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.

2022-RA-376-ESGO CLINICOPATHOLOGICAL CHARACTERISTICS OF 'MULTIPLE-CLASSIFIERS' IN ENDOMETRIAL CANCER

[1]Luigi Antonio de Vitis, [1,2]Gabriella Schivardi, [1]Giulio Bonaldo, [3,4]Caterina Fumagalli, [5]Paola Rafaniello Raviele, [1]Maria Teresa Achilarre, [1]Alessia Aloisi, [1]Annalisa Garbi, [1]Mariateresa Lapresa, [1]Gabriella Parma, [1]Vanna Zanagnolo, [1,6]Giovanni Damiano Aletti, [5,6]Elena Guerini-Rocco, [2]Andrea Mariani, [1]Angelo Maggioni, [5]Massimo Barberis, [1,7]Nicoletta Colombo, [1]Francesco Multinu, [1]Ilaria Betella. *[1]Department of Gynecology, European Institute of Oncology, Milan, Italy; [2]Department of Obstetrics and Gynecology, Mayo Clinic, Rochester, MN; [3]Clinical Unit of Oncogenomics, European Institute of Oncology, Milan, Italy; [4]Department of Diagnostic Services, Division of Pathology, ASST Valle Olona, Gallarate, Italy; [5]Department of Pathology, European Institute of Oncology, Milan, Italy; [6]Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy; [7]Faculty of Medicine and Surgery, University of Milan Bicocca, Milan, Italy*

10.1136/ijgc-2022-ESGO.210

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 instable 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-clasifier clinicopathological characteristics

Table 1a. Multiple-classifier clinicopathological characteristics.

Molecular group	ID	Age (years)	BMI (kg/m2)	LND Metastasis	FIGO stage	Histotype	Grade	LVSI	Risk class* (molecular unknown)	Risk class* (molecular known)	Adjuvant Treatment
POLEmut–MMRd	1	59	21.9		IA	Endometrioid	Grade 1	No	Low	Low	No
	2	47	19.6	ITC	IA	Endometrioid	Grade 2	No	Low	Low	No
POLEmut–p53abn	3	42	25.5		IA	Endometrioid	Grade 1	No	Low	Low	No
	4	51	28.3		IB	Clear cell	Grade 3	Diffuse	High	High	CHT + EBRT + VB
	5	75	22.7		IA	Endometrioid	Grade 3	No	Intermediate	Low	No
	6	44	19.3		IA	Endometrioid	Grade 3	No	Intermediate	Low	No
	7	54	24.2		IA	Endometrioid	Grade 1	No	Low	Low	No
	8	57	19.5		IA	Endometrioid	Grade 2	No	Low	Low	No
MMRd–p53abn	9	76	26.1	Macro	IIIC1	Undifferentiated	Undifferentiated	Diffuse	High	High	CHT*
	10	55	22.7		II	Endometrioid	Grade 3	No	High-intermediate	High-intermediate	EBRT + VB
	11	59	31.1		IA	Endometrioid	Grade 1	No	Low	Low	No
	12	55	33.6		IA	Endometrioid	Grade 1	No	Low	Low	No
	13	69	17.5		IA	Clear cell	Grade 3	Focal	Intermediate	Intermediate	No
	14	84	20.5		IA	Serous	Grade 3	No	Intermediate	Intermediate	No
	15	74	23		IA	Endometrioid	Grade 2	No	Low	Low	No
	16	63	23.3	Macro	IVB	Endometrioid	Grade 3	Focal	Advanced/Metastatic	Advanced/Metastatic	CHT
	17	54	26.7	Macro	IIIC1	Endometrioid	Grade 3	No	High	High	CHT + EBRT + VB
	18	78	28.7		IA	Endometrioid	Grade 3	No	Intermediate	Intermediate	No
	19	64	33.2		IA	Endometrioid	Grade 2	No	Low	Low	No
	20	69	28.6	ITC	IIIA	Endometrioid	Grade 2	No	High	High	CHT
	21	50	32.8	Macro	IIIC1	Endometrioid	Grade 2	No	High	High	CHT + EBRT
	22	60	27.7	Macro	IIIC2	Endometrioid	Grade 3	Diffuse	High	High	CHT + EBRT
	23	67	25.3		IVB	Mixed	Grade 3	No	Advanced/Metastatic	Advanced/Metastatic	CHT + EBRT + VB
POLEmut–MMRd–p53abn	24	58	29.1		IB	Mixed	Grade 3	No	High	High	CHT + EBRT
	25	59	24.9		IA	Endometrioid	Grade 3	No	Intermediate	Low	No

