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Molecular classification of endometrial carcinoma: a clinically oriented review

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Take home messages

Molecular classification defines four subgroups of endometrial carcinoma that are associated with different prognoses.

Although current guidelines recommend molecular classification in all endometrial carcinomas to improve risk-stratification, it is possible to restrict comprehensive molecular workup to 40% of cases without compromising risk-assessment.

The role of molecular subgroups in modifying the effect of traditional prognostic factors and predicting response to adjuvant therapies can be considered key themes in future research.

Abstract

The Cancer Genome Atlas research network performed a genome-wide analysis of endometrial carcinomas in 2013 and classified tumors into four distinct subgroups: polymerase-ϵ ultramutated; microsatellite unstable hypermutated; copy-number low; and copy-number high. These molecular alterations are mostly mutually exclusive as only about 3% of tumors exhibit

more than one molecular signature. Apart from the polymerase-ϵ ultramutated subgroup, molecular classification can be reproduced by utilizing surrogate markers. This has facilitated the implementation of molecular diagnostics into routine patient care. Molecular subgroups are associated with different prognoses; thus, improved risk-assessment is their most obvious clinical application. However, based on their unique molecular architectures, molecular subgroups should not be regarded simply as risk groups but rather as distinct diseases. This has prompted us and others to examine the role of molecular subgroups in modifying the prognostic effect of traditional risk factors, including clinical factors, uterine factors, and tissue biomarkers, and in predicting the response to adjuvant therapies. In the following review, we summarize the current knowledge of molecularly classified endometrial carcinoma and present, based on our own experience, a proposal for implementing molecular classification into daily practice in pathology laboratories.

Introduction

Cancer of the uterine corpus is the most common cancer of the female genital tract in developed countries.[1] Worldwide, more than 400,000 new cases of uterine corpus cancer are diagnosed annually. The vast majority (95%) of uterine cancers are carcinomas that develop in the epithelial compartment of the uterine mucosa (endometrium).

Endometrial carcinomas have been traditionally divided into two main types based on clinical, endocrinological, and metabolic features.[2] Type I cancers (65%) are mostly represented by low-grade endometrioid tumors arising in pre- or perimenopausal women who often show hyperestrogenism, obesity, and other signs of metabolic syndrome. Typically, type I cancers harbor a favorable outcome (five-year survival rate 85.6%). Type II cancers (35%) follow an estrogen-unrelated pathway and generally develop from atrophic endometrium in postmenopausal women in the absence of metabolic disturbances. Type II cancers are typically high-grade endometrioid or nonendometrioid carcinomas and follow an aggressive clinical course (five-year survival rate 58.8%).

The paradigm of endometrial carcinoma dichotomy was profoundly challenged in 2013 when The Cancer Genome Atlas (TCGA) consortium performed a genomic, transcriptomic, and

proteomic characterization of endometrial carcinomas and identified four pathogenetically and prognostically distinct molecular subgroups of the disease.[3] The TCGA analysis changed the landscape of research on endometrial carcinoma so that molecular classification should now be included in predictive and prognostic research models whenever possible.

In this clinically oriented review, we summarize the current knowledge of molecularly classified endometrial carcinoma and propose that molecular subgroups should not be approached merely as risk groups of one disease. Rather, based on findings from our research group and others, molecular subgroups of endometrial carcinoma may be considered distinct disease entities, an argument that, if held true, provides new insights into future research and patient care.

Molecular classification

The 2013 TCGA analysis was performed on 373 endometrioid and serous/mixed endometrial carcinomas.[3] The proportion of grade 1–2 endometrioid carcinomas was lower compared with cases registered in the U.S. Cancer Database between 2004 and 2016 (52% vs. 67%).[4] The following tumor characteristics were investigated: overall mutational burden; somatic copy number alterations and nucleotide substitutions; p53, *POLE,* and phosphatase and tensin homolog (*PTEN*) mutations; microsatellite instability; and histology.[3] Tumors were classified into four distinct subgroups: polymerase-ϵ (*POLE*) ultramutated (7%); microsatellite unstable hypermutated (28%); copy-number low (39%); and copy-number high (26%). Classification was carried out in a stepwise fashion with *POLE* ultramutated as the first subgroup (Figure 1A). *POLE* wild type (wt) tumors were then categorized according to the microsatellite instability status and microsatellite stable tumors according to copy number alterations. The analysis demonstrated an association between molecular subgroups and patient outcome, so that the *POLE* ultramutated and copy-number high subgroups were associated with an excellent outcome and poor outcome, respectively, whereas the microsatellite unstable hypermutated and copy-number low subgroups were associated with an intermediate outcome.

