Title: SENECA Study: **S**taging **E**ndometrial ca**N**cer based on mol**E**cular **C**l**A**ssification

Principal Investigator	**Co-Principal Investigator**
Enrique Chacón MD Gyn Oncology Unit. Clinica Universidad de Navarra. Pamplona. Spain. echaconc@unav.es	**Luis Chiva MD, PhD** Gyn Oncology Unit. Clinica Universidad de Navarra. Madrid. Spain. lchiva@unav.es **Antonio González MD, PhD** Gyn Oncology Unit. Clinica Universidad de Navarra. Madrid. Spain. agonzalezma@unav.es

Co-investigators

- Félix Boria MD. Gyn Oncology Unit. Clinica Universidad de Navarra. Madrid. Spain. F.boria.alegre@gmail.con

-**Luisa Sanchez Lorenzo MD, PhD**. Gyn Oncology Unit. Clinica Universidad de Navarra. Madrid. Spain. lsanchezl@unav.es

-**José Ángel Mínguez MD, PhD**. Gyn Oncology Unit. Clinica Universidad de Navarra. Pamplona. Spain. jaminguez@unav.es

Cancer Center Universidad de Navarra

Version: 2.1 Date: 1/1/2023

1. List of abbreviations

EC: Endometrial Cancer

LN: Lymph Node

SLN: Sentinel lymph node mapping

OS: Overal Survival

DFS: Disease Free Survival

OSNA: One Step Nucleic Acid Amplification

ICG: Indocyanine green

POLEmut: POLE exonuclease domain ultramutated

p53abn: p53 abnormal

MMRd: mismatch repair (MMR) proteins-deficient

NSMP: no specific molecular profile

2. Clinical study summary

Study title:	**S**taging **E**ndometrial ca**N**cer based on mol**E**cular **C**l**A**ssification **(SENECA Trial)**
Study rationale:	The management of endometrial cancer (EC) is currently undergoing a true revolution in terms of diagnosis and treatment. Since 2013 and thanks to the TCGA project (The Cancer Genome Atlas), four distinct molecular subgroups (POLE, MMR-D, Copy number low, Copy number high) with distinct prognostic implications were identified. Currently, thanks to the retrospective analysis of the PORTEC-3 cohort, we know that these four molecular subgroups may present differences in survival in high-risk patients. On the other hand, the surgical management of early EC (stage I/II) has been changing for some years now, especially at the level of nodal staging, from modulation of treatment depending on the risk group (pelvic lymphadenectomy +/- sentinel lymph node (SLN) for low and intermediate risk groups vs. aortopelvic lymphadenectomy +/- sentinel lymph node (SLN) for those at high risk) to a generalization of treatment based on detailed study of the SLN

	following the algorithm described by the group of Abu-Rustum NR et al. It remains unknown (or an open question) if it could be possible to tailor the type of lymph node staging in early EC (the most common and most frequent in our daily clinical practice) depending on the molecular subgroup. We therefore propose a study to evaluate the rate of lymph node involvement depending on the molecular subgroup in early-stage EC (I/II).
Study design:	International, retrospective, multicentre, observational study.
Sites number:	Not defined
Objectives:	**Primary objective** -To evaluate the lymph node involvement rate (sentinel) for each molecular subtype in patients with stage I/II EC
	Secondary objectives -To evaluate the lymph node involvement rate (sentinel and non-sentinel) for each prognostic risk group in patients with stage I/II EC -To evaluate morbidity associated to SLN biopsy -To assess compliance of ESGO quality Indicators
Study population:	Patients with early-stage (FIGO stage I-II) EC that underwent surgical treatment with lymph node evaluation between January 2021 to December 2022, both included. A sample size of 1,032 patients may provide sufficient statistical power to evaluate the association between molecular subgroups and lymph node status. We assumed a 90% power for a 2-sided p-value of 0.05 and a minimum difference of 4.4 percentage points in prevalence rates of positive lymph nodes. We anticipated a potential dropout rate of 10%.

