MOLECULAR CLASSIFICATION GUIDES FOR THE POSTOPERATIVE ADJUVANT THERAPY OF EARLY-STAGE ENDOMETRIAL CARCINOMA

by

Yixin PENG and Xingsheng YANG*

Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, Jinan, China

Original scientific paper
https://doi.org/10.2298/TSCI2403217P

Surgical treatment has been widely used for early-stage endometrial carcinoma patients, but 15-20% of patients had a poor prognosis, requiring a postoperative adjuvant therapy. With the development of molecular classification of endometrial carcinoma, the combination of molecular and clinicopathological factors can guide the prognosis risk assessment and make the adjuvant therapy selection more accurate, as a result, the survival outcome of patients can be greatly improved. In this article, the molecular classification of endometrial carcinoma is reviewed, and its guidance to the postoperative adjuvant therapy for early-stage endometrial carcinoma is discussed. It concludes that the molecular classification opens up the opportunity of creating new ideas for adjuvant treatment strategies for early endometrial cancer.

Key words: *endometrial carcinoma, adjuvant therapy, molecular classification*

Introduction

Recent years have seen a rocketing incidence of the endometrial carcinoma (EC). Most patients showed the corresponding clinical symptoms in the early stage (stages I and II), which was relatively easy to be diagnosed and treated, and the prognosis was relatively good, but there were still 15-20% patients with poor prognosis, requiring a postoperative adjuvant therapy. At present, the choice of the postoperative adjuvant therapy for early EC is still controversial, and there is a large number of undertreatment or overtreatment. In 2013, the *Cancer Genome Atlas* (TCGA) project conducted the molecular classification of EC [1]. Compared with histopathology, the molecular classification improves the accuracy of the diagnosis of endometrial carcinoma and is of great significance in predicting prognosis and guiding treatment. In 2020, European Society of Gynecological Oncology, European Society for Radiotherapy and Oncology, European Society for Pathology (ESGO-ESTRO-ESP) jointly updated the EC patient management guidelines to incorporate the molecular classification for the first time to assess of prognosis risk. In 2022, ESMO [2] revised the content of risk stratification in accordance with the ESGO-ESTRO-ESP Guidelines [3]. Changes in risk stratification only occurred in patients with early endometrioid carcinoma, namely, the molecular classification mainly changed the risk level of these patients, affecting the choice of the postoperative adjuvant therapy.

* Corresponding author, e-mail: 13903850116@163.com

Introduction of the molecular classification of endometrial carcinoma

The method of the early-stage EC postoperative adjuvant therapy is mainly based on the assessment of the risk factors for disease recurrence, including age, surgical pathological stage, pathological grade, lymph-vascular space invasion (LVSI), and myometrial invasion. In clinical practice, it was found that the biological behavior of about 20% of uterine serous carcinoma was similar to that of type I EC, which is not very aggressive [4]. The TP53 mutations, which is more common in uterine serous carcinoma, was also seen in 10% of endometrioid carcinoma [5]. Therefore, the classification of recurrence risk by clinicopathologic characteristics are not accurate enough, which has certain limitations in guiding the adjuvant therapy. The EC can be divided into four types [1]: POLE-mutated, high microsatellite instability (MSI-H), copy number-low (CNL), and copy number-high (CNH). About 25% of high-grade endometrioid carcinoma are POLE-mutated, which may lead to overdiagnosis if determined solely by histomorphology. The identification of patients with POLE-mutated with good prognosis from high-grade EC can avoid clinical overtreatment. The copy number-high EC, despite its morphology as endometrioid carcinoma, requires more aggressive clinical management.

