Title page

Sentinel node detection in cervical cancer.

Date 180914

Table 1. Participating Sites and Site-Principal Investigators

Site	Site-PI	# patients enrolled
1.Department of Obstetrics and Gynecology, Skåne University Hospital and Lund University, Lund, Sweden	Jan Persson MD, PhD	XX
2. Department of Obstetrics and Gynecology, Karolinska University Hospital and Karolinska Institute Stockholm Sweden	Henrik Falconer MD, PhD	YY

Coordinating Center: Department of Obstetrics and Gynecology, Skåne, Lund University Hospital and Lund University, Lund Sweden.

Lead Principal Investigator: Jan Persson MD PhD Associate Professor
Department of obstetrics and Gynecology, Division of Gynecologic Oncology
Skåne University Hospital Lund
SE-22185 Lund, Sweden
(ph) +46733522080
(fax) +4646156868
Email: jan.persson@med.lu.se

Local Principal Investigator: Henrik Falconer MD
Department of Obstetrics and Gynecology Karolinska University Hospital
Division of Gynecologic Oncology
Adress Karolinska University Hospital
Solna, 171 76 Stockholm, Sweden
(ph) +468-517 700 00
Email: henrik.falconer@karolinska.se

Study Coordinator: Linnea Ekdahl MD
Department of obstetrics and Gynecology, Division of Gynecologic Oncology
Skåne University Hospital Lund
SE-22185 Lund, Sweden
(ph)+4646172093
(fax) +4646156868
Email: Linnea.ekdahl@med.lu.se

Study Statistician:
Filip Mörk. Faculty of Engineering, Lund University.

Overview study plan.

Consecutive cervical cancer patients planned for robotic surgery, clinically stage 1A2 to 2A1.

Cervical injection of ICG and a total of 80 MBq Tc99 nanocolloid (the first 75 patients) and if the combination of nanocolloid and ICG after analysisis better than ICG alone a continued combined use. If ICG or radiocolloid alone is equal or superior to the combined use in terms of technical success rates, the study will continue with the use of ICG or radiocolloid alone as tracer according to sample size analysis.

Registration of adverse events during insufflation and placement of port including necessary adhesiolysis.

Pelvic SLN identification with near infrared technique (and gamma probe first 75 patients) for identification of Indocyanine green (ICG) in lymphatics and lymph nodes. Ipsilateral cervical reinjection of ICG in case of non-display of any lymphatic pathway. Separate removal of the upper lymphovascular parametria. Registration of SLN- associated intraoperative adverse events.

Frozen section of SLN and upper lymphovascular parametria.

A full pelvic lymphadenectomy after removal of SLN according to the Swedish National treatment protocol extended to the level of the inferior mesenteric artery in case of metastatic SLN's. Robotic Surgery with a Querleu Morrow B2 to C2 radical hysterectomy as appropriate or an aborted hysterectomy in case of metastatic SLN. Adnexal surgery as appropriate. Registration of associated intraoperative adverse events.

SLN's for ultrasectioning and immunohistochemistry. The remaining nodes for standard pathological bisectioning and staining with hematoxylin/eosine.

Registration of postoperative adverse events until 30 days after surgery using the Clavien Dindo classification.

Continuous data management. Analysis of technical success rates, sensitivity and false negative rates for SLN's comparing ICG with Radiocolloid after first 75 patients. In case of ICG + radiocolloid superior to ICG alone continued enrollment of patients using both tracers. In case of any of ICG or radiocolloid alone equal or superior to the combination continuation of the study for an interim analysis after 36 node positive patients (see section 11).

Table of contents

1. OBJECTIVES

To estimate ICG and Radiocolloid alone and in combination to estimate any advantage of the combination of both tracers in terms of technical success rates/ bilateral mapping, sensitivity and false negative rates for the detection of pelvic SLN (first 75 patients).

To estimate the sensitivity and negative predictive value of the pelvic sentinel lymph node (SLN) concept for determination of lymph node metastases in patients with early stage cervical cancer planned for robotic surgery using either a combination of ICG and Radiocolloid or in case ICG alone is equal to or superior (in terms of technical success rate, bilateral mapping rates) to the combination the use of ICG as tracer for cervical injection using an anatomically based surgical algorithm and definition of sentinel lymph nodes.

To estimate if reinjection of tracer (ICG) will enhance the technical success / bilateral mapping rates.

To estimate the proportion of cervical cancer patients suitable for the SLN concept in conjunction with robotic surgery

To estimate complications associated with the detection and removal of SLN as such.

To estimate complications associated with ICG as tracer.

To estimate the incidence of lymphatic complications by the use of a validated questionnaire

2. BACKGROUND

The surgical approach for cervical cancer staging has changed from conventional laparotomy to a more minimally invasive technique with proven advantages in terms of less perioperative morbidity. Robotic assisted laparoscopic radical hysterectomy with pelvic lymph node dissection has emerged as the approach of choice for an increasing number gyne-oncologists worldwide. Previous data from the department of Obstetrics and Gynecology in Lund, Sweden, shows that robot assisted surgery feasible for 95% of unselected cervical cancer patients selected for primary surgery.

In cervical cancer, nodal involvement is a strong prognostic factor and also determines adjuvant treatment and a pelvic lymphadenecomy is recommended for cervical cancer stages 1a2 and higher stages where primary surgery is recommended. A pelvic lymphadenectomy is associated with an increased risk for lymphatic complications such as lymphedema, lymphocysts and in rare cases lymphatic ascites.

The concept of identifying nodal metastases by detection of sentinel lymph nodes as a marker of nodal metastatic disease or not therefore is appealing. However, during later years many studies have been published, most of the retrospective, using a variety of tracers and with little or no information on used surgical algorithm, used definition of SLN and without reference to lymphatic anatomy. Moreover, technical success rate varies. A high technical success rate is cruzial to avoid the need for a full hemipelvic or pathwaywize LND in case of failure. In pilot studies from Lund University we have shown that reinjection of tracer (ICG) increases the technical success rates both defined per hemipelvis and pathwaywize, defined by detection of at least one SLN per hemipelvis to around 95%. By the use of Indocyanine green, a fluorescent tracer, we have also previously demonstrated two bilateral separate pelvic pathways, the upper paracervical pathway (UPP) running along the upper lymphovascular parametrium usually to external iliac and/ or obturator nodes and the further lateral to the common iliac artery to paraaortic nodes and the lower paracervical pathway (LPP) running via the sacrouterine ligament to nodes medial of the internal iliac artery and/ or presacral nodes then further medial to the common iliac artery to paraaortic nodes. Hence there are two bilateral pelvic pathways draining further to the paraaortic region below as well as above the inferior mesenteric artery.