Inclusion criteria:	-Age 18 years or older -Patient was operated during 2021-2022 -Histological confirmation of Endometrial Cancer with endometrioid histology or high risk histology (serous, clear cell, carcinosarcoma and mixed histologies) -Preoperative FIGO stage I or II by MRI or US -Preoperative CT-Scan or PET-CT without evidence of local or distant disease (could be omitted in low-risk and intermediate risk endometrial carcinoma with low grade histology) -Surgical protocol according to ESGO/ESTRO/ESP guidelines -A detailed sentinel lymph node study protocol must be accredited, either by ultra-staging or OSNA -Molecular analysis performed on the pathology specimen according to ESGO/ESTRO/ESP guidelines (POLE mutation analysis could be omitted in low-risk and intermediate risk endometrial carcinoma with low grade histology).
Exclusion criteria:	-Pregnant women -Previous hysterectomy -Previous pelvic/para-aortic lymphadenectomy -Presence of extra-uterine disease (peritoneal, visceral or suspicious lymph node metastasis) -Past medical history of any invasive tumor -History of previous abdominal or pelvic radiotherapy of any type (including braquitherapy) -History of preoperative neoadjuvant chemotherapy
Statistical considerations:	Quantitative data will be presented as mean and standard deviation and qualitative variables with absolute values and percentages. Additionally, qualitative variables among groups will be compared by chi-square test or Fisher exact test; and quantitative variables

	with t-test and ANOVA test. For the multivariate analysis a logistic regression model will be used. Alpha error will be set at 5%

3. BACKGROUND

The management of endometrial cancer (EC) is currently undergoing a true revolution in terms of diagnosis and treatment. Since 2013 and thanks to the TCGA project[1] (The Cancer Genome Atlas), four distinct molecular subgroups (POLE, MMR-D, Copy number low, Copy number high) with distinct prognostic implications were identified.

Subsequently, PROMISE study[2] brought this "new era" closer to daily clinical practice. These new findings led ESGO[3] (European Society of Gynecologic Oncology) to decide to integrate this molecular classification into the definition of the different risk groups.

Currently, thanks to the retrospective analysis of the PORTEC-3 cohort[4,5], we know that these four molecular subgroups may present differences in survival in high-risk patients depending on the type of treatment proposed, chemoradiotherapy vs. adjuvant radiotherapy. This question will be answered by the RAINBO trial[6] (Refining Adjuvant treatment IN endometrial cancer Based On molecular profile).

On the other hand, the surgical management of early endometrial cancer (stage I/II) has been changing for some years now, especially with regard to nodal staging, from modulation of treatment depending on the risk group (pelvic lymphadenectomy +/- sentinel lymph node (SLN) for low and intermediate risk groups vs. aortopelvic lymphadenectomy +/- SLN for those at high risk) to a generalization of treatment based on detailed study of the sentinel node following the algorithm described by the group of Abu-Rustum NR et al[7].

Just as the RAINBO study will try to clarify the type of adjuvant treatment necessary for each molecular subgroup, we do not currently know if it could be possible to tailor the type of lymph node staging in early EC (the most common and most frequent in our daily clinical practice) depending on the molecular subgroup[8,9].

We therefore propose a study to evaluate the lymph node involvement rate depending on the molecular subgroup in early-stage EC (I/II).

4. OBJECTIVES

Primary objective

-To evaluate the lymph node involvement rate (sentinel) for each molecular subtype in patients with stage I/II EC.

Secondary objectives

-To evaluate the lymph node involvement rate (sentinel and non-sentinel) for each prognostic risk group in patients with stage I/II EC.

-To evaluate morbidity associated to SLN biopsy.

-To assess compliance of ESGO quality Indicators.

We consider that our study will help to:

- ✓ Tailor the type of nodal staging depending on the molecular subgroup in early EC.
- ✓ Take a snapshot of the morbidity in SLN.
- ✓ Evaluate if the quality indicators described by ESGO are met.
- ✓ Know the degree of correlation between the molecular classification of the preoperative and the definitive biopsy.

5. STUDY GENERAL DESIGN

STUDY GENERAL DESIGN

The study is a retrospective multicentric international observational study reviewing data of patients diagnosed with early-stage (FIGO stage I-II) EC and underwent surgical treatment with lymph node evaluation between January 2021 to December 2022, both included.

