



## Impact of the new molecular classification of endometrial cancer: A French cohort study

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1   **IMPACT OF THE NEW MOLECULAR CLASSIFICATION OF ENDOMETRIAL**  
2   **CANCER: A FRENCH COHORT STUDY**

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23     **Abstract**  
24  
25     **Objective:** To evaluate the potential impact of the latest ESGO guidelines for endometrial  
26     cancer with molecular classification on the management strategy in a French cohort.  
27     **Methods:** All patients treated between January 1st, 2014 and December 31, 2020 for an  
28     endometrial cancer at the Centre Hospitalier Intercommunal de Créteil (CHIC, FRANCE)  
29     were selected from our prospectively maintained database. All postoperative samples were  
30     reviewed to confirm histological subtype, myometrial infiltration, cytonuclear grade and  
31     presence of lymphovascular emboli. Analysis of p53, MLH1, MSH2, MSH6, PMS2 genes  
32     was performed by immunohistochemistry first then a systematic POLE sequencing was  
33     performed to identify gene mutation. The impact of the latest ESGO 2020 guidelines was  
34     assessed regarding adjuvant therapy, surgical strategy, and survival.  
35     **Results:** Eighty patients were analyzed, including 70% NSMP (n = 56), 13.75% MSI (n =  
36     11), 10% p53 mutated (n = 8) and 6.25% POLEMut (n = 5). A total of 21 patients (26.3%)  
37     were reclassified using the latest ESGO classification. Patients classified at low risk or with  
38     advanced / metastatic disease were not reclassified using molecular analysis. Molecular  
39     analysis and the latest ESGO classification had the most important impact on patients initially  
40     classified at intermediate – high risk that were reclassified in intermediate (10/23) and in low  
41     (4/23) risk. Nine patients (11.3%) were overtreated according to the 2020 ESGO  
42     classification: six patients in the low – risk group (4 received vaginal brachytherapy and 2  
43     external radiotherapy) and three in the intermediate risk group (3 received external irradiation  
44     and 1 received chemotherapy). None of the patients in our cohort would have been  
45     undertreated using the 2020 ESGO classification. Patients within the p53 mutated group were  
46     the most likely to experience recurrence (37.5%, 3/8) and none of the patients POLE mutated  
47     recurred.

48   **Conclusion:** Around one in 4 patients were reclassified in a more accurate prognostic group  
49   using molecular diagnosis and the latest ESGO guidelines which could decrease the use of  
50   adjuvant therapies to spare morbidity.

51   **Keywords:** endometrial cancer; molecular classification; ESGO guidelines; survival; risk  
52   assessment; prognostic

53  
54

55      **Introduction**

56            Endometrial carcinoma (EC) is currently the most common gynecological pelvic  
57   malignancy in developed countries, accounting for 57.8% of new cases of gynecological  
58   cancers in the US in 2020 (1). Preoperative assessment of the risk of lymph node invasion is  
59   currently based on histotype and grade in patients that do not exhibit lymph node invasion on  
60   preoperative MRI (2). These parameters have been shown to have poor reproducibility (3).  
61   The generalization of the sentinel lymph node procedure even in patients classified  
62   preoperatively at low risk has significantly reduced the complication risk and the morbidity  
63   rates and reshuffled the cards (4,5).However, preoperative accurate assessment of lymph node  
64   invasion risk still matters to both anticipate adjuvant therapies and inform patients accordingly  
65   (6). Besides, lymphovascular space invasion, which could be very relevant to refine risk  
66   group, is hardly assessed on preoperative biopsy (7,8). All of these factors result in partial  
67   preoperative assessment potentially leading to inadequate surgical gestures. Moreover, the  
68   postoperative risk of recurrence assessment has been shown to have a limited predictive value  
69   as some patients at "low – risk" experience recurrences sometimes with a short delay  
70   following treatment (9).

71            In 2013, the Cancer Genome Atlas (TCGA) research network group performed an  
72   integrated genomic characterization of 373 endometrial carcinomas (EC) using sequencing  
73   and array-based technologies (10). Based on these findings, the ProMisE classification has  
74   identified four molecular groups of EC with different prognoses(11): the POLE-mut group  
75   (POLEMut), the mismatch repair-deficient group (MMRd), the p53-abn group is classified as  
76   "high copy number" and the p53-wild-type group (p53-wt) or "non-specific molecular profile"  
77   (NSMP). More than individually, the ProMise classification appears to be a beneficial and  
78   complementary contribution to the 2013 ESMO classification. Talhouk *et al* in 2017 reported  
79   that regarding the main oncological outcomes (OS, DFS and PFS), ProMisE use alone seems  
80   to perform as well as ESMO, or even better when postoperative parameters are

81 considered(12). The new ESTRO ESGO ESP 2020 guidelines have integrated the molecular  
82 classification into the management algorithms, with a modification of the risk groups and  
83 therefore of the medical and surgical management of endometrial cancers (13). The ultimate  
84 goal of applying accurate prognostic classification using molecular subtypes is to eventually  
85 reduce iatrogenic morbidity by decreasing indications of unindicated adjuvant therapies  
86 according to ESGO 2020 guidelines while efficiently reserving these treatments for patients  
87 truly at high risk.

