When guidelines are silent: Prognosis and therapeutic implications in molecular high-risk non-myoinvasive Endometrial Cancer

1. Background

Endometrial cancer is currently classified into four molecular subtypes with distinct prognostic implications: tumours POLE-ultramutated (POLEmut), with an excellent prognosis; mismatch repair deficient (MMR-d) and no specific molecular profile (NSMP) showing an intermediate prognosis and tumours p53-abnormal (p53abn), exhibiting the poorest prognosis (1-5). Among NSMP tumours, further stratification based on estrogen receptor (ER) status has revealed important prognostic differences. Multiple studies have demonstrated that ER-negative NSMP tumours (defined as tumours with <10% ER positivity) exhibit worse outcomes, comparable to those of p53abn tumours (6-11).

Given these prognostic differences, molecular classification is now integrated into recurrence risk stratification (12), which informs adjuvant treatment decisions: patients with early-stage POLEmut tumours should not receive adjuvant therapy, regardless of stage, grade, or histotype, due to their excellent prognosis; conversely, all patients with p53abn tumours and myometrial invasion are recommended to undergo chemoradiotherapy according to the PORTEC-3 protocol.

However, three clinical scenarios remain undefined in current guidelines (12):

1. p53-abn tumours without myometrial invasion.

2. NSMP tumours ER-negative without myometrial invasion

3. High-grade NSMP tumours without myometrial invasion.

According to the 2025 ESGO guidelines, these groups are classified as having an unknown risk of recurrence, with insufficient data to support the use or type of adjuvant therapy (Level IV, Grade C). These subgroups were not included in historical randomized clinical trials. Consequently, decisions on adjuvant treatment in these patients remain highly individualized or guided by institutional protocols.

Several studies have sought to explore outcomes in similar patient populations. In a cohort of 1709 patients with Stage I serous carcinoma without myometrial invasion, Nasioudis et al (13) found that adjuvant chemotherapy was associated with improved survival compared to no therapy or radiotherapy alone. Similarly, Kurnit et al (14) described an improved recurrence-free and overall survival with adjuvant chemotherapy in 238 patients with serous tumours confined to the endometrium. In a recent study published by Cucinella et al (15) including 97 cases of carcinosarcoma without myometrial invasion, chemotherapy did not

significantly improve outcomes, with most recurrences being distant. However, the only study focusing on the molecular subtype rather than histology was published recently by Jamieson et al (16), analysing recurrence rates among p53abn tumours with and without myometrial invasion. They found recurrence rates of 16% in non-myoinvasive tumours and 17% in tumours with <50% myometrial invasion. In patients with stage IA p53abn tumours without myometrial invasion treated with chemotherapy ± radiation, the recurrence rate was only 8%. All but one recurrence in this group were distant, with only one pelvic recurrence and no isolated vaginal vault recurrences—questioning the effectiveness of vault brachytherapy in preventing distant metastases.

For NSMP tumours that are ER-negative or high-grade without myometrial invasion, data are even more limited. No consistent studies have evaluated the prognostic impact or benefit of various adjuvant therapy modalities in this subgroup or the rate of lymph node involvement.

2. Justification

Recent advances in molecular classification have substantially redefined prognostic assessment in endometrial cancer, identifying subgroups with markedly different clinical behaviors.

Among these, p53-abnormal tumours and NSMP tumours that are estrogen receptor–negative or high-grade are associated with significantly worse outcomes. However, because these subgroups without myometrial invasion are relatively uncommon scenarios, they were not adequately represented in randomized trials and, therefore, their risk of recurrence is considered unknown according to the 2025 ESGO–ESTRO–ESP guidelines. Consequently, the current lack of evidence leads to substantial heterogeneity in daily clinical practice regarding surgical staging and adjuvant treatment.

Emerging evidence suggests that some non-myoinvasive tumours with high-risk molecular features may nonetheless show aggressive behavior, including distant recurrences, and may benefit from systemic therapy. Furthermore, the true rate of lymph-node involvement in these molecular subgroups is poorly defined, limiting accurate staging and risk stratification.

A multicentre evaluation is therefore essential to clarify the prognostic impact of these subgroups, determine their likelihood of nodal and extrauterine spread, and compare oncologic outcomes according to adjuvant treatment strategies. Generating robust evidence in this area is critical to guide future recommendations, reduce variability in management, and optimize outcomes for patients with these understudied, high-risk molecular profiles.