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy. The Investigators and other site personnel will not use such data and records for any purpose other than conducting the study.

This protocol will be reviewed by the IRB of Clinica Universidad de Navarra, and the data collection will not start until the IRB approval is obtained. Each institution will be responsible of obtaining its own IRB/EC approval.

RECRUITMENT

A sample size of 1,032 patients may provide sufficient statistical power to evaluate the association between molecular subgroups and lymph node status. We assumed a 90% power for a 2-sided p-value of 0.05 and a minimum difference of 4.4 percentage points in prevalence rates of positive lymph nodes. We anticipated a potential dropout rate of 10%.

Gynaecological Cancer Centres/Units and hospitals regularly performing elective surgeries for EC internationally will be invited to participate in this study. Invitations will be sent through international/national and informal networks. Participating sites will register with the central audit team at Clinica Universidad de Navarra and will be provided with unique user access credentials for the database.

Each participating site (centre/unit/trust/hospital) will identify a principal investigator (PI) who will be responsible for coordinating data entry at their local site. The PI may have a bigger local reporting group/team with as many individuals as felt to be necessary to sustain the project. Team members may include gynaecological oncology trainees/fellows, medical/clinical oncologists, radiologists, pathologists, nurses, medical students, research staff, data managers. Each participating site will be provided a unique site code, individual username and password for database access. Inputting sites will only be able to access/see their own data and will not be able to see another site's data. The central coordinating centre team will be able to see individual data from all sites.

Study IDs are unique to each patient and will be automatically assigned when a patient is registered onto the database. Study IDs will be assigned centrally by the REDCap bespoke database. Only one study ID should be created for each patient and new data registered on the appropriate electronic case record file (eCRF). Individual centres will retain access to their patient data and not provide identifiable information on the international database. Local sites will retain information on patient identifiable data (name, date of birth, address, telephone number) in a separate file but not share this with the central audit team. This information/file that links the study ID to the patient identifiers will be kept on the local centre/unit/hospital server, in case further clarification of data is required.

6. STUDY POPULATION

INCLUSION CRITERIA

A patient will be considered eligible for inclusion in this study if all the following criteria are met:

-Age 18 years or older

-Patient was operated during 2021-2022

-Histological confirmation of Endometrial Cancer with endometrioid histology or high risk histology (serous, clear cell, carcinosarcoma and mixed histologies)

-Preoperative FIGO stage I or II by MRI or US

-Preoperative CT-Scan or PET-CT without evidence of local or distant disease (could be omitted in low-risk and intermediate risk endometrial carcinoma with low grade histology)

-Surgical protocol according to ESGO/ESTRO/ESP guidelines

-A detailed sentinel lymph node study protocol must be accredited, either by ultra-staging or OSNA

-Molecular analysis performed on the pathology specimen according to ESGO/ESTRO/ESP guidelines (POLE mutation analysis could be omitted in low-risk and intermediate risk endometrial carcinoma with low grade histology).

EXCLUSION CRITERIA

-Pregnant women

-Previous hysterectomy

-Previous pelvic/para-aortic lymphadenectomy

-Presence of extra-uterine disease (peritoneal, visceral or suspicious lymph node metastasis)

-Past medical history of any invasive tumor

-History of previous abdominal or pelvic radiotherapy of any type (including braquitherapy)

-History of preoperative neoadjuvant chemotherapy

7. EVALUATION

Definition of variables

Sentinel lymph node: Defined as the first echelon lymph node where the tumor directly flows through the lymphatic duct.

Isolated tumor cells: <0.2mm or less than 200 tumoral cells in a single histologic section

Micrometastases: 0.2mm-0.2cm or more than 200 tumoral cells in a single histologic section.

Macrometastases: Metastases >0.2cm

Data to be abstracted:

Medical history and demographic data will be reviewed. Patients will be grouped in group 1 (POLEmut), group 2 (MMRd), group 3 (NSMP), group 4 (p53abn), depending on the molecular profile. Preoperative FIGO stage, modality of initial treatment, final pathology diagnosis, preoperative pathology analysis, type of surgery, perioperative details and adverse events will be reviewed. Follow up until the date will be reviewed as well. (Appendix).

