for patients? This feasibility study was designed to see if men would accept being randomised in a clinical trial to standard radical prostatectomy or radical prostatectomy with the NeuroSAFE technique. Men in two UK hospitals agreed to be randomised and so the full trial, evaluating the role of the NeuroSAFE technique in the recovery from prostate cancer surgery, is now underway at four UK hospitals.

Introduction

Erectile dysfunction is a common and significant side effect of radical prostatectomy (RP). Nerve-sparing (NS) RP improves recovery of erectile function [1–3]; however, the quality of NS is often balanced against the oncological risks associated with positive surgical margins (PSMs) [4,5]. Clinical suspicion of extraprostatic extension of prostate cancer is a relative contraindication of NS RP [6] as it confers an increased risk of PSMs; however, accurate prediction of prostate cancer stage T3 (i.e. extraprostatic extension) is difficult, requiring methods such as multiparametric MRI (mpMRI) [7], nomograms [8] and DRE). The NeuroSAFE technique is a standardized approach that uses intra-operative frozen section (IFS) analysis of the posterolateral neurovascular structure adjacent prostate margin during RP to assess margin status after NS in real time [9]. If the frozen section demonstrates the surgical margins to be positive, a wider dissection of the neurovascular bundle (NVB) may be performed. In this way, the NeuroSAFE technique seeks to promote optimal NS to maximize the opportunity for functional recovery without jeopardizing oncological safety.

Frozen section is the current ‘gold standard’ for providing intra-operative margin feedback during RP [10]. However, a recent systematic review performed by our group identified no prospectively performed studies, considerable variation in the technical application of IFS, and a wide range in the thoroughness of IFS technical description [11]. The same review found that the NeuroSAFE technique is the only IFS approach that has been disseminated across and reported by more than a single institution [12–14].

The NeuroSAFE PROOF feasibility study was designed to evaluate the role of the NeuroSAFE technique in the recovery from prostate cancer surgery. The aims of the present study were to (1) provide a detailed description of the NeuroSAFE technique as implemented in the NeuroSAFE PROOF study, including reporting concordance between margin diagnosis on frozen section and final section, (2) provide a detailed description of the pathological handling of secondarily resected neurovascular tissue including oncological outcomes of patients who had a PSM on IFS and (3) report on peri-operative outcomes from the NeuroSAFE PROOF feasibility study.

Materials and Methods

NeuroSAFE PROOF Feasibility Study

The NeuroSAFE PROOF feasibility study (NCT03317990) was a prospective, multi-site, 1:1 randomized controlled feasibility study [18]. Patients with localized operable prostate cancer (defined as absence of metastases on staging scans and absence of obvious T3b disease on imaging), who had undergone pre-biopsy mpMRI, who had been discussed at a regional multidisciplinary team meeting and who had selected RARP as their primary treatment, were deemed eligible. Eligibility criteria included good baseline erectile function (defined as International Index of Erectile Function-5 score >21) as has been used previously [19–21]. Recruited patient were randomized to RARP with NS according to the standard-of-care decision-making process (control arm) or RARP with NS.
according to a decision-making process that included the NeuroSAFE technique (intervention arm). The ‘standard of care decision-making process’ (control arm) included available information from the prostate biopsy, the preoperative mpMRI and the DRE under anaesthetic. The primary outcome of the study was satisfactory recruitment of 50 men in order to prove feasibility of the full-scale NeuroSAFE PROOF RCT. Detailed peri-operative and histopathological data were included as secondary outcomes in order to help determine power calculations for the subsequent study.

Ethical approval was acquired prior to study commencement (Regional Ethics Committee reference 17/LO/1978). All patients provided written informed consent and were randomized using an established, centralized, online randomization service [22]. All tissue was handled in accordance with the Helsinki Declaration [23].

NeuroSAFE RARP

The intervention was performed in accordance with previously described methods, as developed at the Martini Klinik, Hamburg, Germany [9]. The surgeon starts their dissection of the NVBs with a bilateral NS RP (partial, inter- or intrafascial [3], as per the surgeon’s discretion) after which they are able to verify the safety of their NS strategy as the neurovascular structure adjacent margin is inspected to check for PSMs by IFS. The prostate is removed from the body through an enlarged umbilical incision as soon as it has been detached from the surrounding structures using the Alexis Laparoscopic System (Applied Medical, Rancho Santa Margarita, CA, USA). Pneumoperitoneum was re-established once the specimen had been removed in order to continue with the remaining steps of the RARP.

