

STUDY PROTOCOL

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| **Title** | Sentinel lymph node mapping in early stage endometrial cancer using indocyanine green and near infra-red fluorescence imaging during minimally invasive surgery – validation of technique in Queensland centres |
| **Short Title** | Sentinel lymph node mapping in early stage endometrial cancer |
| **Principal Investigator** | Dr Nisha Jagasia 07 3163 8111 |
| **Associate Investigator(s)** | Assoc. Professor Lewis Perrin, Dr Naven Chetty |
| **Location** | Mater Hospital South Brisbane |

# **FULL STUDY TITLE**

Sentinel lymph node mapping in early stage endometrial cancer using indocyanine green and near infra-red fluorescence imaging – validation of technique in Queensland centres.

# LAY DESCRIPTION

We propose a prospective study to validate the feasibility and clinical impact of a technique for assessing cancer spread in women with cancer of the uterus (endometrial cancer). This technique involves locating and removing the first draining lymph gland (node) that would be involved with spread of the cancer, from each side of the pelvis, using a fluorescent dye and special cameras during key-hole surgery. This obtains information about the degree of spread (stage) of the endometrial cancer without the need to remove all pelvic lymph nodes from both sides of the pelvis. We hope to show that sentinel lymph node mapping can safely replace traditional full pelvic lymph node removal in selected women with endometrial cancer.

The study will be conducted in such a way that it complies at all times with:

* Mater Hospital Code of Conduct
* Mater Hospital Research Ethics Committee guidelines and all other relevant NHMRC standards
* Australian Medical Association Code of Conduct for Medical Practitioners

**STUDY INVESTIGATOR(S)**

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# **INTRODUCTION**

Systematic pelvic and para-aortic lymphadenectomy has not been shown to be of therapeutic benefit for low and intermediate risk endometrial cancer patients in terms of disease free survival or overall survival1,2. However, knowledge of lymph node status is invaluable for prescribing adjuvant therapies and delivering prognostic information3.

Exposing all patients with endometrial cancer to the risks and morbidity associated with a pelvic and para-aortic lymphadenectomy must be balanced against the potential of missing occult lymph node metastasis in those women that do not undergo systematic surgical lymph node staging. Sentinel lymph node (SLN) mapping allows us to obtain information about lymph node involvement with malignancy, in all suitable patients without exposing them to the risks of comprehensive lymphadenectomy. SLN mapping has now been incorporated into the NCCN guidelines4 for endometrial cancer as there is robust evidence to support its efficacy, accuracy and safety in staging endometrial cancer patients5,6,7.

We propose a prospective, open label, single arm trial of sentinel lymph node mapping in early stage endometrial cancer, using indocyanine green and near infra-red fluorescence imaging during minimally invasive surgery, to validate the feasibility and clinical impact, relating to prescription of adjuvant radiation and chemotherapy, of this technique in our institution as well as other Queensland centres offering this procedure.

All women with clinically uterus confined endometrial malignancy undergoing laparoscopic or robotic surgery for treatment and staging are advised to have SLN mapping as part of their surgical staging. The SLN mapping is performed according to an evidence based protocol. With this research study, we wish to prospectively collect information on the detection rate and localization of mapped sentinel lymph nodes, the utilization of SLN biopsy results in informing decisions on adjuvant therapy, as well as the safety of the technique in our hands.

## **BACKGROUND**

Endometrioid adenocarcinoma of the endometrium is the most common gynaecological cancer seen in the developed world.   In Australia, there are approximately 2,500 cases of endometrial cancer per year giving a crude incidence rate of 20 per 100,000 women. Based on national trends the incidence was set to rise by almost 15% between 2013 and 20188.

Two randomized controlled trials have failed to show a therapeutic benefit of pelvic and para-aortic lymphadenectomy on progression free survival (PFS) or overall survival (OS) in endometrial cancer patients1,2. However, lymph node metastasis is a known poor prognostic factor for patients with endometrial cancer. Stage III patients (those with pelvic or para-aortic lymph node involvement) have 5-year relative survival rates of 59.6% compared with 97.4% for patients with stage I disease (uterus confined)3. Progression free survival drops from 87% to 70 % in those with pelvic lymph node metastasis and to 36% in those with para-aortic nodal disease9. Systemic chemotherapy in patients with positive lymph nodes has been shown to improve PFS and OS compared to radiation alone10,11,12,13. Hence, knowledge of lymph node status is vital as it defines recurrence risk and informs clinician decisions regarding adjuvant therapy.

