Conclusion Expression of TROP-2 in cervical cancer is associated with increased levels of intratumoral TILs, indicating the potential therapeutic target for TROP-2 targeted antibody-drug conjugate alone or in combination with immune checkpoint inhibitors.

2022-RA-803-ESGO FUNCTIONAL CHARACTERIZATION OF NRIP1 IN ENDOMETRIAL CANCER; MUTATIONS ASSOCIATES WITH AGGRESSIVE FEATURES WHILE LOW EXPRESSION LEVELS PREDICT POOR SURVIVAL

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Introduction/Background *NRIP1*, encoding an obligate cofactor to the estrogen receptor alpha (ERα), is a significantly mutated gene in endometrial cancer (EC) with possible implications on hormone signaling and cancer development. We aimed at determining the prognostic impact of *NRIP1* mutations and mRNA expression in patients with ERα-positive EC, and functionally test patient-observed mutations *in vitro* on ERα-dependent transcriptional activity.

Methodology Sanger sequencing of recurring *NRIP1* frameshift mutations was performed on 242 EC patients enriched for ERα-positive tumors (endometrioid histology). Transcriptional profiles of 305 patients, in part overlapping with the sequenced samples, were used to investigate differentially expressed genes between *NRIP1* mutated/non-mutated tumors, and to determine the relation of *NRIP1* mutation status with expression and patient survival. A dual-luciferase reporter assay was used to uncover the effect of NRIP1-mutants on ERα-regulated gene transcription using COS-1 cells.

Results Hotspot *NRIP1* mutations were identified in 5.4% of the samples, and associated with high FIGO-stage, deep myometrial infiltration, and positive lymph-node status. However, there was no significant difference in disease-specific survival between patients with mutated and wildtype *NRIP1*, and mutation status did not associate with *NRIP1* expression levels. Interestingly, reporter assays showed that the repressive effect of wildtype NRIP1 was significantly abolished with NRIP1 mutants. Further, low *NRIP1* expression levels were significantly associated with decreased disease-specific survival, high grade, negative ERα status and low BMI. Gene Set Enrichment Analysis revealed an increase in gene sets related to proliferation in *NRIP1* mutated tumors.

Conclusion The relatively low mutation rate hampers a firm conclusion on the prognostic value of *NRIP1* frameshift mutations in endometrioid EC. However, low *NRIP1* expression may be a marker of poor prognosis. Our functional investigations demonstrates that frameshift mutated *NRIP1*, compared to wildtype *NRIP1*, have a significantly reduced corepressor effect on ERα-transcriptional activity, suggesting a possible effect on estrogen signaling.

2022-RA-807-ESGO MLH1 PROMOTER METHYLATION ANALYSIS IN PATIENTS WITH ENDOMETRIAL CANCER STAGE I-II

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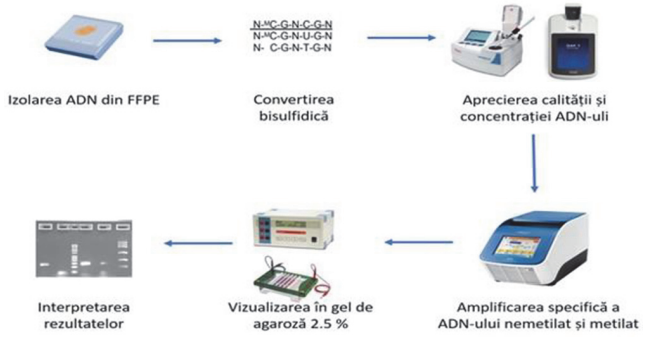
Introduction/Background The study included 50 patients diagnosed with endometrial cancer (EC) stage I-II. The age of these patients, on average, was 49.1 ± 12.1 years and ranged from 54 to 86 years.

Methodology The tumor DNA was extracted from mapped formalin-fixed, paraffin-embedded tissue sections to provide tumor samples for the assays (figure 1).

The methylation status of the MLH1 gene was determined using the Methylation Specific Polymerase Chain Reaction (MS-PCR) method and specific primers for both unmethylated and methylated fragments.

Results The frequency of MLH1 promoter methylation was 20.0% and was determined in 10 patients. The frequency of tumors with MLH1 promoter methylation increases during menopause, reaching 30.0% at the age of 50–59 years and 50, 0% of cases at 60–69 years and decreases in the age periods 70–79 years, reaching 20.0%.The analysis of the obtained results showed that in patients with EC, the presence of MLH1 epimutation was significantly higher in stage I of the disease.



Abstract 2022-RA-807-ESGO Figure 1 Methylation chain-specific polymerization reation workflow

Abstract 2022-RA-807-ESGO Table 1 MLH1 dependence on stage of the disease in patients with endometrial cancer in stages I-II

FIGO stage	MLH1 promoter methylation				P
	Negativ		Positive		
	Abs.	%	Abs.	%	
I	34	68,0	8	22,0	χ2=0,001; gl=1; p>0,05
II	6	8,1	2	4	

The presence of MLH1 epimutation was observed in 22.0% of patients with stage I EC and only in 2 stage II patients. The results of the analysis of overall survival in patients, according to the presence of MLH1 epimutation, showed that 71% of women with MLH1 epimutation and 92.5% without MLH1 epimutation survived at 3 years.

Conclusion MLH1 promoter methylation analysis would play a valuable role as a clinical biomarker.

