

free DNA (cfDNA), circulating free mitochondrial DNA (cfmtDNA) and citH3 were measured by qPCR using one microliter of deactivated serum, and by ELISA assay respectively. Fragmentation pattern of serum cfDNA was analyzed using the Agilent 2100 Bioanalyzer and High Sensitivity DNA Chips. Receiver operating characteristic (ROC) analysis was used to identify a cut off for cfDNA and cfmtDNA values able to discriminate EC from HC. Multiple correspondence analysis (MCA), between cfDNA, mtcfDNA, citH3 and blood parameters were used to identify associations among serum parameters in EC.

**Results** NETosis is activated in all EC grades. In EC sera, elevated cfDNA concentration is associated with citH3 in G1 and G2 EC. Categorizing by ROC cut off cfDNA and cfmtDNA value distributions, we observed that citH3 levels are significantly higher in samples with high values of cfDNA and low values of cfmtDNA. A specific cfDNA fragmentation pattern characterizes EC and correlates with citH3 serum levels.

**Conclusion** NETosis could represent a new therapeutic target concerning EC. The combination of three serum parameters, citH3, cfDNA and cfmtDNA could be useful to monitor NETosis by non-invasive liquid biopsies opening the way for new therapeutic strategies in EC.

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**CLINICOPATHOLOGICAL CHARACTERISTICS OF 'MULTIPLE-CLASSIFIERS' IN ENDOMETRIAL CANCER**

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**Introduction/Background** According to the molecular classification, most endometrial cancers (ECs) can be categorised based on a unique molecular signature (e.g., POLE-mutation, mismatch-repair(MMR)-deficiency, p53-abnormality). However, a small number of cases harbour more than one molecular feature and are referred as 'multiple-classifiers'. The aim of the study is to describe the clinicopathological and molecular characteristics of multiple-classifier ECs.

**Methodology** Among all ECs undergoing a comprehensive molecular analysis at European Institute of Oncology, Milan, between April 2019 and December 2021, 'multiple-classifiers' were identified. Clinicopathological and molecular characteristics were collected from electronic medical records. The molecular analysis consisted of immunohistochemistry for p53 and MMR proteins, microsatellite instability assay, and Next Generation Sequencing (NGS) for POLE exonuclease domain and TP53. ECs were considered p53-abnormal if either immunohistochemistry or NGS resulted altered, MMR-deficient if either proteins expression was abnormal or mismatch-repair unstable and POLE when pathogenic POLE mutations were found. ECs were

molecularly classified according with WHO-endorsed algorithm. To compare continuous and categorical variables Wilcoxon-Mann-Whitney test and chi-square test were used, respectively. Proportions are reported as number (percentage and 95% confidence interval(CI)).