Most cases of endometrial cancer present with early stage disease confined to the uterus5,14. Full surgical staging for endometrial cancer as per FIGO guidelines involves total hysterectomy, bilateral salpingo-oophorectomy, pelvic and para-aortic lymphadenectomy and peritoneal cytology. Clinico-pathological studies have shown that patients with uterus confined disease and low risk histological features (Grade 1 or 2 endometrioid tumour with less than 50% myoinvasion, tumour diameter <2cm and an absence of cervical involvement) are at low risk of lymph node metastasis (incidence 0 to 5%) 15,16. In this group, there are negligible gains made from comprehensive lymphadenectomy as part of staging as these women have very low risk of recurrent disease even if they are observed without surgical staging16. In contrast, patients with high-grade histology (Grade 3 endometrioid, serous/ clear cell) have a 20 to 40% risk of lymph node involvement, with highest risk in those with deep myometrial invasion16. The method and extent of lymph node dissection have not been uniformly defined and remain controversial. In seeking the optimal care for women with endometrial cancer, the aim is to avoid both overtreatment (i.e. sparing a patient unnecessary surgery or radiotherapy) and under-treatment (i.e. not treating a patient with occult involvement of her lymph nodes).

Many centres in Australia and internationally, including those in Queensland, have used intra-operative assessment of the uterus (frozen section) to assess a priori risk of lymph node involvement and decide if a patient requires staging lymphadenectomy. This is to avoid those at very low risk of lymph node metastasis being exposed to the not insignificant risk associated with pelvic and para-aortic lymphadenectomy. These risks include potential intra-operative complications of injury to major vessels, pelvic nerves (genitofemoral nerve and obturator nerve) and viscera as well as post-operative morbidity including lower limb lymphedema, lymphocyst formation, increased risk of deep venous thrombosis, and small bowel obstruction16,17. Furthermore, pelvic lymphadenectomy is a technically difficult procedure particularly in obese patients, that represent a substantial proportion of patients with endometrial cancer.

Our practise in Queensland has been to conduct intraoperative assessment of the uterus with frozen section by a pathologist to provide information on grade, depth of myoinvasion and tumour volume. If this deems the patient to be at low risk for lymph node metastasis, based on a Grade 1 or 2 tumour with less than 50% myoinvasion, lymphadenectomy has been omitted from surgical staging. Those women with Grade 3 tumours, deep myoinvasion, non-invasive serous or clear cell histology or large tumour volume undergo pelvic +/- para-aortic lymphadenectomy for staging. Women considered very high risk because of serous or clear cell tumours with myoinvasion or Grade 3 deeply invasive tumours are routinely prescribed adjuvant radiation and chemotherapy to reduce risk of loco-regional and distant recurrence respectively. Hence in this group, lymph node dissection is avoided unless there are any suspicious or bulky lymph nodes (*Appendix A: Current Staging Protocol for Endometrial Cancer*).

However, there are inaccuracies with using pre-operative and intra-operative assessment to triage whether patients require lymph node staging. Case et al. found significant discordance between frozen and permanent section for depth of invasion and tumour grade18. Overall, 10% to 15% of patients believed to have Grade 1 tumours on pre-operative endometrial biopsy will be upgraded on final pathology and up to 25% will have more extensive invasion than predicted19. Ballester et al. found that 21% of presumed low and intermediate risk patients were upstaged on final histology, of whom between 15% and 25% had positive lymph nodes. Even when definitive histology is considered as the reference, up to 9% of low risk patients had positive nodes20. Hence, assessment of lymph nodes even in those with apparent low risk uterine features will identify a cohort of patients with occult lymph node disease, who would benefit from adjuvant systemic treatment.

Sentinel lymph node (SLN) mapping has emerged as a feasible and safe technique for obtaining information about a patient’s lymph node status and directing adjuvant therapy, whilst avoiding the risks associated with comprehensive lymphadenectomy. The sentinel node is defined as the first lymph node to receive drainage from a primary tumour and is the most likely to harbour metastasis in cancers with lymphatic spread. SLN biopsy involves the selective and limited removal of this tumour specific or organ specific lymph node that is identified by injection of a tracer dye into, or in proximity to the primary tumour. It is a technique that has been used widely in melanoma and breast cancer staging. It has also been applied successfully in vulvar cancer. SLN mapping in the endometrial cancer setting entails following lymphatic channels to a single SLN, compared to a comprehensive pelvic lymph node dissection where the entire pelvic nodal bundle (external iliac, common iliac and obturator fossa nodes) is removed.

At our institution, the disparity between pre-operative or intra-operative assessment and final histology results in up to 15% of patients, who could have potentially benefited from full surgical staging, being under-staged based on our current staging and treatment protocol21. These patients receive external beam radiation to the pelvis to reduce local recurrence risk which could be omitted if they were known to be node negative. Evidence suggest that treatment algorithms that do not include lymph node staging for all patients rely upon an increased use of external beam radiation to treat patients with intermediate to high risk uterine features, to reduce local recurrence risk16,20. The serious complication rate of external beam radiation is 3%22, including late bowel and bladder toxicity. SLN mapping in all endometrial cancer patients with apparent early stage disease, avoids this under-staging of women who are upgraded on upstaged on final histology.

