MOLECULAR CLASSIFICATION OF ENDOMETRIAL CANCER

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Abstract

For endometrial cancer, the current risk stratification remains problematic and cannot be fully assigned until after surgery. A new framework for understanding and classifying endometrial carcinomas (ECs) was provided by the Cancer Genome Atlas findings of four prognostic associated molecular subgroups: *ultramutated, hypermutated, copy number (CN) high* and *CN low*. For clinical implementation analogous subgroups have been identified: POLE mutated (analogous to *ultramutated*), mismatch repair deficient (analogous to *hypermutated*), abnormal p53 (analogous to *CN high*) and those lacking the above-mentioned molecular features (analogous to *CN low*). This review investigates the incorporation of molecular based classification into routine practice. The analogous subgroups are applicable to biopsies/curetting, thus providing earlier accessibility to prognostic information to guide EC management. Patients with POLE mutated EC have a favorable prognosis and treatment de-escalation may be considered, while patients with p53 abnormal EC have a poor prognosis and escalated treatment may be considered. However, challenges regarding inconsistencies in analysis and interpretation of POLE mutations were identified and a solution has recently been suggested by a group of researchers. Likewise, challenges regarding interpretation of ECs with more than one molecular classifying feature have been addressed. Exactly how the molecular information should be incorporated into the risk-based approach has yet to be determined and the molecular classification is currently being evaluated in a clinical setting. It may soon be ready for implementation into routine practice and facilitate a more objective risk stratification and more homogeneous subgroups of EC for clinical use and future research.

Key words: Endometrial carcinoma, molecular classification, genomic subtype, risk stratification, ProMisE

Introduction

Endometrial cancer is the sixth most common cancer accounting for over 2% of all cancer related deaths in women worldwide (1). Currently, risk stratification is mainly based on clinical and histopathological factors, for instance integrated in European Society for Medical Oncology (ESMO) risk groups as presented in table 1. The 2013 version of ESMO risk stratification of endometrial cancer (2) is considered the frame of reference in the included studies and likewise in this review, despite of more recent versions (3).

"Low risk"	Stage IA (grade 1 and grade 2) with endometrioid type
"Intermediate risk"	Stage IA grade 3 with endometrioid type Stage IB (grade 1 and 2) with endometrioid type
"High risk"	Stage IB grade 3 with endometrioid type all stages with non-endometrioid type

Table 1 – ESMO 2013 risk stratification for endometrial cancer (2)
IA: <50% myometrial invasion, IB: ≥50% myometrial invasion. Grade 1: <5% solid component, grade 2: 5-50% solid component, grade 3: >50% solid component. ESMO: European Society for Medical Oncology

The histopathological evaluation remains problematic due to high interobserver variability, particularly in high-grade endometrial carcinomas (ECs) (4), and patients with histologically similar ECs may have different outcomes (5). For these reasons among others there is room for improvement of the current risk stratification. Incorporation of molecular based classification of EC might add a level of objectivity which will yield biologically more homogeneous subgroups.

The aim of this review is to investigate the application of molecular classification into EC management, including determination of polymerase epsilon (POLE), mismatch repair (MMR) and p53 status due to the fact that they have shown great promise in a classifier, that recreates the molecular subgroups of EC from The Cancer Genome Atlas (TCGA) research findings. The molecular classification may change clinical practice for EC diagnostics in the next few years and thus it is an interesting research topic.

TCGA molecular subtypes of EC

In 2013 TCGA research project discovered four distinct molecular subtypes of EC based on genomic alterations. The subtypes are associated with clinical outcome and thus provides a new framework for understanding and classifying EC. The project preformed a combination of whole genome sequencing, exome sequencing, copy number analysis, DNA methylation analysis as well as RNA and protein expression analysis. Based on the molecular information from these tests, the subtypes could be identified: *ultramutated* with frequent POLE mutations, *hypermutated* with microsatellite instability (MSI), *copy number high (CN-high)* with frequent mutations in the TP53 gene and *copy number low (CN-low).* (6)

The *CN-high* subtype primarily consists of serous ECs and the *hypermutated* subtype of endometrioid ECs (6), yet both low-grade and the currently challenging high-grade endometrioid EC occur in all four molecular subtypes (7, 8). This indicates that ECs within the molecular subtypes may be classified differently using the current classification system.

Unfortunately, due to methodological challenges, such as the need for fresh frozen tissue, long turn-around time, cost, and lack of applicability to biopsies or curetting, the TCGA approach is not currently applicable to routine practice. Hence identification of more clinical applicable methods are needed.

TCGA molecular subtypes of EC subsequently proved to be reproducible using clinically applicable surrogate markers (9). One classifier in particular is dominating the research field. It uses a combination of MMR, POLE and p53 analysis to detect ECs analogues to the TCGA subgroups. The classifier was developed (9), confirmed (10), and validated (11) according to the Institute of Medicine Guidelines for biomarker development (12) and is currently being evaluated in clinical trials for clinical utility (13). It may soon be ready for incorporation into clinical practice and thus this review focuses on this classifier in particular.