88 To date, potential impact of these new guidelines on prognostic assessment and  
89 management of patients with endometrial cancers has not been evaluated in a French cohort to  
90 assess its external validity.

91

92

93 **Materials and methods**

94 The protocol was validated by the Research Organization Committee of the Centre  
95 Hospitalier Intercommunal de Créteil on September 26, 2019. Written consent was obtained  
96 for all patients as part of the PELVIMASS protocol (CPP No. 2016-A01381-42)

97

98 Population

99 All patients treated between January 1<sup>st</sup>, 2014 and December 31, 2020 for an  
100 endometrial cancer at the Centre Hospitalier Intercommunal de Créteil (CHIC, FRANCE)  
101 were selected from our prospectively maintained database. Patients for whom the tissue was  
102 not usable due to alterations during preservation or due to poor quality of DNA's extractions  
103 were excluded. Young patients < 18 years, those with rare histological forms, and those with  
104 numerous missing data were not included.

105 Data of interest were abstracted from patients' chart, including socio demographic  
106 characteristics, preoperative imaging and pathological analysis, prospective management  
107 including surgery and adjuvant therapies as well as survival data.

108

109 Prospective management

110 Patients were treated in accordance with European recommendations at the time of  
111 prospective management(14,15). Preoperative management included clinical examination,  
112 pelvic ultrasonography and abdomino-pelvic MRI to determine loco-regional extension,  
113 lymph node involvement and distant metastases. Tumors' markers such as cancer antigen 125  
114 (CA125) were measured in patients with type II tumors. The 2009 - FIGO classification was  
115 used to classify tumors (16).

116 Follow-up consisted of a clinical examination every 4 months for 3 years, then every 6  
117 months for 2 years and then annually. Depending on the clinical findings, the histological  
118 type of the tumor and the initial extension of the tumor, a thoraco-abdomino-pelvic CT scan

119 could be requested as well as a biological evaluation including tumor markers CA125 for  
120 non-endometrioid tumors.

121

122 Pathological et molecular analysis

123 All postoperative samples were reviewed to confirm histological subtype, myometrial  
124 infiltration, cytonuclear grade and presence of lymphovascular emboli. A systematic analysis  
125 of p53, MLH1, MSH2, MSH6, PMS2 genes was performed first by immunohistochemistry.  
126 Immunohistochemical staining was performed on a Ventana BenchMark Ultra<sup>©</sup> machine,  
127 according to the protocols of the various antibody suppliers. The Thermo Fisher<sup>©</sup> monoclonal  
128 antibody (DO-7 clone) was used for p53 testing. Results were characterized in 2 categories: a  
129 heterogeneous positivity classified the sample as wild type. A strong and diffuse positivity (over-expression)  
130 or a complete absence of marking (negative) classified the sample as abnormal. A systematic POLE sequencing was performed to identify gene mutation. This was  
131 first screened by HRM (High Resolution Matching) PCR to select samples with suspected  
132 POLE gene mutation. In order to precisely characterize the type of mutation, a gene  
133 sequencing technique (Next-Generation Sequencing or NGS) was performed on the samples  
134 previously selected by HRM.

136 In cases of loss of expression of immunophenotypic markers or ambiguity of the  
137 immunostaining, a molecular technique was used using Idylla<sup>©</sup> (Biocartis, Mechelen,  
138 Belgium). Eight cases had microsatellite instability searched using PCR prior the initiation of  
139 this study.

140

141 Assessment of the new ESGO 2020 classification impact

142 All patients were reclassified according to the new ESGO 2020 classification, using  
143 molecular analysis. The new risk group was then compared with the initial risk assessed

144 during prospective management. The impact of the new ESGO 2020 guidelines was assessed  
145 by comparing adjuvant therapy and surgical strategy.

146 Survival of patients according to histological characteristics, prognostic risk group, and by  
147 molecular group were analyzed.