The survival differences have subsequently been recapitulated in two classifiers, *i.e.* the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE)[5] and the Leiden classifier.[6] Apart from *POLE* mutational analysis, they utilize surrogate markers that are

clinically more feasible than the original genome-wide TCGA analysis. The classifiers are based on a combination of microsatellite instability analysis and/or mismatch repair (MMR) protein immunohistochemistry; *TP53* mutational testing and/or p53 immunohistochemistry; and *POLE* mutational analysis. The resulting subgroups are generally referred to as p53 wt/no specific molecular profile (NSMP) (surrogate to copy-number low in the TCGA classification system[3]); mismatch repair deficient (MMRd, surrogate to microsatellite unstable hypermutated); p53 abnormal (p53 abn, surrogate to copy-number high); and *POLE* mutant (*POLE*mut).

Similar to the original TCGA algorithm, ProMisE is a decision tree analysis but the order of subcategorization differs from the original TCGA analysis. Molecular analyses are performed sequentially in the order of MMR protein immunohistochemistry, *POLE* sequencing (in MMR proficient cases), and p53 immunohistochemistry (in *POLE* wt cases) (Figure 1B). After its development,[5] the ProMisE classifier was confirmed[7] and validated[8] according to the Institute of Medicine Guidelines for the development of 'omics-based biomarkers. The study cohorts were unselected regarding stage and histology but weighed towards higher-risk tumors. In the Leiden classifier, developed in a cohort of early-stage endometrioid carcinomas with high-risk uterine features, all molecular markers are determined for each sample and multiple classifiers are discarded (Figure 1C).[6] Multiple classifiers include tumors with more than one molecular classifying feature, found in about 3% of cases,[6] and denote those with combined *POLE*mut and MMRd (*POLE*mut–MMRd); combined *POLE*mut and p53 abn (*POLE*mut–p53 abn); combined MMRd and p53 abn (MMRd–p53 abn); and all three alterations (*POLE*mut–MMRd–p53 abn).

Our research in the field is based on an unselected cohort of patients who underwent surgical treatment for stage I–IV endometrial carcinoma at the Department of Obstetrics and Gynecology, Helsinki University Hospital, between January 1, 2007 and December 31, 2012. Appropriate approvals were obtained from the Institutional Review Board of the Helsinki University Hospital (journal number 135/13/03/03/2013, date 29 May 2013) and the National Supervisory Authority for Welfare and Health (journal number 753/06.01.03.01/2016, date 9 February 2016).

Our cohort has been classified both by ProMisE (n = 604)[9] and Leiden algorithms (n = 515).[10] The disease-specific survival curves created are very similar to the progression-free survival curves in the TCGA study (Figure 2).[3]

As a modification to ProMisE, we attempted to perform comprehensive molecular characterization on all primary tumor samples. Consequently, 20 cases with multiple molecular features were identified. Based on clinical outcomes associated with multiple classifiers, a combination of *POLE*mut with MMRd and/or p53 abn was classified as *POLE*mut (n = 4), and a combination of MMRd with p53 abn was classified as MMRd (n = 16).[11 12]

Minor adjustments were also introduced to the Leiden protocol. First, while our findings on p53 and MMR status were solely based on immunohistochemistry of the respective proteins, the original Leiden classifier uses a combination of *TP53* mutational testing and p53 immunohistochemistry to determine p53 status, and primarily the Promega microsatellite instability analysis for determination of microsatellite instability status. For tumors exhibiting low levels of instability, or from which extracted DNA quality is poor, immunohistochemistry of MMR proteins is performed. Second, the Leiden classifier detects *POLE* exonuclease domain hotspot mutations by Sanger sequencing of exons 9 and 13, whereas we performed sequencing of exons 9, 13, and 14. Lastly, we did not exclude cases with multiple classifying alterations.