8. STATISTICAL ANALYSIS

Quantitative data will be presented as mean and standard deviation and qualitative variables with absolute values and percentages. Additionally, qualitative variables among groups will be compared by chi-square test or Fisher exact test; and quantitative

variables with t-test and ANOVA test. For the multivariate analysis a logistic regression model will be used.

Survival analysis will be performed using the Kaplan-Meier method and log-rank tests. Alpha error will be set at 5%.

9. ETHICAL, LEGISLATIVE AND REGULATORY CONSIDERATIONS

Ethical considerations

The investigator will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. All data will be collected on a customised, secure, password protected central REDCap database hosted by Clinica Unviersidad de Navarra. REDCap is used internationally to securely collect research data. All collected data will be transmitted and held anonymously.

Investigator responsibilities

The principal investigator of each site must conduct the study in accordance with the research protocol and with current regulations.

The investigator should not implement any deviation from, or changes of the protocol without authorized in writing by the principal investigator and without the previous authorization of the Ethics Committee and competent authorities of proposed changes.

The principal investigators are responsible of respecting the study confidentiality.

Confidentiality of study documents and patient records

The investigator must ensure that patients' anonymity will be maintained and that their identities are protected from unauthorised parties. On CRFs or other documents submitted to the principal investigator, patients should not be identified by their names, but by an identification code.

Funding and conflict of interest

This study has not received funding from any public or private for-profit entity, nor will it admit any during the development of the study. None of the investigators or patients will receive any financial or material compensation for the development of the study, nor do they have any financial conflict of interest in the conduct of the study.

10. PUBLICATIONS

We are currently in the design and approval phase of the study and expect to complete this phase by the end of 2022.

Between January and February 2023 we will start the phase of dissemination of the project and recruitment of institutions wishing to participate in it, once the total number of participating centres is obtained, we will start recruiting between March and May 2023. Throughout this process, several virtual meetings will be held to inform the heads of each institution of the status of the project as well as to resolve any doubts that may arise. In the same way, several reminder e-mails will be sent to ensure that the recruitment process is carried out on time.

Once recruitment has been completed, we will proceed to the analysis of the data by our statistical team and the research committee. Preliminary results are intended to be presented at the ESGO Congress in ISTANBUL in October 2023.

It will be the responsibility of the Principal Investigator to obtain and publish the results of this study in high impact journals indexed in the Journal Citation Report (Pubmed). The tentative deadlines for publication of the main results will be December 2023.

With respect to authorship in the different papers, the following rules will be followed, the acceptance of which is considered implicit to participation in this study. We will always try to include the maximum number of authors, as well as the maximum number of participating centers, even those with the lowest participation, as follows:

- The number of authors per publication will be a maximum of 20 authors (unless the journal is flexible, in which case the maximum allowed will be included). The general order of authors will be established by individual order of inclusion of cases in the study. If any author considers that another researcher from his/her institution should participate as a co-author in his/her place, this will be communicated in time for inclusion in the corresponding publication.
- In case the limitation of authors makes it necessary to reduce the number of authorships, it will be reduced following the same rule of inclusion of cases in the study.

- There will be 4 fixed positions in the publications corresponding to the persons in charge of the writing and elaboration of the articles (first and second author), coordination and development of the study (last author - PI), statistical study and methodology (penultimate author).

-For the rest of the positions, half will correspond to the authors with the highest number of cases introduced in all the publications derived; and the other half consecutively to the authors of the institutions that follow them in number of cases.
For example, in the case of 32 collaborating researchers arranged in order of participation and 3 articles to be written, the order of authorship would be:

- Article 1: 4 fixed positions + authors centers of 1--8 + Authors 9-16
- Article 2: 4 fixed positions + authors centers of 1--8 + Authors 17-24
- Article 3: 4 fixed positions + authors centers of 1--8 + Authors 25-32

In this way, the inclusion of the maximum and most varied number of authors will be encouraged while giving priority to the greatest participation and work of the study's collaborators.