The final histology including a review of uterine features and lymph node assessment (in those that underwent lymph node staging) is used to determine which patients require adjuvant therapy with radiation +/- chemotherapy (*Appendix B: Adjuvant Therapy Protocol*). Women with Stage 1 endometrial cancer that fall into the “intermediate risk” group can potentially avoid adjuvant radiation if they have negative lymph node staging or be prescribed vaginal vault brachytherapy to reduce risk of vaginal recurrence, which as a lower morbidity compared to external beam pelvic radiation23. Those intermediate risk patients found to have positive lymph nodes can be appropriately prescribed chemotherapy and pelvic radiation to reduce risk of systemic and local recurrence respectively10,11,12. There is a category of women that fall into a “high – intermediate risk” group based on age and uterine features (Grade 2-3 tumour, outer 1/3 myoinvasion and evidence of lymphovascular space invasion) that warrant attention. These women benefit from pelvic radiation and chemotherapy 13,24 to reduce recurrence risk. With SLN mapping replacing comprehensive pelvic lymph node dissection, both lymph node positive patients and those with high-intermediate risk uterine features are being spared the combined morbidity of lymphadenectomy followed by external beam radiation whilst recommendations for chemotherapy can be more accurately triaged. The 4-year complication rate of comprehensive surgical staging followed by pelvic radiation being estimated at 13%17.

Until recently the “SENTIENDO” study represented the largest multi-institution prospective study on endometrial cancer SLN biopsy. The SENTIENDO group7,25 have shown that up to 10% of patients with low-risk and 15% of patients with intermediate risk endometrial cancer will be upstaged based on SLN biopsy. This study showed a detection rate of 89%, sensitivity of 84% and a negative predictive value of 97% for SLN biopsy in Stage 1 and 2 endometrial cancer patients with the use of patent blue dye and technetium radioisotope. In addition, there was a significant difference in rates of adjuvant therapy in those with detected SLN depending on SLN status. This data is supported by a meta-analysis of 26 studies including more than 1000 SLN procedures that demonstrated a pooled detection rate of 78% and sensitivity of 93%26.

Data support that sentinel lymph node detection rate and sensitivity in endometrial cancer approach those observed in breast cancer and melanoma, malignancies in which SLN mapping is the standard of care.

Several studies have now reported improved detection rates and negative predictive values with the following refinements to technique:

1. Use of indocyanine green (ICG) dye and near infra-red fluorescence (NIRF) imaging
2. Intracervical tracer injection
3. Application of a sentinel lymph node mapping algorithm to decrease false negative rate
4. Ultra-staging

ICG is a water soluble tricarbocyanine dye that emits a fluorescent signal in the near –infrared light range and is FDA approved for vascular and hepatobiliary imaging but is off-label when utilized for lymphatic mapping. Studies comparing various tracers and injection techniques have found that the use of ICG and NIRF imaging has superior detection rates compared to the use of blue dye alone (particularly in obese patients) and similar or higher detection rates compared to the combination of blue dye and radioisotope technetium (99mTc-SC). How et al. demonstrated that ICG had a significantly higher SLN detection rate when compared to blue dye in both overall (87% vs 71%, respectively; p = 0.005) and bilateral (65% vs 43%, respectively; p = 0.002) detection27. Buda et al found that bilateral optimal mapping rate for ICG was 85%, significantly higher than the 58 % obtained with 99mTc-SC with blue dye (p=0.003) and the 54 % for blue dye (p=0.001)28. Use of ICG mitigates the need for pre-operative injection of technetium, which can cause patient discomfort, as well as the utilization of nuclear medicine resources which may not be available at all facilities.

The FIRES trial was the first prospective cohort study to report on the use on ICG and near infrared imaging on the robotic platform. This study reported successful mapping using this technique in 86% of patients and bilateral mapping in 52% of cases. This study found the technique was 97% sensitive for detection of nodal metastatic disease, the false negative rate was 2.4% and negative predictive value 99%, consistent with data previously published6.

Despite the disparity in para-aortic sentinel lymph node detection rates compared to myometrial or endometrial injection, cervical injection of tracer has shown consistently high SLN detection rates including sentinel nodes identified in the para-aortic region in 5 to 15%5,6. Furthermore, the incidence of para-aortic metastasis in the absence of pelvic lymph node metastasis is exceedingly low (1-3%). Hysteroscopic endometrial injection or intra-operative myometrial injection of tracers and dye is a technically difficult skill to learn and perform. The meta-analysis by Kang et al in fact found that sub-serosal myometrial injection was associated with decreased sensitivity of SLN biopsy26.

By comparison cervical injection is safe and easy to perform with high detection rates (bilateral detection rate averaging 56% with intracervical tracer injection) and acceptable false negative rates5,6,7. Recent meta-analysis show that injection site is not associated with detection rate or the sensitivity of sentinel lymph nodes for detection of metastatic disease.

Studies have consistently shown the importance of an ultra-staging protocol for the pathological processing of sentinel nodes. 35% to 50% of sentinel nodes are found to be positive on immunohistochemistry (IHC) even when Haematoxylin and Eosin (H&E) sections are negative5,6,7,25,26. While the significance of low volume SLN metastasis is not fully understood, literature to date would indicated that micro metastasis in LN are a negative prognostic factor29 and an independent risk factor for recurrence30. SLN mapping with ultra-staging allows us to detect low volume lymph node disease in up to 5% of patients who would otherwise have lymph node involvement undetected. Treatment with adjuvant chemotherapy in those with low volume lymph node disease (micro-metastases or isolated tumour cells) results in recurrence free survival equivalent to that of patients who are node negative31.