Materials and methods

To identify relevant articles, a literature retrieval was conducted in Medline and Embase from their interception to February 2020. The combined search terms and an overview of the selection process is illustrated in figure 1. Relevant Subject Headings were inclu ded, and truncations were used to include various word endings and spellings. The search was conducted in accordance with recommendations made by specialist librarians at University of Southern Denmark. Only studies investigating molecular classification of EC based on interpretation of MMR, POLE and p53 status were included. The inclusion was limited to articles in English, German, Danish, Norwegian, and Swedish language. Case reports and letters to editor/author were a priori defined as exclusion criteria. References from each full-text screened article were also considered. A control search was conducted in Google Scholar to identify additional relevant articles.

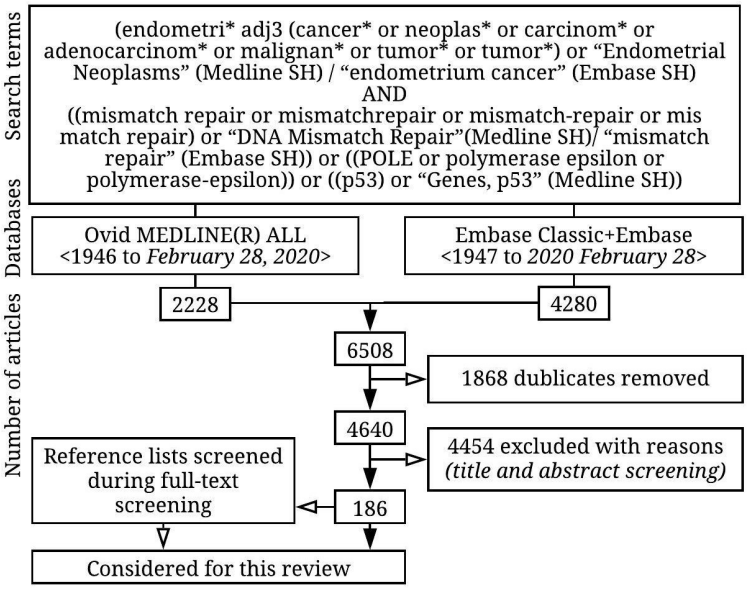

Figure 1 – Overview of literature search and inclusion

Adj3: adjacency operator threshold; the search terms are within two words of each other. SH: Subject Heading

Results

The combined search yielded 6508 articles, and after removal of duplicates, 4640 titles and abstracts were screened. 186 articles initially qualified for full-text screening. Due to the number of relevant articles, the most relevant, largest, and newest were considered for this review and were continually included - likewise for articles identified through reference lists. No further relevant articles were identified through the additional Google Scholar search.

Proactive Molecular Risk Classifier for Endometrial Cancer

Most relevant articles identified concern a classifier called Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE). In 2015 Talhouk and colleagues developed ProMisE, which applies MMR, POLE and p53 status for risk stratification of ECs. Analogous to TCGA *CN-high* subgroup with frequent TP53 mutations is the p53 abnormal subgroup (*p53abn*). *P53abn* ECs is identified by abnormal p53 expression in immunohistochemical (IHC) staining. MMR deficiency leads to MSI (14) and ECs analogous to TCGA *hypermutated* subtype with MSI is the MMR deficient (MMRd) ECs. The *MMRd* subgroup is identified by absence of MMR proteins in IHC staining. Analogous to TCGA *ultramutated* subtype with frequent POLE mutations, is the POLE mutated subtype (*POLEmt*), identified by exonuclease domain mutations (EDM) of the POLE gene. Analogous to the TCGA *CN-low* subtype is the ECs lacking the above-mentioned molecular features; referred to as *Non-Specific Molecular Profile (NSMP).* (9)

An overview of the TCGA and ProMisE subgroups is presented in table 2. Please note that the prevalence of the molecular subgroups may vary depending on the histological subgroup of EC (e.g. *POLEmt* subtype present in up to 21% of grade 3 endometrioid ECs (8)).