The sequencing cost of the TCGA molecular classification is high and time-consuming, which is not conducive to clinical promotion. Subsequently, several research teams proposed some improved molecular classification methods, including the proactive molecular risk classifier for EC (ProMisE) molecular classification [6] and the TransPORTEC molecular classification [7], see figs. 1 and 2. The core molecular features were similar for both molecular classification methods, varying in the order of detection of POLE-mutations and mismatch repair (MMR) proteins. It was found that the genome was in a hyper-mutational state after POLE-mutation, and MMR or TP53 gene mutation might be a secondary event during tumor progression [8]. The first step of ProMisE is MMR protein detection, which may misjudge the POLE-mutated type with MMR protein deletion as mismatch repair-deficient (MMR-d) type, thus overtreating these patients. Therefore, the POLE detection is recommended as the first step in molecular classification methods. The above modified molecular classification methods have the same prognostic value as the TCGA molecular classification, reproducing the survival difference observed in TCGA and are widely used in current clinical studies. Leon-

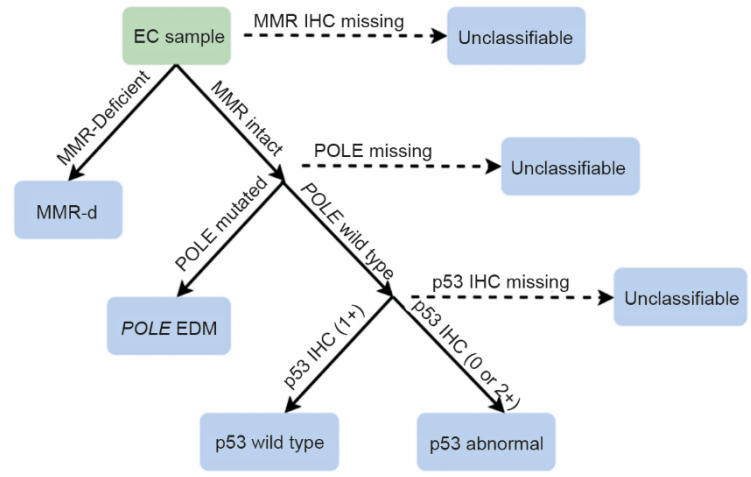

Figure 1. The ProMisE molecular classification [6]

Castillo *et al.* [8] reported tumors with POLE pathogenic mutations combined with MMR-d or TP53 mutations, are consistent with POLE-mutation in terms of morphological, molecular characteristics and clinical behavior, suggesting that EC with both MMR-d and TP53 mutations should be classified as MMR-d EC, and EC with both POLE-mutations and TP53 mutations should be classified as POLE-mutated EC.

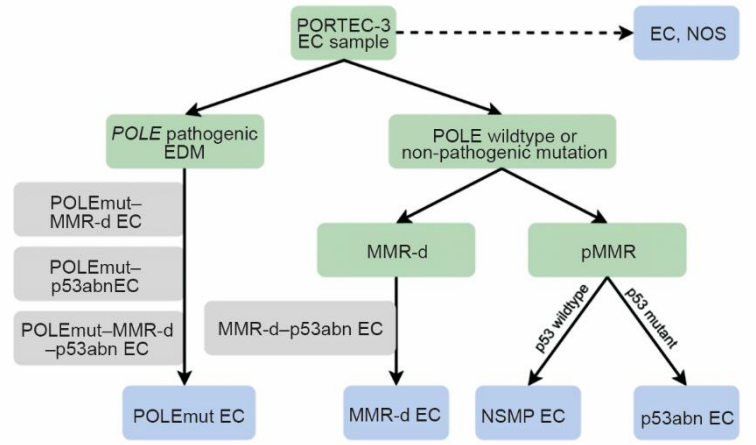

Figure 2. The TransPORTEC molecular classification [7]

In 2020, the TCGA molecular classification was first included in the National Comprehensive Cancer Network (NCCN) guidelines. In the same year, the molecular classification of EC was included in the 5th edition of the WHO classification of female genital tumors [9]. Comparison for molecular typing of endometrial carcinoma in different studies or guidelines are shown in tab. 1.