Finally, incorporation of SLN mapping algorithm improved performance of mapping procedure and significantly reduced the false negative rate of SLN mapping from 15% to 2% in the Memorial Sloan Kettering (MSK) cohort32. Despite a shift from full lymphadenectomy to SLN mapping with application of the MSK algorithm the rates of detection of Stage 3C endometrial malignancy have remained stable33. Furthermore, a comparison of SLN mapping versus selective lymphadenectomy in patients with high risk uterine features, which compared cohorts from MSK and Mayo clinic, suggests that SLN mapping offers better detection of Stage 3C1 disease (pelvic lymph node involvement) and as a result more patients in the SLN cohort received adjuvant chemotherapy with no significant differences in 3-year OS or PFS between cohorts but statistically significant improvement in 3-year disease specific survival in the SLN cohort34.

## **AIM(S) OF STUDY**

### Primary Aim(s)

The aim of this study is to show that a sentinel lymph node (SLN) mapping and biopsy can be safely incorporated into our current staging and treatment protocol for women with apparent early stage endometrial cancer. With this study, we wish to collect data on how successfully we can detect sentinel lymph nodes with the indocyanine green (ICG) dye and near infra-red fluorescence (NIRF) imaging technique, the percentage of low and intermediate risk women (based on pre-operative and intra-operative assessment of uterine features) who are found to be lymph node positive (Stage 3) and how the results of SLN biopsy influences management decisions regarding adjuvant therapy. We also aim to document any complications associated with SLN mapping at our institution.

## **OBJECTIVE(S)**

### Primary Objective(s)

1. To compare our unilateral and bilateral detection rates (the proportion of eligible patients in whom sentinel lymph nodes are successfully identified at the time of the surgery using ICG and NIRF) and mapped locations for sentinel lymph nodes in women with apparent early stage endometrial cancer, with internationally published standards.
2. To assess the utility of sentinel lymph node results in informing management decisions regarding adjuvant therapy:
   1. Determine the percentage of low and intermediate risk women (based on pre-operative and intra-operative assessment of uterine features) who are found to be lymph node positive based on SLN biopsy
   2. Determine the proportion of women who have decisions regarding adjuvant therapy altered based on results of SLN biopsy (compared to adjuvant therapy prescribed based on uterine features alone)
   3. To compare rates of adjuvant pelvic radiation and chemotherapy delivered in our Stage 1 -2 endometrial cancer patients after incorporation of the SLN biopsy staging protocol compared to historical controls

### Secondary Objective(s)

1. To identify and document any adverse effects associated with ICG injection or the SLN mapping procedure
2. To identify the ideal timing of ICG dye injection prior to commencement of SLN mapping
3. To assess the percentage of positive sentinel lymph nodes identified on ultra-staging with immunohistochemistry (IHC) versus routine haematoxylin and eosin (H&E) stained sections.

## **HYPOTHESI(E)S**

### Primary Hypothesis

With strict implementation of our sentinel lymph node mapping protocol and algorithm we hypothesise that our centre can achieve detection rates for sentinel lymph nodes that are comparable to international standards with detection of at least one SLN in over 85% of patients and bilateral successful SLN mapping in 60% of cases. The location of identified SLNs will add to the growing international body of literature on the lymphatic drainage patters of uterine malignancy.

Knowledge of lymph node status based on SLN biopsy will allow for better tailoring of adjuvant therapies while avoiding the intra and post-operative complications associated with comprehensive lymphadenectomy. We believe that sentinel lymph node mapping will identify a cohort of otherwise low and intermediate risk women with occult Stage 3 disease who will benefit from adjuvant chemotherapy in addition to pelvic radiation, while avoiding the morbidity associated with a comprehensive pelvic lymph node dissection. We also anticipate that fewer women with low and intermediate risk uterine features will require adjuvant external beam pelvic radiation based on negative SLN biopsies, compared to historical controls who were managed according to a staging protocol utilizing intra-operative frozen section and hence susceptible to under-staging if the final histology upgraded or upstaged the tumour compared to frozen section results.

### Secondary Hypothesis

We believe that intracervical injection of ICG is safe with a very limited adverse effect profile. Furthermore, nodal dissection when limited to the selective removal of a sentinel lymph node will decrease rates of intra-operative complications related to full pelvic lymph node dissection.

By auditing our ICG injection time and commencement of SLN mapping dissection we hope to identify an optimal time window for SLN mapping to be conducted after ICG has been injected.

Consistent with existing literature, we believe that ultra-staging with immunohistochemistry (IHC) will be integral to identifying at least 35 to 50% of patients with positive sentinel lymph nodes that have low volume disease (isolated tumour cells and micro-metastasis) not able to be detected with routine haematoxylin and eosin (H&E) staining.

