MOLECULAR CLASSIFICATION OF ENDOMETRIAL CANCER

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Introduction

Endometrium cancer (EC) is the 6th most common tumor in women worldwide, and it is the most common tumor of the female genital tract. According to the recently published reports, 382,069 new cases were detected worldwide in 2018, and 89,929 deaths occurred due to diseases (1). The incidence of EC has increased in recent years due to reasons such as the expected increase in human life span, obesity, and metabolic syndrome. Unlike other malignancies, there is an increase in EC-related mortality rates (2). By 2025, new case and mortality rates are expected to increase by 20.3% and 17.4%, respectively (1).

The most important prognostic factors associated with EC are tumor grade, histological subtype, deep myometrial invasion, cervical involvement, tumor size, lymphovascular area invasion (LVSI) and lymph nodes status (3).

EC is conventionally divided into two groups based on clinical, pathological and molecular characteristics. The most common of these is type 1 or endometrioid subgroup (ECC) and it includes endometrioid histological types and has a good prognosis, while type 2 or non-endometrioid (NEEC) type, serous (10%), clear cell (3%), adenocarcinoma and other rare types and have a worse prognosis (4). Although its prognostic value is limited; this dual classification is used for preoperative evaluation and surgical planning (5). There are clinically important shortcomings of this classification. Using this classification, approximately 20% of ECC relapses, while almost 50% of NEEC cases do not (4). Besides 15-20% of ECC are high-grade lesions and they are not included in this classification (6).

With the consensus established by ESGO, ESMO and ESTRO, patients were classified according to their risk status using clinical, molecular and pathological characteristics to determine outcomes, recurrence risk and to plan treatment (7). Albeit this system has the highest efficacy in classifying EC patients to determine the risk of recurrence, 9% of patients in the low-risk group can develop recurrence. On the other hand, 60% of the patients in the high-risk group do not develop recurrence (8). This is not an uncommon condition because of the heterogeneous nature of the EC tumor. However practical translation of this risk system may result in inadequate treatment for a part of the patients in the low-risk group and unnecessary adjuvant therapy for some patients in the intermediate-high risk group. This was the main indication to research and investigate for a more precise classification system, that eventually added molecular biomarkers (6).

Classification	**Histopathology-Stage**	**5-Year Survival**	**Treatment**

Low	Type 1, FIGO 1A, grade ½ EC	93.4%	Total abdominal hysterectomy + bilateral salpingooferectomy
Intermediate	Type 1, FIGO 1B, grade ½ EC	86.3%	Total abdominal hysterectomy + bilateral salpingooferectomy + Lymphadenectomy + Brachytherapy
High-Intermediate	EC with Type 1, FIGO 1A, grade 3 or Type 1, FIGO 1A / B, grade ½ LVSI	82%	Total abdominal hysterectomy + bilateral salpingooferectomy + Lymphadenectomy + Brachytherapy +/- Teletherapy
High	EC advanced than Type 1, FIGO 1B, grade 3, Type 2 or stage 1	Less than 74%	Total abdominal hysterectomy + bilateral salpingooferectomy + Lymphadenectomy + Brachytherapy +/- Teletherapy +/- Sitereductive surgery +/- Chemotherapy

Table 1. ESMO endometrial cancer classification (7). EC: Endometrial cancer; LVSI: Lymphovascular area invasion.

In 2013, The Cancer Genome Atlas (TCGA) Research Network proposed a new classification based on the molecular characterization of the tumor (9). In this study; 373 EC cases (307 endometrioid, 53 serous, 13 mix histology) were evaluated in terms of their genomic, transcriptomic and proteomic properties using array and sequencing techniques, and EC was divided into 4 different subgroups.
POLE ultra-mutated (Ultramutated group with pathological variants of DNA polymerase epsilon (POLE) exonuclease domain - 7%
Microsatellite stability instable (MSI) hypermutated group - 28%
Copy number low (microsatellite stable, MSS) group characterized by low mutation load - 39%
Copy number high (serous like) group mostly characterized by the p53 mutation - 26%
Pole ultra-mutated group of tumors; in the POLE gene, which is involved in the replication and repair of nuclear DNA and is the catalytic subunit of the polymerase enzyme; they contain mutations in the exonuclease domain. It is characterized by high mutation rates (232 × 10-6 mutation per Mb). It is found in a small group of all EECs and some serous ECs and is characterized by an excellent prognosis (10). Progression-free survival in TCGA in tumors with POLE mutation has been shown as 100%.