Table 1. Comparison for molecular classification of endometrial carcinoma in different studies or guidelines

TCGA [1]	ProMisE [6]	TransPORTEC [7]	ESGO-ESTRO-ESP [3]	WHO [9]	NCCN [10]
POLE	POLE-EDM	POLE-mutant	POLE-mutant	POLE-mutant	POLE
MSI	MMR-d	MSI	MMR-d	MMR-d	MSI-H
CNL	p53 wildtype	NSMP	NSMP	NSMP	CNL
CNH	P53-abnormal	p53-mutant	P53-abnormal	P53-abnormal	CNH

Note: CNL – copy number-low, CNH – copy number-high, MMR-d – mismatch repair-deficient, MSI-H – high microsatellite instability, NSMP – non-specific molecular profile, POLE EDM – polymerase epsilon exonuclease domain mutation

Molecular classification in guiding postoperative adjuvant therapy for early-stage endometrial carcinoma

The POLE-mutated

Van Gool *et al.* [11] created a POLE-mutated mouse embryonic stem cell line and demonstrated that POLE-mutation does not increase sensitivity to radiation or chemotherapy

compared to POLE wildtype, that is, the excellent prognosis of POLE-mutated patients cannot be explained by strong sensitivity to adjuvant therapy, clarifying that overtreatment for early POLE-mutated tumors (including EC and colorectal cancer) can be reduced by omitted adjuvant therapy. After meta-analysis of 359 patients with POLE-mutated EC, Mcalpine *et al.* [12] showed that none of the clinicopathological factors except stage were associated with recurrence, progression or death of disease, that is, adjuvant therapy was not associated with survival outcome in POLE-mutated EC patients.

A prospective cohort study Tailored Adjuvant therapy in POLE-mutated and p53 wildtype early-stage Endometrial cancer (TAPER) (NCT04705649) plans to include 276 patients with early POLE-mutated or NSMP EC for downgraded or omit adjuvant therapy to assess a low (<5%) pelvic (including vaginal) recurrence rate to clarify the safety of adjuvant downgrade in such patients.

An international multi-center clinical trial is underway for modified adjuvant therapy for endometrial carcinoma based on molecular classification of TransPORTEC-Refining Adjuvant treatment in endometrial cancer based on molecular features (RAINBO) (NCT05255653) [13]. Among them, RAINBO-BLUE included stage I-III POLE-mutated EC patients to assess the impact of postoperative adjuvant therapy downgrade (or without adjuvant therapy) on patient outcome.

The results of Eggink *et al.* [14] showed that PD-1 expression in POLE-mutated EC tissues was 73%, PD-L1 expression was 100%, indicating that POLE-mutated EC is sensitive to immunotherapy and suitable for the treatment of PD-1/PD-L1 inhibitors. However, due to the excellent outcome of POLE-mutated patients, only individual cases have reported the efficacy of immunotherapy in this part of the population.

The MMR-d

Radiotherapy plays an important role in MMR-d EC. In a multicenter retrospective cohort study of stage Ib/II high-grade endometrioid carcinoma, Reijnen *et al.* [15] showed that adjuvant radiotherapy was associated with improved disease-specific survival in patients with MMR-d (HR = 0.19, 95%CI 0.05-0-0.77) and not in patients with mismatch repair proficient (pMMR) patients, suggesting that MMR status could be used as a biomarker to select the population most able to benefit from adjuvant radiotherapy, but requiring further validation in prospective studies.

So far, for EC, the US Food and Drug Administration (FDA) approved pembrolizumab and dostarlimumab alone for advanced or recurrent MMR-d EC, and pembrolizumab combine lenvatinib for advanced or recurrent pMMR EC. Ongoing clinical studies try to determine the optimal timing of immunotherapy for MMR-d EC patients, as postoperative adjuvant therapy in combination with chemotherapy in newly diagnosed high-risk EC patients, or for salvage therapy in advanced or relapsed patients.