## **STUDY DESIGN**

This is a prospective, open label, single arm trial of sentinel lymph node mapping in early stage endometrial cancer using indo-cyanine green and near infra-red fluorescence imaging during minimally invasive surgery. This study will collect patient information and data on the systematic implementation of a new evidence based surgical staging protocol for patients with endometrial cancer.

To assess whether sentinel lymph node status helps inform management decisions regarding adjuvant therapy, we will utilize comparative data from historical controls (treated according to staging protocol utilizing intra-operative frozen section), recorded on the Queensland Centre for Gynaecological Cancer (QCGC) database. All patients are consented for data collection on the QCGC database when treated at a gynaecological oncology unit in Queensland.

## **STUDY SETTING/LOCATION(S)**

This study is to be conducted at Mater Hospital under the Gynaecological Oncology service (public and private).

Locations:

* Mater Hospital Department of Gynaecological Oncology (public)
* Private Practices of Assoc. Prof Lewis Perrin, Dr Naven Chetty and Dr Nisha Jagasia (operating through Mater Private Hospital)

With ethics approval and the successful implementation of the SLN mapping protocol at Mater Hospital we then intend to seek ethics approval from other gynaecological oncology services in Queensland that offer sentinel lymph node mapping for endometrial cancer staging (i.e. Royal Brisbane and Women’s Hospital, Greenslopes Private Hospital, Wesley Hospital and St Andrew’s War Memorial Hospital) in order to collect state-wide data on the detection rate, mapped location of sentinel nodes, prescription of adjuvant therapies based on sentinel lymph node mapping as well as the safety of the technique. This would allow us to compare our data with other centres that offer SLN mapping for endometrial cancer, in Australia and internationally.

## **STUDY DURATION**

Accrual period November 2017 to December 2019 to recruit 350 patients with apparent early stage endometrial cancer. Follow-up will be completed December 2022, allowing at least 36-month follow-up of all recruited patients.

## **STUDY POPULATION**

### Recruitment Process

All patients diagnosed with histologically confirmed endometrial malignancy presenting to Mater Hospital Department of Gynaecological Oncology or the private practices of Dr LP, Dr NC and Dr NJ will be screened for inclusion. Patients that satisfy the inclusion criteria will be approached for participation and will have a verbal explanation of the SLN mapping technique and study protocol delivered by the attending Gynaecological Oncologist (investigator) and be provided with a copy to the patient information and consent form (PICF) to consider. They may wish to discuss this with another health professional and family. Those women who agree to participate after reading the PICF will be asked to sign a written informed consent either at the pre-operative consultation or on the day of surgery. The patient will then be allocated a study number and a Registration Form (Form A) will be completed.

### Inclusion criteria

All patients with newly diagnosed, histologically confirmed, endometrial malignancy deemed to be suitable for treatment with laparoscopic or robotic hysterectomy, bilateral salpingo-oophorectomy and staging procedure.

* Clinically FIGO Stage 1 or Stage 2 disease (disease that is clinically and radiologically confined to the uterus with no evidence of nodal or distant metastatic disease).
* Patient over 18 years and able to give informed consent.

### Exclusion criteria

* .
* Current pregnancy or desire to retain fertility.
* Clinical or radiological evidence of extra-uterine disease including lymphadenopathy.
* Previous hysterectomy or treatment of endometrial cancer by radiotherapy, chemotherapy or hormonal therapy.
* Any contra-indication to comprehensive lymph-node staging.
* Patient declining any form of lymph node assessment or staging.
* Contra-indication to receiving indocyanine green dye including history of hepatic impairment or an iodine allergy.
* Inaccessible to follow-up.

If a patient who has been recruited to the study is found to have gross extra-uterine disease at the time of surgery (Stage 3 or Stage 4 disease), they will be withdrawn from the study as it is no longer appropriate for these patients to have sentinel lymph node mapping as part of their surgical staging. These patients will be identified as enrolled on the study but subsequently withdrawn due to disease status, for the purposes of the CONSORT flow diagram.

### Potential for Risk, burdens and benefits

This is a prospective study collecting patient information and data on the systematic implementation of a new evidence based surgical staging protocol for patients with endometrial malignancy. As such is represents a change from the current staging protocol, detailed in Appendix A. We wish to offer all women with clinically and radiologically uterus confined endometrial malignancy, lymph node staging as per the sentinel lymph node mapping protocol outlined in this study.

Current practise dictates that those women with low risk uterine features based on intra-operative frozen section of hysterectomy specimen and no evidence of macroscopic lymph node involvement, do not require a staging pelvic lymphadenectomy. In this cohort, participation in the study would entail the additional injection of ICG dye and exploration of retroperitoneal spaces to detect the sentinel lymph node. The benefits include identification of a group of women with low risk uterine features who harbour occult lymph node metastasis, which would otherwise have gone undetected as they would not have undergone lymphadenectomy. These women have proven survival benefit if chemotherapy is prescribed as part of their adjuvant therapy. Furthermore, those women who are upgraded or upstaged on final histology (occurring in up to 15% of cases) will also benefit from information on their lymph node status obtained on SLN biopsy, as they too would have otherwise gone un-staged based on intra-operative assessments.