148

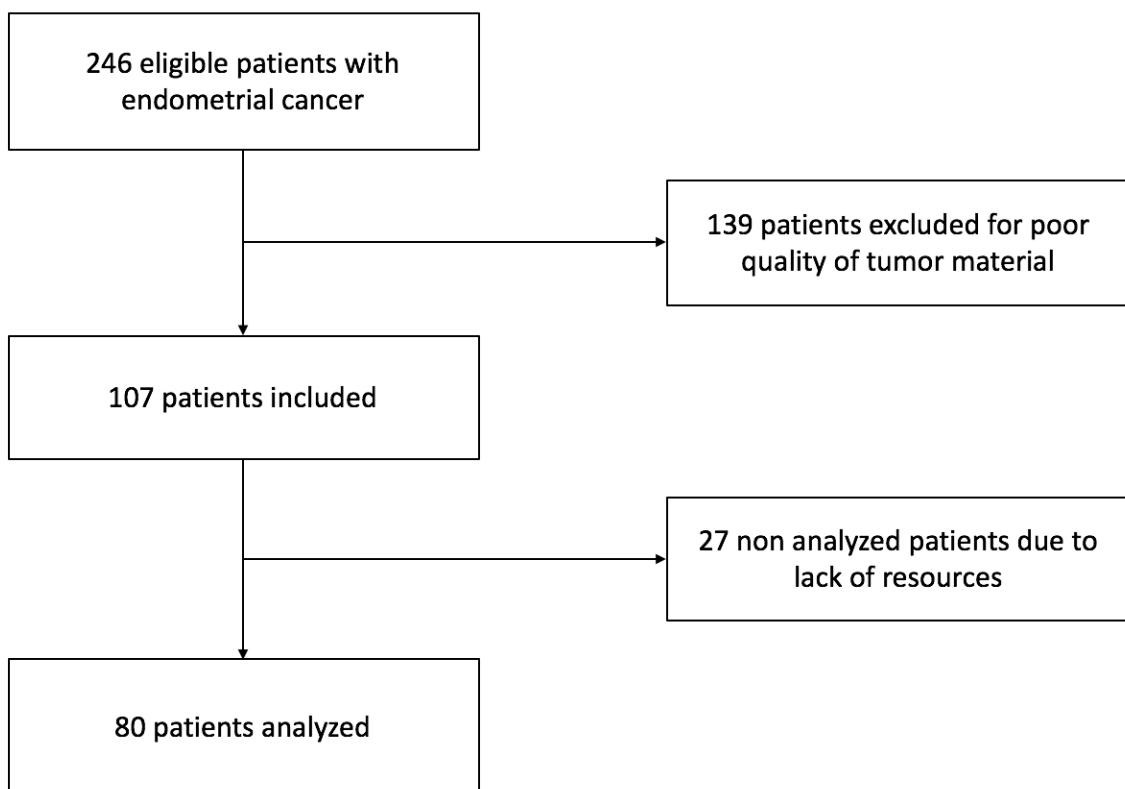
149 Statistical analysis

150 The data used were collected on a secure Excel sheet (Microsoft Corporation, Redmond, WA,  
151 USA) and all statistical analyses were performed using the freely available online R software  
152 (version 1.3.1093). For all analyses performed, a p-value < 0.05 was considered to indicate a  
153 statistically significant difference. Categorical variables were compared using a Chi<sup>2</sup> or  
154 Fisher test according to the number of participants, and quantitative variables were compared  
155 using a Student's t test. Kaplan-Meier survival curves were generated to assess recurrence-free  
156 survival and overall survival according to the groups determined. The log-rank test was used  
157 to compare survivals.

1    **Results**

2       Among the 246 eligible patients, 107 patients were included and 139 patients were  
3       excluded due to poor quality of tumor material. Eventually, 27 unselected patients did not  
4       undergo molecular analysis due to lack of resources during the COVID 19 pandemic and thus  
5       were excluded leading to a total of 80 patients analyzed (Figure 1).

6



7

8       Figure 1: Flow chart of the study

9

10      Characteristics of the population

11     The main characteristics of the patients included are displayed in Table 1. The mean age was  
12     66 years old (range 34-87 years old) with an average BMI of 31kg/m<sup>2</sup> (range 20-51kg/m<sup>2</sup>).  
13     Diagnosis was obtained through endometrial biopsies in 76% (61/80) cases and operative  
14     hysteroscopy in 24% (19/80) cases. Patients that could not undergo molecular analysis due to  
15     COVID 19 pandemic were similar to those that did (Supplementary Table 1 and 2).

16 On preoperative MRI, 13.8% of patients (11/80) had pelvic lymph node involvement and  
17 6.3% (5/80) had para-aortic lymph node involvement (Table 1). Most patients had stage I  
18 endometrial cancer 72.5% (58/80).

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Characteristics	Final population <b>N = 80 (%)</b>
Age in years (mean ± sd)	66 ± 11.6
Body mass index (kg/m <sup>2</sup> ) mean (± sd)	31 (± 7.1)
Nulliparity	18 (26.5)
High blood pressure	43 (54)
Diabetes	14(18)
Menopausal	70 (87.5)
Bleeding	70 (87.5)
FIGO MRI stage IA IB II III IV	25 (31.25) 33 (41.25) 5 (6.25) 7 (8.75) 6 (7.5)

Surgery	
HBSO	78 (97.5)
Total Hysterectomy and ovarian sparing	2 (2.5)
Omentectomy	11 (13.8)
Appendectomy	6 (7.5)
Pelvic sentinel node	3 (3.8)
Pelvic lymphadenectomy	30 (37.5)
Para-aortic lymphadenectomy	26 (32.5)
Inguinal lymphadenectomy	3 (3.8)
External beam radiotherapy	32 (40)
Neoadjuvant chemotherapy	1 (1.3)
Adjuvant chemotherapy	21 (26.3)
Brachytherapy	56 (70)
Preoperative ESMO	
Low	22 (27)
Intermediate	29 (39)
High	26 (32.5)
NA	3

29 Table 1: Characteristics of the study population, treatments received by patients and  
30 ESMO/ESGO 2013 preoperative classification. HBSO:  
31 Total hysterectomy with bilateral salpingo-oophorectomy  
32 NA: Not assessed

33 Patients' management

34 Lymph node staging was performed by sentinel node procedure in 3.8% cases, by pelvic  
35 lymphadenectomy in 37.5% and para-aortic lymphadenectomy in 32.5% of the cases. No  
36 lymph node staging was performed in 56% (45/80) and 17.5% of the patients (14/80)  
37 underwent secondary surgery for lymph node staging.