Surgical competence and experience is necessary to achieve a high technical success rate and a low false negative rate for SLN.
This study aims to evaluate the pelvic sentinel node concept based on a defined surgical algorithm and with a clear definition of SLN based on described uterine lymphatic anatomy. The study setting including only high volume surgeons at two of more tertiary high volume centers enables an evaluation of the true potential of the pelvic SLN concept in cervical cancer.

This study aims to include consecutive early stage (Stage 1a2 to 2a1) cancer patients fulfilling criteria to ensure the results is representative for the cervical cancer population. Planned surgery may be a radical hysterectomy or a radical trachelectomy including a full LND after removal of identified SLN's or an aborted procedure in favour of radiochemotherapy in case of metastatic SLN's.

3. PATIENT ELIGIBILITY

Inclusion Criteria

-Women of age 18 years and older at the time of informed consent.

-Women with a pathologically proven cervical carcinoma of any histologic subtype, clinically stage 1a2- 2a1 planned for primary surgery

-Absence of any exclusion criteria

Exclusion Criteria

-Non consenting patients

-Ongoing pregnancy

-Inability to understand written and/or oral study information

-Who performance status III or more

-Previous lower limb lymphedema

-Surgical contraindication to a laparoscopic approach or lymphadenectomy at surgeons discretion.

-Anesthesiologic contraindication to a laparoscopic approach at the anesthetist's discretion

- Locally advanced disease or intraabdominal/distant metastases at preoperative CT, MRI or ultrasonography

- Radiologically suspect pelvic nodal metastatic disease according to the RECIST criteria (>= 1 node with >=16 mm short axis diameter)

-Allergy to Iodine

-Patients with a known liver disease

-Patients with a significant bleeding disorder or mandatory antithrombotic treatment.

Before surgery patients will be allocated to:

Primarily: Removal of pelvic SLN's followed by a complete pelvic lympadenectomy with boundaries as described below. SLN and the separately removed upper paracervical tissue will be sent for frozen section and later for ultrasectioning and immunohistochemistry.
A Querleu Morrow B2 to C2 radical hysterectomy or a robotic abdominal radical trachelectomy will be performed and at surgeons' discretion an abortion of those procedures in case of metastatic SLN's. Adnexal surgery / transpositioning of the adnexae will be performed at surgeons' discretion and patients wish.

Surgeon eligibility

All included surgeons outside the primary investigating center must have had a case observation at the primary investigating center followed by a an approved site visit by the principal investigating surgeon at their home center ensuring adherence to protocol. All included surgeons at the primary investigating center were approved by the principal investigating surgeon.
All included surgeons must have a previous experience of at least 100 robot-assisted procedures.

The departments of pathology were coordinated in terms of principles for ultrasectioning and immunohistochemistry of SLN and management on non-sentinel lymph nodes.

4.0 STUDY MODALITIES

Surgical Procedures

- Patients with stage 1a2- 2a1 cervical cancer planned for primary surgery with either a Querleu-Morrow B2- C2 radical hysterectomy or a robotic radical trachelectomy.
- Injection of tracer (a total of 80 MBq Technetium nanocolloid and 2,5mg ICG solution / 2,5 mg/mL) will be injected submucosally into the cervix ad 2-4-8-and 10 O'clock respectively at onset of surgery (first 75 patients).
- Scanning of pelvic side walls after a minimum of 20 minutes after injection and a transperitoneal or retroperitoneal evaluation of ICG uptake per pathway (UPP and LPP bilaterally)
- Detection and removal of SLN will follow a strict protocol with a proximal- presacral dissection before the distal external iliac-obturator nodal compartment dissection and with careful opening of the presacral, paravesical and pararectal planes to avoid leaking of tracer. SLN will be defined as describe below.
- Identified SLN's and the upper paracervical tissue will be sent for frozen section and further for final ultrasectioning and immunohistochemistry as described below.
- A full pelvic nodal dissection will be performed after the removal of SLN's.
- The described procedures in this protocol will be performed with robot-assisted laparoscopy and with removal of SLN and non-SLN's before the radical hysterectomy/ trachelectomy.

Drug Information:

Indocyanine Green solution (ICG) 2.5mg/mL.

Description: ICG (Pulsion medical system, PICG0025SE, Feldkirchen Germany) is a sterile, lyophilized green powder containing 25 mg of Indocyanine green with no more than 5% sodium iodide.

The ICG solution is prepared immediately before surgery and intended for single patient use. For preparation, 10mL of sterile water is injected directly into the lyophilized ICG in its glass vial. Invert the vial multiple times to ensure thorough mixing. Draw up 0,25 mL in six 1 mL syringes from the vial with ICG solution (2,5mg/mL) for the cervical injection. A 0.6x38mm 23Gx1 ½ needle. The content of four of the syringes are used for the initial

injection and in case of non-display of any pathway one or two of the other are used for an ipsilateral re-injection.
The ICG solution is stored at room temperature. The solution is active for 6 hours, and should be discarded after that period of time.
Manufacturer: Pulsion medical system, Feldkirchen Germany
Availability: ICG will be provided by the manufacturer to each site.

Adverse Effects if ICG: All adverse effects are allergic in nature and occur in <1% of patients. Anaphylactic or urticarial reactions have been reported in patients with or without a history of allergy to iodides. If such reactions occur, treatment with the appropriate agents (e.g. adrenalin, antihistamines, corticosteroids) should be initiated.
Contraindications: Known hypersensitivity to iodine containing compounds. Known liver failure. Radioactive iodine uptake studies should not be performed for at least 1 week following the use of ICG.

Please refer to the current ICG package insert for complete prescribing information

Injection of Indocyanine Green (ICG)
- The ICG is prepared by thorough mixing of 10mL of sterile water with the lyophilized ICG in its vial creating a 2.5mg/mL concentration. The lot number, expiration date and dose injected (mg) will be recorded.
- Six separate sterile 1mL syringes is prepared with 0.25 mL ICG solution (0.625 mg ICG)in each syringe is prepared.
- A 0.6x38 mm 23G needle is attached to each syringe for the injection. A separate back table is used for the syringes.
- The ICG injection will be performed immediately before placement of surgical port and docking the robot.
- Half the ICG volume in each syringe is injected submucosally and half the volume 3 cm into the cervical stroma at 2-4-8-and 10 O'clock respectively to a total dose of 2.5mg ICG and a total volume of 1 mL. Time for injection is recorded.
- After injection of dye, a fornix presenter without an intracervical device is placed
- A second ipsilateral injection of 0,25mL ICG is performed in case of non-display of

either of the upper (UPP) or lower (LPP) paracervical pathways after a minimum of 10 minutes observation time after ICG injection. The injection is done at 3 and 9 O'clock respectively, half the volume submucosally and half the volume 3 cm into the cervix.
- Display of the separate lymphatic pathways (UPP, LPP and IPP will be recorded after the first and if performed after the second injection).