Conversely, in women with intermediate and high risk uterine features, successful sentinel lymph node mapping mitigates the need for comprehensive lymphadenectomy. These patients can proceed with adjuvant therapy if required whilst having avoided the combined morbidity associated with a full pelvic lymph node dissection and pelvic radiation.

The risk of adverse events is extremely low for ICG (1/42,000 anaphylaxis); however, it should be avoided in patients with severe iodine allergy or hepatic impairment. Risks of the SLN mapping include bleeding and damage to pelvic side wall nerves (genitofemoral and obturator nerves) or the ureter from retroperitoneal dissection. These risks are comparatively less than those of comprehensive lymphadenectomy as minimal retroperitoneal dissection is conducted during SLN mapping.

1. STUDY OUTCOMES

### Primary Outcome

Descriptive data will be collected on the following outcome measures:

* Rates of SLN identification – per patient and per hemi-pelvis.
* Sites and laterality of detected SLNs.
* Rate of successful removal of SLN – determined by the presence of nodal tissue on histopathological assessment of submitted specimen.
* Percentage of low and intermediate risk patients (based on assessment of uterine features) who are found to have lymph node positive disease based on SLN biopsy.
* Proportion of patients that have recommendation for adjuvant therapy altered based on knowledge of SLN biopsy (detailed according to MDT discussion once final histopathology is available).
* Percentage of low and intermediate risk patients who have undergone successful SLN mapping prescribed adjuvant pelvic radiation and chemotherapy compared with matched historical low and intermediate risk controls (extracted from QCGC database).

### Secondary Outcome(s)

Descriptive data will be collected on the following outcome measures:

* Intra-operative or post-operative complications related to SLN mapping / biopsy procedure.
* Time interval between injection if ICG and commencement of pelvic side wall dissection and detection of SLN (recorded for each side of pelvis) to determine if there is an optimal time interval between injection and dissection which optimises detection rate.
* Rate of patients deemed to have positive SLN based on routine histology (H&E staining) versus ultra-staging (IHC).

## **STUDY PROCEDURES**

Ethics approval will be sought from the institutional review committee (Mater Human Ethics Research Committee.

Informed consent will be sought from women with apparent early stage endometrial cancer undergoing surgical management.

### Recruitment and consent of participants

Patients will be recruited from the Gynaecological Oncology outpatient clinic at Mater Hospital or the private practices of Drs LP, NC and NJ. Patients with a confirmed diagnosis of endometrial malignancy that is believed to be confined to the uterus and who are suitable for surgical management (laparoscopic or robotic) will be approached.

Patients that satisfy the inclusion criteria will be approached for participation and will have a verbal explanation of the SLN mapping technique and study protocol delivered by the attending Gynaecological Oncologist (investigator) and be provided with a copy to the patient information and consent form (PICF) to consider. They may wish to discuss this with another health professional and family. Those women who agree to participate after reading the PICF will be asked to sign a written informed consent. The patient will then be allocated a study number and a Registration Form (Form A) will be completed on the day of the pre-operative consultation with the surgeon.

The participant consent process will include the following information:

* This is a new technique that has been incorporated into the standard practise for staging women with endometrial cancer believed to be confined to the uterus
* This study allows us to collect demographic, clinical, surgical and pathology information about the patient and their cancer
* Explanation of the SLN mapping procedure and risks associated with the SLN mapping procedure
* The procedure for staging in those who fail to map a SLN i.e. SLN not detected on one or both sides of pelvis
* Information to be collected at enrolment, during surgery and at each follow-up visit
* Participation being optional and voluntary with participants able to withdraw from the study at any time
* Management in those who do not participate in the study will include SLN mapping unless the patient specifically indicates that they do not consent to any form of lymph node staging (after discussion with their treating surgeon).
* Patients not consenting to participation will not have their information collected as part of the study even if they undergo SLN biopsy as part of their treatment.

### Withdrawal of participants from a study

#### 11.2.1 Participant withdrawal from study procedures

Patients who withdraw or are withdrawn due to disease status, from the study procedure (SLN mapping or any follow-up procedures) will revert to standard management and follow-up as per institutional protocols. The data collected up to that point will be used. If possible data will continue to be collected from patient records and departmental databases, unless the patient has indicated that they wish to withdraw from study participation.

#### 11.2.2 Participant withdrawal from a study

As described on the patient consent and withdrawal form, withdrawal from the study can be at any time, at no penalty, or change in follow up. The data collected up to that point will be used to uphold scientific integrity of the study. This information will be specified in the PICF.

### Randomisation

There will be no randomization of patients. All eligible patients (as per inclusion criteria) undergoing the SLN mapping procedure as part of the surgical staging of their endometrial malignancy will be offered enrolment on the study.