If a PSM is noted on the IFS, secondary resection (SR) may be performed. One of two types of SR was performed by the surgeon. ‘Partial SR’ was defined as an excision of a focus of Denonvilliers’/periprostatic fascia based on the surgeon’s best effort to cognitively localize the focus of prostate cancer that had given rise to the PSM, also taking into account which section was reported as having a PSM and calculating distance from the apex. ‘Full SR’ was defined as the entire en bloc excision of the ipsilateral NVB from base to apex [18]. The decision to perform full or partial SR was at the discretion of the surgeon based on the length and grade of the PSM on the NeuroSAFE frozen section. If IFS demonstrated a negative surgical margin, the ipsilateral NVB was left intact. Detailed timings for operation duration and complications were collected for all patients.

NeuroSAFE Technique

Immediately after removal of the prostate, the posterolateral neurovascular structure adjacent surfaces were painted in theatre by the console surgeon (Fig. 1A). The inked prostate was then transported to the histopathology department where the entire inked margin was cleaved by a straight blade. This posterolateral portion of prostate with ink on the outer margin surface was further divided by perpendicular cuts at intervals of 5 mm from apex to base. A minimum of four and a maximum of seven pieces from the posterolateral prostate on each side were submitted for freezing in this manner according to the size of the prostate. Each piece of prostate tissue was then embedded into optimal cutting temperature compound on a cryostamp, and frozen (Fig. 1B). The frozen prostate tissue was then transferred to the cryostat for sectioning at

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Fig. 1 Images showing the performance of intra-operative frozen section as per the NeuroSAFE technique. (A) Ink stains the left (yellow) and right (green) neurovascular structure adjacent prostate margin, respectively. (B) After cleaving the right side and slicing perpendicularly a 5-mm piece of prostate tissue sits on the cryostat before freezing. (C) Once embedded in optimal cutting temperature compound and frozen, 5-μm sections are prepared on the microtome before staining.
a tissue thickness of 5 µm before staining with haematoxylin and eosin (Fig. 1C).

Stained frozen sections were examined in the histopathology department by a dedicated uropathology consultant (A.F., A.H., I.B.-S., M.R., J.O. or R.B.). A PSM was defined as a single neoplastic gland present at the inked margin. Negative margins were reported as ‘clear’ if tumour was not seen or was >0.5 mm from the inked margin, or ‘narrowly clear’ if tumour was seen <0.5 mm away from the ink (Fig. 2A–C). Frozen section diagnoses were recorded prospectively on the day of surgery. Once the reporting pathologist had given a frozen section margin assessment, each piece of prostate tissue was defrosted in warm water and placed individually in formalin for subsequent paraffin embedding. Careful attention to the orientation of each piece in the processing cassette was given to ensure that the final slides examined the same surface. All prostatectomy specimens were processed in accordance with the standard procedures recommended by the International Society of Urological Pathology (ISUP) [24] and reported as per the standards outlined by the Royal College of Pathologists (June 2016) [25].

Secondary Resection

The SR NVB (when performed) was received in formalin after the completion of the operation and processed with the rest of the main prostatectomy specimen. Further IFS was never performed on the SR NVB. When the entire NVB was submitted en bloc following full SR, the specimen was annotated by the surgeon with clips and sutures to orientate the prostate adjacent side, the resection margin and apical end. When a partial SR was carried out, orientation of the
tissue was not performed. All tissue was formalin-fixed, painted and sectioned at 5-mm intervals. Where possible the specimen was embedded from apex to base in separate cassettes. If no tumour was identified in the SR specimen, adjacent levels were taken and examined.

Concordance Assessment

After formalin fixation, paraffin embedding, haematoxylin and eosin staining, each frozen section margin was reassessed by one of three reporting pathologists (A.F., A.H. or J.O.). This assessment of the postero-lateral margin was considered the reference test for the purposes of the concordance study. The margin was classified again as clear, narrowly clear or positive (Figs 2, 3 and 4).