38 Discrepancy between pre and postoperative histology occurred in 11.6% (7/60) and 10.5%  
39 (2/19) of patients diagnosed by endometrial biopsy and operative hysteroscopy, respectively.

40 Regarding adjuvant therapies, brachytherapy, external radiotherapy and chemotherapy were  
41 used in 70% (56/80), 40% (32/80), and 26.3% (21/80), respectively (Table 1).

42

43 **Comparison of ESGO 2020 and ESMO 2016**

44 The NSMP group was the most represented (70%, 56/80), followed by the MSI (13.75%;  
45 11/80), the mutated P53 (10%, 8/80) and the POLEmut (6.25%, 5/80) groups (Table 2).  
46 Morphological characteristics of tumors according to molecular group are described in  
47 Supplementary Table 3.

48

	N = 80 (%)
<b>Histological type</b>	
Endometrioid	70 (87.5)
Serous	7 (8.75)
Serous + Endometrioid	1 (1.25)
Clear cell	2 (2.5)
<b>Grade</b>	
Low grade	62 (77.5)
High grade	18(22.5)
<b>LVSI</b>	
0	37 (46)
<5	13 (16)
>5	30 (38)
<b>Molecular group</b>	
POLE	5(6.25)
MSI	11(13.75)
NSMP	56 (70)
P53	8 (10)

49

50 Table 2: Histological and molecular characteristics of the study population

51

52 A total of 21 patients (26.3%) were reclassified following application of the new  
53 ESGO 2020 classification (table 3). Concordance between the two classifications regarding  
54 postoperative risk was observed in 73.7% (59/80). Patients classified at low risk or with  
55 advanced / metastatic disease were not reclassified using molecular analysis. Molecular  
56 analysis and the latest ESGO classification had the most important impact on patients initially  
57 classified at intermediate – high risk that were reclassified in intermediate (10/23) and in low  
58 (4/23) risk.

59 Two patients with clear cell adenocarcinoma classified NSMP were considered at high – risk.

60

ESMO 2016 ESGO 2020	Low	Intermediate	Intermediate high	High	Advanced/metastatic
Low	18 (75%)	2 (8%)	4 (17%)	0	0
Intermediate	0	10 (50%)	10 (50%)	0	0
Intermediate high	0	0	9 (64%)	5 (36%)	0
High	0	0	0	15 (100%)	0
Advanced metastatic	0	0	0	0	7 (100%)

61

62 Table 3: Number of patients classified into risk groups according to ESMO 2016 and ESGO  
63 2020 recommendations. Proportions are calculated based on the ESGO 2020 group size.  
64

## 65 Impact of the molecular classification

66 Twelve patients (15%) had lymph node involvement on final analysis. Of these patients, 50%  
67 were p53 mutated and 33% had no specific molecular profile. All of these patients were  
68 classified as high risk (58%) or advanced/metastatic (42%). Patients in the p53mutated group  
69 had lymph node involvement in 75% cases (6/8). The distribution of patients with lymph node  
70 involvement by molecular group and prognostic classification is presented in Table 4.

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	Pelvic lymph node involvement (N=7)	Para-aortic lymph node involvement (N=9)	Lymph node involvement (N=12)
Molecular group			
<ul style="list-style-type: none"> <li>• POLE mutated (N=5; 6.25%)</li> <li>• MSI (N=11; 13.75%)</li> <li>• NSMP (N=56; 70%)</li> <li>• P53 mutated (N=8; 10%)</li> </ul>	0  0  3 (5.4%)  4 (50%)	0  2 (18%)  2 (3.6%)  5 (62.5%)	0  2 (18%)  4 (7.1%)  6 (75%)
ESGO Risk Group 2020			
<ul style="list-style-type: none"> <li>• Low (N=24; 30%)</li> <li>• Intermediate (N=20; 25%)</li> <li>• Intermediate-high (N=14; 17%)</li> <li>• High (N=15; 19%)</li> <li>• Advanced/metastatic (N=7; 9%)</li> </ul>	0  0  0  4 (27%)  3 (43%)	0  0  0  4 (27%)  5 (71%)	0  0  0  7 (47%)  5 (71%)

76

77 Table 4: Node involvement by molecular group and ESGO 2020 risk group. The proportions  
78 of lymph node involvement were calculated according to the size of the molecular groups or  
79 the 2020 risk groups

80

81

82 **Impact of the ESGO 2020 classification**

83 The 2020 ESGO classification could have spared secondary surgery for staging in 21.4%  
84 (3/14) of patients classified at low or intermediate risk. According to the latest ESGO 2020  
85 guidelines, 86% (69/80) of our patients could have benefited from the sentinel lymph node  
86 procedure (only intermediate-high risk or high - risk patients with FIGO stage >2 are not  
87 eligible).