### Measurement tools used

Following informed consent and the allocation of a study number, a registration **Form A** describing patient demographics and characteristics, tumour histology and radiological findings will be completed by the attending Gynaecological Oncologist.

On the day of surgery, the attending Gynaecological Oncologist will be required to complete **Form B** outlining the mode of SLN mapping (laparoscopic or robotic), number and anatomical location of sentinel lymph nodes mapped in each hemi-pelvis as well as timing of ICG injection, commencement of dissection and detection of sentinel lymph nodes per hemi-pelvis. In cases where a unilateral or bilateral comprehensive pelvic LN dissection is performed this will be documented. Operative time, estimated blood loss and any intra-operative complications will be detailed on this form. All data will be recorded in pre-set fields or on the pre-formatted diagram. Categorical data fields will list options to circle and continuous data will have prescribed units of measurement.

One week after the patient’s surgery the principle investigator will review histopathology results and complete **Form C** which details the final staging and grading of the tumour as well as the status of sentinel lymph nodes based on the ultra-staging synoptic report. The status of any non–sentinel lymph nodes removed will also be documented. Recommendations made at the Mater Gynaecological Oncology Tumour Board MDT for the prescription of adjuvant therapies will be recorded on this form along with any relevant changes made to recommended adjuvant therapy based on knowledge of SLN status.

**Form D** will be completed by the attending Gynaecological Oncologist at the 3-month post-operative visit and details length of post-operative hospitalization, immediate or delayed post-operative complications, post-surgical therapies (radiotherapy / chemotherapy) and the presence or absence of any recurrent disease. If the patient is unable to attend the 3 month – post-operative visit the clinician will go through the form with the patient over the phone.

Subsequent section**s of Form D** will be completed at the 6, 12, 24 and 36 month post-operative visits and again details any delayed complications, development of recurrent disease since last follow-up and treatment thereof.

### Study involvement by participants

Once patients have signed the written consent form they will be required to:

* Have intra-cervical injection of ICG dye on the day of surgery and have NIRF imaging directed sentinel lymph node mapping and excision of these lymph nodes during their surgery (as per *Appendix C: SLN mapping protocol*)
* Allow comprehensive lymph node dissection on the side of the pelvis where there is failure to map a SLN, if they meet criteria for lymph node staging as per current staging protocol (*Appendix A*)
* Allow documentation of their clinical details, surgical and pathology findings as well as follow-up details on the study forms
* Attend prescribed study follow-up visits at 3, 6, 12 24 and 36 months
* Subsequent visits will be as per institutional protocol for the follow-up of endometrial cancer patients (3 monthly visits for first 2 years, 6 monthly visits till 5 years, then yearly visits till 10 years)

There will be no compensation to participants for the time, transport or other expenses as the study related visits will all coincide with standard follow-up protocol for endometrial cancer patients.

### Data management

On all study forms, any clinical details and patient data will be identified by Study Number only. Only the principal investigator and associate investigators will have access to the list of patients registered and their allocated study numbers. All forms will be completed by the Gynaecological Oncologist attending to the patient at their enrolment visit, during surgery and at each subsequent follow-up visit. All study forms will be collected by the principal investigator (NJ) and stored in locked cabinets within the Department of Gynaecological Oncology. Any electronic data files will be stored on a secure password protected computer in the Gynaecological Oncology office. All electronically stored data will have patients de-identified. Any publication or presentation of results will be such that all patient data is de-identified. Data will be stored for 15 years (as per protocol for clinical trials) and disposed of at the end of the study in a legal manner abiding by Mater Health information Services policy.

### Safety considerations/Patient safety

The protocol (*Appendix C*) describes a specified technique and algorithm for SLN mapping which will be adhered to during surgical staging to ensure consistency among surgeons regarding detection of sentinel lymph nodes. All surgeons in the unit are experienced in comprehensive pelvic lymph node dissection if this is required. A pre-specified protocol for ultra-staging of all removed sentinel lymph nodes is described in the protocol and has been agreed upon by the Mater Department of Anatomical Pathology to ensure consistent processing and reporting of SLN biopsy results.

Any adverse events (AE) or serious adverse events (SAE) related to participation in this study will be reported to the Mater Human Research Ethics Committee (HREC).

All public patients will have direct access to their surgical team at Department of Gynaecological Oncology (including the unit fellow and staff specialist) to discuss any issues relevant to participation in the study and to address and manage any complications. Private patients will be attended to by their attending Gynaecological Oncologist (Drs LP, NC or NJ).

The Mater Hospital also has a pathway for patients to discuss issues or lodge complaints related to their care with the Mater Patient Representative, independent of the study investigators.

### Data monitoring

This will be administered by the Manager, Mater Research Office, and Mater Ethics Committee. While there are no pre-specified stopping or early discontinuation rules, any adverse events related to patients participating in the study will be immediately reported to the Mater HREC.

Form B will be audited by the principal investigator on a quarterly basis to ensure compliance with the ICG injection and mapping protocol. Any issues identified in adhering with the study protocol will be addressed during quarterly investigator meetings.