Technetium ^{99} Nanocolloid

The nanocolloid (GitPharma S.R.L. Saluggia Italy) will be prepared at the radionuclide unit at Skåne University Hospital, Lund, 1-2 hours before injection in four separate syringes each contacting 0,5mL and 20 MBq nanocolloid. The injection was performed the same was as described above and performed before the injection of ICG. A laparoscopic gamma-probe (Neo2000© laparoscopic probe, Neoprobe Corporation Dublin OHIO) will be used for detection of the SLN's defined by radiotracer.

Sentinel Node Identification

The bilateral technical success rate of SLN identification is important to determine if this technique can be transferred to clinical practice. Previous studies at our institution using Technetium ^{99} Nanocolloid as single tracer have shown a bilateral mapping rate of 65% in tumors smaller than 20 mm and in all size cervical cancer of 59%. Based on pilot studies at our institution on cervical and endometrial cancer with the use of ICG we expect that the bilateral pelvic SLN identification (defined as at least one SLN per hemipelvis) will be 90% or more: We intend to separately study if reinjection of ICG will enhance the bilateral detection rate. A lower bilateral detection rate will lead to the need of full lymphadenectomy in a higher proportion of patients.
A clear anatomically definition of what is a SLN and a strict surgical algorithm as described in this study is important.
Importantly, accreditation of surgeons will be performed as described and is likely important to achieve a similar success rate as in pilot studies.

The sentinel nodes are defined as the juxtauterine ICG positive / radiopositive node with an afferent ICG positive lymphatics in each of the UPP and LPP respectively on each pelvic side with the potential of parallel lymphatics in the UPP to the external and obturator areas . These SLN are defined as **SLN type 1.**

In case of a ICG positive lymph vessel where no nodes are ICG positive in that pathway, the node where the ICG positive lymphatic channel ends is defined as **SLN ICG type 2**.

The definitions based on ICG positive lymphatic channels were only attributed for nodes defined by ICG but does not exclude that nodes were also defined by radiopositivity.

Nodes macroscopically suspect of metastatic disease will be defined as **SLN Macro** regardless of ICG uptake or radiopositivity but with information on ICG and radiopositivity.

Importantly, to secure accuracy, the positions and types of SLN will be marked on an anatomical chart, recorded on a list with anatomical locations and placed in pre-labeled jars with corresponding names and numbers. The SLN will be defined as **SLNICG, SLNICG+Radio,** and **SLNRadio** and for SLN defined by ICG also as type 1 and 2. This list is used by the department of pathology for reporting the results to minimize the risk of errors in location of nodes and which nodes are SLN's, the types of SLN as of above and non-SLN's. A copy of the list is kept in the patients study file. Nodes defined as SLN will have red labels on the jars, other nodes will have black labels.

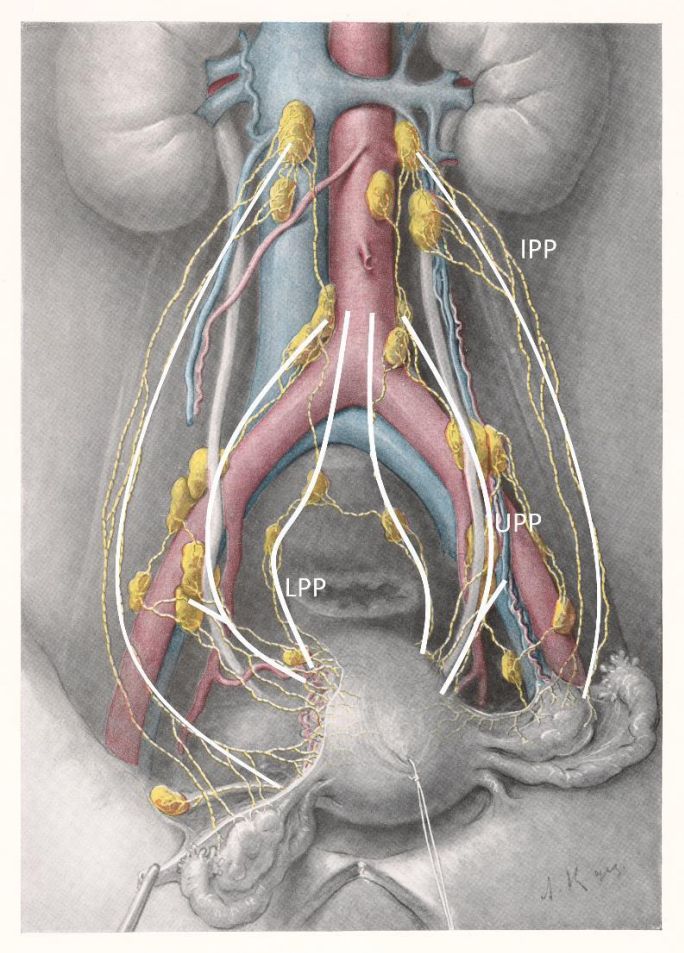
SLN's will be sent for frozen section and final histological evaluation including ultrasectioning and immunohistochemistry as described. SLN's will be sent for frozen section during surgery for intraoperative decisions on the extent of lymphadenectomy (paraaortic or not), transposition of ovaries and abortion of the hysterectomy in favour of radiochemotherapy..

Before final removal of SLN, the pelvic side walls will be scanned with the gamma-probe to get an overview of radiopositivity per hemipelvis including the presacral area. After opening of the avascular planes as of below a renewed scanning with the probe will be performed. Finally, in case no SLN radio have been identified per pathway, a final scan with the probe will be performed after removal of SLN's. The display of ICG in the respective pathways will be evaluated a minimum 10 minutes after the injection of ICG, first transperitoneally, and if not seen, after opening of the retroperitoneal avascular planes

starting with the presacral plane, then the paravesical and pararectal planes leaving the lymphatics intact with the aid of switching between white light and the ICG mode (Firefly mode). In case of a non-display of a pathway an ipsilateral injection of ICG as described will be performed. (the comparison of bilateral mapping rates between ICG and radiotracer will be performed before any second injection of ICG).Another 10 minutes will be alloved for awaiting distribution of ICG in the respective pathways. To minimize disturbance by leaking ICG or radiotracer identification and removal of SLN's starts cranially, presacrally (along the LPP) and continues at the pelvic side walls along the UPP. After removal of SLN's the upper lymphovascular parametrial tissue is removed separately (defined as the tissue along the uterine artery, medial to the obliterated umbilical artery and caudal of the supravesical artery) as it may contain lymph nodes that may not be separated from the green lymphovascular tissue and hence be the juxtauterine lymph node.
After removal of sentinel nodes a complete compartmentwize pelvic lymphadenectomy is performed followed by the hysterectomy as appropriate.

