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Relationship between molecular markers and lymphadenectomy and lymphovascular space invasion in endometrial cancer

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Abstract

Purpose Relationship between pathologic parameters, surgical parameters, or lymph node status with oncologic outcomes is not fully elucidated in endometrial cancer (EC). We want to investigate the molecular classification of uterine cancer in the Turkish population and its relationship between lymphadenectomy and lymph node metastasis.

Methods In this study, 100 patients' clinical and pathologic data diagnosed with EC were analyzed. Pathologic and molecular parameters were investigated and compared them with clinical parameters.

Results According to the molecular analysis, 16 patients (16%) had p53 mutation, 3 patients (3%) were classified as POLE mutant group, 38 (38%) patients in the MSI group, and the remaining 43 patients (43%) into the no specific mutation profile (NSMP) group. Lymph node metastasis rate was significantly higher in copy number high (CNH) group compared to the others. In the CNH group, 29 of 437 (6.6%) dissected lymph nodes had metastasis. The median OS was the highest in the POLE group (72 months) and lowest in the CNH group (36 months).

Conclusion Endometrial cancer patients showed significantly different overall and disease-free survival according to the molecular subtypes and it was consistent with the literature, Lymph node metastasis risk was the highest in CNH group. MSI status is important for the lymph node metastasis risk but not all abnormalities, especially PMS2 and MLH1 expression changes showed the highest risk.

Keywords Endometrial cancer · Lymph node metastasis · Molecular subgroups · Lymphovascular space invasion

Introduction

The second most prevalent gynecologic cancer is endometrial cancer (EC), and it affects thousands of women worldwide. In 2018, nearly 100 000 individuals died, and more than 400 000 new cases were diagnosed globally [1]. Even, though more than 80% of EC patients were diagnosed with stage I–II, some of the EC cases that appear to be in an early stage showed recurrence and have catastrophic results [2]. After the curative surgery adjuvant chemotherapy is controversial, especially in early-stage EC, that is why we need new criteria for the adjuvant treatment indications as well as extended surgery.

The Cancer Genome Atlas was the first to introduce the molecular endometrial cancer (EC) classification (TCGA) [3]. POLE-ultramutated (POLEmut), mismatch repair deficient (MMRd), p53-abnormal (p53abn), and No Specific Molecular Profile Subgroup (NSMP) are the four subgroups of this molecular classification. The molecular

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EC classification has demonstrated a good predictive value using this method in clinical trials and unselected cohorts [4]. Moreover, PORTEC 3 trial showed that molecular classification of EC has a strong prognostic value in high-risk uterine cancer, and adjuvant chemotherapy and radiation significantly improved recurrence in p53abn tumors, regardless of histologic subgroup [5]. These findings prompted the inclusion of molecular subgroups in the existing classification system. Nevertheless, the relationship between pathologic parameters, surgical parameters, or lymph node status with oncologic outcomes is not fully elucidated.

In this study, we want to investigate the molecular classification of uterine cancer in the Turkish population and its relationship between lymphadenectomy and lymph node metastasis.

Methods

In this study, 100 patients' clinical and pathologic data diagnosed with EC were analyzed. All patients were operated on between 2008 and 2020 period in the Gynecological Oncology Clinic. Clinical data and demographic features were obtained from the hospital records and via phone call. Pathologic parameters included histological diagnosis, tumor size, myometrial invasion, cervical invasion, grade, abdominal washing cytology, lymphovascular invasion, excised lymph node, and metastatic lymph nodes. Institutional Review Board approved the study.

Sequence analysis of the 9 and 13 exons of the POLE gene: For the identification of POLE mutations, genomic DNA was isolated from FFPE (formalin-fixed, paraffin-embedded) samples using the relevant DNA isolation procedures (Invitrogen™ PureLink™ Genomic DNA Mini Kit, Cat No.: K182002, USA). The amount and purity were determined with Promega QuantiFluor E6090 (Promega, Madison, USA) and stored at – 20 °C until use. Sequence analyzes of relevant regions from genomic DNA were performed using the Applied Biosystems 3130XL Genetic Analyzer (USA). The amplification was performed by Biorad T100 Thermal Cycler. MyTaq™ HS DNA Polymerase (Bioline, Meridian Bioscience, Tennessee, USA) was used in the reaction mixture, primers were designed by Sentebiolab (ANKARA).