**Abstract 2022-RA-376-ESGO Table 1 Multiple-classifier clinicopathological characteristics**

Table 1a. Multiple-classifier clinicopathological characteristics.											
Molecular group	ID	Age (years)	BMI (kg/m <sup>2</sup> )	LND Metastasis	FIGO stage	Histotype	Grade	LVS	Risk score Endometrial cancer	Risk score Endometrial Adjuvant Treatment	
POLEmut-MMRd	1	59	21.9	NA	Endometrial	Grade 1	No	Low	Low	No	
	2	47	19.6	EC	Endometrial	Grade 2	No	Low	Low	No	
	3	42	25.5	NA	Endometrial	Grade 1	No	Low	Low	No	
	4	31	29.1	IB	Clear cell	Grade 1	Diffuse	High	High	CHT + EBRT + V8	
	5	75	22.7	NA	Endometrial	Grade 3	No	Intermediate	Low	No	
	6	44	28.8	NA	Endometrial	Grade 2	No	Intermediate	Low	No	
	7	54	24.2	NA	Endometrial	Grade 1	No	Low	Low	No	
	8	57	18.5	NA	Endometrial	Grade 2	No	Low	Low	No	
	9	76	26.5	Macro	EC2	Undifferentiated	Diffuse	High	High	CHT	
	10	55	22.7	II	Endometrial	Grade 3	No	High-intermediate	High-intermediate	EBRT + V8	
MMRd-p53abn	11	79	19.1	NA	Endometrial	Grade 1	No	Low	Low	No	
	12	55	18.6	NA	Endometrial	Grade 1	No	Low	Low	No	
	13	69	17.5	NA	Clear cell	Grade 1	No	Intermediate	Intermediate	No	
	14	84	20.5	NA	Serous	Grade 3	No	Intermediate	Intermediate	No	
	15	74	21	NA	Endometrial	Grade 2	No	Low	Low	No	
	16	63	21.3	Macro	EC2	Undifferentiated	Focal	Advanced/Metastatic	Advanced/Metastatic	CHT	
	17	54	26.7	Macro	EC2	Endometrial	Grade 3	No	High	High	CHT + EBRT + V8
	18	78	28.7	NA	Endometrial	Grade 2	No	Intermediate	Intermediate	No	
	19	64	33.2	NA	Endometrial	Grade 2	No	Low	Low	No	
	20	69	28.8	EC	EC2	Endometrial	Grade 2	No	High	High	CHT
POLEmut-MMRd-p53abn	21	50	32.8	Macro	EC2	Endometrial	Grade 2	No	High	High	CHT + EBRT
	22	60	27.7	Macro	EC2	Endometrial	Grade 3	Diffuse	High	High	CHT + EBRT
	23	67	25.3	NA	Macro	Grade 3	No	Advanced/Metastatic	Advanced/Metastatic	CHT + EBRT + V8	
	24	58	29.1	IB	Mixed	Grade 3	No	High-intermediate	High	CHT + EBRT	
	25	62	24.8	NA	Endometrial	Grade 3	No	Intermediate	Low	No	

Table 1b. Multiple-classifier molecular characteristics.										
Molecular group	ID	POLE mutation (NGS)	MMR protein immunohistochemistry	MSS	TP53 mutation (NGS)	P53 immunohistochemistry				
POLEmut-MMRd	1	POLE exon 9 c.3627G>T (p.R1211C) (480C-T)	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	WT	NA				
	2	POLE exon 9 c.3627G>T (p.R1211C) (480C-T)	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	WT	absent				
	3	POLE exon 13 c.4481G>A (p.V1494I) (1311G-C)	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
	4	POLE exon 13 c.4481G>A (p.V1494I) (1311G-C)	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	WT				
	5	POLE exon 9 c.3627G>T (p.R1211C) (480C-T)	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS	TP53 exon 7 c. Gln271P (p.G271S) (477G-C)	equivocal				
	6	POLE exon 9 c.3627G>T (p.R1211C) (480C-T)	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	equivocal				
	7	POLE exon 9 c.3627G>T (p.R1211C) (480C-T)	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	equivocal				
	8	POLE exon 9 c.3627G>T (p.R1211C) (480C-T)	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
	9	POLE exon 9 c.3627G>T (p.R1211C) (480C-T)	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
	10	POLE exon 9 c.3627G>T (p.R1211C) (480C-T)	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
MMRd-p53abn	11	WT	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	WT				
	12	WT	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	WT				
	13	WT	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
	14	WT	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
	15	WT	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
	16	WT	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
	17	WT	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
	18	WT	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
	19	WT	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
	20	WT	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
POLEmut-MMRd-p53abn	21	POLE exon 13 c.4481G>A (p.V1494I) (1311G-C)	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS-high	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
	22	POLE exon 13 c.4481G>A (p.V1494I) (1311G-C)	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
	23	WT	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
	24	WT	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				
	25	WT	Mut 1 +, PMS2 +, MSH2 +, MSH3 +	MSS	TP53 exon 8 c. Arg273P (p.R271W) (484C-T)	absent				

**Abstract 2022-RA-376-ESGO Table 2 Clinicopathological characteristics comparison between single- and multiple-classifier**