-At the end of the list of authors of each publication, "on behalf of SENECA group collaborative" will be written. Authors who cannot be included as such in each publication will be included as collaborators or in the acknowledgements according to the possibilities of each journal.

11. REFERENCES

1. *Kandoth C, Schultz N, Cherniack AD et al. Integrated genomic characterization of endometrial carcinoma. Nature 2013; 497(7447): 67–73.*
2. *Talhouk A, McConechy MK, Leung S et al. A clinically aplicable molecular-based classification for endometrial cancers. Br J Cancer 2015; 113(2): 299–310*
3. *Concin N, Matias-Guiu X, Vergote I et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. Int J Gynecol Cancer. 2021 Jan;31(1):12-39. doi: 10.1136/ijgc-2020-002230. Epub 2020 Dec 18. PMID: 33397713.*
4. *León-Castillo A, de Boer SM, Powell ME et al. Molecular Classification of the PORTEC-3 Trial for High-Risk Endometrial Cancer: Impact on Prognosis and Benefit From Adjuvant Therapy. J Clin Oncol. 2020 Oct 10;38(29):3388-3397. doi: 10.1200/JCO.20.00549. Epub 2020 Aug 4. PMID: 32749941; PMCID: PMC7527156.*
5. León-Castillo A, Gilvazquez E, Nout R , et al . Clinicopathological and molecular characterisation of 'multiple-classifier' endometrial carcinomas. J Pathol 2020;250:312–22
6. *https://clinicaltrials.gov/ct2/show/NCT05255653*
7. *Abu-Rustum NR. Sentinel lymph node mapping for endometrial cancer: a modern approach to surgical staging. J Natl Compr Canc Netw. 2014 Feb;12(2):288-97. doi: 10.6004/jnccn.2014.0026. PMID: 24586087.*
8. *Moloney K, Janda M, Frumovitz M, et al. Development of a surgical competency assessment tool for sentinel lymph node dissection by minimally invasive surgery for endometrial cancer. Int J Gynecol Cancer. 2021 May;31(5):647-655. doi: 10.1136/ijgc-2020-002315. Epub 2021 Mar 4. PMID: 33664126.*
9. Betella I, Fumagalli C, Rafaniello Raviele P, *et al* A novel algorithm to implement the molecular classification according to the new ESGO/ESTRO/ESP 2020 guidelines for endometrial cancer *International Journal of Gynecologic Cancer* 2022;**32:**993-1000.

11. APPENDICES:

Table 3 Operation guide

Surgical step	Descriptor	Consensus recommendation
Tracer	ICG	Mandatory
	Blue dye	Optional
	Radio-labeled technetium	Optional
Injection location	Ectocervix in two or four positions	Mandatory
Injection technique	Superficial injection into the ectocervix Transperitoneal injection into the uterus Hysteroscopic injection into the uterus Surgeon appreciation of resistance at tracer injection	Mandatory Prohibited Prohibited Mandatory
Injection needle	Gauge between 20G and 25G Length sufficient to ensure easy and accurate access to the cervix	Mandatory Mandatory
Uterine manipulator	If being used, insert uterine manipulator after tracer injection	Mandatory
White light inspection	Prior to SLN mapping, conduct an inspection of the pelvic areas	Mandatory
Round ligament & Infundibulopelvic ligament	Preserve	Optional
	Divide	Optional
External vessels	Identify the external iliac vessels	Mandatory
Internal iliac artery	Identify the internal iliac artery	Mandatory
Ureter	Identify the ureter	Mandatory
Obliterated umbilical ligament	Identify the obliterated umbilical ligament	Mandatory
Uterine artery	Identify the uterine artery (medial to the ureter)	Optional
Paravesical space	Open the paravesical space	Mandatory
Direction of dissection	Start sentinel lymph node mapping at the level of the uterine artery and continue dissection LATERALLY away from the uterus	Mandatory
	Start sentinel lymph node mapping at the level of the uterine artery and and continue MEDIALLY toward the uterus	Optional
	Start sentinel lymph node mapping at the level of the uterine artery and and continue toward the pre-sacral area	Optional
	Start sentinel lymph node mapping at the most highlighted node and dissect proximally (TOWARD cervix)	Optional
	Start sentinel lymph node mapping at the most highlighted node and dissect cephalad (AWAY from cervix)	Optional
Dissection technique	Use blunt or electrosurgical technique Avoid disrupting lymphatic channels during dissection Ensure isolation of node from local anatomy	Mandatory Mandatory Mandatory
Definition of the sentinel node	A sentinel node is defined as ... ▶ The most proximal node (The most proximal node is defined as the node closest to the uterus, regardless of location), irrespective of the nodal station in which the node is found ▶ A single mapped node or a single node plus its next station echelon node(s).	Mandatory Mandatory
SLN dissection	SLN dissection should be completed in one hemi-pelvis before proceeding to the contralateral side	Mandatory
Troubleshooting	Troubleshooting when no nodes are mapping includes any one, or combination of, the following options: ▶ Wait, undertake dissection on the contralateral side before returning to original side ▶ Extend retroperitoneal dissection to encompass common, pre-sacral and/or paraaortic areas ▶ Re-inject ICG ▶ Undertake a side-specific lymphadenectomy	Mandatory
Specimen extraction	Removal of nodes without using a containment device	Prohibited
Proof of sentinel node	Use ex-vivo green fluorescence to prove the sentinel node	Mandatory
Specimen labeling	Label specimens according to laterality (right/left) AND nodal station (obturator/external iliac/internal iliac/presacral/common iliac/aortic/caval)	Mandatory
Ultrastaging	Use enhanced pathology techniques, such as immunohistochemistry, for ultrastaging of sentinel nodes	Mandatory