Anatomical boundaries of lymph node compartments in the female pelvis				
Lymph node compartment	Proximal limit	Lateral limit	Distal limit	Medial limit
External iliac area	Bifurcation of external and internal iliac artery	Genitofemoral nerve	Cloquets lymph node	External iliac vein
Obturator fossa	Internal iliac vein	Ileopsoac muscle	Os pubis, obturator nerve	Obliterated umbilical artery
Common iliac	Aortic bifurcation	Genitofemoral nerve	Bifurcation of external and internal iliac artery	Common iliac artery
Presacral	Aortic bifurcation	Common iliac artery	Lower promontory	Hypogastric nerve (as distinction between right and left)
Lower paraaortic	Inferior mesenteric artery	Ureter	Aortic bifurcation	
Higher paraaortic	Left renal vein	Ureter	Inferior mesenteric artery	

Anatomic description of lymphatic pathways draining the uterus



The upper paracervical pathway (UPP) follows the uterine artery to the pelvic side wall draining primarily to the external iliac and obturator nodal compartments, then running lateral to the common iliac artery further to the paraaortic area.

The lower paracervical pathway (LPP) follows the ventral rim of the sacrouterine ligament, primarily to internal iliac and presacral nodes, then running medial of the common iliac artery further to the paraaortic area.

The infundibulopelvic ligament pathway (IPP) runs via the Ip-ligament further to the supramesenteric paraaortic area.

Assuming that lymph runs from the uterus and then cranially, supramesenteric paraaortic true paraaortic SLN's logically can only be defined when inframesenteric lymph are not ICG positive: Theoretically this may occur in situation when no pelvic nodes are dyed or when ICG have dyed pelvic nodes but not have been distributed further to the inframesenteric node, i.e that ICG goes directly to the supramestenteric nodes via the IP-ligament.
Alternatively, when inframesenteric paraaortic nodes are ICG positive via the UPP and /or the LPP but no pelvic nodes in these pathways are dyed.

For the pelvic SLN concept, ideally one SLN should be identified per LPP and UPP per pelvic side wall.

Sentinel node in cervical cancer
Version 1.2 180914

Anatomic plan for localization of sentinel lymph nodes

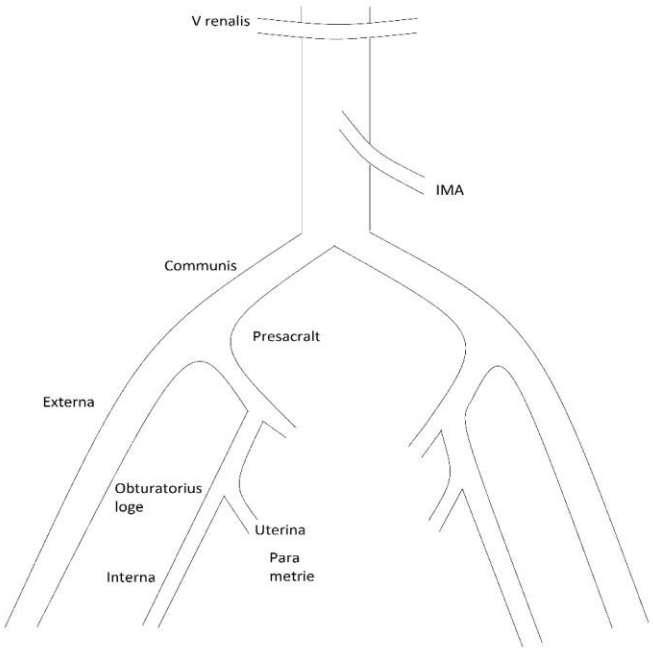
Injection site of ICG and Tc99nanocolloid ☐ cervix Reinjection ICG cervix: ☐ yes ☐ no

Display after first injection

	UPP	LPP	IP-ligament
Right			
Left			

Display after second injection

	UPP	LPP	IP-ligament
Right			
Left			



Mark position and type of SLN on anatomical chart with number corresponding to position and number at list and on separate jars for each SLN.

O = ICG positive juxtauterine Sentinel node **(SLN1**)

☐ = ICG neg juxtauterine lymph node with afferent lympahtic vessel **(SLN 2)**

X = Tumor suspect lymph nodes regardless of mapping **(SLN makro)**

Sentinel node in cervical cancer
Version 1.2 180914

List of nodal specimens (swedish) Cervical cancer ICG+TC99 ID date

Om preparat saknas från station stryks raden i listan. Burknumrering behålls för övriga prep.

KK Burk nr	Körtelposition	Patol burknr	Dosa nr	Antal bitar	Mikro Antal körtlar	Varav med metast
1	Uterus, höger ovarium & tuba, vänster ovarium & tuba					
2	Höger tuba					
3	Vänster tuba					
4	SLN Parametrium höger					
5	SLN Parametrium vänster					
6	Lgl Externa höger ICG NEG Techn NEG					
7	Lgl Externa höger ICG POS Techn NEG					
8	Lgl Externa höger ICG POS Techn POS					
9	Lgl Externa höger ICG NEG Techn POS					
10	Lgl Obt. höger ICG NEG Techn NEG					
11	Lgl Obt. höger ICG POS Techn NEG					
12	Lgl Obt. Höger ICG POS Techn POS					
13	Lgl Obt. Höger ICG NEG Techn POS					
14	Lgl Comm höger ICG NEG Techn NEG					
15	Lgl Comm höger ICG POS Techn NEG					
16	Lgl Comm höger ICG POS Techn POS					
17	Lgl Comm höger ICG NEG Techn POS					
18	Lgl Presacralt hö ICG NEG Techn NEG					
19	Lgl Presacralt hö ICG POS Techn NEG					
20	Lgl Presacralt hö ICG POS Techn POS					
21	Lgl Presacralt hö ICG NEG Techn POS					
22	Lgl Externa vä ICG NEG Techn NEG					
23	Lgl Externa vä ICG POS Techn NEG					
24	Lgl Externa vä ICG POS Techn POS					
25	Lgl Externa vä ICG NEG Techn POS					
26	Lgl Obt. vänster ICG NEG Techn NEG					
27	Lgl Obt. vänster ICG POS Techn NEG					
28	Lgl Obt. vänster ICG POS Techn POS					
29	Lgl Obt. vänster ICG NEG Techn POS					
30	Lgl Comm vänster ICG NEG Techn NEG					
31	Lgl Comm vänster ICG POS Techn NEG					
32	Lgl Comm vänster ICG POS Techn POS					
33	Lgl Comm vänster ICG NEG Techn POS					
34	Lgl Presacralt vä ICG NEG Techn NEG					