MSI hyper-mutated group tumors have promoter methylation in the MLH1 gene and a smaller number of mutations (18 × 10−6 mutations per Mb). They usually consist of tumors in endometrioid histology and show a moderate prognostic course.
Copy number low (CNS, endometrioid-like group) tumors have a lower mutation rate (2.9 × 10−6 mutations per Mb). This group consists primarily of microsatellite stable endometrioid tumors. CTNNB1 mutation can be observed more frequently than average in this group of tumors (52%) (6).

The MSI hyper-mutated group is distinguished by containing most of the high-grade ECs that show genomic instability, the copy number low (MSS) group is distinguished by a lower rate of somatic copy number changes (6).
Copy number high (serous like) is characterized by the worst prognosis and has lower mutation rates (2.3 × 10−6 mutations per Mb) but a high number of somatic copy number changes.

	POLE-ultramutaed	**MSI-hypermutated**	**Copy number low, CNS endometrioid**	**Copy number high, serous like**
Prevalence	5-15%	25-30%	30-40%	5-15%
Clinical feature	Diagnosis at a young age	May be associated with Lynch syndrome	High BMI	Advanced stage at diagnosis
Histology	Endometrioid in general	Endometrioid in general	Endometrioid in general	Serous and endometrioid
Grade	G3> G1, G2	G3> G1, G2	G1, G2> G3	G3
Stage	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4
Histological feature (11th)	Mixed morphology	Mucinous differentiation, MELF type invasion, LVSI involvement	Squamous differentiation, ER and PR diffuse positive	Diffuse cytonucleer atypia
Tp53 mutation	35%	5%	one%	>90%
Specific molecular changes	Hotspot mutations in the POLE gene	DNA MMR protein loss	CTNNB1 (52%)	Tp52, 25% ERBB2 amplification
Protein-IHQ		MMR-loss: MLH1, MSH2, MSH6, PMS2	MMR-proficient, Tp 53 wild type mutation	Tp 53 mutation, Tp 53 abnormal
Progression free survival	Excellent (phase independent)	Middle	Medium (stage independent)	Bad (stage independent)

Researched treatment modalities (12.20-23)	Immune checkpoint inhibitors (The ultramuteted condition present in tumors with POLE mutation creates a highly immunogenic environment with intra-peritumoral lymphocyte infiltration, PD-1 + PD-L1 expression and the addition of T cell markers to the situation and may become targets for immune checkpoint therapy (12, 20- 23).	Immune checkpoint inhibitors	PI3K / AKT / mTor inhibitor, hormonal therapy	Cell cycle regulators, PI2K / AKT / Mtor inhibitor, hormonal therapy

Table 2. Clinical features, risk factors, molecular features, diagnosis, prognosis and treatment modalities associated with each subgroup of the TCGA classification system (6,11,12,20-23). MSI: microsatellite stability instable; MSS: microsatallite stable; IHQ: immunohistochemistry; MMR: miss-match repair proteins.

The addition of the TCGA classification to the currently used EC histopathological classification has a prognostic significance. When the results of the TCGA study are examined, POLE mutation can be seen in all grades, and mutation frequency increases as grade increases. However, progression of the disease is not observed in any of the patients with high-grade POLE mutation.