Determination of molecular subgroup; All patient's pathologic specimens were analyzed by immunohistochemistry (IHC) for Microsatellite instability (MSI) status including MLH1, MSH2, MSH6, and PMS2. According to guidelines POLE mutant patients were diagnosed as POLEmt (ultramutated) group. If a patient had no POLE mut but abnormal staining of the MSI parameters diagnosed as MSI-mutant group (hypermutated). p53 abnormal results (without POLE or abnormal MSI staining) diagnosed as p53-Copy number high (CNH) group. The remaining all these 3 groups are categorized as Non-specific molecular profiles (NSMP).

Statistical analyses: All analyses performed by the SPSS for Windows 22.0 software and $P < 0.05$ was accepted as significant. Categorical variables relationships were tested with a chi-square test. The Kaplan–Meier test and log rank test were used for survival analysis. Cox Regression analysis was tested for the determination of parameters that affect the oncologic outcomes.

Results

In the present study, 100 patients were included, the mean age was 59 (std. 9.1) and the body mass index was 33 (std 5.6). The mean CA 125 level was 158 units/mL. According to the molecular analysis, 16 patients (16%) had p53 mutation, 3 patients (3%) were classified as POLE mutant group, 38 (38%) patients in the MSI group, and the remaining 43 patients (43%) into the no specific mutation profile (NSMP) group.

Based on the histological classification 83 (83%) were in the endometrioid group, 7 (8.5%), 37 (44,5%), 38 (46%), and 1 (1%) patients were classified as CNH, MSI, NSMP and POLEmt group, respectively. Moreover, 17 patients were in the non-endometrioid cancers, 9 (53%), 1 (6%), 5 (29%), and 2 (12%) were classified as CNH, MSI, NSMP, and POLEmt groups, respectively. Only 2 patients had clear cell carcinoma and the remaining 15 had serous carcinoma in the non-endometrioid group.

POLEmt patients had NM_006231.4(POLE):c.1320C > G, NM_006231.4(POLE):c.1231G > A and NG_033840.1:g.18919 T > A. The last patient's missense variant had not been previously reported in the literature.

We investigated the characteristics of the stage, tumor size, myometrial invasion, cervical invasion, grade, abdominal washing cytology, and lymphovascular invasion. According to our analysis, stage, cervical invasion, LVSI, tumor grade, abdominal washing cytology, and histological subtype had significant relationship with the molecular subtype. All these data are summarized in Table 1. Briefly, p53 mutation rates was the highest in stage IV, NSMP rate was the highest in stage I disease, and MSI high status was detected 63% and 52% in stage II and stage III, respectively. NSMP was significantly higher in the cervical invasion compared to negative group, contrary MSI high disease was significantly higher in the cervical invasion than the positive group. Grade III disease showed significantly higher (40%) p53mt rate compared to NSMP (33%) group. Abdominal washing cytology was significantly positive CNH group (30% vs

Table 1 Descriptive statistics of general characteristics and clinicopathological with molecular subgroups

	Total group	p53mt	MSI	NSMP	POLEmt	*P* value
Stage						
I	59 (59%)	6 (10%)	20 (34%)	31 (53%)	2 (3,5%)	< 0.001
II	8 (8%)	0	5(63%)	3 (37%)	0	
III	21 (21%)	2 (10%)	11 (52%)	7 (33%)	1 (5%)	
IV	12 (12%)	8 (66%)	2 (17%)	2 (17%)	0	
Tumor size, cm	4,1	3,6	4,6	3,9	3	0.2
Myometrial invasion						
< 1/2	41 (41%)	6 (15%)	13 (32%)	21 (51%)	1 (2%)	0.07
> 1/2	59 (59%)	10 (17%)	25 (43%)	22 (36%)	2 (4%)	
Cervical invasion						< 0.001
Negative	75 (75%)	12 (16%)	26 (34%)	35 (47%)	2 (3%)	
Positive	25 (25%)	4 (16%)	12 (48%)	8 (32%)	1 (4%)	
Grade						< 0.001
I	32 (32%)	3 (10%)	12 (37%)	16 (50%)	1 (3%)	
II	38 (38%)	1 (3%)	20 (53%)	17 (44%)	0	
III	30 (30%)	12 (40%)	6 (20%)	10 (33%)	2 (7%)	
Abdominal cytology						< 0.001
Negative	77 (77%)	9 (12%)	33 (43%)	32 (41%)	3 (4%)	
Positive	23 (23%)	7 (30%)	5 (22%)	11 (48%)	0	
Histological subtype						< 0.001
Endometrioid	83 (83%)	7 (8%)	37 (45%)	38 (46%)	1 (1%)	
Non-Endomet	17 (17%)	9 (53%)	1 (6%)	5 (30%)	2 (11%)	
LVSI						< 0.001
Negative	71 (71%)	8 (50%)	11 (29%)	8 (19%)	2 (67%)	
Positive	29 (29%)	8 (50%)	27 (71%)	35 (81%)	1 (33%)	
Lymph node met						0.003
Negative	79 (79%)	10 (62%)	31 (82%)	35 (81%)	3 (100%)	
Positive	21 (21%)	6 (38%)	7 (18%)	8 (19%)	0 (0%)	