Sentinel node in cervical cancer
Version 1.2 180914

35	Lgl Presacralt vä ICG POS Techn NEG					
36	Lgl Presacralt vä ICG POS Techn POS					
37	Lgl Presacralt vä ICG NEG Techn POS					
38	Lgl Ned aortabif ICG NEG TECHN NEG					
39	Lgl Ned aortabif ICG POS TECHN NEG					
40	Lgl Ned aortabif ICG POS TECHN POS					
41	Lgl Ned aortabif ICG NEG TECHN POS					
42	Lgl Paraaortal nedom IMA ICG NEG Techn NEG					
43	Lgl Paraaortal nedom IMA ICG POS Techn NEG					
44	Lgl Paraaortal nedom IMA ICG POS Techn POS					
45	Lgl Paraaortal nedom IMA ICG NEG Techn POS					
46	SLN typ 1 ICG POS Techn Neg					
47	SLN typ 1 ICG POS Techn Neg					
48	SLN typ 1 ICG POS Techn Neg					
49	SLN typ 1 ICG POS Techn POS					
50	SLN typ 1 ICG POS Techn POS					
51	SLN typ 1 ICG POS Techn POS					
52	SLN typ 2 ICG NEG Techn NEG					
53	SLN typ 2 ICG NEG Techn NEG					
54	SLN typ 2 ICG NEG Techn POS					
55	SLN Makro ICG pos radio pos ICG neg radio neg					
56	SLN Makro ICG pos radio pos ICG neg radio neg					
57						

SLN anges i fritext i tabell och med tryck RÖD klisteretikett på burk. Vid behov av sep histologi (tex bulky nodes) används fritext i tabell och numrering 50 ff samt etikett+ fritext på burk avseende lokal / orsak

Sentinel node definieras som den mest uterusnära ICG pos körteln i respektive kompartment där man identifierar en separat afferent lymfbana

Sentinel node in cervical cancer
Version 1.2 180914

List of nodal specimens (Swedish) cervical cancer Tracer ICG pat-id date

KK Burk nr	Körtelposition	Patol burk nr	Dosa nr	Antal bitar	Mikro Antal körtlar	Varav med metast
1	Uterus, höger ovarium & tuba, vänster ovarium & tuba					
2	Lgl Iliaca Externa höger ICG NEG					
3	Lgl Iliaca Externa höger ICG POS					
4	Lgl Obturatorius höger ICG NEG					
5	Lgl Obturatorius höger ICG POS					
6	Lgl Iliaca Communis höger ICG NEG					
7	Lgl Iliaca Communis höger ICG POS					
8	Lgl Presacralt höger ICG NEG					
9	Lgl Presacralt höger ICG POS					
10	Lgl Iliaca Externa vänster ICG NEG					
11	Lgl Iliaca Externa vänster ICG POS					
12	Lgl Obturatorius vänster ICG NEG					
13	Lgl Obturatorius vänster ICG POS					
14	Lgl Iliaca Communis vänster ICG NEG					
15	Lgl Iliaca Communis vänster ICG POS					
16	Lgl Presacralt vänster ICG NEG					
17	Lgl Presacralt vänster ICG POS					
18	Lgl Paraaortalt nedom IMA ICG NEG					
19	Lgl Paraaortal nedom IMA ICG POS					
20	Lgl Paraaortal ovan IMA ICG NEG					
21	Lgl Paraaortalt ovan IMA ICG POS					
22	SLN Parametrium höger					
23	SLN Parametrium vänster					
24	SLN typ 1 presacralt höger					
25	SLN typ 1 presacralt vänster					
26	SLN typ 1 iliaca externa höger					
27	SLN typ 1 obturatorius höger					
28	SLN typ 1 iliaca externa vänster					
29	SLN typ 1 iliaca obturatorius vänster					
30	SLN typ 1					
31	SLN typ 1					

32	SLN typ 2					
33	SLN makro ICG pos ICG neg					
34	SLN makro ICG pos ICG neg					
35						

om preparat saknas från station stryks raden i listan. Burknumrering behålls för övriga prep.

Numbers 30-34 will be used for describing locations outside the most common sites and for SLN type 2 and SLN macro as appropriate. The locations will be written by hand on list and labels for jars.

Histopathologic evaluation of the sentinel nodes and the non-sentinel nodes.

At final pathological examination, all macroscopically identified SLN lymphoid tissue will be embedded and bisected at the minimum thickness exceeded 3 mm. Ultrastaging using hematoxylin/ Eosin staining will be performed in five sections at three different levels , separated by 200 µm. First and second level immunohistochemistry (pan-cytokeratin, MNF 116) will be performed if the maximum diameter of the SLN tissue exceeds one mm. If no macroscopically lymphoid tissue is identified in SLN or parametrial tissue the most suspicious find will be embedded and microscopically investigated. ITC can be detected by Hematoxylin/Eosin or by Immunohistochemistry alone.

Non- SLN nodes less than 3 mm in thickness will be embedded whole, and from nodes thicker than 3 mm at least half the node will be embedded. The slides will be evaluated after hematoxylin/Eosin staining.

Classification of tumor size in SLN's

Sentinel nodes will be classified according to a modification of the AJCC staging for axillary nodes from breast cancer as follows:

Macrometastases = tumor greater than 2.0 mm in diameter.

Micrometastases = tumor cell aggregates between 0.2 and 2.0 mm in diameter.

Isolated tumor cells =individual tumor cells or aggregates that are less than 0.2mm in diameter, usually detected by immunohistochemistry.

Tumor absent – no tumor cells identified in H&E (or immunohistochemically, if applicable) stained sections.

Non-sentinel lymph nodes will be reported as positive or negative for metastases based upon routine sectioning and examination of a single H&E stained section.

5.0 TREATMENT PLAN

-Intraoperative cervical injection of Tc 99 nanocolloid and ICG (immediately before onset of surgery)

-Intraoperative lymphatic mapping and sentinel node identification with robotic NIR/fluorescence imager and gamma probe will be used for an initial transperitoneal, then retroperitoneal evaluation of display of ICG fluorescence/ radiopositivity. The retroperitoneal dissection with opening of the avascular planes saving the lymphatic vessels will start presacrally then further with the pelvic side walls. The upper lymphovascular parametria will be kept intact by a careful opening of the pararectal and paraveiscal spaces.