Statistical Analysis

All analyses were performed using SPSS (version 26). Baseline clinical and pathological characteristics are presented as descriptive statistics. Proportions were compared using the two-tailed t-test for continuous variables and the chi-squared test for categorical variables. For frozen section (diagnostic test) compared to paraffin-embedded section (reference test), 2 x 2 contingency tables were used to present the results and calculate the diagnostic accuracy estimates with 95% CIs. The 2 x 2 contingency tables are presented, with the unit of assessment being both each single section of margin submitted and each neurovascular structure adjacent laterality (two NVB sides per patient). The diagnostic performance was also evaluated using the receiver-operating characteristic (ROC) curve and the area under the curve (AUC) to describe the relationship between the sensitivity and specificity of the test. P values < 0.05 were taken to indicate statistical significance.

Results

Forty-nine patients underwent RARP at two UK NHS participating centres (University College Hospital London and North Bristol Trust) between 29 May 2018 and 25 March 2019 (see CONSORT Diagram in Appendix S1). Twenty-five men underwent NeuroSAFE RARP and 24 underwent standard RARP according to randomization. One patient had treatment deferred as a result of cardiological comorbidities identified as part of his preoperative assessments and was withdrawn from the trial. Baseline clinical and pathological characteristics of the patients included in the study are shown in Table 1.

Pathological Concordance for the NeuroSAFE Technique

From 25 patients undergoing NeuroSAFE RARP, 250 postero-lateral neurovascular structure adjacent prostate margin sections were submitted for IFS. Table 2A shows the
concordance between the frozen section and the final section assessment for each individual section. For the purposes of this analysis, the diagnosis of ‘clear’ and ‘narrowly clear’ were considered together. Frozen section sensitivity was 100%, specificity 98.7%, positive predictive value 97.1%, and negative predictive value 100% ($P < 0.001$). The ROC curve is shown in Fig. 3, with an AUC of 0.994 (95% CI 0.985 to 1; $P < 0.001$).

In analyses considering frozen section results with the 50 NVBs as the denominator (two per NeuroSAFE patient), frozen section had a sensitivity of 100%, a specificity of 92.7%, a positive predictive value of 75% and a negative predictive value of 100% ($P < 0.001$; Table 2B). The ROC curve is shown in Fig. 4, with an AUC of 0.963 (95% CI 0.914 to 1; $P < 0.001$). There were three instances when a PSM on intra-operative frozen section was subsequently considered narrowly clear on final section assessment.

Secondary Resection

In total, 11/25 patients had PSMs identified by the NeuroSAFE technique. Bilateral PSMs were identified in one patient. Seven out of 11 patients underwent SR (Table 3). Of the seven patients who had SR, four underwent partial and three patients full SR of the ipsilateral NVB. Cancer was found in the SR in three out of seven patients on final

Table 1 Clinical and pathological characteristics of the NeuroSAFE (intervention) and standard (control) arms.

| Variable                          | NeuroSAFE | Standard | $p^*$ |
|-----------------------------------|-----------|----------|-------|
| Number of patients                | 25        | 24       | 0.66  |
| Mean (range) age, years           | 57 (51-66)| 55.9 (44-63)| 0.99  |
| Mean (range) preoperative PSA, ng/mL| 10.4 (1.2-39.2)| 9.5 (4-35)|       |
| MRI stage, n (%)                  | 2         | 2        | 0.52  |
|                                   | 3         | 3        |       |
| Biopsy ISUP, n (%)                | 1         | 3 (13)   | 0.5   |
|                                   | 2         | 17 (70)  |       |
|                                   | 3         | 3 (13)   |       |
|                                   | 4         | 1 (4)    |       |
|                                   | 5         | 0        |       |
| Mean (range) prostate weight, g   | 43.9 (21-75)| 42.2 (15-59)| 0.71  |
| LND performed, n (%)              | 6 (24)    | 5 (21)   | 0.78  |
| Pathological stage, n (%)         | 2a/2b     | 0 (0)    | 0.25  |
|                                   | 2c        | 16 (67)  |       |
|                                   | 3a        | 7 (28)   |       |
|                                   | 3b        | 1 (4)    |       |
| Mean (range) tumour volume, mL    | 5.0 (0.25-22.27)| 4.7 (0.7-12.89)| 0.17  |
| Final ISUP grade, n (%)           | 1         | 2 (8)    | 0.27  |
|                                   | 2         | 17 (68)  |       |
|                                   | 3         | 5 (20)   |       |
|                                   | 4         | 0        |       |
|                                   | 5         | 1 (4)    |       |
| Mean (range) operation length, h:min†| 3:16 (2:05-4:20)| 2:13 (1:30-3:06)| <0.001|
| First 5 cases                     | 3:20 (3:05-3:30)| 2:15 (1:30-2:35)|       |
| Last 5 cases                      | 3:07 (2:40-3:30)| 2:06 (1:45-2:45)|       |
| Clavien-Dindo complications grade > 1| 1         | 2        |       |