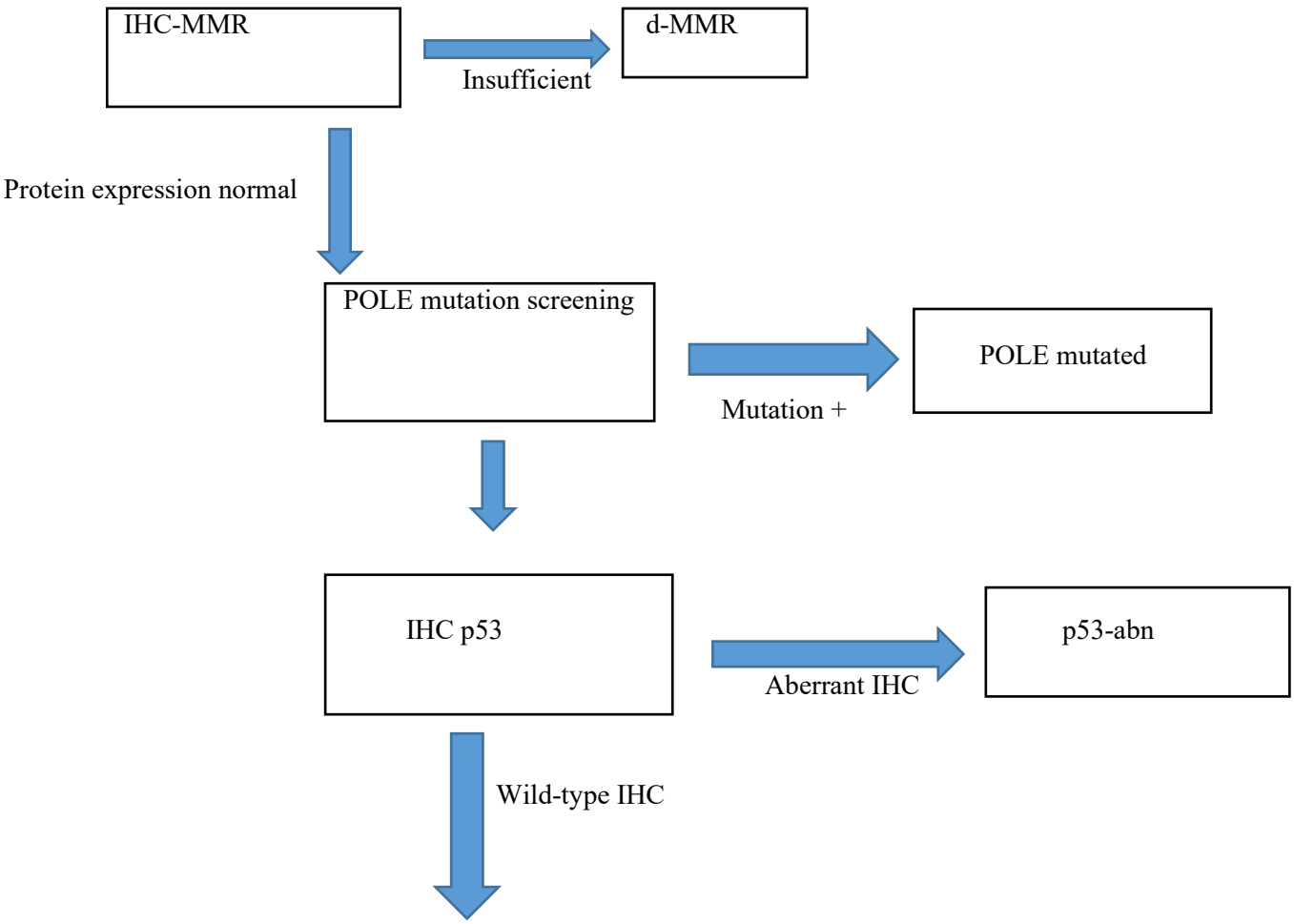
In conventional classification, all grade 1 EECs have an excellent prognosis and low recurrence rates, and adjuvant treatment is not required. However, only 7% of POLE mutations were detected in the grade 1 ECC histopathological group and it was stated that they would show an excellent prognosis and has been observed that the presence of high copy numbers, which is seen at a rate of approximately 2%, is associated with a poor prognosis (9). Therefore, the group that needs adjuvant therapy due to the high risk of recurrence may receive incomplete treatment. Again, in this study, it was determined that the group with POLE mutation, which constitutes approximately 6-13% of the whole ECC, progressed with an excellent prognosis independent of the factors that determine the treatment and survival for EC such as stage, grade, myometrial invasion.