-An ipsilateral reinjection of ICG will be performed in case of non-display of either of UPP or LPP. If the sentinel node cannot be identified in a particular pathway following a 15-20 minute search, the case should be described as a ICG-negative mapping event for that pathway. A initial scanning of the pelvic side walls and presacral area will be performed

- A reinjection of ICG will be performed in case of non-display of any of the UPP and LPP bilaterally.

-The results of the lymphatic mapping will be marked on the anatomic plan and the specimens and labeling of those will be according to descriptions above.

-The complete SLN algorithm should be adhered to, i.e in case of a ICG negative mapping (for **SLN type 1 and 2, ICG defined**) and / or radio negative mapping any macroscopically metastase suspect nodes should be removed and defined as **SLN macro**. All patients will be subject for at least a full pelvic lymphadenectomy after removal of SLN.

-The upper lymphocascular parametrial tissue (the tissue medial of the obliterated umbilical artery, with the supravesical artery as distal/ventral border, the ureter as dorsal border and including tissue adjacent to the inner side of the broad ligament) will be removed and evaluated the same way as SLN's. This is to investigate the presence of lymph node in the upper parametria, theoretically the most juxtauterine nodes, hence the SLN.

- The SLN's and separate upper parametria will be sent for frozen section.

-All SLN and the upper parametrial tissue will be subject for later ultrasectioning and IHC Histopathologic evaluation.

- In case of metastatic SLN the lymphadenectomy will be extended to the level of the inferior mesenteric artery and the hysterectomy aborted in favour of radiochemoptherapy. In case of a planned trachelectomy this will be transformed to a radical hysterectomy.

-A Querleu-Morrow type B2 or C2 hysterectomy or robotic radical trachelectomy will be performed as appropriate or aborted in case of metastatic LSN at surgeons discretion and patients' wish.

- Adnexal surgery or transpositioning of the adnexa shall be performed at surgeons discretion and patient' wish.

- Complications will be recorded and subdivided to enable association with parts of the procedure, most importantly, the SLN procedure as such and the lymphadenectomy. The classification described under the adverse events tables will be used.

NOTE: Surgeon skill and adherence to protocol will be verified as described.

6.0 TREATMENT MODIFICATIONS

In case of no ICG uptake (including reinjection according to protocol) or radiopositivity the overall SLN algorithm should be followed, i.e removal of any macroscopically suspect nodes and as planned a full compartmentwize lymphadenectomy.

In case of conversion to open surgery before identification and removal of SLN's the patients should be included in evaluation of adverse events and feasibility on an intention to treat basis but not included in the calculation of sensitivity and negative predictive values for the sentinel node concept.

In case of metastatic SLN on frozen section, the lymphadenectomy should be extended to the level of the inferior mesenteric artery, a planned trachelectomy converted to a radical hysterectomy and at surgeons discretion and patient wish a planned radical hysterectomy completed or aborted in favour of radiotherapy. Adnexal surgery / transposition of ovaries shall be performed at surgeons' discretion and patient wish.

7.0 STUDY CRITERIA

Observations and Tests

The following observations and tests are to be performed and, where appropriate, recorded on the designated form(s) (Appendix A):

PARAMETER	Pre-operative	Intraoperative	Postoperative
Log on approached patient	X		
Log on included patients and patients' withdrawal from study before surgery	X		
History & Physical examination Examination and evaluation by surgeon and anesthetist	X		
Laboratory test Hb, S-electrolytes, S-kreatinine CRP, Trc, coagulation test when appropriate	X		X
Injection data for ICG including drug related adverse events	X	X	X
Sentinel nodes identification		X	
Recording of intraoperative adverse events		X	

Histologic evaluation of sentinel nodes and non-sentinel nodes			X
Recording of post-op complications until 30 days after surgery			X
Recording of lymphatic complications on validated questionnaire at symptoms and at one year after surgery			

Adverse Events will be captured from time of ICG administration until30 days after surgery

8.0 EVALUATION CRITERIA

-Technical success rates, sensitivity and false negative rates with ICG and Tc99 nanocolloid will be compared per patient, per hemipelvis and per pathway, UPP and LPP.

- All patients who are injected with tracer and not converted to open surgery before detection and removal of SLN's will be included for evaluation of technical success rate (described per patient, per hemipelvis and per separate pathway) after first and in case of non-dispay of any of the LPP and UPP after reinjection of ICG as described.

- Patients with at least one SLN type1-2 identified will be included for determining the sensitivity and the negative predictive value for the SLN ICG concept. In addition the overall SLN concept, including SLN macro will be evaluated as of above.

- All patients included, regardless of conversion to open surgery and mapping of ICG (or the combination of ICG and Tc99 nanocolloid) will be included in the evaluation of feasibility and safety.

- Intraoperative adverse associated with the detection and removal of SLN will be evaluated and reported separately on all patients who have at least one SLN removed regardless of type, i.e all patients in whom SLN were removed separately.

- Postoperative complications until 30 days after surgery will be reported.

-The incidence of lymphatic complications defined by the questionnaire will be evaluated

9.0 DURATION OF STUDY

The study includes a radical hysterectomy/ radical abdominal trachelectomy with detection of SLN followed by a full pelvic lymphadenectomy along both the UPP and LPP.

The patient may withdraw from the protocol at any time prior to surgery or at any time until retrieval of postoperative data until one year after surgery.

The study is initiated at Lund University hospital with a planned later inclusion of the second investigating center which may motivate a revision of the study protocol related estimated time for enrollment of patients and for accreditation of the second centre and surgeons.

Assuming that the prevalence of pelvic lymph node metastases is 15 % 227 patients will be needed to obtain 34 lymph node positive observations. Given a 95% bilateral detection rate of SLN's approximately 15 patients more will need to be included. On the other hand, ultrasectioning and IHC of SLN's is estimated to increase the proportion of patients with nodal metastases by identifying micro metastases. As there will be a continuous report of node positive patients to the study coordinator an interim analysis will start after 36 node positive patients regardless of the total number of patients included. (see 11, statistical considerations) Accrual will not be suspended during the interim analysis as the intervention is estimated to pose minimal risk to patients and the national treatment protocol, recommending a full lymphadenectomy, will be followed, even in the event of futility.
If the trial continues to stage 2, another 28 node positive patients (total 187 patients) will be required. (see 11, statistical considerations)
Given an estimate that the accrual rate is 2,5 cervical cancer patients per month per center, a total of 45 months will be required to reach 226 cervical cancer patients and a total of an additional 37 months to reach 413 patients.

10 STUDY MONITORING AND REPORTING PROCEDURES

ADVERSE EVENT REPORTING

The study protocol has been revised related principles study monitoring and for reporting adverse events to the principal investigating center after inclusion of the second center.