12%). Endometrioid carcinoma had significantly lower p53 mt (8% vs 53%) and higher MSI status (45% vs 6%).

Lymph node metastasis rate was significantly higher in CNH group compared to the others. In the CNH group, 29 of 437 (6.6%) dissected lymph nodes defined as metastatic disease. In the MSI group 18 of 645 (2.7%) dissected lymph nodes had metastasis, and in the NSMP group, 11 of 714 (1.5%) dissected lymph nodes had metastasis. POLEmt group did not have lymph node metastasis in 52 dissected lymph nodes. Age, tumor size, and myometrial invasion did not have a statistically significant relationship with the molecular groups.

In the MSI group, 5 of 38 patients had MSH2 mutation, 3 of 38 patients had MSH 6 mutation, 35 of 38 patients had PMS2 mutations and 22 of 38 patients had MLH1 mutation. 23 patients had doubled mutation, and 92% of them showed combination of PMS2 and MLH1 mutation, and 8% of the remaining had PMS2 with MSH6 mutations. Only one patient showed triplet mutation including PMS2, MSH2, and MSH6 mutations. According to the lymph node metastasis status in MSI group all metastatic patients showed either PMS2 and MLH1 or both of these mutations. Seven of all lymph node metastatic patients did not show MSH2 and/or MSH6 mutations.

In the NSMP group, none of the pathologic parameters showed a significant relationship with lymph node metastasis. Interestingly, NSMP group with lymph node metastasis did not have a recurrence during the follow-up period, on the other hand, 7 of 35 patients without lymph node metastasis in the NSMP group had recurrence (0% vs 20%, $p < 0.001$).

In the study, the mean follow-up time was 40 months, the median overall survival (OS) was 37 months (7–156), the median disease-free survival (DFS) was 36.5 months (4–140), and the progression-free survival rate was 23 months(3–36) in stage 4 disease. During the study follow-up period, 32 (32%) patients died and 14 (14%) patients had recurrence disease. The log-rank test was used to interpret the prognostic value of four molecular groups. Figures 1 and 2 show the OS and DFS graph in each group. The median OS was the highest in the POLE group (72 months) and lowest in the CNH group (36 months). The median DFS was highest in the POLE group (72 months) and lowest in the CNH group (28 months).