Association with uterine risk factors

p53 abn subgroup typically shows aggressive characteristics such as nonendometrioid or high-grade endometrioid histology, deep myometrial invasion, large tumor size, and lymphovascular space invasion.[7–9] p53 abn did not stand out as a consistently unique subgroup in studies restricted to endometrioid carcinomas.[6 13]

Although associated with a favorable outcome, about 50% of *POLE*mut tumors were grade 3 endometrioid carcinomas in the original TCGA study[3] and in the ProMisE confirmation cohort.[7] The proportion was up to 35% in subsequent studies[6 8 13] which is more comparable to our finding at 13%.[9] This variation may be due to underrepresentation of the more common low-grade carcinomas in the earlier studies.[3 7]

Association with clinical factors

Several studies have shown that *POLE*mut is associated with younger age and lower body mass index, and p53 abn is associated with older age.[5 7 10 14] The prevalence of type 2 diabetes is similar between molecular subgroups.[10]

Old age has a negative impact on the survival of endometrial carcinoma patients.[15 16] Reports on the prognostic significance of body mass index and diabetes are inconsistent.[17–19] This may be explained by differences in study design and selection of study subjects, methods of body mass index and diabetes assessment, lack of power, and choice of the outcome of interest. By merely assessing overall survival, the impact of potential risk factors on cancer-related survival may be unnoticed.

Importantly, the prognostic studies were mostly conducted prior to the development of the molecular classification system for endometrial carcinoma; therefore, they did not address the role of molecular subgroups in modifying the prognostic effect of clinical factors. We examined the prognostic significance of age, body mass index, and type 2 diabetes among the molecular subgroups.[10] Overweight/obesity (body mass index ≥25 kg/m^2) had no effect on survival outcomes in the whole cohort of 515 patients but was associated with decreased overall and cancer-related mortality in the NSMP subgroup and increased overall and non-cancer-related mortality in the MMRd subgroup. Overweight/obesity effect on cancer-related mortality in the NSMP subgroup remained unchanged after controlling for confounders (hazard ratio 0.32, 95% confidence interval 0.11–0.92; P = 0.034). These findings suggest that the metabolic consequences of adiposity play different roles in the aggressiveness of endometrial carcinoma, depending on the molecular subtype. Clinical factors should be assessed as prognostic variables in conjunction with the molecular subgroup.

Association with tissue biomarkers

Molecular subgroups in endometrial carcinoma are associated with rather modest hazard ratios for poor outcome when controlled for various clinicopathologic covariates. This emphasizes the need to develop molecular subgroup-specific prognostic tools. We wanted to elucidate whether

the prognostic impact of various tissue biomarkers can be specific to a certain molecular subgroup. For this purpose, the prognostic effects of L1 cell adhesion molecule (L1CAM), estrogen and progesterone receptor, beta-catenin, p16, E-cadherin, and KRAS were compared between NSMP and MMRd, *i.e.* two largest subgroups that harbor an intermediate outcome.[20] Strong and diffuse staining for p16 was associated with poor disease-specific survival in NSMP but not MMRd, a finding that was confirmed in a multivariable model (hazard ratio for NSMP 6.7, 95% confidence interval 1.3–35; P = 0.024). The prognostic effect of p16 also differed between the subgroups in an interaction analysis (hazard ratio 0.2, 95% confidence interval 0–0.9; P = 0.033), which further supports the idea that its impact is modified by subgroup type.

Several retrospective studies have found that L1CAM expression is associated with poor outcome in women with endometrial carcinoma.[21–24] Kommoss *et al.* determined the subgroup-specific prognostic significance of aberrant L1CAM expression in a population-based endometrial carcinoma cohort.[25] Univariable survival analyses of L1CAM within each subgroup showed that L1CAM status had a significant prognostic impact only among NSMP tumors. L1CAM remained a significant prognosticator for disease-specific survival in the NSMP subgroup after multivariable analyses that included clinicopathologic risk factors available preoperatively (hazard ratio 3.8, 95% confidence interval 1.1–12; P = 0.035) and postoperatively (hazard ratio 4.0, 95% confidence interval 1.1–14; P = 0.035).

Many tissue biomarkers have been proposed as molecular determinants of outcome in endometrial carcinoma. However, none of the biomarkers are widely used in daily practice, mainly because data on clinically validated outcomes are lacking. Enhancement of biomarker performance characteristics by molecular classification could eventually contribute to the more general utilization of biomarkers in gynecologic oncology clinics.