ISUP, International Society of Urological Pathology; LND, lymph node dissection. *Statistical tests; two sample t-test for means, Fisher’s exact test for proportions. Analysis performed using SPSS version 26. †Missing data for two patients, both in the NeuroSAFE arm.

Table 2A Concordance between frozen section and final section per slide generated.

| Frozen section-negative | Frozen section-positive | Total |
|-------------------------|-------------------------|-------|
| Final section-negative  | 234                     | 3     | 237  |
| Final section-positive  | 0                       | 13    | 13   |
| Total                   | 234                     | 16    | 250  |

Sensitivity = 100%, specificity = 98.7%, positive predictive value = 97.1%, negative predictive value = 100%.

Table 2B Concordance between frozen section and final section per neurovascular bundle.

| Frozen section-negative | Frozen section-positive | Total |
|-------------------------|-------------------------|-------|
| Final section-negative  | 38                      | 3     | 41   |
| Final section-positive  | 0                       | 9     | 9    |
| Total                   | 38                      | 12    | 50   |

Sensitivity = 100%, specificity = 92.7%, positive predictive value = 75%, negative predictive value = 100%.
histological examination (two full SR and one partial SR). Of the four patients who underwent partial SR, one had cancer present in the SR specimen and three had RP failure (defined as requiring adjuvant therapy or a PSA of >0.2 ng/mL) at 12-month follow-up. Of the three patients who underwent full SR, two out of the three had cancer present in the SR specimen and two went on to have RP failure at 12-month follow-up.

Four patients (patients 5, 6, 7 and 11 in Table 3) had five PSMs on IFS, and no SR was performed because of short length of Gleason Grade Group 1 cancer at the PSM. In all three patients where there was a single PSM of <1 mm, no RP failure was noted at 12-month follow-up. Moreover, three patients men (patients 5, 7 and 9 Table 3) had a single PSM of <1 mm in length, all three of which were converted to 'clear' on final section analysis.

Four men (patients 2, 3, 6 and 8 in Table 3) who had multiple section PSMs during IFS had RP failure regardless of whether they had SR. Of these four patients, two had full SR (of whom one had cancer observed within it), one man had a partial resection with cancer observed within it, and the last had no SR.

### Operation Timings and Peri-operative Outcomes

The mean length of NeuroSAFE RARP was 3 h 16 min (95% CI 3 h 2 min to 3 h 30 min) compared to 2 h 14 min (95% CI 2 h 2 min to 2 h 25 min; \(P < 0.001\)) in the standard RARP arm. Incorporating the additional time in the operating room and the pathology laboratory expense incurred by performing the NeuroSAFE technique, the estimated additional cost of the procedure was £1000.

There were no serious adverse events in the study. All patients were discharged home the day after surgery. There were three complications of Clavien–Dindo grade >1 in two patients (one NeuroSAFE and one standard arm), including one readmission within 90 days. One patient in the NeuroSAFE arm developed bladder neck stenosis and required bladder neck dilatation under general anaesthetic (Clavien–Dindo grade 3b). One patient in the standard arm was readmitted to hospital with urosepsis and treated with antibiotics (Clavien–Dindo grade 2). The same patient had a urinary leak seen on cystogram and required a prolonged period with a urinary catheter (Clavien–Dindo grade 3a). There were no postoperative complications recorded in the intervention arm on account of the additional time spent under general anaesthesia.