88 A total of 9 patients (11.3%) were overtreated according the 2020 ESGO classification: Six  
89 patients in the low – risk group (4 received vaginal brachytherapy and 2 external  
90 radiotherapy) and three in the intermediate risk group (3 received external irradiation and 1  
91 received chemotherapy).

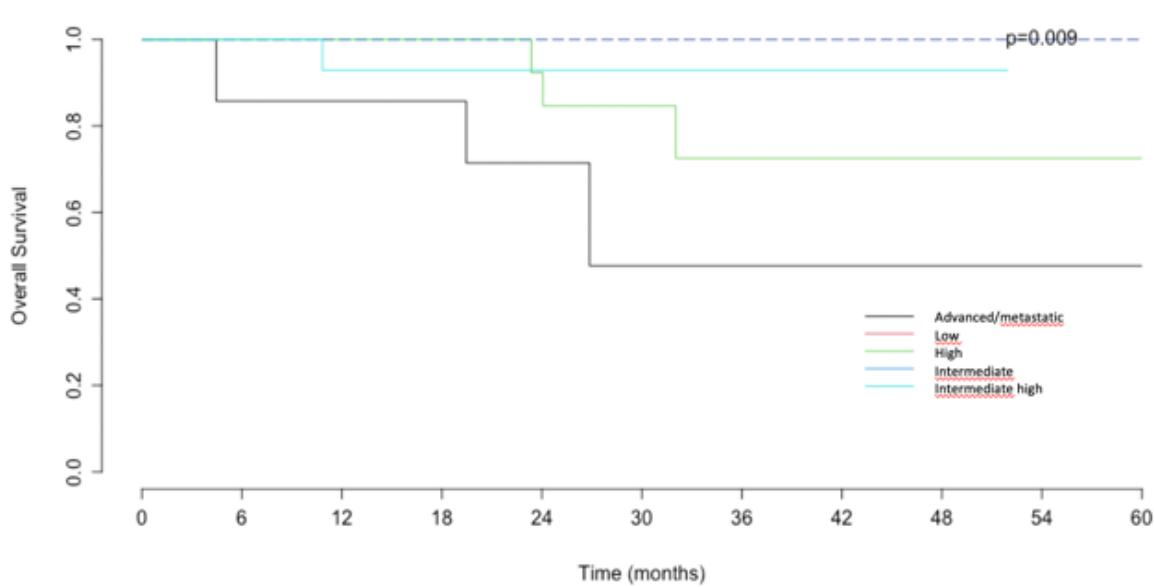
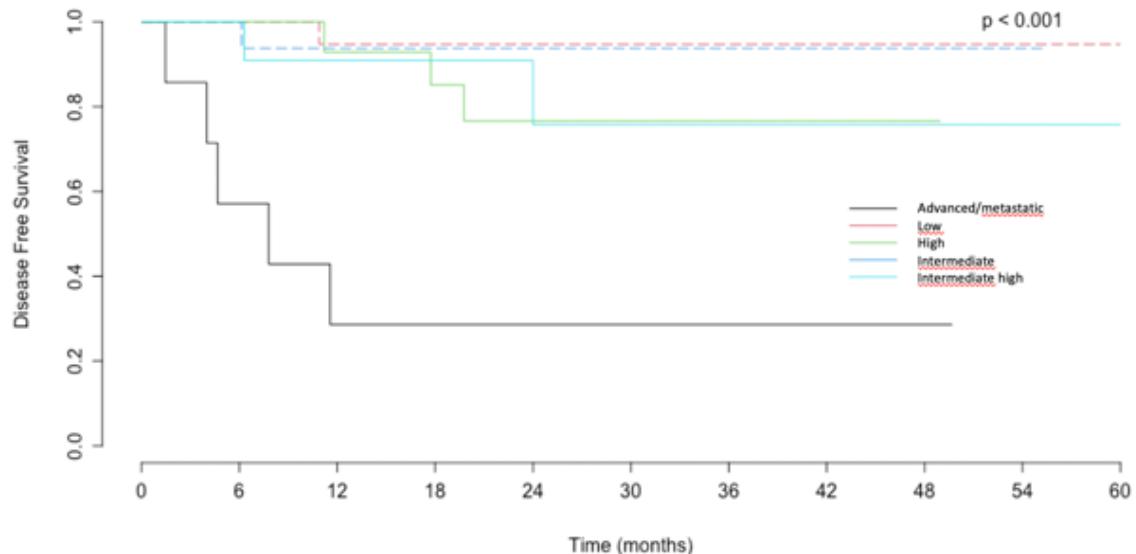
92 None of the patients in our cohort would have been undertreated.