Association with stage

Tumor stage plays an important role in determining the prognosis of patients with endometrial carcinoma. Compared with the five-year survival rate of 78–90% for stage I disease, the survival rate is 74% for stage II, and only 21–57% for advanced stages (III–IV).[26]

The stage distribution of endometrial carcinoma has been found to differ across molecular subgroups.[5 7 8 13 27] As molecular classification can be achieved on diagnostic endometrial samples and is highly concordant with hysterectomy specimens,[28 29] preoperative molecular classification could potentially play a role in the triage of patients to different types of staging surgery.

We assessed the capability of molecular classification to predict lymph node and distant metastasis in our cohort. In an unadjusted analysis, p53 abn was associated with an increased risk for stage IIIC–IV cancer (odds ratio 4.6, 95% confidence interval 2.3–9.2; $P < 0.0005$). In a multivariable analysis, uterine risk factors independently predicted stage IIIC–IV cancer but the effect of p53 abn was no longer significant. However, p53 abn was invariably associated with increased odds for the presence of high-risk uterine factors. It could be suggested that molecular data, when examined preoperatively, could aid in lymphadenectomy decisions when data on traditional risk factors are inconsistent or unavailable.

Prognostic significance

The distinct survival curves associated with molecular subgroups prompted many research teams to study the independent effect of subgroups on patient outcome.[5–8 13 27] The studies differed regarding various aspects, such as stage, histologic subtypes, outcomes of interest, and selection of confounders (Table 1). Nevertheless, the studies uniformly found that molecular factors provide independent prognostic information beyond established clinicopathologic risk factors. In a meta-analysis providing pooled data, prognosis of p53 abn was worst and was further worsened by unfavorable clinicopathologic factors.[30] Prognosis of MMRd overlapped with NSMP but was worsened by unfavorable clinicopathologic factors. Prognosis of *POLE*mut was best and did not seem to be affected by clinicopathologic factors.

For the current review, we performed multivariable survival analyses on our unselected cohort, molecularly classified by the Leiden schema. Distinct from earlier studies, a comprehensive set of clinicopathologic confounders was included in the analyses. With all-cause mortality as the outcome of interest, MMRd was associated with poor outcome in stage I and all stages (Table 2). By contrast, with endometrial cancer-related death as the outcome of interest, molecular

subgroups showed no independent prognostic effect (not shown). Thus, although molecular subgroups show independent prognostic effect in endometrial carcinoma, the findings may be significantly modified by the design of multivariable models.

In agreement with the view that molecular subgroups are distinct disease entities, it could be speculated that the prognostic effect of clinicopathologic risk factors may vary for each subgroup. We tested this hypothesis in a sample of NSMP and MMRd endometrial carcinomas and found that grade of differentiation has a stronger prognostic impact on NSMP.[20] We also explored the prognostic effect of MMR status in the absence and presence of established risk factors, including age, uterine risk factors, peritoneal cytology finding, and L1CAM.[31] MMRd was invariably associated with an increased risk for disease-related death in the absence of any individual risk factor, but the risk was similar for NSMP and MMRd when such factors were present. For example, the hazard ratio for MMRd was 2.8 (95% confidence interval 1.4–5.6, P = 0.003) in the subset of grade 1–2 endometrioid carcinomas, and 0.70 (95% confidence interval 0.35–1.4, P = 0.332) in grade 3 endometrioid and nonendometrioid carcinomas. This further supports the idea that MMRd subtype carcinomas at risk for relapse and poor outcome are less adequately identified by traditional risk factors. Accordingly, compared with the MMRd subgroup, NSMP was associated with worse survival in grade 3 endometrioid carcinomas[27] but improved survival in endometrioid carcinomas of all grades of differentiation.[6]

The association of MMR status on types of relapses was studied in stage I endometrial carcinoma.[31] Compared with the NSMP subgroup, the proportion of pelvic relapses was higher in the MMRd subgroup (2.4% vs. 8.6%), which may be explained by a poor response of MMRd carcinomas to adjuvant radiotherapy.[32] Lymphatic dissemination, defined as primary lymph node involvement or relapses in regional lymph nodes, was more common in the MMRd subgroup compared with NSMP (19.9% vs. 10.6%),[31] in agreement with a study where MMRd endometrial carcinomas were more likely to recur in retroperitoneal lymph nodes.[33]