### Discussion

A recent systematic review found no prospectively performed studies on IFS or the NeuroSAFE technique [11]. Our feasibility study, which is the first prospective and
randomized study of its kind, shows that the technique is safe, that the results of the frozen section are reproducible on final section analysis, and that the technique can be carried out in more than one institution as part of the same trial. Additional detailed analysis of oncological outcomes up to 1 year after surgery in men who had PSMs on IFS has allowed alteration of the NeuroSAFE technique that is now being employed in the full NeuroSAFE PROOF trial.

In our feasibility study, the NeuroSAFE technique contributed an additional hour to the length of RARP. This is considerably longer than the Martini Klinik experience, where Beyer et al. [26] noted that, although the results of the NeuroSAFE took 35 min until communication to the surgeon, the additional step only added 4 min to the length of the operation. Possible explanations for this discrepancy include greater pathology laboratory capacity at the Martini Klinik where there are five cryostats in the laboratory and the fact that, in the present study, lymph node dissection (LND) was only performed in 11/49 patients (22%) [26]. If the proportion of patients in both arms undergoing LND was higher, the difference in the length of RARP between arms may have lessened as the procedure would take longer on average in the standard arm. It should also be noted that, in both our participating study centres, the pathology laboratories were in different buildings from the operating room and were equipped with two cryostats each. van der Slot et al. [27] describe a reduction in the duration of NeuroSAFE RARP with increasing experience. This finding is also familiar to our group, as system familiarity and planning, transfer to the laboratory and technical experience all shorten times. Despite this, within the number of NeuroSAFE RARPs performed in the intervention arm during the feasibility study \((n = 25)\) the difference between the mean duration of the first five cases (3 h 20 min) and the last five cases (3 h 7 min) did not meet statistical significance \((P = 0.49; \text{Table 1})\).

In spite of the additional time taken to perform the NeuroSAFE technique, the completion of the feasibility study at two NHS UK hospitals shows that the technique is feasible and that it can be performed at units that have access to routinely available frozen section equipment (cryostat, cryostamp, staining apparatus). Since the completion of the feasibility study, two further centres (Sheffield University Teaching Hospitals NHS Trust and Glasgow and Clyde NHS Trust) have also joined the NeuroSAFE PROOF RCT. Importantly, our experience shows that there were no intra-operative or immediate postoperative complications for patients associated with the longer time spent under general anaesthesia.

To date, only two other groups who use the NeuroSAFE technique have published their frozen to final section concordance. Our results, showing excellent diagnostic accuracy and agreement between IFS and final section, are consistent with these two aforementioned retrospective case-control studies. At the Martini Klinik, Hamburg, Germany, where the technique was developed, Schlomm et al. [12] noted that frozen section had a sensitivity of 93.5% and a specificity of 98.8% when compared to final section. Similarly, Mirmilstein et al. [13] reported a sensitivity of 90% and a specificity of 97.4% when comparing frozen to final section at the neurovascular structure adjacent margin only. It is noteworthy that in the present study no PSM was missed by IFS that was subsequently found on final section. For these reasons, IFS may still be deemed the ‘gold standard’ for real-time resection margin assessment during uro-oncological surgery [10]. The accuracy of the NeuroSAFE technique can be increased by reducing the slice thickness at the postero-lateral margin to 3 mm (from 5 mm), but we have demonstrated excellent concordance with our current process and so it remains unchanged in the protocol in order to manage resources well with no compromise on detail. The emergence of promising fast digital imaging techniques [28] and the ongoing development of new platforms to incorporate preoperative mpMRI information intra-operatively via augmented reality also hold considerable promise for future strategies to reduce the risk of PSMs [29].

Our finding of cancer in 42% of the SR tissue submitted is at the upper end of what has previously been reported in the IFS in the RP literature (0–42.4%) [11,14,30,31]. This may be because our patients represent a higher risk group.