TCGA (6)	ProMisE (9-11)	Prevalence (6, 9-11)	Prognosis (15)	Dominating histotype (6)
Hypermutated	Mismatch repair deficient (*MMRd*)	20-29%	Intermediate	Endometrioid
Ultramutated	POLE mutated (*POLEmt*)	7-9%	Favorable	-
Copy number high (*CN-high*)	p53 abnormal (*p53abn*)	12-27%	Unfavorable	Serous
Copy number low (*CN-low*)	Non-Specific Molecular Profile (*NSMP*)	39-50%	Intermediate	-

Table 2 – Overview of TCGA and ProMisE subgroups of endometrial carcinomas

TCGA: The Cancer Genome Atlas, ProMisE: Proactive Molecular Risk Classifier for Endometrial Cancer, POLE: polymerase epsilon

The ProMisE algorithm for classifying ECs is illustrated in figure 2. ECs with deficient MMR expression is considered *MMRd* and those with intact MMR is examined for POLE-EDM and if present, considered *POLEmt*. Those without POLE mutations can be classified as *p53abn* or *NSMP* EC depending on p53 status. (9)

This surrogate-model for TCGA classification is more cost-effective and practical, allowing testing on formalin-fixed paraffin-embedded tissue (9) and with results based on biopsy/curetting being highly concordant with results based on the hysterectomy specimen *(kappa=0.88 (95% CI: 0.79-0.94)* (11).

ProMisE analysis methods

- MMR status was examined by IHC for MLH1, MSH2, MSH6 and PMS2 proteins in the discovery study (9), while an approach with only MSH6 and PMS2 was used subsequently (11).
  Tumors were considered *MMRd* in the case of complete absence of nuclear staining combined with positive staining of control tissue (9). In the validation cohort, the tumors were considered *MMRd* if both MSH6 and PMS2 had abnormal IHC, otherwise they were considered MMR proficient (11).

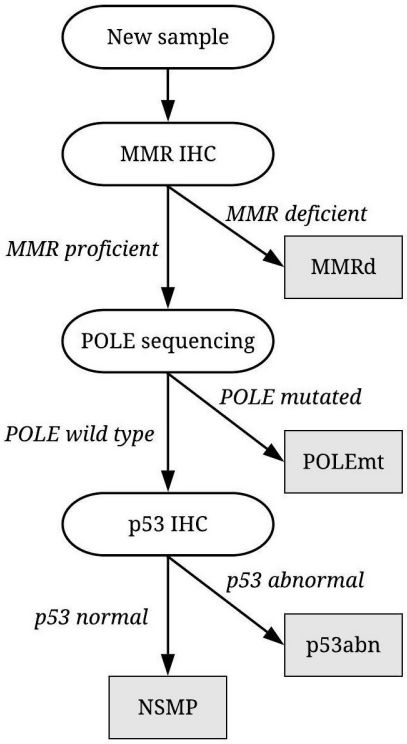

Figure 2 – ProMisE algorithm (9)

MMR: mismatch repair, IHC: immuno-histochemistry, POLE: polymerase epsilon, NSMP: Non-specific molecular profile, ProMisE: Proactive Molecular Risk Classifier for Endometrial Cancer

MMRd can be as a result of Lynch syndrome and subsequent identification of lack of MLH1 promotor methylation might be considered to detect these cases (14), but it will not be discussed in this review.
MMR IHC evaluation was shown to be highly concordant with MSI assay for detection of the TCGA *hypermutated* subtype *(kappa=0.854 (95% CI 0.811-0.897)* (14) and more cost-effective than MSI assay (9).

- P53 status was examined by IHC and considered abnormal in the complete absence of nuclear staining or when staining was strong and diffuse (>80%). Normal expression was defined as week to moderate (1-80%) and on-slide positive and negative controls were used (9).
  The concordance between p53 (protein) IHC and TP53 (gene) mutation is high (92-95%) (16).

- For detection of *POLEmt* subtype, ProMisE used next-generation sequencing (NGS) targeting POLE exonuclease domain exons 9-14 for "validated mutations", and hotspot regions (P286 and V411) were adequately covered (9-11). The mutations were confirmed by Sanger sequencing, and the outcome was binary: mutated or wild type (11). No IHC surrogate was identified.
  In the TCGA cohort, P286R and V411L were present in 76% of the tumors in the *ultramutated* subtype (6).

Insufficient molecular classification was present in 6-11% of the cases in the confirmation and validation studies, and data were missing among all molecular features (10, 11).

ProMisE concordance and discriminative ability

ProMisE demonstrated a high level of interlaboratory concordance *(kappa=0.82),* which increased when correcting some technical challenges of week p53 IHC stating in one site *(kappa=0.96).* No significance level is available. (17)

ProMisE's ability to discriminate survival outcomes is illustrated by Harrel's C-index. A C-index of 0.5 indicates that the model has no discriminative ability and a value of 1 indicates that the model perfectly distinguishes who have an event and who do not. Values >0.7 indicates a good model and values >0.8 indicates a strong model (18). ProMisE failed to demonstrate a significant ability to discriminate survival outcomes in the discovery cohort (9), yet the ability was significant in the confirmation and validation cohorts. Depending on the endpoint selected the C-index ranged from 0.67-0.74 (lowest limit 0.6) in the confirmation cohort and 0.63-0.67 (lowest limit 0.57) in the validation cohort (10, 11). In the confirmation cohort, the ability of ProMisE and ESMO risk stratification were similar and seemed to improve slightly when combined (10). In the validation cohort, the ability of ProMisE seemed lower than that of ESMO risk stratification, but the difference did not reach statistical significance (11).