	Multiple-classifier N = 25	Single-classifier N = 253	P-value
<b>AGE AT DIAGNOSIS</b> Median (years) ± SD	60.9 ± 10.9	61.4 ± 12.2	0.645
<b>BMI</b> Median (kg/m <sup>2</sup> ) ± SD	25.4 ± 4.5	27.6 ± 7.3	0.271
<b>Histotype</b>			
Endometrioid	19 (76%; 57.1-89.3%)	227 (90%; 85.5-93.0%)	0.040
Other	6 (24%; 10.7-42.9%)	26 (10%; 7.0-14.5%)	
<b>Miometrial invasion</b>			
<50 %	18 (72%; 52.7-86.5%)	167 (66%; 60.0-71.6%)	0.545
≥50 %	7 (28%; 13.5-47.3%)	86 (34%; 28.4-40.0%)	
<b>LVS</b>			
None or focal	22 (88%; 71.3-96.5%)	225 (89%; 84.6-92.4%)	0.888
Diffuse	3 (12%; 3.5-28.7%)	28 (11%; 7.6-15.4%)	
<b>Low grade (G1-2)</b>	11 (44%; 26.1-63.2%)	183 (72%; 66.6-77.6%)	0.003
<b>High grade</b>	14 (56%; 36.8-73.9%)	70 (28%; 22.4-33.4%)	
<b>Lymph nodes metastasis</b>			
Negative	18 (72%; 52.7-86.5%)	214 (85%; 79.8-88.6%)	0.266
ITC	2 (8%; 1.7-23.3%)	10 (4%; 2.0-6.9%)	
Micro- or macro-metastasis	5 (20%; 8.1-38.4%)	29 (12%; 8.0-15.8%)	
<b>FIGO stage</b>			
I	17 (68%; 48.5-83.6%)	166 (66%; 59.6-71.3%)	0.810
II - IV	8 (32%; 16.4-51.5%)	87 (34%; 28.7-40.4%)	
<b>ESGO/ESTRO/ESP (2020) Molecular classification unknown</b>			
Low	9 (36%; 19.5-55.5%)	115 (46%; 39.4-51.6%)	0.318
Intermediate	6 (24%; 10.7-42.9%)	30 (12%; 8.3-16.3%)	
High-Intermediate	1 (4%; 0.4-17.2%)	30 (12%; 8.3-16.3%)	
High	8 (32%; 16.4-51.5%)	65 (26%; 20.6-31.3%)	
Advanced/metastatic	1 (4%; 0.4-17.2%)	13 (5%; 2.9-8.4%)	
<b>ESGO/ESTRO/ESP (2020) Molecular classification known</b>			
Low	12 (48%; 29.5-66.9%)	112 (44.3%; 38.2-50.4%)	0.919
Intermediate	3 (12%; 3.5-28.7%)	19 (7.5%; 5.2-10.4%)	
High-Intermediate	1 (4%; 0.4-17.2%)	24 (10%; 6.3-13.6%)	
High	8 (32%; 16.4-51.5%)	75 (30%; 24.3-35.5%)	
Advanced/metastatic	1 (4%; 0.4-17.2%)	13 (5%; 2.9-8.4%)	
<b>FOLLOW UP</b>			
Mean (months) ± SD	6.6 ± 6.1	6.4 ± 6.3	
Number of recurrences	1 (4%; 0.4-17.2%)	15 (6%; 3.5-9.4%)	

NOTE: Data reported as Count (Column%; 95% Confidence interval) unless otherwise indicated. Abbreviations: ITC, isolated tumour cells; MMRd, mismatch repair-deficient; p53abn, p53 abnormal; BMI, body mass index; SD, standard deviation; LVS, lympho-vascular space invasion.

**Results** A total of 278 ECs underwent molecular analysis, of which 253 (91.0%, CI 87.8–94.2) harboured a unique molecular signature, while 25 (9.0%, CI 5.8–12.2) were 'multiple-classifiers'. Among them, we identified 15 (5.4%, CI 2.9–8.3) MMRd-p53abn, 2 (2.2%, CI 0.7–4.0) POLEmut-p53abn, 2 (0.7%, CI 0.0–1.8) POLEmut-MMRd, and 2 (0.7%, CI 0.0–1.8)

POLEmut-MMRd-p53abn. Clinicopathological and molecular characteristics of 'multiple-classifiers' are shown in table 1.

'Multiple-classifiers' were more frequently high-grade (56% vs 28%, $p=0.003$ ), non-endometrioid (24% vs 10%, $p=0.04$ ) ECs when compared to 'single-classifier'(table 2).

**Conclusion** Multiple-classifier ECs represent 9% of the entire study population. Compared to previous studies, the higher proportion of 'multiple-classifiers' could be related to the extensive molecular analysis, comprising the evaluation of both p53 expression and TP53 mutations. More studies addressing the clinical implications on prognosis of 'multiple-classifiers' are needed.