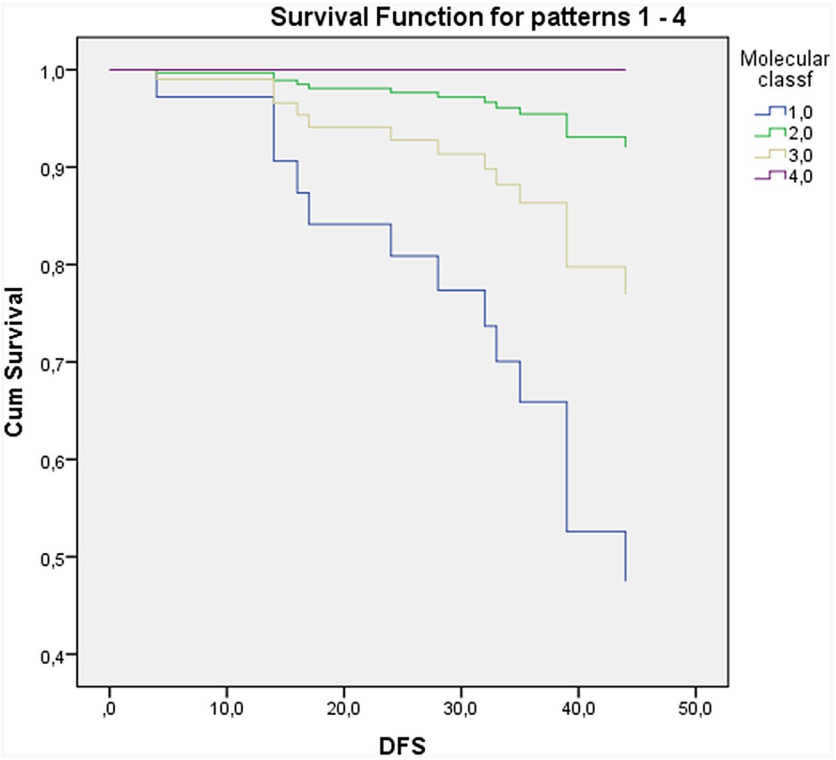

Fig. 1 The median overall survival rates of molecular subgroups. Legends: 1. POLE mutant group, 2. MSI group, 3. Nonspecific molecular profile, 4. P53 copy number high group

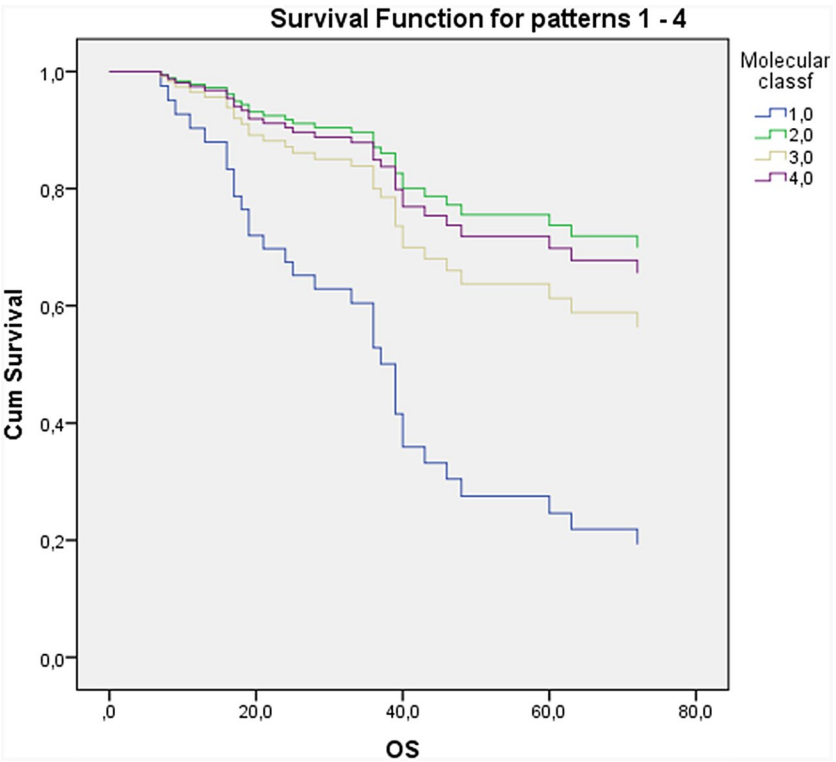

Fig. 2 The median disease free survival rates of molecular subgroups. Legends: 1. POLE mutant group, 2. MSI group, 3. Nonspecific molecular profile, 4. P53 copy number high group

The lymph node metastatic group had significantly lower OS and DFS (28 vs 39 months, *P*: 0.006 and 25 vs 38 months *P*: 0.003). LVSI positive group had significantly lower OS and DFS rates (27 vs 39 months, *P*: 0.003 and 36 vs 25 months *P*: 0.003).

Discussion

In the present study, we found that EC patients showed significantly different overall and disease-free survival according to the molecular subtypes and it was consistent with the literature, lymph node metastasis risk was the highest in CNH group (6.6%), and it was 1.5% in the NSMP group. LVSI positive group had significantly lower oncologic outcomes as well as patients who had lymph node metastasis.