Prognostic value of ProMisE subgroups

In the ProMisE studies most survival outcomes were associated with molecular subtype and the ProMisE cohorts consisted of patients with endometrioid, serous, clear cell, undifferentiated and mixed histology ECs (9-11). Pooled data of survival outcomes in the molecular subgroups are presented in table 3. The results are reported in a meta-analysis including 2818 patients from six studies (15). The meta-analysis included the discovery, confirmation, and validation studies of ProMisE along with three other studies (containing 80% of the included patients), which were restricted to endometrioid EC (7, 8, 19), of which one was restricted to grade 3 endometrioid EC (8). A single study used MSI (assay) instead of MMR (IHC) analysis for detection of the *hypermutated/MMRd* subtype (7).

	MMRd	POLEmt	p53abn
Univariable analysis			
OS	**1.522** (1.101-2.104; I^2 = 65.63)	**0.589** (0.376-0.921; I^2 = 18.15)	**3.179** (1.946-5.193; I^2 = 83.22)
DSS	**1.965** (1.278-3.023; I^2 = 20.66)	0.552 (0.257-1.187; I^2 = 0)	**5.052** (3.242-7.872; I^2 = 38.22)
PFS	1.354 (0.813-2.255; I^2 = 74.28)	**0.287** (0.152-0.542; I^2 = 0)	**3.512** (1.838-6.710; I^2 = 83.97)
Multivariable analysis*			
OS	1.192 (0.943-1.508; I^2 = 29.98)	0.795 (0.514-1.230; I^2 = 13.34)	**1.986** (1.517-2.600; I^2 = 22.87)
DSS	1.068 (0.720-1.585; I^2 = 0)	**0.325** (0.111-0.949; I^2 = 0)	**2.133** (1.352-3.365; I^2 = 0)
PFS	0.817 (0.530-1.257; I^2 = 0)	**0.217** (0.104-0.452; I^2 = 0)	**1.833** (1.379-2.436; I^2 = 55.95)

Table 3 - Pooled data of prognosis in the molecular subgroups of endometrial cancer (15) [HR (95% CI; I^2)]
Non-specific molecular profile (NSMP) subgroup is considered reference for HR analysis. (continued)

**Multivariable survival analysis considered prognostic factors available from time of diagnosis and post-surgical staging, such as age, BMI, grade, histotype, stage, nodal status, myometrial invasion, LVSI, and adjuvant treatment status in addition to TCGA group.*
HR: Hazard ratio, MMRd: mismatch repair deficient, POLEmt: polymerase epsilon mutated, p53abn: p53 abnormality, OS: overall survival, DSS: disease specific survival, PFS: progression free survival.

Patients with *POLEmt* subtype have the most favorable prognosis and it does not seem to be affected by clinicopathological factors. Prognosis of patients with *p53abn* subtype is the most unfavorable and is further worsened by unfavorable clinicopathological factors. The prognosis of the *MMRd* subgroup overlaps with that of *NSMP* subgroup but is worsened by unfavorable clinicopathological factors. (15)

Histopathological characterization of ProMisE subgroups

Pooled data from a meta-analysis of prevalence of histopathological characteristics in the ProMisE subgroups are shown in table 4. The analysis included the discovery, confirmation, and validation studies of ProMisE with a total of 912 ECs, and the heterogenicity among the studies were mainly moderate or high. (20)

		MMRd	**POLEmt**	**p53abn**	**NSMP**
Histological grade	3	47.4% (14.4-82.8)	39.6% (11.0-77.6)	90% (77.5-95.9)	15.6% (6.1-34.5)
	1-2	52.6%	60.4%	10%	84.4%
Histotype	Endometrioid	85.8% (70.5-93.9)	86.1% (76.5-92.1)	27% (17.9-38.6)	96.7% (86.4-99.3)
	Non-endometrioid	14.2%	13.9%	73%	3.3%
ESMO 2013 risk category	“Low risk”	30.1% (15.0-51.4)	44.1% (15.0-77.9)	7.2% (2.4-19.6)	59.5% (53.4-65.4)
	“Intermediate risk”	19.9%	22.5%	8.1%	17.3%
	“High risk”	50% (30.8-69.2)	33.4% (16.1-56.6)	84.7% (73.4-91.7)	23.2% (13.6-36.9)

Table 4 - Prevalence of histological characteristics in ProMisE subgroups (20) [prevalence (95% CI)]
MMRd: mismatch repair deficient, POLEmt: polymerase epsilon mutated, p53abn: p53 abnormality, NSMP: Non-Specific Molecular Profile, ESMO: European Society for Medical Oncology, ProMisE: Proactive Molecular Risk Classifier for Endometrial Cancer.