93

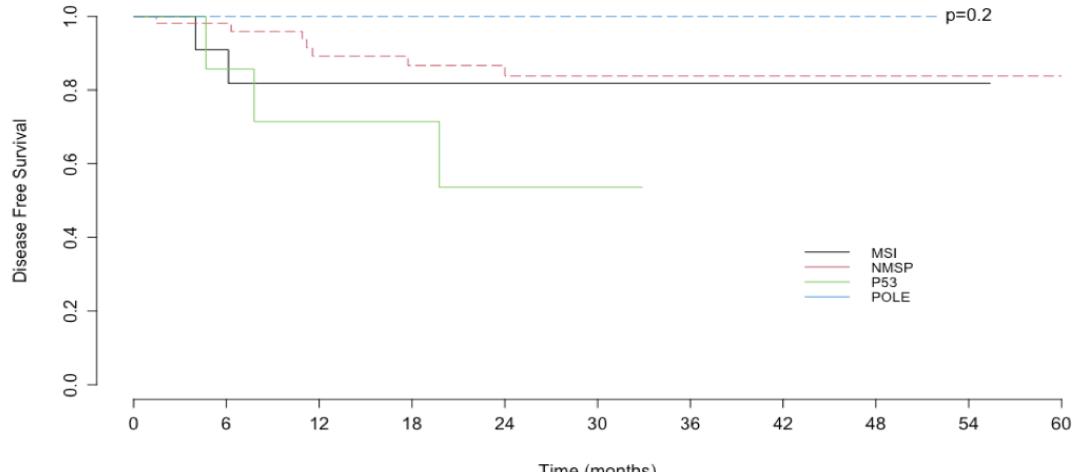
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95 Survival analysis

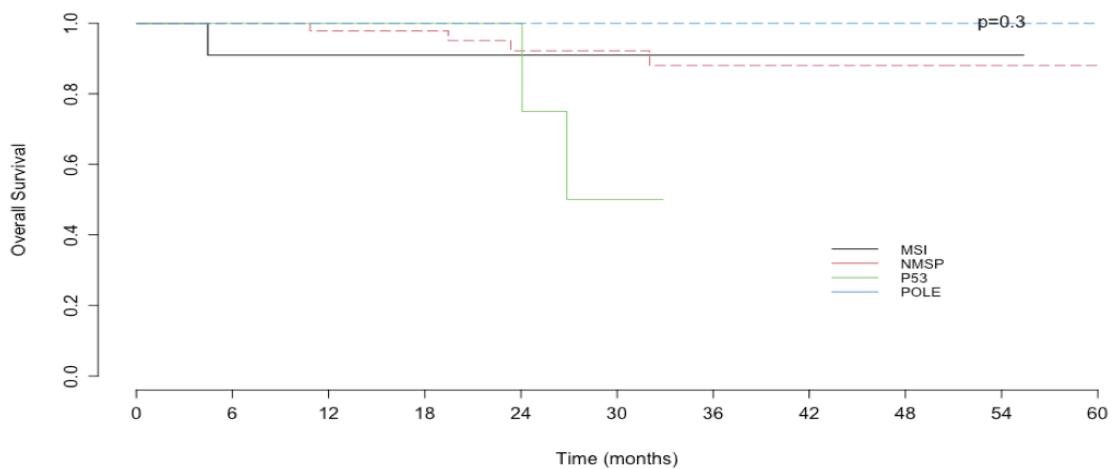
96 The median follow-up time was 25 months (0-64). During follow-up, 12 patients relapsed  
97 (15%) and 7 patients died (9%). The 2020 ESGO postoperative risk groups but not molecular  
98 subtypes were significantly associated with disease free survival ( $p < 0.001$ ) and overall  
99 survival ( $p = 0.005$ ) (Figure 2 and 3). Survival curves according to the histological type, the  
100 FIGO stage, the cytonuclear grades, the presence of lymphovascular emboli and the ESMO  
101 2016 classification are available in the Supplementary Figure 1



104  
105 Figure 2: Recurrence-free survival (left) and overall survival (right) stratified by risk groups  
106 according to ESGO/ESTRO/ESP 2020 (in months). There were a significative difference  
107 between groups (p-value 0.001 and 0.009 respectively).  
108



109



110

111 Figure 3: Recurrence-free survival (left) and overall survival (right) stratified by molecular  
 112 groups according to ESGO/ESTRO/ESP 2020 (in months). There was not a significant  
 113 difference between groups neither for recurrence – free nor overall survival (p-value =  
 114 0.2 and 0.3, respectively)  
 115

116 Relapses occurred on average at 9 months (1 - 23) and were localized as follow: locoregional  
 117 (vagina = 1, rectum = 3, parametrium = 2, pelvic non - specified = 6), lymph node (para-  
 118 aortic, n = 6) and distant (peritoneum = 5, lung = 5, liver = 5 and bone = 1). Patients within  
 119 the p53 mutated group were the most likely to experience recurrence (37.5%, 3/8), followed  
 120 by those MSI (18%, 2/11) and NSMP (12.5%, 7/56). None of the patients with a POLE  
 121 mutation recurred. Location of recurrence varied with the molecular subtype. In patients with  
 122 NSMP, 86% (6/7) had distant recurrence. All p53 patients had a distant relapse and one  
 123 patient also had locoregional recurrence. Patients in the MSI group had pelvic recurrences  
 124 without distant lesions.

125 Patients were most likely to die during follow – up when p53 mutated (25%; 2/8) than when  
126 part of the NSMP (7%, 4/56) or MSI (9%, 1/11) groups (p=0,3)

127 The distribution and characteristics of patients who recurred or died according to their risk  
128 group or molecular status are presented in Tables 5 and Supplementary Table 4.