Several risk classification schemes have been put out in EC over the past few decades also, and further description of the heterogeneity that distinguishes this tumor type [6]. However, the availability of numerous classification schemes frequently makes it challenging to choose the best one and adds to the clinical difficulties associated with patient management. Based on genomic, transcriptomic, and proteomic characterization, EC is divided into four categories: p53mut EC (mutation in p53), MMRd EC (mismatch repair protein deficiency), NSMP EC (nonspecific molecular profile), and POLEmut EC (pathogenic polymerase–epsilon variations). The term NOS (not otherwise specified) should be used if a molecular test is inconclusive or not performed [7].

About 7% of all EC have POLE mutations, and although patients have poor pathologic characteristics (high grade and deep myometrial invasion), this variety of tumors has an excellent prognosis and good progression-free survival (PFS). In the intermediate and high intermediate EC POLE mut and POLE wild type (wt) groups, the 10-year cancer-specific survival rates were 98% and 90%, respectively [4]8. The first Turkish population study was just published in 2022 and the investigators found that POLE mut rate was 30% in 97 EC patients. This is a high rate compared to the literature and our results showed a 3% of POLE mut rate. All these studies confirm that favorable prognosis in this molecular subtype.

The integration of LVSI, a significant prognostic factor linked to the risk of lymph node metastases, recurrence, and poor survival, and genetic characteristics have been demonstrated to improve risk assessment in EC with intermediate (high) risk. Independent of TCGA groups, age, and adjuvant therapy, LVSI has predictive value. Particularly, the risk of death from any cause, death from EC, and recurrent or progressive illness increases by 1.5–2 times in the presence of LVSI [9]. In a recent study, 367 patients with EC were divided into four molecular subgroups: 38 POLEmut EC (10.4%), 161 p53abn (43.9%), 107 MMRd (29.2%), and 61 NSMP EC (16.6%). Patients with p53abn and NSMP EC had poor prognoses: the 5-year recurrence rate for p53abn was 41.5% and 37.9%, respectively. Even if lymph nodes were negative and the cancer was stage I, patients with p53abn EC had a poor clinical outcome. Women with POLEmut EC had a high survival rate, even without adjuvant therapy. Finally, regardless of molecular subgroups, LVSI was accepted as a strong prognostic factor [10]. In our study, we found that LVSI positive group had significantly lower OS and DFS rates compared to LVSI negative group. Interestingly, LVSI rate was significantly higher in the MSI and NSMP groups compared to the POLE mut and p53 mt groups.

The Mayo criteria are the most extensively used algorithm in the EEC for estimating the probability of lymph node metastases. They are primarily based on preoperative and intraoperative clinicopathological results and use the parameters such as tumor diameter, grade, and myometrial invasion [11]. According to the Mayo criteria, the reported lymph node involvement risk for individuals classified as low and high risk was 1.4% and 6.4%, respectively [12]. In a recent study, pathological and molecular characteristics of EC with LN metastases (FIGO stage IIIC) were investigated; the mutational status of p53 and DNA mismatch repair (MMR) proteins in the primary lesion, as well as all positive LNs, were analyzed. 33 patients were analyzed in the study, and immunohistochemically, 12 patients (36.4%) had abnormal p53 expression in metastatic lesions, with a 93.4% concordance rate between primary and metastatic lesions. p53 expression in metastatic LNs was strongly linked with recurrence ($p=0.013$) [13].To our knowledge our results are concordant with the literature as worse outcomes in p53mt patients and lymph node metastasis risks are correlated with recurrence risk but some of them are the first findings; In the CNH group, lymph node metastasis risk was 6.6%, in the MSI group it was 2.7%, and in the NSMP group it was 1.5%. POLEmt group did not have lymph node metastasis in 52 dissected lymph nodes. So, we can speculate that sentinel lymph node biopsy or lymph node dissection can be essential for CNH and MSI group but for the NSMP and POLE mt group less invasive techniques can be an option if these findings should be validated by prospective trials.

Lastly, we found an interesting result, MSI status was significantly correlated with lymph node metastasis. Abnormal staining of MSH2 and MSH6 did not show a prognostic effect on lymph node metastasis, on the other hand, all lymph node metastatic patients showed PMS2 and MLH1 abnormal staining. This was the first data in the literature and we did not know exact data about the possible mechanism. However, we know that PMS2 and MLH1 form a heterodimer that directs the excision of the newly synthesized, error-containing strand and gap repair by DNA ligase and DNA polymerase. Furthermore, PMS2 has been demonstrated to be required for cisplatin-induced activation of P73,

a P53 family transcription factor with proapoptotic activity [14]. MSH2 and PMS2 protein expression were significantly correlated with tumor size, tumor invasion depth, and lymph node metastases in a colon cancer study [15].