Overlap of cases were found in the *p53abn* subgroups and ESMO “high risk” group, and the prevalence differ significantly from that of *NSMP* subgroup, but otherwise great diversity of risk-group assignment within the ProMisE subgroups, with both ESMO “low risk“ and “high risk” present in all subtypes. Likewise, high prevalence of aggressive pathologic features (e.g. grade 3 and non-endometrioid) was noted in *p53abn* tumors. Contrarily, a relatively high prevalence of low grade and endometrioid histotype in *POLEmt* and *NSMP* subgroups were present (20).

Discussion

Applicability to diagnostic specimen

The high concordance of ProMisE classification in diagnostic (biopsy/curetting) and postsurgical staging specimen (hysterectomy) found in the validation study (11) was confirmed in other studies (21, 22). The obvious advantage to successful molecular classification with ProMisE in diagnostic specimen is earlier accessibility of prognostic information, thus providing information that could be used to plan the timing and extent of surgery. This is a considerable advantage compared to ESMO risk stratification, which cannot be fully assigned until after surgery *(e.g. stage requires hysterectomy specimen for evaluation of myometrial invasion (2)).*
Another advantage to diagnostic over postsurgical specimen is a superior antigen preservation due to a more rapid fixation (21). However, the associated stronger IHC staining can complicate the interpretation of p53 and MMR IHC staining (16). Likewise, in case of a sparse amount of tissue in the biopsy/curetting, the interpretation of IHC might be challenging too.

Detection of *hypermutated/MMRd* subtype

MMR IHC seems like a reasonable surrogate method for detection of the TCGA *hypermutated* subtype based on the cost-effectiveness and high concordance with MSI assay (9, 14).
The utility of MSH6 and PMS2 IHC testing for *MMRd* have been confirmed. It is as efficient as the four-antibody panel in detecting *MMRd*, yet it has been suggested that it can be followed by MLH1 and MSH2 in case of loss of MSH6 or PMS2 (14).

Pathologists should be aware of sub-clonal loss, and it is suggested that cases with sub-clonal loss (>10%) of MMR protein expression should be classified as MMR deficient (14). For interpretation of MMR status, on-slide positive control tissue should be used as in ProMisE studies to ensure high quality of staining (9).

Detection of *CN-high/p53abn* subtype

The concordance of p53 IHC and TP53 mutation is high *(92%)* and improved after excluding *MMRd* and *POLEmt* ECs *(95%)* (16), and it has been shown that p53 IHC is only a surrogate for TCGA *CN-high* subtype if *POLEmt* and *MMRd* tumors are excluded (6, 9, 11). As for MMR IHC, positive on-slide control tissue increases the credibility of the interpretation. Regarding the interlaboratory concordance for ProMisE, the laboratory with weak p53 staining did not use on-slide control tissue (17). The lower cost and wide availability of p53 IHC in pathology departments support MMR IHC as the preferred tool for detection of the TCGA *CN-high* subtype. (9)

Detection of *ultramutated/POLEmt* subtype

Nowadays, the *POLEmt* subtype is mainly detected by NGS or Sanger sequencing (7, 11, 17, 23), but it is not widely available and is time-consuming compared with IHC procedures. Thus, it represents a challenge for implementation into routine practice, that no IHC surrogate for *POLEmt* has been identified. However, a greater challenge at present is the lack of transparency and evidence of analysis and interpretation of POLE mutations. The "validated mutations" mentioned in ProMisE publications are not specified and it needs to be illustrated which mutations interpreted as pathogenic other than those of hotspot regions *(P286 (exon 9) and V411 (exon 13))* (11).

It is important to note that the presence of a POLE mutation alone is insufficient to classify tumors as *POLEmt* since not all POLE mutations are pathogenic (24). There is no clear consensus of the interpretation of the POLE mutations among studies and thereby the definition of the *POLEmt* molecular subtype of EC (23-26). The latter might represent a problem regarding comparability within research results if they do not stratify patients on the same terms. To define the prognostically favorable group of *POLEmt* it is essential to carefully select the pathogenic POLE mutations (23, 24). However, the most frequent (66-76%) POLE mutations (P286R and V411L) are both considered pathogenic (6, 27) and represents a consistent basis.

To overcome the challenge with uncertain interpretation of POLE mutations and definition of the *POLEmt* subgroup of EC, a research group developed a pathogenicity scoring system for POLE mutations (POLE-score) (24) based on TCGA findings. The principle of POLE-score is illustrated in figure 3. 11 POLE mutations were identified as pathogenic according to POLE-score, and should be classified as *POLEmt* (24). These findings may be helpful uniformizing the *POLEmt* subgroup of EC in future research and subsequent clinical implementation.