	POLE	**MSI**	**CN-low**	**CN-high**

ECC-G1	7%	26%	65%	2nd%
ECC-G2	5%	31%	56%	8%
ECC-G3	17%	54%	9%	20%
SEC			2nd%	98%

Table 3. Distribution of histopathological groups into subgroups according to the TCGA classification (6). ECC: Endometrioid Carcinoma, SEC: Serous Carcinoma, MSI: Microsatellite stability instable, CN: Copy number, POLE: DNA polymerase epsilon.

To increase the clinical usability and prognostic accuracy of the TCGA study, the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE, Vancouver) and transPORTEC (Leiden) classification systems were created (15). In these studies, 3 main biomarkers were examined (Figure 1). In the analysis; by sequencing the POLE exonuclease domain mutation, MMR (MLH1, MLH2, MSH6, PMS2) immunohistochemical examination (IHC-MMR) were performed and the presence of deficiency of d-MMR, again by immunohistochemically p53 protein analysis and evaluation of p53-wt (wild type) and p53-abn (abnormal) were made (13-15).



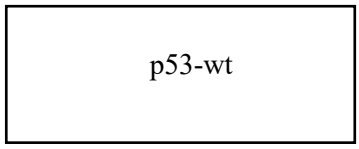


Figure 1. Application of ProMisE molecular classification. IHC: Immunhistochemistry, MMR: mismatch repair preotein, wt: wild type, abn: abnormal, POLE: DNA polymerase epsilon.

In a study conducted on 452 patients; 28.1% of the patients had d-MMR, 9.3% had POLE-mutation, 12.2% had p53-abn and 50.4% had p53-wt, and the prognoses of the 4 subgroups examined were different from each other in the analysis (18).

In 2020, an up-to-date meta-analysis consisting of 3 studies and a total of 912 patients trying to reveal the histopathological characteristics of ProMisE groups was published (19). In this meta-analysis, characteristics of ProMisE groups according to histopathological type, grade, myometrial invasion, lymphovascular area invasion and ESGO-ESMO-ESTRO risk groups were determined. In this analyzes, it was found that when patients were treated by clinical, histopathological and risk group characteristics, many could have under or overtreatment, particularly in the POLE mutation and d-MMR groups. The authors had emphasized the necessity of making molecular and histopathological evaluations together.

TransPortec is a classification system that is created by the addition of independent prognostic factors such as L1CAM, LVSI, CTNNB1 to the ProMise system aims to differentiate tumor positively and negatively (24). Data on the clinical usability of these subgroups will be revealed with the on-going PORTEC-4 study. The PORTEC-4 study is a randomized phase 3 study aiming to compare the efficacy of adjuvant radiotherapy in moderate to high-risk patients according to molecular characteristics. It is planned to randomize 500 patients into a) brachytherapy as the standard treatment, b) brachytherapy and/or external beam radiotherapy (EBRT) in the study arm. In the study arm; if the presence of POLE mutation- MSS- CTNNB1-wt is found, observation is chosen; if MSI or CTNNB1 mutant is found, brachytherapy will be performed; In case of extensive LVSI presence - 10% more staining with LCAM1, p53 mutant is detected, EBRT will be applied. With the results of this study; the effect of planning adjuvant therapy according to molecular features will be revealed.

Systemic analysis is necessary for detecting pathological POLE mutation, while immunohistochemical analysis of p53 protein cannot reflect Tp53 copy-number changes exactly. Classification of tumors that may contain more than one genomic variants is difficult and the system does not contain the heterogeneity observed in copy-number low group and these factors appear to be the limiting factors for non-TCGA classification in EC (16,17).

However, despite all these limitations; currently available scientific data support the use of this system.

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