2022-RA-449-ESGO

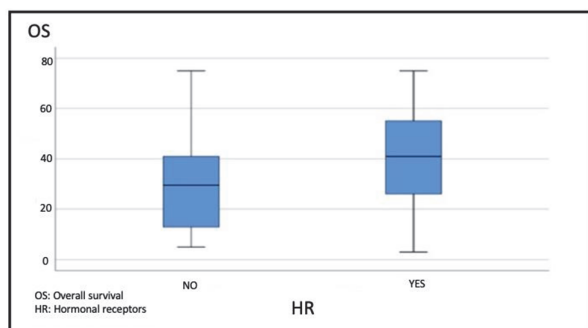
### CLINICAL-MOLECULAR CORRELATIONS OF ENDOMETRIAL CANCER. RETROSPECTIVE STUDY

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**Introduction/Background** Endometrial cancer (EC) is the most common gynecological tumor in developed countries, with more than 75% diagnosed at early stages. It is associated in 20–30% with microsatellite instability (MSI) due to mutations in the MMR genes, which can be sporadic (80–90%) or hereditary (10–20%) such as Lynch syndrome (LS). Objective: To establish the clinical-molecular profile of endometrial carcinoma and its implication for treatment.

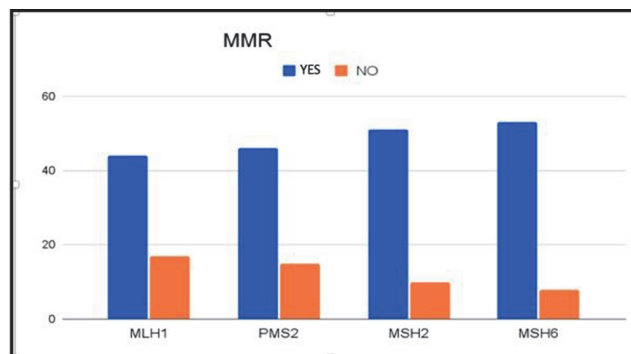
**Methodology** Retrospective cross-sectional observational study. 162 patients diagnosed with EC during the period 2010–2020 and treated in Medical Oncology Service at the HUNSC were studied. Study variables: age, histology grade and type, stage, hormone receptors (HR), MSI, LS, overall survival. SPSS 25 was used for statistical analysis.



Abstract 2022-RA-449-ESGO Figure 1

**Results** The median age was 64,51 years. The most frequent stages at diagnosis were IA (25.3%) and IB (24.7%). Histologically, endometrioid adenocarcinoma accounted for 51.2% and

grade 3 for 41.4% of cases. Patients with LS were mainly diagnosed at stage III, being endometrioid or serous adenocarcinomas, and mainly grade 3 (60%). Overall survival was longer in the HR+ group (40.5 months). MSI-H was observed in 36.1% of the sample and the dMMR distribution: MLH1 (27.9%) and PMS2 (24.6%), MSH2 (16.4%), MSH6 (13.1%). Ten patients were diagnosed with LS.



Abstract 2022-RA-449-ESGO Figure 2

**Conclusion** Our EC and LS results are comparable to those published for other settings. There is a significant association between HR+ and longer overall survival. The percentage of dMMR/MSI-H is higher than reported in other studies. Further studies with a larger sample would be needed.

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### BENEFIT OF ADJUVANT RADIOTHERAPY DEPENDS ON MOLECULAR CLASS OF EARLY-STAGE ENDOMETRIAL CANCER

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**Introduction/Background** The endometrial cancer (EC) molecular class is predictive for response to chemotherapy. Little is known about its' predictive value for response to radiotherapy. We investigated benefit of adjuvant vaginal brachytherapy (VBT) and external beam radiotherapy (EBRT) across the four molecular classes.

**Methodology** Participants of the randomized PORTEC-1 (n=714) and PORTEC-2 (n=427) trials were eligible if their EC were molecularly profiled according to the WHO 2020 classification. PORTEC-1 included intermediate risk EC and compared EBRT to no adjuvant treatment. PORTEC-2 included high-intermediate risk EC and compared VBT to EBRT. Locoregional recurrence-free survival (LRFS) was estimated and compared using Kaplan-Meier's methodology and log-rank tests. Correction for confounding by predefined clinicopathological factors was done using Cox proportional hazards models.