Abbreviations: BMI, body mass index; CHT, chemotherapy; EBRT, external beam radiotherapy; FIGO, The International Federation of Gynecology and Obstetrics; ITC, isolated tumor cells; LND, Lymph node; LVSI, lymph-vascular space invasion; MMRd, mismatch repair-deficient; na, not available; POLEmut, POLE-mutated; p53abn, p53 abnormal; VB, vaginal brachytherapy; *According to ESGO/ESTRO/ESP 2020. * Relapsed.

Table 1b. Multiple-classifier molecular characteristics.

Molecular group	ID	POLE mutation (NGS)	MMR protein immunohistochemistry	MSI	TP53 mutation (NGS)	P53 immunohistochemistry
POLEmut–MMRd	1	POLE exon 9 p.Ser2979Phe (p.S2979F) c.890C>T	MLH 1 +, PMS2 +, MSH6 –, MSH2 +	MSI-high	WT	NA
	2	POLE exon 9 p.Pro286Arg (p.P286R) c.857C>G	MLH 1 –, PMS2 –, MSH6 +, MSH2 +	MSS	WT	NA
POLEmut–p53abn	3	POLE exon 14 p.Ala1456Pro (p.A456P) c.1366G>C	MLH 1 +, PMS2 +, MSH6 +, MSH2 +	MSS	TP53 exon 8 p.Arg306Ter (p.R213*) c.916C>T	aberrant
	4	POLE exon 13 p.Val411Leu (p.V411L) c.1231G>C	MLH 1 +, PMS2 +, MSH6 +, MSH2 +	MSS	TP53 exon 6 p.Arg213Ter (p.R213*) c.637C>T	WT
	5	POLE exon 9 p.Ser2979Phe (p.S2979F) c.890C>T	MLH 1 +, PMS2 +, MSH6 +, MSH2 +	MSS	TP53 exon 7 p.Cys238Gly (p.C238G) c.712T>G	equivocal
	6	POLE exon 14 p.Tyr458His (p.Y458H) c.1372T>C				
	7	POLE exon 9 p.Pro286Ala (p.P286A) c.857C>G	MLH 1 +, PMS2 +, MSH6 +, MSH2 +	NA	TP53 exon 5 p.Arg175Cys (p.R175C) c.523C>T	WT
	8	POLE exon 9 p.Pro286Arg (p.P286R) c.857C>G	MLH 1 +, PMS2 +, MSH6 +, MSH2 +	MSS	TP53 exon 6 p.Arg213Ter (p.R213Ter) c.637C>T	equivocal
MMRd–p53abn	9	WT	NA	NA	TP53 exon 6 p.Arg213Ter (p.R213Ter) c.637C>T	Not Evaluable
	10	WT	MLH 1 –, PMS2 –, MSH6 –, MSH2 +	MSI-high	NA	aberrant - over expression pattern
	11	WT	MLH 1 –, PMS2 –, MSH6 +, MSH2 +	MSI-high	TP53 exon 8 p.Arg282Trp (p.R282W) c.844C>T	WT
	12	WT	MLH 1 –, PMS2 –, MSH6 –, MSH2 +	MSI-high	TP53 exon 7 p.Cys229Tyrfs*10 (p.C229Ifs*10) c.686_687del	Not Evaluable
	13	WT	MLH 1 +, PMS2 +, MSH6 –, MSH2 +	MSS	TP 53 exon 6 p.Gly199Ter (p.G199*) c.595G>T	WT
	14	WT	MLH 1 +, PMS2 +, MSH6 –, MSH2 +	MSI-high	TP53 exon 8 p.Asp281Gly (p.D281G) c.842A>G	aberrant
					TP53 exon 7 p.Arg248Gln (p.R248Q) c.743G>A	
					TP53 exon 8 p.Arg282Trp (p.R282W) c.844C>T	aberrant
	15	WT	MLH 1 –, PMS2 –, MSH6 +, MSH2 +	MSI-high	TP53 exon 8 p.Pro301GlnfsTer44 (p.P301Qfs*44) c.902del	aberrant
	16	WT	MLH 1 –, PMS2 –, MSH6 +, MSH2 +	MSI-high	TP53 exon 4 p.Val73fs (p.V73fs) c.216delC	
					TP53 exon 6 p.His214Arg c.641A>G	aberrant
	17	WT	MLH 1 –, PMS2 –, MSH6 +, MSH2 +	MSI-high	TP53 exon 8 p.Arg273Cys (p.R273C) c.817C>T	NA
	18	WT	MLH 1 +, PMS2 +, MSH6 –, MSH2 +	MSI-high	TP53 exon 5 p.Ala159Val (p.A159V) c.476C>T	aberrant
	19	WT	MLH 1 –, PMS2 –, MSH6 +, MSH2 +	MSI-high	NA	aberrant - over expression pattern
	20	WT	MLH 1 +, PMS2 +, MSH6 –, MSH2 –	MSI-high	TP53 exon 8 p.Pro301GlnfsTer44 c.902del	Not Evaluable
	21	WT	MLH 1 –, PMS2 –, MSH6 +, MSH2 +	MSI-high	TP53 exon 8 p.Arg273Cys (p.R273C) c.817C>T	equivocal
	22	WT	MLH 1 +, PMS2 +, MSH6 –, MSH2 +	MSI-high	TP53 exon 5 p.Pro177Ser (p.P177S) c.529C>T	equivocal
	23	WT	NA	MSI-high	TP53 exon 8 p.Arg273Cys (p.R273C) c.817C>T	Not Evaluable
POLEmut–MMRd–p53abn	24	POLE exon 13 p.Val411Leu (p.V411L) c.1231G>T	MLH 1 +, PMS2 +, MSH6 –, MSH2 +	MSI-high	TP53 exon 4 p.Val73fs (p.V73fs) c.216delC	
					TP53 exon 7 p.Asn239Ser c.716A>G	
					TP53 exon 8 p.Arg273His c.818G>A	aberrant
	25	POLE exon 13 p.Val411Leu (p.V411L) c.1231G>T	MLH 1 +, PMS2 +, MSH6 –, MSH2 +	MSS	TP53 exon 8 p.Arg273Cys (p.R273C) c.817C>T	
					TP53 exon 8 p.Arg282Gln (p.R282Q) c.845G>A	equivocal