129

	Patients that had recurrence during follow - up (N=12)	Patients that died during follow - up (N=7)
Molecular group		
• POLE mutated (N=5)	0	0
• MSI (N=11)	2 (18%)	1 (9%)
• NSMP (N=56)	7 (12.5%)	4 (7%)
• P53 mutated (N=8)	3 (37.5%)	2 (25%)
ESGO Risk Group 2020		
• Low (N=24; 30%)	1 (8.3%)	0
• Intermediate (N=20; 25%)	1 (8.3%)	0
• Intermediate-high (N=14; 17%)	2 (16.7%)	2 (28.6%)
• High (N=15; 19%)	3 (25%)	2 (28.6%)
• Advanced/metastatic (N=7; 9%)	5 (41.7%)	3 (42.8%)
ESGO Risk Group 2016		
• Low (N=18; 23%)	1 (8.3%)	0
• Intermediate (N=12; 15%)	0	0
• Intermediate-high (N=23; 28%)	2 (16.7%)	1 (14.3%)
• High (N=20; 25%)	4 (33.3%)	3(42.8%)

● Advanced/metastatic (N=7; 9%)	5 (41.7%)	3 (42.8%)
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130 Table 5: Distribution in molecular groups and prognosis of recurrence and death  
131  
132

133      **Discussion**

134      In this first report of a French cohort following the latest issue of ESGO guidelines for  
135      endometrial cancer, around ¼ of the patients were reclassified into a more accurate group of  
136      prognosis. Molecular analysis and the latest ESGO classification had the most important  
137      impact on patients initially classified at intermediate – high risk that were reclassified in  
138      intermediate (10/23) and in low (4/23) risk. The 2020 ESGO classification could have spared  
139      secondary surgery for staging in 21.4% of patients classified at low or intermediate risk. A  
140      total of 9 patients (11.3%) were over-treated according to the 2020 ESGO classification: six  
141      patients in the low – risk group (4 received vaginal brachytherapy and 2 external  
142      radiotherapy) and three in the intermediate risk group (3 received external irradiation and 1  
143      received chemotherapy). None of the patients in our cohort were undertreated. The 2020  
144      ESGO postoperative risk groups but not molecular subtypes were significantly associated  
145      with disease free survival ( $p < 0.001$ ) and overall survival ( $p = 0.005$ ).

146      In our cohort, the molecular group distribution included a higher proportion of NSMP tumors  
147      than the study of Kommooss et al.(17) and the meta-analysis of Raffone et al. (18) that  
148      included 2818 patients, but with an equivalent proportion of POLEmut and p53 mutated.  
149      Patients diagnosed either in the POLEmut group or in the p53 mutated group were little  
150      represented (16.75%). These two groups are associated with extreme prognoses with very low  
151      and high risk of recurrence / death, respectively. Such discrepancy in the repartition of the  
152      molecular groups could be explained by the limited number of patients included, leading to an  
153      over-representation of patients classified NMSP that have mild benefit of the molecular  
154      subtype assessment. Regarding pathological characteristics, our findings were consistent with  
155      previous study(12,19). In the studies by Talhouk et al, the POLEmut group was composed of  
156      92% of endometrioid tumors including 58% of low-grade tumors and 58% with LVSI. Of  
157      note, a significant proportion of POLEmut patients in our cohort had a myometrium  
158      infiltration > 50% (80%) with 40% LVSI which are poor prognostic factors for recurrence

159 and survival. Our findings highlight the limited value of these parameters to assess the risk of  
160 recurrence and advocate for molecular diagnosis use to decrease adjuvant therapies in patients  
161 with excellent prognosis. On the other side, patients with p53 mutation usually have  
162 numerous factors associated with bad prognosis with 75% serous tumors with 88% of LVSI  
163 and infiltration of myometrium > 50%. In our cohort, significant survival differences existed  
164 by ESGO 2020 groups but not by the different molecular groups. These results are conflicting  
165 with those reported by Talhouk et al. (12,20,21) that found that compared to the "non -  
166 specific molecular profile" group, the risk was reduced by 77% in overall survival rates and  
167 84% in recurrence-free survival rates for the POLE group, whereas the risk of death or  
168 recurrence was multiplied by 3.29 and 2.19 times respectively for the p53 mutated group. The  
169 main issue with molecular analyses remain the availability of the technic, limited by both the  
170 cost and the time – consuming procedure. In the case of the POLE mutation research, High  
171 Resolution Melting (HRM) screening of candidates for gene sequencing by NGS allows to  
172 limit the final cost of the analysis, with a unit cost of 10 € for HRM against 120 € for Next  
173 Generation sequencing (NGS). The time required for molecular biology analysis of POLE  
174 mutations or microsatellites can be long when confirming cases in NGS or for microsatellite  
175 analysis. McConechy et al. reported a concordance rate of more than 93% for the diagnostic  
176 performance of immunohistochemistry and molecular biology (22). This problematic is  
177 relative for p53 analysis as IHC has a high performance (Se: 90-100%, Sp: 94%, PPV: 98%,  
178 NPV: 74%)(3). When adjuvant therapy decision relies on molecular analysis, the delay to  
179 obtain results is crucial. This is all the more important since these patients could exhibit bad  
180 prognostic factors that could encourage clinicians to prescribe unindicated adjuvant therapies.  
181 In our cohort, 9 patients had unindicated adjuvant treatment according to ESGO 2020  
182 guidelines. In the PORTEC 3 study, side effects (neuropathy, alopecia, hematological,  
183 gastrointestinal, auditory side-effects, pain etc.) were significantly more important in the  
184 group treated by chemotherapy in combination with radiotherapy (23). In the study by De