Final consensus on mandatory and prohibited steps of sentinel lymph node dissection (SLND) by minimally invasive surgery in endometrial cancer. ICG, indocyanine green.

Figure 1 Operation guide

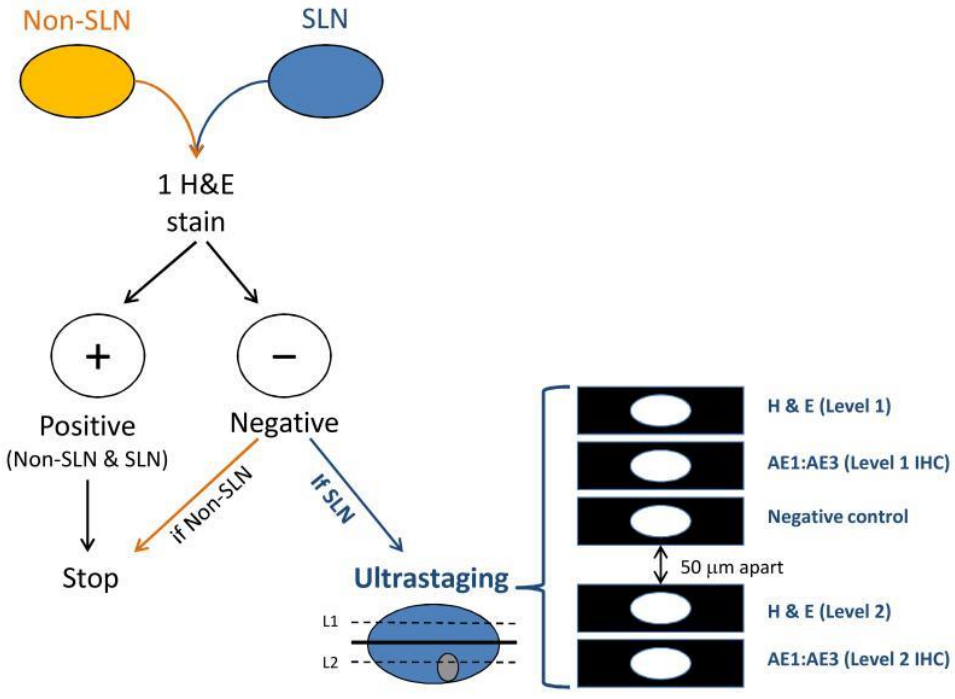

Figure 2 Memorial Sloan-Kettering Cancer Center's Pathologic Ultrastaging Algorithm for Sentinel Lymph Nodes