Abbreviations: MMR-d, mismatch repair-deficient; MSI, microsatellite instability; MSS, microsatellite stable; NA, not available; NGS, next generation sequencing; P53abn, P53 mutated; p53abn, p53 abnormal; WT, wild type.

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
AGE AT DIAGNOSIS Median (years) ± SD	60.9 ± 10.9	61.4 ± 12.2	0.645
BMI Median (kg/m²) ± SD	25.4 ± 4.5	27.6 ± 7.3	0.271
Histotype			
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%)	
Miometrial invasion			
<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%)	
LVSI			
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%)	
Grade			
Low grade (G1-2)	11 (44%; 26.1-63.2%)	183 (72%; 66.6-77.6%)	**0.003**
High grade	14 (56%; 36.8-73.9%)	70 (28%; 22.4-33.4%)	
Lymph nodes metastasis			
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%)	
FIGO stage			
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%)	
ESGO/ESTRO/ESP (2020) Molecular classification unknown			
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%)	
ESGO/ESTRO/ESP (2020) Molecular classification known			
Low	12 (48%; 29.5-66.9%)	112 (44.3%; 38.2-50.4%)	0.919
Intermediate	3 (12%; 3.5-28.7%)	29 (12%; 8.0-15.8%)	
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%)	
FOLLOW UP 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; LVSI, lymph-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, 6 (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)

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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.