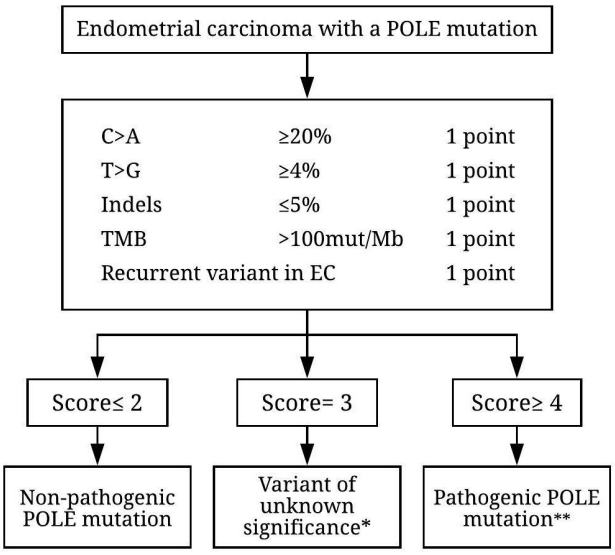

Figure 3 – Pathogenicity scoring system for POLE mutations (POLE-score) (24)

** A465V, L424V, T278M, A428T. ** P286R, V411L, S297F, S459F, A456P, F367S, L424I, M295R, P436R, M444K, D368Y*

TMB: tumor mutational burden, mut: mutations, Mb: megabase

A different approach of identifying POLE mutations called BaseScope has been suggested (26). BaseSco pe uses in situ hybridization assays for identification of POLE mutations P286R and V411L on RNA level. The method has reached a sensitivity of 95% and a specificity of 100% when compared with Sanger sequencing in a study of 51 high grade ECs of which 39 had POLE mutations. Despite the small study size and the high-grade only, it may be interesting as it can be applied to samples with a small amount of tissue and the positive signals can be counted under microscopy by eye while morphology can be directly combined as reference. For a more objective interpretation, computers can count the positive signals as well. (26)

Quality of ProMisE as a classifier

Although ProMisE's ability to discriminate survival outcomes are significant, it is not a "strong model" and probably not even a "good model" to discriminate survival outcomes. It is unclear whether the currently used ESMO risk stratification has a similar or slightly higher discriminative ability. However, a major benefit of ProMisE over ESMO risk stratification is it can provide prognostic information preoperatively.

The near-perfect interlaboratory concordance of ProMisE subgroup assignment indicates great promise for a more homogeneous risk-group assignment among pathology departments and comparability in future clinical trials regarding EC. However, it should be noted that no significance levels are available, so it only is an indication for the time being.

The extent of insufficient molecular classification may represent a challenge, but it is unclear whether the missing data is due to failed analysis or interpretation, or due to practical circumstances regarding the scientific work (10, 11). However, in any case, the ECs with insufficient molecular classification should not be misinterpreted as *NSMP* subtype since the tumors have not been completely profiled. They should be classified as *not otherwise specified (NOS)* and risk stratified according to current standards.

Outcomes in ProMisE subgroups

The survival outcomes in the molecular subgroups are variable among the included studies in the meta-analysis, thus the meta-analysis provided estimates, that may better reflect the actual prognostic significance of the molecular subgroups. Although not all survival data are significant in the meta-analysis, the significant outcomes confirms the prognostic value of the molecular subgroups and their suitability for risk stratification of EC.

When pooling the data of prognosis to better detect the actual prognosis of the molecular subgroups in general, it may be distorted when half of the included studies *(containing 80% of the included patients)* were restricted to endometrioid EC (15). Fortunately, the endometrioid histotype represents the majority of ECs (2).

The wide prognostic overlap found between *MMRd* and *NSMP* subgroups may be due to the heterogenicity of the latter one, and it may be a general problem, using *NSMP* subgroup as reference since it is the largest and least molecularly defined subgroup (15). However, the *MMRd* subgroup should be investigated further to confirm the prognostic value of this specific molecular subgroup.

It is an interesting finding that the prognosis of *POLEmt* subgroup of EC seems quite unaffected by unfavorable clinicopathological factors. It may indicate that in the case of *POLEmt* subtype of EC in biopsy/curetting, no further diagnostic initiatives are needed regarding risk stratification.

Changing risk stratification

Apart from the high prevalence of ESMO "high risk" in the *p53abn* subgroup, no predictable associations between ProMisE and ESMO risk groups were found, indicating that they stratify ECs differently. This is supported by the fact that all molecular subgroups include both ESMO "low risk" and "high risk" ECs. (20)

Besides the association with ESMO "high risk", *p53abn* tumors are associated with aggressive pathologic features (e.g. grade 3 and non-endometrioid histotype) (20), which is consistent with the poor prognosis in this subgroup of EC (15). A high prevalence of low grade and endometrioid histotype is observed in *POLEmt* and *NSMP* subgroups ECs, which is consistent with the relatively good prognosis of these subgroups (15, 20).