The NRG-GY020 Study (NCT04214067) plans to enroll 168 newly diagnosed early high-intermediate risk MMR-d endometrioid carcinoma, and the results of this clinical trial will clarify whether adding pablizumab to radiotherapy is more effective in reducing disease recurrence than radiotherapy alone. Similarly, Rainbo-Green [13] plans to enroll stage II with LVSI or III MMR-d EC patients to compare the efficacy of adjuvant radiotherapy with the sequential PD-1 inhibitor dostarlimab or placebo.

The NSMP

This group mainly includes endometrioid cancers with estrogen and progesterone receptor (ER, PR) positive and sensitive to hormone therapy. A randomized Phase III clinical trial initiated by Fudan University (NCT05454358) to evaluate letrozole as maintenance therapy for NSMP EC is underway. The study plans to include 299 NSMP EC patients with intermediate or high risk factors for postoperative adjuvant therapy according to the latest NCCN Guidelines or ESGO-ESTRO-ESP Guidelines, comparing progression-free survival between the letrozole group and the observation group. Rainbo-Orange [13] will include patients with stage II-III NSMP EC to compare whether there is a difference in the efficacy of chemoradiotherapy or radiotherapy combined with endocrine therapy.

The P53-abnormal

The p53-abnormal (p53abn) EC is the relatively worst prognosis subtype. To assess the prognostic value of molecular classification for high-risk EC, the investigators regrouped tissue samples from participants in the PORTEC-3 trial according to the molecular classification [16]. The results showed that p53abn patients had the worst prognosis and significant benefit from chemotherapy, and the 5-year recurrence-free survival between chemoradiotherapy and radiotherapy groups was 59% and 36% (P = 0.019), respectively. A randomized controlled trial of CAN-STAMP (NCT04159155) to evaluate first-line therapy for serous or p53abn EC is ongoing, in which an early cohort will include stage I-II eligible EC patients to compare the effects of chemotherapy alone (carboplatin plus paclitaxel) versus chemoradiotherapy on patient survival. For the application of immune-targeted therapy, RAINBO-RED [13] will include EC patients with stage I-IV p53abn EC, planned to evaluate the efficacy of adjuvant chemoradiotherapy combined with the PARP inhibitor niraparib or placebo.

The PORTEC-4a trial [17] (NCT03469674) is the first to use ProMisE molecular classification to guide adjuvant therapy in patients with high-intermediate risk endometrioid cancer. Integrating molecular classification with L1 cell adhesion molecule (L1CAM) overexpression, catenin Beta1 (CTNNB1) mutation, and substantial LVSI defined a molecular integrated risk profile, figs. 3 and 4. On the one hand, the PORTEC-4a Study hopes to avoid

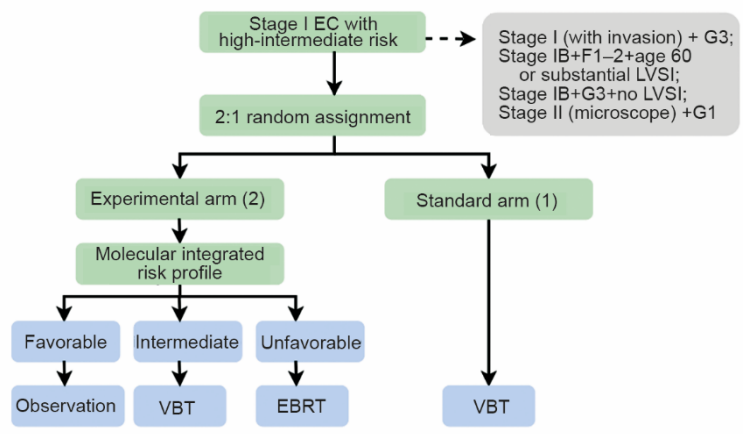

Figure 3. Study design of PORTEC-4a [17]; *EBRT – external beam radiation therapy, G – grade, LVSI – lymph-vascular space invasion, VBT – vaginal radiotherapy*

overtreatment through molecular classification, for example, some patients with high-risk factors in traditional clinicopathologic characteristics can be follow-up from a molecular point of view; on the other hand, the study also aims to apply external beam radiation therapy to avoid undertreatment in a few patients with high-risk factors (such as substantial LVSI, TP53 mutations, or L1CAM overexpression). If the PORTEC-4a shows that the local control rate is similar between experimental and control groups, then this model will be a standard method to guide adjuvant therapy.