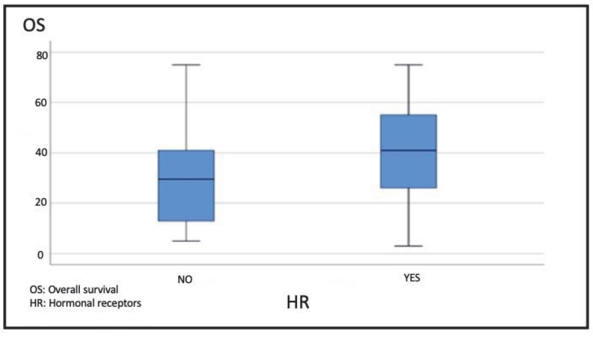
2022-RA-449-ESGO CLINICAL-MOLECULAR CORRELATIONS OF ENDOMETRIAL CANCER. RETROSPECTIVE STUDY

[1]Natalia Pérez Rodríguez, [2]Alfonso Quesada López, [3]María López Acosta, [2]Jose Antonio Pérez Álvarez, [4]Mónica Vilar Chesa, [5]Vanesa Concepción Martín, [6]Jose Javier Martin Ortega, [7]Pablo Martín Vasallo. *[1]Medical Oncology, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain; [2]Gynecology, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain; [3]Nuclear Medicine, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain; [4]Pathological Anatomy, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain; [5]Surgery, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain; [6]Oncology, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain; [7]Biochemistry and Molecular Biology, University de la Laguna, Santa Cruz de Tenerife, Spain*

10.1136/ijgc-2022-ESGO.211

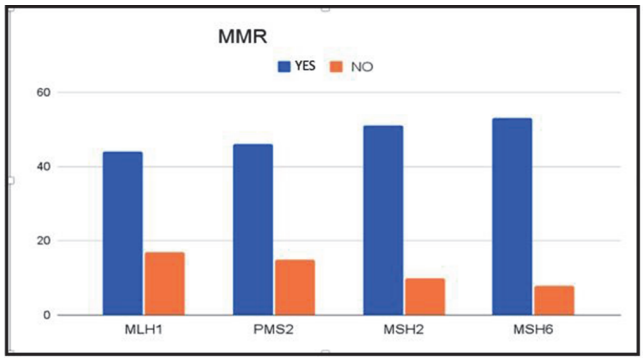
Introduction/Background Endometrial cancer (EC) is the most common gynecological tumor in developed countries, with more than 75% diagnosed at early stages. It is associated in 20–30% with microsatellite instability (MSI) due to mutations in the MMR genes, which can be sporadic (80–90%) or hereditary (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.

2022-RA-450-ESGO BENEFIT OF ADJUVANT RADIOTHERAPY DEPENDS ON MOLECULAR CLASS OF EARLY-STAGE ENDOMETRIAL CANCER

[1]Nanda Horeweg, [2]Remi A Nout, [3]Ellen Stelloo, [4]Ludy CHW Lutgens, [5]Jan J Jobsen, [6]Ina M Jürgenliemk-Schulz, [7]Elsbieta M van der Steen-Banasik, [2]Jan-Willem M Mens, [8]Annerie Slot, [3]Vincent THBM Smit, [3]Tjalling Bosse, [1]Carien L Creutzberg. *[1]Radiation Oncology, Leiden University Medical Center, Leiden, Netherlands; [2]Radiotherapy, Erasmus MC Cancer Center, Rotterdam, Netherlands; [3]Pathology, Leiden University Medical Center, Leiden, Netherlands; [4]Maastricht Radiation Oncology Clinic, Maastricht, Netherlands; [5]Radiotherapy, Medisch Spectrum Twente, Enschede, Netherlands; [6]Radiation Oncology, University Medical Center Utrecht, Utrecht, Netherlands; [7]Radiotherapiegroep, Arnhem, Netherlands; [8]Radiotherapeutic Institute Friesland, Leeuwarden, Netherlands*

10.1136/ijgc-2022-ESGO.212

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.

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