2022-RA-818-ESGO BIUXX

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Introduction/Background We found cyclin B1 immunohistochemistry (IHC) expression is different between polymerase epsilon exonuclease (*POLE*) and copy number low (CN-low) subtype in endometrial cancer. The objective is to examine whether *POLE* can be distinguished from CN-low subtype using clinicopathologic factors and cyclin B1 IHC.

Methodology For 240 patients with endometrial cancer who underwent hysterectomy at Seoul National University Bundang Hospital from 2006 to 2013, *POLE* gene sequencing and IHC for hMLH1, hMSH2, hMSH6, PMS2 and p53 were perfomed. For 155 patients with *POLE* or CN-low subtype, clinicopathologic factors were abstracted from medical record, and cyclin B1 IHC was performed using primary monoclonal antibody (RBT-B1, 1:50; LSBio, Seattle, WA, USA). Cyclin B1 expression level (cyclin B1 score) was determined by intensity of staining. Decision tree classifiers encompassing clinicopathologic factors and cyclin B1 IHC were constructed using accuracy from 5-fold cross-validation. Hyperparameters (max_depth, min_samples_leaf) were tuned using GridSearch.

Results 24 with *POLE* and 131 with CN-low were included. Median age was 56 and median weight was 61.6kg. Number of patients with stage 3, 4 were 14 and those with LVSI were 41. In the final model, weight (cutoff 54.3kg) and cyclin B1 IHC (cutoff score 1.5) were selected. With the *POLE* subtype, the mean validation accuracy were 84%. The model divided the whole cohort into 3 groups. Of 25 patients with weight ≤ 54.3 kg (group 1), 10 patients with *POLE* subtype were included (40%); Of 51 patients with weight > 54.3 kg and cyclin B1 score > 1.5 (group 2), 8 patients with *POLE* subtype were included (16%); Of 48 patients with weight > 54.3 kg and cyclin B1 score ≤ 1.5 (group 3), 1 patients with *POLE* subtype were included (2%).

Conclusion *POLE* vs. CN-low cannot be distinguished but can be enriched using clinicopathologic factors and cyclin B1 IHC.

2022-RA-846-ESGO PREDICTIVE VALUE OF DNA METHYLATION MARKERS AS AN INVASIVE DIAGNOSTIC TOOL FOR PATHOLOGICAL UPGRADING CERVICAL LESIONS

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Introduction/Background Due to bleeding, cervical atrophy, cervical type III transformation zone and other factors, resulting in the coincidence rate of pathology between colposcopy guided biopsy and conization/surgery was only 42%-57%,it may be even lower in countries with poor health care.The study aimed to evaluate the diagnostic accuracy and agreement between pathologists by performing methylated PAX1 and ZNF582 gene tests in colposcopy guided biopsy and surgical pathology.

Methodology 217 patient's medical records and pairs of wax blocks of biopsy and conization/surgery were collected from Xiangya Hospital, Changsha, China. After DNA extraction and bisulfite conversion process, methylated PAX1 and ZNF582 genes were detection by methylated real-time PCR system before surgery. The results of methylation, cytology, high-risk human papillomavirus (HR-HPV), colposcopy, and pathology of colposcopy biopsy and surgical specimens were evaluated.

Results The mean age of cases was 42.9 years. The positivity rates for hr-HPV,PAX1(+), ZNF582(+), TCT (≥HSIL), and colposcopy (≥HSIL) were 95.4%(n=207),47.86% (n = 56), 38.46% (n = 45), 26.50% (n = 31), and 39.31% (n = 46) in the CIN2+ pathological results. The pathological results of the punch biopsy and LEEP were not statistically significant in terms of positivity rate for CIN2+ (p = 0.545). Of all the punch biopsy results, 29.03% were upgraded to higher pathological grades and 34.10% were downgraded to lower pathological grades by LEEP. PAX1 was found in 26 patients (59.09%) with the final pathology of upgraded CIN3+.

Conclusion The noninvasive methylated gene test could indicate the cervical CIN3+ misdiagnosis in punch biopsy and increase the accuracy of biopsy results.

2022-RA-902-ESGO NOVEL METHYLATED GENES AS A SECOND TRIAGE STEP AFTER HRHPV TESTING TO IMPROVE COLPOSCOPY REFERRAL IN HPV INFECTED WOMEN WITH CERVICAL LESIONS (CANCER)

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Introduction/Background High-risk human papillomavirus (hrHPV) testing to triage women with abnormal cervical lesions (cancer) generates many referrals. hrHPV infection and excessive colposcopy referrals may lead to panic in patient and bring waste of medical resources.

Methodology From 2019 to 2022, a prospective study of outpatient opportunistic cervical cancer screening was conducted with multiple centers. More than 20,000 subjects were collected and be follow-up for one year. The research team of Peking Union Medical College Hospital is responsible for preliminary experiment, clinical study planning, and process quality control. The analysis of methylation level was determined by using the CisCer methylation real-time system (CISPOLY Co., China). Positive rate, sensitivity, specificity, accuracy for cytology, hrHPV, and the methylation level of PAX1 and JAM3 genes were analyzed.

Results A system set-up study in 2210 hrHPV infection subjects including normal uterine cervix (n=1230), CIN1(n=514), CIN2(n=69), CIN3/CIS(n=194), SCC (n=50), and adenocarcinoma (n=6) of the uterine cervix diagnosed according to histological results. The CIN2, CIN3, and Cancer immediate risk with HPV 16/18 (n=810) and non-16/18 hrHPV (n=1400) were 33.83%, 20.99%, 6.17% and13.71%, 5.71%, 0.43% respectively. The sensitivity and specificity of CisCer