However, since in average 33% of the *POLEmt* ECs with a favorable prognosis are identified as ESMO "high risk", incorporation of molecular classification may avoid several overtreatments in this subgroup of EC. On the other hand, in average 7% of the *p53abn* with a poor prognosis are identified as "low risk" and undertreatment may be avoided in this subgroup (20).

From a pathologist point of view, it can be interesting to see how the molecular subtypes allocate amongst histopathological subtypes of EC, but this approach has not been systematically investigated.

ECs with more than one molecular classifying feature

While most ECs can be classified into a single molecular subtype, 2-4% have more than one classifying feature (11, 28, 29) and the interpretation of these are interesting, as one feature may recommend intensified treatment (*p53abn*) and another one treatment de-escalation (*POLEmt*).

The "multiple classifiers" have been investigated in 3518 ECs of which 3% (107) had *p53abn* subtype in addition to another molecular subtype of EC (29) and 0.9 % had M*MRd-POLEmt* ECs (30 cases) (24). The findings of these studies are presented in table 5. The subtype clustering was based on clinical outcomes as well as known histopathological and molecular features *(e.g. TCG>TTG substitutions, which are frequent in POLEmt EC and absent in p53abn EC)* (29).

"Multiple classifier"	Clustered with	Prevalence [%]
MMRd-p53abn	*MMRd*	1.8
POLEmt-p53abn	*POLEmt*	0.9
MMRd-POLEmt	*	0.9-3.4**
MMRd-POLEmt-p53abn	*	0.3

Table 5 – Clustering and prevalence of "multiple classifiers" in ProMisE (24,25)

**Clustering not significant **The prevalence in the presented study is 0,9 %, yet the prevalence is 3,4% in the TCGA study (6) MMRd: mismatch repair deficient, p53abn: p53 abnormality, POLEmt: polymerase epsilon mutated, ProMisE: Proactive Molecular Risk Classifier for Endometrial Cancer*

Based on selected molecular features like proportions of single nucleotide variants and somatic copy number alterations, the *MMRd-p53abn* and *POLEmt-p53abn* mostly clustered with single classifier *MMRd* ($p \leq 0.001$) and *POLEmt* ($p \leq 0.001$), respectively. For instance the clinical outcome of patients with *MMRd–p53abn* and *POLEmt–p53abn* ECs (*5-year recurrence free survival (RFS) of 92% and 94%, respectively)* was significantly different from "single classifier" *p53abn* EC *(RFS 71%, p=0.024 and p=0.050, respectively)* (29). These findings suggests that p53 mutations in "multiple classifier" ECs are probably passenger mutations not affecting clinical behavior, which is supported by the fact, that they often show sub-clonal p53 overexpression (29).

Regarding multiple classifier *MMRd-POLEmt* ECs, the researchers did not conduct the same kind of clustering (based on clinical outcome, and molecular and histopathological features). However, patients with *MMRd-POLEmt* seems to have worse prognosis *(5-year RFS and OS of 81-83%)* than patients with single classifier *POLEmt* EC *(5-year RFS and OS of 92-100% (6, 7, 9)* – but no statistics were made on the difference (24), thus it cannot be evaluated. The researchers did not compare the survival outcome of *MMRd-POLEmt* EC with that of single classifier *MMRd*, which for frame of reference is a 5-year OS of approximately 70-75% and a 5-year PFS of approximately 75-80% (9-11) – i.e. slightly lower than of *MMRd-POLEmt* EC. The researchers found that POLE mutations coexisting with *MMRd* are more likely to be non-pathogenic than pathogenic (evaluated by POLE-score), though it is not universally valid (24). When dividing the POLE-EDMs into pathogenic or non-pathogenic in the case of *MMRd-POLEmt*, the 5-year RFS was 92% and 76%, respectively, but the difference was not significant (p=0.40) (24). Even though some of the above-mentioned findings suggest that *MMRd-POLEmt* should be classified as single classifier *MMRd*, the latter findings indicate that *MMRd* coexisting with pathogenic POLE-EDM should be classified as *POLEmt*, while those with non-pathogenic POLE-EDM variants should be classified as *MMRd*. This underlines that the presence of a POLE-EDM alone is insufficient to classify tumors as *POLEmt* molecular subtype and the importance of only classifying the pathogenic variants of POLE-EDMs as the *POLEmt*/*ultramutated* subtype of EC (24).