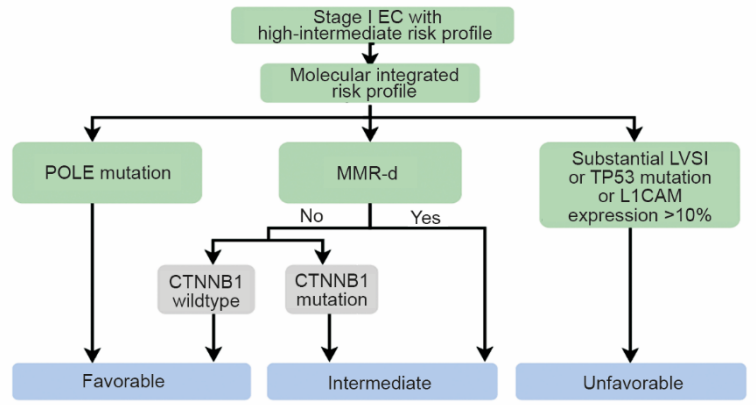

Figure 4. Molecular integrated risk profile [17]

Prospect of postoperative adjuvant therapy for early-stage endometrial carcinoma

The NSMP EC need to further refine the risk stratification

The NSMP is the most common molecular subtype, accounting for about half of all EC. The lack of predictive biomarkers to identify patients at high-risk of disease recurrence in this heterogeneous population has been described as *the most challenging molecular subtype*. Recent studies have found that many biomarkers can further refine this subgroup. After analyzing 342 EC patients, Kurnit *et al.* [18] found that CTNNB1 mutation EC often had low grade, low incidence of myometrial invasion and LVSI, which clinically indicated a lower risk of recurrence, but CTNNB1 mutation was associated with worse recurrence-free survival (HR = 5.97, 95%CI 2.69-13.21), suggesting that mutation analysis of CTNNB1 can help identify early stage and low-grade EC patients at high risk of recurrence.

In addition, NSMP/L1CAM positive patients often present with early recurrent [19], with a significantly shorter platinum-free interval after adjuvant platinum-based chemotherapy, and L1 CAM overexpression has been shown to be an independent prognostic marker for distant recurrence and overall survival in NSMP EC. Moreover, the expression of FGFR2c was significantly associated with the shorter disease-specific survival and progression-free survival in patients with endometrioid carcinoma [20]. The inclusion of FGFR2c into future molecular subtypes could further refine the risk stratification.

Potential therapeutic targets for TP53 mutation need to be further explored

Re-analysis of the TCGA data revealed HRD in 15% of the high copy number EC. The analysis by de Jonge *et al.* [21] found HRD in 46% of non-endometrioid carcinomas.

RAINBO-RED [13] plans to include patients with stage I-IV p53-abnormal EC to evaluate the efficacy of chemoradiotherapy combined with PARP inhibitor. Furthermore, combining trastuzumab on the basis of chemotherapy significantly improved survival in late or recurrent serous EC with HER-2 overexpression [22], and the current findings support the extension of HER-2-targeted therapy to early stage serous EC [23]. Vermij *et al.* [24] found that the correlation between p53-abnormal and HER-2 status was significantly stronger than that between serous tissue type and HER-2 status, suggesting that molecular classification-oriented HER-2 testing is better than tissue type-oriented detection.

The potential of immunophenotype for immunotherapy needs to be explored

In recent years, immunophenotype has unique advantages in predicting the effect of the immunotherapy in patients, which can provide a reference for the selection of postoperative adjuvant therapy in EC patients. After analysis of stage I EC pathological specimens in PORTEC-1 and 2 [25], Horeweg found that CD8+ cell density was the strongest predictor of EC recurrence, and a proportion of TP53 mutation and NSMP EC were also enriched with immune cells. It suggests that using molecular classification alone as a molecular marker for immune checkpoint blockade therapy may miss part of EC patients who can benefit from it. Although immunophenotype does not have independent prognostic significance [24], it can better judge the effect of immunotherapy in patients. The combination of molecular classification and immunophenotype facilitates the chose of patients more suitable for immunotherapy.