Definitions

An adverse event (AE) is any new medical problem or exacerbation of an existing problem experienced by a subject enrolled in the study, whether or not it is considered drug-related by the investigator.

This study will utilize the Adverse Events Logs (Tables 10.1-5). Any SAE will be reported to the study coordinator linnea.ekdahl@med.lu.se or jan.persson@med.lu.se

Adverse events related to the study drug (ICG).
All adverse events occurring from the first dose of study drug until hospital discharge (whether or not attributed to the study drug) will be reported on the Adverse Event Log. In addition, any adverse event reported by the subject to the investigator after discharge and determined to be reasonably associated with the study drug should also be captured and followed until resolution.

Adverse events related to the sentinel node procedure as such (excluding AE related the study drug, ICG)
All intraoperative events related to the SLN procedure will be reported on the adverse events log.

Adverse events related the surgical procedure (excluding the SLN part) including AE until 30 postoperative days.
All adverse events will be reported on the adverse events log.

Serious adverse event (SAE):
An adverse event that results in one or more of the following:
-Any death occurring prior to the postoperative outpatient evaluation 30 days postoperatively.
-Any life-threatening event until and including 30 postoperative days.
-Any medical event requiring inpatient hospitalization or prolongation of existing hospitalization beyond five postoperative days

NOTE: Hospitalizations that are not considered SAE are:
-Hospitalization planned prior to first administration of study drug
-Hospitalization for elective treatment of a pre-existing condition unrelated to the study medication
- Hospitalization due to social / practical reasons such as an untimely coordination with local community home care services.

Attribution: Attribution is the determination of whether an adverse event is related to a medical treatment or procedure. The categories of attribution are:

Definite: The adverse event is clearly related to the study drug
Probable: The adverse event is likely related to the study drug.
Possible: The adverse event may be related to the study drug.
Unlikely: The adverse event is doubtfully related to the study drug.
Unrelated: The adverse event is clearly NOT related to the study drug.

Unexpected Adverse Event: An unexpected adverse event is an event not mentioned in the package insert/ manufacturer's instructions or the specificity or severity of which is not consistent with the package insert/ manufacturer's instructions.
The grading described beneath and the attribution described above will be used for cathegorization of unexpected adverse events.

Participating Center Reporting Responsibilities

Reporting to the study coordinator

Any SAE's must be reported to study Coordinator at Lund University Hospital within 3 working days of discovery of the incident, using the study-specific SAE Form.
Email: linnea.ekdahl@med.lu.se

The lead and local principal investigators and the study coordinator shall conduct continuous review of data and patient safety for a monthly summary of the included number of patients, patient safety and significant AE's described in the protocol.

All SAE's potentially associated with the study drug, the sentinel node procedure as such or deaths shall be evaluated by a

Reporting to the IRB:

Each participating center will report adverse events to their IRB per local guidelines.

Coordinating Center Reporting Responsibilities

Reporting to the study coordinator, Lund University Hospital
Same criteria as above.

Reporting to the IRB:

Same criteria as above.

The Study Coordinator will distribute reports which are serious, unexpected and associated with the study intervention (possibly, probably or definitely) to all participating investigators. Copies of all serious adverse event reports will be kept on file the department of Obstetrics and Gynecology, Lund University Hospital.

The study coordinator will also report all individual SAE's related to study drug, the

sentinel node procedure as such, are life threatening or resulting in death (defined above and in table 10.6) to the respective Safety Monitoring Committees (SMC) for clinical studies at Skåne University Hospital or local investigating centre for an independent evaluation.

At any time during the conduct of the trial, if it is the opinion of the investigators that the risks (or benefits) to the patient warrant early closure of the study, this recommendation should be made in writing to the SMC. Alternatively, the SMC may initiate suspension or early closure of the study based on its review of the investigator reports.

10.1.4. Study Monitoring / study accrual oversight

The study principal investigator, local principal investigator and study coordinator will conduct meetings (teleconferenced) every 6 months to discuss the protocol. The s-PI and l-PI can call a meeting to convene at additional times if deemed necessary, for example following statistical review at the interim period if stopping the study for either achieved goals or futility. Apart from the monitoring described below, the number of node positive patients and potential false negative SLN's will be monitored continuously by e-mail to the study coordinator by the use of the study number assigned to each patient.

In case of identified inconsistencies or missing data, additional source documents (identified only by unique patient number) will be requested from the site to resolve ongoing inconsistencies.

The principal investigator and/or the study coordinator will, if deemed necessary by the principal or second participating center perform audits of informed consents and subject eligibility.

Data Management

Preoperative, intraoperative and postoperative data from each surgery will be recorded on the standardized study sheets.These study sheets will be made available to each study site. Each study site will be allocated a study number which will serve as the prefix to the case number. For example, Lund university Hospital will be allocated the prefix "1" and the first

study patient will have the study number "1-001. Each investigating center will hold a record with the full identification of patients whereas data otherwise should only identify the patient by the study number (see above).

Upon interim analysis the full data will be monitored by the study coordinator, the principal investigator, local principal investigator and study statistician.

Separate analysis of patient data from individual sites can only be performed with the written permission of the study principal investigator.

Kommenterad [PJJ1]: Staff at the individual centers will be responsible for completing the data collection sheet for each patient and all data will continuously and all be entered into a common secured web-based database (https://data.dynareg.se/slnicgec) using the designated study numbers.

Early Study Closure

Death will be reported according to section 10.1 above and per local IRB reporting guidelines. The SMC will review all reported deaths monthly. Early closure of the study will be based on judgement of the SMC.

The study will be stopped for futility reasons as described under the statistics section.

10.1.8. Protocol Deviations

Major protocol deviations shall be reported by mail to the study coordinator. linnea.ekdahl@med.lu.se, or Jan.persson@med.lu.se and filed at Lund University Hospital using the designated study number. Major protocol deviations include, but are not limited to, violations to inclusion/ exclusion criteria, erroneous preparation of ICG or surgery by a non-accredited surgeon.

Table 10.1. Adverse Events before docking the robot (during injection of ICG, insufflation, port placement and adhesiolysis) including conversions to open surgery.