The order of the algorithm

Since the order of the classification algorithm dictates the order in which tumors are assigned to a specific subtype, it is important that the dominant subtype determining tumor phenotype and clinical outcome is assigned as the first one. Assessment of p53 should indisputably be the last one. Unfortunately, due to the non-evident findings regarding multiple classifier *MMRd-POLEmt* EC, the order of assessment of MMR and POLE status remains unclear. An argument of MMR assessment as the first one is one should not risk de-escalating treatment (due to favorable prognosis of *POLEmt* EC) considering the woman could carry a germline MMR mutation (Lynch syndrome). Lynch syndrome accounts for 3% of all ECs (30) and is associated with an increased risk of colon, uterine and ovarian carcinomas (31). Despite that *MMRd-POLEmt* only account for 0.9-3.4% of ECs (6, 29), it is a clinically relevant subgroup of ECs and further research is needed to establish the appropriate order of the classification algorithm to correctly classify these cases.

Other molecular based classifiers

Contemporary with ProMisE, a similar classifier was developed, identifying TCGA molecular subtypes with distinct outcomes and high diagnostic reproducibility. Unlike ProMisE, the *hypermutated/MMRd* subtype was identified by use of MSI assays (7, 32) instead of MMR IHC, yet MMR IHC is more suitable for routine practice (9).

Surrogates of the TCGA subgroups with a different approach than ProMisE have also been suggested. For instance, a classifier using a multigene NGS panel and MSI determination has recently been suggested (33). By examining POLE, PTEN, TP53, ARID1A, KRAS, ARID5B, FBXW7, PPP2R1A, CTCF, CTNNB1, RPL22, PIK3CA, and PIK3R1 EC subtypes analogous to those of TCGA can be identified. An advantage to this approach is avoiding the intrinsic inter-observer subjectivity within immunohistochemical evaluation.

Sub-stratification of *NSMP* subgroup

As previously discussed, the *NSMP* group is large and heterogeneous, and thus the benefit of sub-stratification is obvious. Molecular markers such as CTNNB1 and L1CAM mutations were recently identified as independent prognostic markers for worse prognosis (34, 35). Remarkably, the CTNNB1 gene encodes the protein β-catenin, which has shown high diagnostic accuracy as an IHC surrogate marker for CTNNB1 mutation (36). It is present in 52% of the ECs in the *NSMP* subgroup (6) and may gain impact for further prognostic sub-stratification of *NSMP* subgroup in the nearby future.

Targeted treatment

Interestingly, MMR deficiency appears to predict clinical benefit of anti-PD-1 immunotherapy in both colorectal and non-colorectal cancers (37), indicating that it may be the case in *MMRd* ECs as well. However, no studies focusing on *MMRd* EC were identified and such studies should be conducted to confirm the response in *MMRd* EC.

Clinical implementation of ProMisE

Regardless of the fact that TCGA study only included serous and endometrioid histotypes of EC, the ProMisE classification should be applicable to endometrial carcinomas in general with similar findings as those of TCGA (10, 11). The molecular subtypes have shown prognostic significance in unselected groups of ECs (10, 11). However, the utility of molecular classification might vary depending on histotype, which have not been investigated.

How the additional molecular information should be incorporated into the risk-based approach has still to be determined. It seems prudent, however, that treatment de-escalation is considered in *POLEmt* EC and intensified treatment is considered in *p53abn* EC.

The ongoing PORTEC-4a (Post-Operative Radiation Therapy in Endometrial Carcinoma) trial (13) is comparing individualized adjuvant treatment based on a molecular classification to standard adjuvant treatment. It has been suggested that the *POLEmt* subtype is considered as “favorable” risk profile, *MMRd* as “intermediate” risk profile and *p53abn* as “unfavorable” risk profile. The *NSMP* subgroup is divided into “favorable” and “intermediate” risk profiles depending on CTNNB1 status. In addition to the *p53abn* subtype, ECs with substantial lymph-vascular space invasion or >10% L1CAM expression are considered “unfavorable” as well. Women with high-intermediate risk EC are randomized to receive vaginal brachytherapy *(standard adjuvant treatment)* or individualized adjuvant treatment where women with “unfavorable” risk profile receive external beam radiotherapy, women with “intermediate” risk profile receive vaginal brachytherapy and women with “favorable” risk profile are only observed. The PORTEC-4a trial was initiated in 2016 and the primary outcome is 5-year cumulative incidence of recurrence (13). Regarding clinical implementation of molecular classification of EC, it is a very relevant trial to be attentive to in the years to come.

In conclusion, this review shows that changing the EC classification to include molecular characteristics provides promising opportunities but will also create certain challenges with respect to incorporation into clinical practice.

Limitations

A pertinent limitation to this review is that deviation form a systematic literature inclusion was made due to the number of relevant articles and the formal requirements given by University of Southern Denmark regarding this master thesis. The quantity of relevant research achievements with uncertain and varying results caused that this review did not achieve in dept presentation and analysis of all aspects of the research topic *(e.g. the divergence regarding POLE mutations in EC).*

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