3. Hypothesis

1. We hypothesize that **the rate of pelvic and para-aortic lymph node involvement in these subgroups is below 10%.** This hypothesis is based on the biological premise that the absence of myometrial invasion limits access to lymphovascular pathways, thereby reducing the likelihood of nodal dissemination, even in molecular subgroups traditionally associated with aggressive behavior.

2. We hypothesize that **the addition of adjuvant chemotherapy in these patients confers a significant prognostic benefit in terms of progression-free survival and overall survival.** Given the aggressive behavior of these tumours, systemic treatment could reduce the risk of early distant recurrence, which is not adequately controlled by local therapies. If confirmed, this would support the use of adjuvant chemotherapy as part of a personalized therapeutic strategy guided by molecular biology in this population.

3. We hypothesize that **the pattern of recurrence in these patients is predominantly distant rather than locoregional.** This hypothesis is supported by the intrinsic aggressive biology of these molecular subtypes, which may favor early systemic dissemination over local spread. If confirmed, these findings would further question the effectiveness of local adjuvant strategies alone, such as vaginal brachytherapy, and reinforce the potential role of systemic therapy in reducing distant relapse in this population.

4. Objectives

Primary Objective

To determine the rate of pelvic and para-aortic lymph node involvement in patients with:

- p53-abnormal endometrial cancer without myometrial invasion.
- ER-negative NSMP tumours without myometrial invasion.
- High-grade NSMP tumours without myometrial invasion.

Secondary Objectives

- To describe the recurrence rate, progression-free survival, overall survival, and recurrence patterns in patients with p53abn tumours, ER-negative NSMP tumours, or high-grade NSMP tumours without myometrial invasion (FIGO 2023 stage IC).
- To compare recurrence rate, progression-free survival, overall survival, and recurrence patterns in patients with p53abn tumours, ER-negative NSMP tumours, or high-grade NSMP tumours without myometrial invasion according to adjuvant treatment received:
  - No adjuvant treatment,
  - Radiotherapy or brachytherapy.

- Chemotherapy ± radiotherapy or brachytherapy.

5. Methods & Study Design

This is a **multicentre, observational cohort study** including consecutive patients diagnosed between 2010 and 2025 with:

- p53-abnormal (p53-abn) endometrial cancer without myometrial invasion
- ER-negative NSMP tumours without myometrial invasion
- High-grade NSMP tumours without myometrial invasion

All patients must have undergone primary surgical treatment and have a molecular profile including, at minimum, immunohistochemical analysis of MMR proteins and p53, performed either on the preoperative biopsy or on the surgical specimen. POLE analysis is not mandatory. Molecular profiling performed by next-generation sequencing (NGS) is also accepted.

Given that the molecular subgroups under study are rare clinical scenarios, a multicentre design is proposed to ensure an adequate sample size. Reference centres with expertise in endometrial cancer and molecular profiling will be included.

Clinical and pathological data will be registered in a REDCap standardized database (the access will be granted to all participating centres) enabling a reliable and structured further data analysis.

Pathological and Molecular Assessment

Molecular profiling may be performed either on the preoperative biopsy or on the hysterectomy specimen, interchangeably. Assessment of MMR proteins, p53, and estrogen receptor expression (the latter only in NSMP tumours) is mandatory. POLE analysis is not mandatory. Molecular profiling may also be determined by next-generation sequencing (NGS).

Definitions

- **MMR-deficient:** Loss of expression of one or more MMR proteins. The two-antibody approach is equivalent to the four-antibody approach. In case of equivocal or heterogeneous mismatch repair immunohistochemistry results, it should be supplemented by microsatellite PCR.
- **p53-abnormal:** Aberrant p53 staining pattern (overexpression, null, or cytoplasmic). *TP53* mutational analysis is a good alternative to p53 testing by immunohistochemistry and should be used when immunohistochemistry is equivocal or heterogeneous.

- **ER (Estrogen Receptors):**
  - **ER-negative:** Defined as **<10% nuclear staining in tumour cells by immunohistochemistry**.
  - **ER-positive:** Defined as **≥10% nuclear staining in tumour cells**.