Pathology reports will be audited on a quarterly basis by the principal investigator to ensure compliance with processing and reporting of SLN biopsy results according to protocol specifications.

If our detection rates are comparable to published series we will endeavour to recruit patients from other centres and clinicians in Queensland that offer this procedure to develop a state-wide database on the utilization and efficacy of sentinel lymph node mapping in endometrial cancer. Site specific approvals will be sought from additional centres and for any amendments to current study protocol, including if information collected as part of the study is retained for longer than the prescribed 15 years.

1. SAMPLE SIZE AND DATA ANALYSIS

### Sample size and statistical power

Clinico-pathological characteristics will be evaluated using basic descriptive statistics. We anticipate a learning curve of 30 cases per surgeon to acquire adequate expertise in the technique of SLN mapping. This is based on the work of Abu-Rustum et al. who showed an increase in SLN detection from 77% to 94% after 30-case experience35. Using an anticipated overall SLN detection rate of 80% and node positivity rate of 15% (across all grades of histology), we need to accrue approximately 250 patients to calculate an accurate lymph node positive detection rate (with 95% confidence level) for SLN mapping at our institution. Hence total sample size required is estimated at 340 patients (250 + 90 {30 cases per surgeon to gain expertise x3 surgeons}).

### Data analysis plan

The false positive rate is defined as zero. Overall and bilateral detection rates as well as sensitivity and false negative rate will be calculated using Fischer’s exact test. The overall detection rate will be derived by dividing the number of procedures where at least one SLN is identified by the total number of procedures performed. The bilateral detection rate will be calculated by dividing the number of procedures where at least one SLN was identified on each side of the pelvis by the total number of procedures performed.

We will not be performing side specific lymphadenectomy in all patients, but rather restrict side specific lymphadenectomy to those patients that fail mapping and meet our current criteria for lymph node staging (*Appendix A*). The false negative rate of SLN mapping will be calculated only or patients that undergo full pelvic lymphadenectomy after SLN mapping. False negative SLN mapping is defined as bilateral negative SLN or failed mapping bilaterally in combination with metastatic non-sentinel lymph nodes as determined by full pelvic lymph node dissection.

Rates of Stage 3 disease and prescription of adjuvant therapies between the SLN mapping cohort and historical controls will be calculated using a two sample t-test.

## **ETHICAL CONSIDERATIONS**

The project will be registered with the Mater Health Services Human Research Ethics Committee.

Agreement from the Mater HREC supervision will be sought for all their stated supervisory responsibilities regarding researchers:

(a) Conducts the trial in compliance with the approved protocol;

b) Provides reports of the progress of the trial to the HREC, at a frequency directed by the HREC (but at least annually), related to the degree of risk to participants;

(c) Informs the HREC, and seeks its approval, of amendments to the protocol including amendments that:

(i) Are proposed or undertaken to eliminate immediate risks to participants;

(ii) May increase the risks to participants; or

(iii) Significantly affect the conduct of the trial;

(d) Notifies, in the manner and form specified by the HREC, any serious adverse events at any of those trial sites;

(e) Informs the HREC as soon as possible of any new safety information from other published or unpublished studies that may have an impact on the continued ethical acceptability of the trial or may indicate the need for amendments to the trial protocol;

(f) Informs the HREC, giving reasons, if the trial is discontinued before the expected date of completion.

## **DISSEMINATION OF RESULTS AND PUBLICATIONS**

Proposed presentation to staff at Mater Gynaecological Oncology multi-disciplinary team meeting and Queensland Centre for Gynaecological Cancer Quality Assurance Committee meetings.

Poster and Scientific Meeting presentations

Publication of data in a peer-reviewed scientific journal

Individual patient feedback at follow-up visits

## **OUTCOMES AND SIGNIFICANCE**

The significance of this study will be to verify that the detection rate of sentinel lymph node mapping for endometrial cancer patients in our centre, using ICG intra-cervical injection and NIRF imaging, is equivocal to internationally published standards with an acceptably low complication rate. Presuming successful incorporation of the technique into our treatment pathway for endometrial malignancy, women will be spared the morbidity associated with comprehensive lymphadenectomy while obtaining valuable information on lymph node status that can be used to more precisely tailor the prescription of adjuvant therapies.  Furthermore, the reduced requirement for intra-operative frozen sections will decrease the cost incurred and burden on the pathology department staff and resources.

1. BUDGET

Stationary including photocopying / printing - $500

Data storage – Mater Hospital Department of Gynaecological Oncology secure password protected computer

## **GLOSSARY OF ABBREVIATIONS**

SLN – Sentinel Lymph Node

ICG – Indocyanine green

NIRF – Near infra-red fluorescence

NCNN – National Comprehensive Cancer Network

MDT – Multidisciplinary team

PFS – progression free survival

OS – overall survival

NPV – negative predictive value

FNR – false negative rate

LP – Lew Perrin

NC – Naven Chetty

NJ – Nisha Jagasia

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