Table 4 Previously published studies on the NeuroSAFE (*for neurovascular bundle-focused intra-operative frozen section) technique including details on pathological stage and presence of cancer in the secondary resection specimen, where given.

| Year | n      | pT2 (%) | pT3a (%) | pT3b (%) | SR +ve cancer (%) |
|------|--------|---------|----------|----------|-------------------|
| 2012 | 11 069 | 70      | 20       | 10       | 23                |
| 2016 | 458    | 81      | 11.6     | 6.8      | 16.1              |
| 2017 | 277    | 83      | 14       | 3        | 42.4              |
| 2019 | 346    | 60      | 40*      | U        | NA                |
| 2020 | 407    | 57      | 42*      | U        | NA                |
| 2020 | 50     | 63      | 28       | 9        | 42                |

NA, information not available in manuscript; SR, secondary resection; U, unknown. *Value for pT3a and pT3b not provided separately.
than those in previous studies on the NeuroSAFE technique (Table 4). This is the first study in the literature that gives a detailed account of individual patient oncological outcomes according to the type of SR performed. Our analysis has led to the development of our protocol for the response to PSM on IFS within the full NeuroSAFE PROOF RCT. Firstly, our protocol now instructs that only full SR is performed. Secondly, the indications for full SR are now: (1) any PSM on multiple sections on a side; (2) any Gleason grade 4 or grade 5 adenocarcinoma at the margin; and (3) any single section PSM >2 mm of Gleason Grade Group 1.

The impact of PSM on cancer control outcomes is not necessarily an all-or-none phenomenon, i.e. grade, length and patient tumour characteristics independent of margin status all impact long-term oncological outcomes and the likelihood of RP failure [32]. Various studies, including our feasibility data, have informed the development of our approach to the positive NeuroSAFE as described above. van der Slot et al. [27] found that in patients with <1 mm of Gleason pattern 3 at the margin, adenocarcinoma was never seen in the SR specimen. Moreover, the presence of Gleason pattern 3 only at the surgical margin has been associated with a decreased risk of biochemical recurrence [33,34], and several studies have demonstrated that men with ≤3 mm PSM length on final prostatectomy specimen have similar biochemical recurrence-free survival as compared to those with negative surgical margin status [4,35,36]. Conversely, Choi et al. [37] found that Gleason pattern 4 and higher on IFS was predictive of biochemical recurrence. We have presented 1-year oncological outcomes to help further explain the rationale behind the development of the protocol for the NeuroSAFE technique within the full NeuroSAFE PROOF RCT. We do not present long-term outcomes for all patients, as the full trial is ongoing, and we do not want to compromise the conduct of this definitive study.

Our feasibility study has some considerable strengths. It is the first report to have collected information on the NeuroSAFE technique that is either prospective or randomized. It also represents a dual-site, multi-surgeon and multi-pathologist experience, and therefore, it is a realistic account of the use of the technique in the wider clinical setting.

The study also has some limitations. Firstly, we cannot yet draw conclusions about oncological outcomes because this was a small study, which was powered only to demonstrate the feasibility of conducting a larger study. Secondly, we have not reported on the grade of the adenocarcinoma contributing to the PSM on IFS. Similarly, we did not record whether PSM on IFS may have been considered capsular incision, or whether there were benign non-cancer intracapsular margins. These features have been incorporated into the reporting of the NeuroSAFE technique in the definitive study as we continue to learn from our experience.

In conclusion, the present study demonstrates the safety, reproducibility and excellent histopathological concordance of the NeuroSAFE technique in the NeuroSAFE PROOF trial. Although the technique increases the duration of RARP, this does not cause short-term harm to patients. These results have been used to aid the development of the full NeuroSAFE PROOF RCT, which will, in turn, provide further long-awaited information as to whether NeuroSAFE RARP can improve outcomes for men undergoing surgery for prostate cancer cure.

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Conflict of Interest

The authors have no personal conflicts of interest to disclose. Within NeuroSAFE PROOF, laparoscopic ports are supplied by Applied Medical. Applied Medical had no role in the design, analysis, or collection of the data, in writing of the manuscript, or in the decision to submit the manuscript for publication.

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Abbreviations: AUC, area under the curve; IFS, intra-operative frozen section; LND, lymph node dissection; mpMRI, multiparametric MRI; NS, nerve-sparing; NVB, neurovascular bundle; PSM, positive surgical margin; RARP, robot-assisted radical prostatectomy; RCT, randomized controlled trial; ROC, receiver-operating characteristic; RP, radical prostatectomy; SR, secondary resection.

Supporting Information
Additional Supporting Information may be found in the online version of this article:
Appendix S1. CONSORT Flow Diagram.