It is important that **the exact percentage of ER expression is specified in the pathology report**, rather than a qualitative interpretation (i.e., positive/negative only), to ensure consistent molecular stratification across participating centres. Whenever ER expression has not been quantified, participating institutions are kindly encouraged to **review the original immunohistochemistry and provide the percentage** if feasible.

Endometrial carcinoma with multiple classifier features should be classified according to their genomic driver, such as a pathogenic *POLE* mutation (combination of *POLE*mut with p53abn or MMRd, or both) or mismatch repair deficiency (combination of MMRd and p53abn) (12).

Positive nodes will be subclassified into macrometastasis (>2 mm) and micrometastasis (0.2 mm–2 mm). Isolated tumour cells (ITCs) will not be considered N1.

6. Patients

Inclusion criteria

1. Patients over 18 years old.
2. Patients with:
   a. p53-abnormal (p53-abn) endometrial carcinoma.
   b. ER-negative NSMP tumours.
   c. High-grade NSMP tumours.

   In whom:
   - No myometrial invasion is present in the final surgical specimen (nor cervical or adnexal invasion).
   - Tumours are identified by endometrial biopsy or hysteroscopic polypectomy with no residual tumour in the final surgical specimen (these cases will also be considered tumours without myometrial invasion).

3. Patients who underwent nodal staging, either with pelvic sentinel lymph node biopsy, associated with pelvic and/or para-aortic lymphadenectomy, or staged by lymphadenectomy alone.
   a. To assess the rate of lymph node involvement, we will analyze tumors with the previous molecular characteristics mentioned and no myometrial invasion, regardless of whether lymph node involvement is present. Stage IIIC tumours will not be excluded; cases will be selected based on the absence of myometrial infiltration in the final pathology specimen.

b. On the other hand, for the survival statistical analyses, only patients with a final stage IC (FIGO 2023) will be included.

Exclusion criteria:

Patients who do not meet the above inclusion criteria and who additionally present with the following conditions will be excluded:

a. Molecular profile not available.
b. Absence of nodal staging (sentinel lymph node biopsy or lymphadenectomy)
c. Inadequate follow-up.
d. Incomplete information regarding adjuvant treatment.

7. Statistical analysis

The statistical analysis will be composed by the following stages:

a) Description of lymph node involvement rates.
b) Description of recurrence, progression-free survival (PFS), and overall survival (OS) in p53abn tumours, ER-negative NSMP tumours, or high-grade NSMP tumours without myometrial invasion.
c) Comparison of recurrence, progression-free survival (PFS), and overall survival (OS) in p53abn tumours, ER-negative NSMP tumours, or high-grade NSMP tumours without myometrial invasion according to adjuvant treatment.
d) Multivariable models to identify independent prognostic factors.
e) Analysis of recurrence patterns.

Demographic and clinical data of the patients will be summarized through descriptive statistics. Quantitative continuous variables will be expressed as means and standard deviation or median and interquartile rank (IQR) depending on the distribution of the variable, while categorical variables will be described as relative frequencies. Imputation of missing values will not be performed.

The oncologic outcomes will be analyzed using the Kaplan-Meier method and the log-rank test along with the median and the 95% CI.

Univariate and multivariate adjusted Cox regression models that will be used for the comparison between groups. For the construction of the multivariate model, a selection method based on maximum likelihood estimation and Akaike information criterion (AIC) will be used, considering all relevant variables related to the primary end point. Differences will be statistically significant when two-sided P value < 0.05. The STATA statistical program (version 18.0) will be used for data analysis.

For the assessment of lymph node involvement, all patients in whom the final surgical specimen confirms the absence of myometrial infiltration will be included, regardless of

whether lymph node involvement is ultimately present or not. For survival analyses, only patients with a final FIGO 2023 stage IC will be included.

8. Ethics

Patients do not undergo any direct risk in this study beyond the exposure of private data. To minimize the risk, the personal information regarding the patients will be pseudonymized, and only the data described in this protocol will be acquired. Data will be collected in a REDCap Database, whose access will be restricted with username and password. The identification of the patient will be only possible at its reference centre.

The technical and functional roles (who collects the data and assigns the pseudonyms) and manager (who custodies the database that allows the reidentification of pseudonyms) are assumed by the same person, and the research role (who works with the pseudonyms data) is assumed by another person who will be in charge of processing the data and carrying out the planned statistical analyses.