Conclusion

This review summarizes how molecular classification guides postoperative adjuvant therapy for early-stage endometrial carcinoma, and integrates the molecular classification with clinical practice and prospects, in order to provide reference and guidance for clinicians, help them to develop more accurate treatment plans for patients, improve the survival rate and the quality of life. The proposal of molecular classification is of great significance, showing potential in explaining patient differences in clinical efficacy, accurately predicting prognosis and guiding precise treatment. It is gradually widely used in clinical practice, but relevant treatment recommendations cannot meet the clinical needs. Gynecological oncologists need to keep pace with The Times. It is recommended to conduct molecular classification of all confirmed EC patients, continuously accumulate high-quality data through multi-center joint research, and apply high-level medical evidence to provide a basis for the chose of adjuvant therapy. The clinicopathological factors and molecular classification are rationally applied in the process of treatment decision-making to realize the individuality, precision and standardization of EC treatment.

References

[1] Getz, G., *et al.*, Integrated Genomic Characterization of Endometrial Carcinoma, *Nature*, *497* (2013), May, pp. 67-73
[2] Oaknin, A., *et al.*, Endometrial caNcer: ESMO Clinical Practice Guideline for Diagnosis, Treatment and Follow-up, *Annals of Oncology*, *33* (2022), 9, pp. 860-877
[3] Concin, N., *et al.*, ESGO/ESTRO/ESP Guidelines for the Management of patIents with Endometrial Carcinoma, *Virchows Archiv*, *478* (2021), 2, pp. 153-190
[4] Prueksaritanond, N., Chantape, W., Comparative Survival Outcomes of Uterine Papillary Serous Carcinoma, Clear Cell Carcinoma, Grade 3 Endometrioid Adenocarcinoma, and Carcinosarcoma of Endometrial Cancer in Rajavithi Hospital, *J. Med. Assoc. Thai.*, *99* (2016), Suppl. 2, pp. S75-S83