STUDY NUMBER	AE/SAE	DESCRIPTION	Grade	Attribution
Example LU001	AE	Converted, disseminated disea	1	
Example LU002	AE	Converted massive adhesions	1	

Table 10. 2. Adverse Events during sentinel node dissection

STUDY NUMBER	AE/SAE	DESCRIPTION	Grade	Attribution
LU001 examples	AE	Bleeding requiring suture	2	
LU002	AE	Obturator nerve damage	3	

Table 10.3. Adverse events during completory pelvic lymphadenectomy

STUDY NUMBER	AE/SAE	DESCRIPTION	Grade	Attribution
LU001examples	AE	Bleeding requiring suture or	2	
LU002	AE	Obturator nerve damage	3	

Table 10.4. Adverse events during hysterectomy/ trachelecomy

STUDY NUMBER	AE/SAE	DESCRIPTION	Grade	Attribution
LU001 examples	AE	Ureteral damage	3	
LU002	AE	Bowel damage	3	

Table 10.5. Postoperative adverse events until 30 postoperative days (all events)

STUDY NUMBER	AE/SAE	DESCRIPTION	Grade	Attribution
LU001 examples	AE	Port hernia	3	
LU002	AE	Fever of unknown origin	2	

Table 10.6 Intraoperative Adverse events will be graded according to the following scale:

Grade	Description
Grade 1	Mild; asymptomatic; not interfering with function.
Grade 2	Moderate; symptomatic; interfering with function but not ADL; medical intervention indicated.
Grade 3	Severe; symptomatic; interfering with ADL; operative intervention indicated; IV intervention indicated
Grade 4	Life-threatening; major urgent intervention indicated; disabling.
Grade 5	Death

Postoperative adverse events will be graded according to the Clavien Dindo classification

Grade I	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions Allowed therapeutic regimens are: drugs as antiemetics, antipyretics, analgetics, diuretics and electrolytes and physiotherapy. This grade also includes wound infections opened at the bedside.
Grade II	Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusionsand total parenteral nutritionare also included.
Grade III	Requiring surgical, endoscopic or radiological intervention
- IIIa	Intervention not under general anesthesia
- IIIb	Intervention under general anesthesia
Grade IV	Life-threatening complication (including CNS complications)* requiring IC/ICU-management
- IVa	single organ dysfunction (including dialysis)
- IVb	multiorgandysfunction
Grade V	Death of a patient

11. STATISTICAL CONSIDERATIONS

This study is designed to evaluate the application of the sentinel node (SLN) technique in determining the pelvic lymph node (LN) status in patients with early stage cervical cancer (stage 1a2 to 2a1) planned for primary surgery with either a radical hysterectomy or radical abdominal trachelectomy.

The main statistical endpoint will be sensitivity and false negative rates /negative predictive value calculated per patient. The sensitivity is the proportion of patients who test as SLN (+) among the patients who have LN metastases (LN+). The patients will, according to the Swedish national guidelines for early stage cancer undergo a full pelvic lymphadenectomy, however with separate removal of SLN as defined above, hence serve as their own controls. As lymph node status (lymph node metastases or no lymph node metastases) is a definite parameter (false positive result can by definition not occur) specificity and positive predictive values will not be reported in this study.

The time for accrual may have to be revised revised after inclusion of additional participating centers.

We used the Fleming two stage design for evaluation of study termination for evaluating if the technique is not working well, as expected or better (Fleming, 1982).

The null hypothesis that the sensitivity is 85% will be tested against one-sided alternative. The study is terminated after stage one if it is unlikely that the sensitivity is $\geq$ 95% or if sensitivity is significantly higher than 85%. In the first stage, 34 LN+ patients will be accrued. If there are 30 or fewer responses in these 34 patients, the study will be stopped for futility. If there are at least 33 responses in 33 patients, the study will be stopped and the null hypothesis rejected. Otherwise, 28 additional patients will be accrued for a total of 62 node positive patients. The null hypothesis will be rejected if 58 or more responses are observed in 62 patients. This design yields a type I error rate of 0.05 and power of 0.8 when the true sensitivity is 92.5

For the first stage we estimated a total of 226 patients to be included taking into consideration a 95% bilateral SLN detection rate, and an estimated 15% rate of node positivity including an increase in node positivity rates due to ultrasectioning and immunohistochemistry. For the second stage, another 187 patients have to be included. As the number of node positive patients will be reported continuously to the study coordinator this will be decisive of the number of patients that has to be included for each stage.

BIBLIOGRAPHY

1. Querleu D, Morrow CP. Classification of radical hysterectomy.
Lancet Oncol. 2008 Mar;9(3):297-303. doi: 10.1016/S1470-2045(08)70074-3.

2. Cibula D, Abu-Rustum NR, Benedetti-Panici P, Köhler C, Raspagliesi F, Querleu D, Morrow CP.New classification system of radical hysterectomy: emphasis on a three-dimensional anatomic template for parametrial resection. Gynecol Oncol. 2011 Aug;122(2):264-8. doi: 10.1016/j.ygyno.2011.04.029. Epub 2011 May 17.

3.Darlin L[1], Persson J, Bossmar T, Lindahl B, Kannisto P, Måsbäck A, Borgfeldt C. The sentinel node concept in early cervical cancer performs well in tumors smaller than 2 cm. Gynecol Oncol. 2010 May;117(2):266-9. doi: 10.1016/j.ygyno.2010.01.035. Epub 2010 Feb 18.

4. Persson J, Kannisto P, Bossmar T. Robot-assisted abdominal laparoscopic radical trachelectomy. Gynecol Oncol. 2008 Dec;111(3):564-7. doi: 10.1016/j.ygyno.2008.05.034. Epub 2008 Jul 11.

5. Rossi EC, Ivanova A, Boggess JF. Robotically assisted fluorescence-guided lymph node mapping with ICG for gynecologic malignancies: A feasibility study. Gynecol Oncol. 2011. Oct 11.

6. Geppert B, Lönnerfors C, Bollino M, Arechvo A, **Persson J.**
A study on uterine lymphatic anatomy for standardization of pelvic sentinel lymph node detection in endometrial cancer. Gynecol Oncol. 2017 May;145(2):256-261. doi: 10.1016/j.ygyno.2017.02.018. Epub 2017 Feb 10.

7. Fleming TR (1982). One-sample multiple testing procedure for phase II clinical trials. Biometrics 38: 143-151.

8. Jung SH, Lee TY, Kim KM, George S (2004). Admissible two-stage designs for phase II cancer clinical trials, Statistics in Medicine 23: 561-569.

Appendix 1 SAE events log

Serious adverse events log. Mail to linnea.ekdahl@med.lu.se or jan.persson@med.lu.se

Per definitions of SAE and attributions as outlined in protocol

Patients study number **Date for SAE**		
Type of SAE	**Yes /no**	**Attribution**
Death		
Life Threatening		
Drug /ICG related		
Intraoperative related the SLN procedure as such		
Intraoperative related the full LND/ hysterectomy		
Postoperative		
Unexpected AE		

Description / outcome of the SAE

Sentinel node in cervical cancer
Version 1.2 180914