The information will be handled in a pseudonymized way in accordance with Institutional policies and supervisory authority guidelines, Regulation (EU) 2016/679 of 27th April 2016 on the General Data Protection Regulation and the free movement of such data, as well as any national rules that may apply. This protocol will be reviewed by the ethics committee of each participant centre and data will not be loaded until the approval is obtained.

9. Relevance

This study addresses a relevant gap of knowledge (recognized by the European clinical guidelines) in the management of non-myoinvasive endometrial cancers with high-risk molecular features—specifically p53-abnormal tumours and NSMP tumours that are ER-negative or high-grade. Current clinical guidelines, identify these subgroups as having "*unknown recurrence risk"* due to the absence of robust data, resulting in substantial variability in the day-to-day practices and adjuvant treatment recommendations.

Nowadays, as reflected before, in this scenario clinical decisions are based on individual physician experience or institutional protocols rather than robust evidence, leading to significant variability in patient management and potentially adverse oncologic outcomes. By analyzing a systematically collected data on nodal involvement, patterns of recurrence, and survival outcomes across different adjuvant treatment strategies, this study has the potential to ensure future guideline updates and standardize clinical decision-making. Moreover, these results may help redefine risk stratification, identify patients who could benefit from adjuvant chemotherapy, and prevent both under and overtreatment in this poorly characterized population.

We will contribute to more personalized and evidence-based care, improving oncologic outcomes and reducing therapeutic uncertainty for women with high-risk molecular subtypes of non-myoinvasive endometrial cancer whom currently have lack clear therapeutic guidance.

10. Collection of clinical data

Clinical data will be recorded in a RedCap (R) database. Patient records will be reviewed using the hospital's electronic medical records. The following variables will be collected:

INITIAL VARIABLES

Demographic data:

- Date of birth (age, month and year).

Medical history:

- Height (cm).
- Weight (kg).
- ECOG performance status at diagnosis (0-5).
- Medical historical cancer (yes/no).
- Menopause (yes/no).

Surgery:

- **Date of sugery (**DD/MM/YYYY).
- **Approach:**
  - Laparoscopy.
  - Robotic.
  - Open.
- **Procedures performed:**
  - Hysterectomy (yes/no/non specified)
  - Bilateral adnexectomy (yes/no/non specified)
  - SLN biopsy (yes/no/non specified)
    - Type of SLN technique
      - Tc 99
      - ICG
      - Blue dye
      - Non specified
    - Drainage of SLN
      - Unilateral
      - Bilateral
      - Absence of drainage
      - Non specified
  - Pelvic lymphadenectomy (yes/no/non specified)
  - Paraaortic lymphadenectomy
    - Up to the mesenteric inferior artery
    - Up to the left renal vein
    - Non specified
  - Omentectomy (yes/no/non specified)
  - Peritoneal biopsies (yes/no/non specified)

- **Surgical complications** (yes/no)
    - Clavien-Dindo (I–V)
    - Type of complication (vascular, bladder, ureteral, bowel injury, etc.)
- **Blood loss volume** (mL)

Definitive histopathology

Grade

- G1
- G2
- G3
- Unknown

Histology

- Endometrioid
- Serous
- Clear-cell
- Carcinosarcoma
- Squamous
- Mucinous
- Mixed
- Others
- Unknown

LVSI

- Negative
- Positive
    - Focal
    - Substantial
    - Positive but not specified
- Non specified
- Unknown

Size of the tumour (mm)

Type of the tumour

- In a polyp
- Limited to endometrium but not a polyp
- Unkown

Number of sentinel lymph nodes

Number of positive sentinel lymph nodes

- Size of metastasis in positive sentinel lymph nodes
    - Macrometastasis
    - Micrometastasis
    - ITCs
    - Non specified

Number of pelvic lymph nodes (lymphadenectomy)

Number of positive pelvic lymph nodes (lymphadenectomy)

- Size of metastasis in positive pelvic lymph nodes
    - Macrometastasis
    - Micrometastasis
    - ITCs
    - Non specified

Number of paraaortic lymph nodes (lymphadenectomy)

Number of positive paraaortic lymph nodes (lymphadenectomy)

- Size of metastasis in positive paraaortic lymph nodes
    - Macrometastasis
    - Micrometastasis
    - ITCs
    - Non specified