[5] Schultheis, A. M., *et al.,* TP53 Mutational Spectrum in Endometrioid and Serous Endometrial Cancers, *Int J Gynecol Pathol, 35* (2016), 4, pp. 289-300
[6] Kommoss, S., *et al.,* Final Validation of the ProMisE Molecular Classifier for Endometrial Carcinoma in a Large Population-Based Case Series, *Ann Oncol, 29* (2018), 5, pp. 1180-1188
[7] Stelloo, E., *et al.,* Improved Risk Assessment by Integrating Molecular and Clinicopathological Factors in Early-stage Endometrial Cancer-Combined Analysis of the PORTEC Cohorts, *Clin. Cancer Res., 22* (2016), 16, pp. 4215-24
[8] Leon-Castillo, A., *et al.,* Clinicopathological and Molecular Characterisation of 'Multiple-Classifier' Endometrial Carcinomas, *J. Pathol., 250* (2020), 3, pp. 312-322
[9] *** WHO Classification of Tumours Editorial Board, *WHO Classification of Tumours: Female Genital Tumours,* 5thed Lyon: IARC Publications, 2020, pp. 252-267
[10] Abu-Rustum, N., *et al.,* Uterine Neoplasms, Version 1.2023, NCCN Clinical Practice Guidelines in Oncology, *J. Natl. Compr. Canc. Netw., 21* (2023), 2, pp. 181-209
[11] van Gool, I. C., *et al.,* Adjuvant Treatment for POLE Proofreading Domain-Mutant Cancers: Sensitivity to Radiotherapy, Chemotherapy, and Nucleoside Analogues, *Clin. Cancer Res., 24* (2018), 13, pp. 3197-3203
[12] Mcalpine, J. N., *et al.,* Evaluation of Treatment Effects in Patients with Endometrial Cancer and POLE Mutations: An Individual Patient Data Meta-Analysis, *Cancer, 127* (2021), 14, pp. 2409-2422
[13] Jamieson, A., *et al.,* The Emerging Role of Molecular Pathology in Directing the Systemic Treatment of Endometrial Cancer, *Ther. Adv. Med. Oncol., 13* (2021), 13, 17588359211035959
[14] Eggink, F. A., *et al.,* Immunological Profiling of Molecularly Classified High-Risk Endometrial Cancers Identifies POLE-Mutant and Microsatellite Unstable Carcinomas as Candidates for Checkpoint Inhibition, *Oncoimmunology, 6* (2017), 2, e1264565
[15] Reijnen, C., *et al.,* Mismatch Repair Deficiency as a Predictive Marker for Response to Adjuvant Radiotherapy in Endometrial Cancer, *Gynecol. Oncol., 154* (2019), 1, pp. 124-130
[16] Leon-Castillo, A., *et al.,* Molecular Classification of the PORTEC-3 Trial for High-Risk Endometrial Cancer: Impact on Prognosis and Benefit From Adjuvant Therapy, *J. Clin. Oncol., 38* (2020), 29, pp. 3388-3397
[17] Van den Heerik, A., *et al.,* PORTEC-4a: International Randomized Trial of Molecular Profile-Based Adjuvant Treatment for Women with High-Intermediate Risk Endometrial Cancer, *Int. J. Gynecol. Cancer, 30* (2020), 12, pp. 2002-2007
[18] Kurnit, K. C., *et al.,* CTNNB1 (Beta-Catenin) Mutation Identifies Low Grade, Early Stage Endometrial Cancer Patients at Increased Risk of Recurrence, *Mod. Pathol., 30* (2017), 7, pp. 1032-1041
[19] Ravaggi, A., *et al.,* Integrated Biomarker Analysis Reveals L1CAM as a Potential Stratification Marker for No Specific Molecular Profile High-Risk Endometrial Carcinoma, *Cancers (Basel), 14* (2022), 21, 5429
[20] Sengal, A. T., *et al.,* FGFR2c Mesenchymal Isoform Expression Is Associated with Poor Prognosis and Further Refines Risk Stratification within Endometrial Cancer Molecular Subtypes, *Clin. Cancer. Res., 26* (2020), 17, pp. 4569-4580
[21] de Jonge, M. M., *et al.,* Frequent Homologous Recombination Deficiency in High-grade Endometrial Carcinomas, *Clin. Cancer Res., 25* (2019), 3, pp. 1087-1097
[22] Fader, A. N., *et al.,* Randomized Phase II Trial of Carboplatin-Paclitaxel Compared with Carboplatin-Paclitaxel-Trastuzumab in Advanced (Stage III-IV) or Recurrent Uterine Serous Carcinomas that Overexpress Her2/Neu (NCT01367002): Updated Overall Survival Analysis, *Clin. Cancer Res., 26* (2020), 15, pp. 3928-3935
[23] Erickson, B. K., *et al.,* Human Epidermal Growth Factor 2 (HER2) in Early Stage Uterine Serous Carcinoma: A Multi-Institutional Cohort Study, *Gynecol. Oncol., 159* (2020), 1, pp. 17-22
[24] Vermij, L., *et al.,* HER2 Status in High-Risk Endometrial Cancers (PORTEC-3): Relationship with Histotype, Molecular Classification, and Clinical Outcomes, *Cancers (Basel), 13* (2020), 1, 13010044
[25] Horeweg, N., *et al.,* Prognostic Integrated Image-Based Immune and Molecular Profiling in Early-Stage Endometrial Cancer, *Cancer Immunol. Res., 8* (2020), 12, pp. 1508-1519

Paper submitted: March 16, 2023
Paper revised: May 21, 2023
Paper accepted: May 21, 2023