185 Boer et al., toxicities and quality of life scores were higher (with more severe symptoms) in  
186 the radiochemotherapy group than in the radiotherapy alone group ( $p<0.001$ ) and seemed to  
187 improve over time (non-significant results at 12 months from the end of treatment) (24).  
188 While survival has improved over the years, recent research has focused on quality of life  
189 after treatment. It seems essential to adapt adjuvant therapies to the molecular profile by  
190 limiting indications to selected subtypes

191 Molecular diagnosis also impacts surgical staging strategy. De kerdiel et al. (25)  
192 found surgical under-staging occurred in 26% of the cases according to the 2010 guidelines.  
193 Older patients ( $>70$  years) were more often under-staged than younger patients ( $<70$  years)  
194 ( $p=0.037$ ). In a recent meta-analysis, He et al. reported a 6% rate of positive lymph nodes (7 /  
195 118) in POLE mutated patients and no significant association between the POLE mutated  
196 status and the risk of lymph node involvement (OR 0.41;  $p=0.47$ ) (26). These results are in  
197 line with our findings that no POLEmut patients had lymph node involvement that could  
198 benefit from less morbid procedure such as sentinel lymph node. Similarly, patients p53  
199 mutated are at high risk of lymph node involvement and could benefit from per-operative  
200 lymph node analysis to decide immediate complete lymphadenectomy, avoiding secondary  
201 surgery. The search for MSI status by immunohistochemistry (more accessible and faster)  
202 and the efficacy of antiPD-1 (27) treatments on these tumors in case of treatment failure  
203 reinforces the necessity for MSI systematic testing. The RAINBO (Refining Adjuvant  
204 treatment IN endometrial cancer Based On molecular profile) program, led by the  
205 TransPORTEC study group, will bring interesting insight on the value on molecular subtype-  
206 based strategy.

207 Some limitations of our work deserve to be mentioned. This is a retrospective,  
208 observational, single-center study with a limited number of patients included. Our follow up  
209 could have been too short to diagnose some recurrences or death which might have bias the  
210 results. However, it has been demonstrated that the higher rate of recurrence is within the first

211 two years of follow up (28,29). The proportions of patients with POLE, p53 and MSI were  
212 insufficient which have limited the full exploration of their prognostic impact. Eventually, a  
213 significant number of patients did not undergo lymph node staging at all which clearly limit  
214 the extent of our conclusions, especially as many cancer centers now perform sentinel lymph  
215 node procedures even in high-risk patients. This also underline the benefit of molecular  
216 subtype assessment was more likely underestimated in this cohort. One issue with molecular  
217 diagnosis is that it depends of the quality of the DNA used, which is directly impacted by cold  
218 ischemia duration, transport duration, delay prior fixation and the quality of the latter (29).  
219 The retrospective inclusion of the cases limited the control of the conditions of conservation  
220 of the slides.

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225     **Conclusion**

226         Around one in 4 patients were reclassified in a more accurate prognostic group using  
227         molecular diagnosis and the latest ESGO guidelines. which would significantly impact the use  
228         of adjuvant therapies and help plan surgical strategy. Systematic molecular subtype  
229         assessment will require easier and faster access to genetic platforms to enable short circuits  
230         useful to impact endometrial cancer strategy. Eventually, it will help plan therapeutic strategy  
231         and decrease the use of adjuvant therapies to spare morbidity.

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339 **Figures and table captions**

340 Figure 1: Flow chart of the study.

341 Figure 2: Recurrence-free survival (left) and overall survival (right) of relapse risk groups  
342 according to ESGO/ESTRO/ESP 2020 (in months)

343 Figure 3: Recurrence-free survival (left) and overall survival (right) of molecular groups by  
344 ESGO/ESTRO/ESP 2020 (in months).

345 Table 1: Characteristics of the study population, treatments received by patients and  
346 ESMO/ESGO 2013 preoperative classification

347 Table 2: Histological and molecular characteristics of the study population

348 Table 3: Number of patients classified into risk groups according to ESMO 2016 and ESGO  
349 2020 recommendations. Proportions are calculated based on the ESGO 2020 group size.

350 Table 4: Node involvement by molecular group and ESGO 2020 risk group. Pelvic lymph  
351 node involvement may be associated with para-aortic lymph node involvement. Para-  
352 aortic involvement may be associated with pelvic involvement. A total of 12 patients  
353 were classified as N+. Here are calculated the proportions of lymph node involvement  
354 according to the size of the molecular groups or the 2020 risk groups

355 Table 5: Distribution in molecular groups and prognosis of recurrence and death

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