We have some limitations in the study; this was a retrospective study and included all stages of EC but, we aimed to have a long-term follow-up period to detect possible long-term effects of molecular subtypes in this common gynecologic cancer. A single center of the study included a small region of the patient population.

In conclusion, endometrial cancer patients showed significantly different overall and disease-free survival according to the molecular subtypes and it was consistent with the literature, lymph node metastasis risk was the highest in CNH. MSI status is important for the lymph node metastasis risk but not all abnormalities, especially PMS2 and MLH1 expression changes had this risk.

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Declarations

Conflict of interest We do not have conflict of interest.

References

1. (2020) Corpus uteri Source: Globocan 2020
2. Siegel RL, Miller KD, Jemal A (2020) Cancer statistics, 2020. CA Cancer J Clin 70:7–30. https://doi.org/10.3322/CAAC.21590
3. Alexa M, Hasenburg A, Battista MJ et al (2021) Cancers the TCGA molecular classification of endometrial cancer and ıts possible ımpact on adjuvant treatment decisions. https://doi.org/10.3390/cancers13061478
4. Talhouk A, McConechy MK, Leung S et al (2017) Confirmation of ProMisE: a simple, genomics-based clinical classifier for endometrial cancer. Cancer 123:802–813. https://doi.org/10.1002/CNCR.30496
5. Lé On-Castillo A, De Boer SM, Powell ME et al (2020) Molecular classification of the PORTEC-3 trial for high-risk endometrial cancer: impact on prognosis and benefit from adjuvant therapy. J Clin Oncol 38:3388–3397. https://doi.org/10.1200/JCO.20
6. Kommoss S, McConechy MK, Kommoss F et al (2018) Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series. Ann Oncol Off J Eur Soc Med Oncol 29:1180–1188. https://doi.org/10.1093/ANNONC/MDY058
7. Bidzinski M, Danska-Bidzinska A, Rychlik A, et al (2022) Molecular classification of endometrial carcinoma, is it the new era of precision medicine? Ginekol Pol 93:. https://doi.org/10.5603/GP.a2021.0216
8. Church DN, Stelloo E, Nout RA et al (2015) Prognostic significance of POLE proofreading mutations in endometrial cancer. JNCI J Natl Cancer Inst 107:402. https://doi.org/10.1093/jnci/dju402
9. Raffone A, Travaglino A, Raimondo D et al (2022) Lymphovascular space invasion in endometrial carcinoma: a prognostic factor independent from molecular signature. Gynecol Oncol 165:192–197. https://doi.org/10.1016/J.YGYNO.2022.01.013
10. Leon-Castillo A, Horeweg N, Peters EEM et al (2022) Prognostic relevance of the molecular classification in high-grade endometrial cancer for patients staged by lymphadenectomy and without adjuvant treatment. Gynecol Oncol 164:577–586. https://doi.org/10.1016/J.YGYNO.2022.01.007
11. Li X, Cheng Y, Dong Y et al (2021) Development and validation of predictive model for lymph node metastasis in endometrial cancer: a SEER analysis. Ann Transl Med 9:538–538. https://doi.org/10.21037/ATM-20-5034
12. Vargas R, Rauh-Hain JA, Clemmer J et al (2014) Tumor size, depth of invasion, and histologic grade as prognostic factors of lymph node involvement in endometrial cancer: a SEER analysis. Gynecol Oncol 133:216–220. https://doi.org/10.1016/J.YGYNO.2014.02.011
13. Okamoto K, Nakamura K, Haraga J, Masuyama H (2022) Molecular characteristics of metastatic lesions have superior prognostic ımpact on endometrial cancer. Anticancer Res 42:4535–4543. https://doi.org/10.21873/ANTICANRES.15956
14. Wang Z, Sun Y, Gao B et al (2014) Two co-existing germline mutations P53 V157D and PMS2 R20Q promote tumorigenesis in a familial cancer syndrome. Cancer Lett 342:36–42. https://doi.org/10.1016/J.CANLET.2013.08.032
15. Zhao L (2018) Mismatch repair protein expression in patients with stage II and III sporadic colorectal cancer. Oncol Lett 15:8053–8061. https://doi.org/10.3892/ol.2018.8337