FIGO 2009 stage

- Stage I
    - IA
    - IB
- Stage II
- Stage III
    - IIIA
    - IIIB
    - IIIC1
    - IIIC2
- Stage IV
    - IVA
    - IVB

FIGO 2023 stage (if available)

- Stage I
    - IA1
    - IA2

    - IA3
    - IB
    - IC
- Stage II
    - IIA
    - IIB
    - IIC
- Stage III
    - IIIA
    - IIIB
    - IIIC1i
    - IIIC1ii
    - IIIC2i
    - IIIC2ii
- Stage IV
    - IVA
    - IVB
    - IVC

Molecular profile

- P53abnormal
- NSMP estrogen-negative
- NSMP high grade

Adjuvant treatment

- **No adjuvant treatment**
- **Brachytherapy**
    - Total Dose (Greys)
    - Number of fractions
    - Date of end of brachytherapy (DD/MM/YYYY)
- **External beam radiotherapy**
    - Pelvic field
    - Pelvic and paraaortic field
    - Field non specified
    - Total Dose (Greys)
    - Number of fractions
    - Date of end of brachytherapy (DD/MM/YYYY)
- **Chemotherapy**
    - Date of first chemotherapy cycle (DD/MM/YYYY)
    - Date of last chemotherapy cycle (DD/MM/YYYY)
    - Number of cycles
    - Type of chemotherapy
        - Platinum based (yes/no)
        - Others (yes/no)

- **Concurrent chemoradiotherapy** (yes/no)
- **Inmunotherapy** (yes/no)

Recurrence

- Did the patient present a recurrence? (yes/no)
- Date of recurrence (DD/MM/YYYY)
- Location of the recurrence
    - Vaginal (yes/no)
    - Pelvic (yes/no)
    - Carcinomatosis (yes/no)
    - Nodal (pelvic) (yes/no)
    - Nodal (aortic) (yes/no)
    - Nodal (other sites than pelvic and aortic nodes) (yes/no)
    - Distance (yes/no)
        - Lungs
        - Bones
        - Liver
        - Spleen
        - Brain
        - Others
- Treatment of recurrence
    - Surgery
    - Brachytherapy
    - Radiotherapy
    - Chemotherapy
    - Inmunotherapy
    - Other treatments
- Disease after treatment
    - Absence of disease (complete response)
    - Partial response
    - Progression of disease

Survival

- Date of last follow-up (DD/MM/YYYY)
- Status of patient
    - Alive without disease
    - Alive with disease
    - Dead of disease
    - Dead of other causes

11. Dissemination and applicability

The coordinator centre will be responsible for the publication of the results (European (ESGO) and American (SGO) congresses). Publications at high-impact international journals will be developed in close collaboration with the participating centres. By participating in this study, all contributors acknowledge and agree to the following authorship guidelines. Acceptance of these terms will be implicit upon participation.

Immediate applicability of the project will include:

- To provide a real-world data on the prevalence of lymph node involvement in these subgroups.
- To define a prognosis according to therapeutic strategy (no adjuvant therapy, radiotherapy, or chemotherapy ± radiotherapy).
- To identify specific patterns of recurrence.

Expected clinical and scientific impact by contributing evidence to standardize recommendations in future European clinical guidelines and reducing heterogeneity in therapeutic management and supporting more precise, data-driven clinical decision-making by establishing the largest published series to date in these rare molecular subgroups.

12. Authorship Policy

Authorship of all publications will follow the recommendations of the International Committee of Medical Journal Editors (ICMJE) and standard practices for multicentre clinical research, ensuring transparency, fairness, and appropriate recognition of scientific contributions.

Given the academic nature and institutional coordination of this project, authorship order will be structured as follows:

- The **first and second authorship positions are reserved for the PhD candidate and the principal investigator of the project.**
- One **intermediate authorship position will be reserved for the study statistician.**
- **Collaborating investigators** from participating centres will be ranked according to the number of patients contributed, in descending order.
- The **last author position is reserved for the senior project supervisor from the coordinating centre**, who assumes overall scientific oversight and institutional responsibility.

If a participating centre seeks representation of more than one contributor and all proposed members meet authorship criteria, the establishment of a **collaborative group (group authorship)** may be considered to ensure appropriate recognition while maintaining a manageable author list.

Final authorship decisions will be made by the study steering committee based on objective contributions.

13. Bibliography

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