Treatment response

The mainstay of the initial treatment for endometrial carcinoma is surgery with total hysterectomy and bilateral salpingo-oophorectomy, supplemented with pelvic sentinel node

biopsy or regional lymphadenectomy in selected cases. Adjuvant therapy is tailored according to stage and final pathology findings, *i.e.* histology and grade, depth of myometrial invasion, and lymphovascular space invasion. Six randomized trials established the role of adjuvant radiotherapy in decreasing the risk of pelvic and vaginal relapse without improving overall survival in early-stage endometrial carcinoma.[34–39] For most patients with stage I or occult stage II disease, vaginal brachytherapy has replaced whole pelvic radiotherapy as it provides similar vaginal control with a lower risk of gastrointestinal toxicity and improved quality of life.[40] A trade-off with vaginal brachytherapy includes a greater risk of nonvaginal pelvic recurrence compared with pelvic radiation (3.8% vs. 0.5%). The use of adjuvant chemotherapy to treat stage I–II endometrial carcinomas is not supported by available evidence.[41] However, decisions regarding early-stage nonendometrioid carcinoma remain challenging as individual studies[42–44] were not adequately powered for subgroup analyses.[40] Given these uncertainties, adjuvant chemotherapy is often recommended, with or without radiotherapy. Multimodality treatment with chemotherapy and whole pelvic radiotherapy is recommended for advanced-stage carcinomas because it may offer superior outcomes compared with single-modality treatment.[45] [46]

Standard therapies

Knowledge of the relationship between molecular subgroups and benefit from standard adjuvant therapies is restricted by the lack of randomized trials. Léon-Castillo *et al.* compared chemoradiotherapy versus whole pelvic radiotherapy for each molecular subgroup using tissue samples from the PORTEC-3 trial.[47] The participants mainly corresponded to high-risk patients as defined by the joint guidelines of the European Society of Gynaecological Oncology (ESGO), European SocieTy for Radiotherapy and Oncology (ESTRO), and European Society of Pathology (ESP).[45] Adjuvant chemotherapy improved recurrence-free survival for p53 abn carcinomas. Of them, 73% were nonendometrioid or mixed, and 34% stage III. Patients with NSMP and MMRd carcinomas did not benefit from adjuvant chemotherapy. Those with *POLE*mut carcinomas had an excellent recurrence-free survival in both trial arms.

We determined the value of MMR protein status in predicting response to adjuvant therapies in a retrospective cohort that was annotated by p53 and MMR protein staining and *POLE* mutation status.[32] Although unadjusted analysis indicated that adjuvant therapies are associated with poor

disease-specific survival in the MMRd subgroup, this finding disappeared after controlling for clinicopathologic risk variables.[32] In the study by Reijnen *et al.*, radiotherapy improved disease-specific survival in MMRd endometrial carcinomas.[48] However, this study may not be similarly applicable in the context of TCGA because tumors were dichotomously categorized into MMRd and MMR proficient subgroups with the latter including NSMP, *POLE*mut, and p53 abn cases. Moreover, vaginal brachytherapy and whole pelvic radiotherapy were combined into a single treatment group.

As for the NSMP subgroup, whole pelvic radiotherapy (hazard ratio 0.092, 95% confidence interval 0.016–0.54; P = 0.008) and chemotherapy combined with radiotherapy (hazard ratio 0.18, 95% confidence interval 0.038–0.89; P = 0.035) were associated with improved disease-specific survival when adjusted for age, stage, and high-risk uterine factors.[32] Of the patients who received adjuvant radiotherapy without chemotherapy, 87.5% had stage I cancer.

As randomized trials failed to show overall survival benefit from adjuvant radiotherapy in early-stage endometrial carcinoma,[34–39] it seems counterintuitive that whole pelvic radiotherapy improved disease-specific survival in the NSMP molecular subgroup. It should be remembered, however, that the randomized adjuvant therapy trials were conducted prior to the TCGA era. Thus, a significant survival advantage in one subgroup may have been obscured.

Hormonal therapy

For younger women who wish to preserve fertility, hormonal therapy with progestins is a suitable alternative treatment to definitive surgery in early-stage low-grade endometrial carcinoma. Chung *et al.* evaluated the prognostic significance of ProMisE in the fertility-sparing management of endometrial cancer.[49] Compared with NSMP (n = 45), patients with MMRd (n = 9) had a significantly lower best overall response (82.2% vs. 44.4%) or complete response rate (53.3% vs. 11.1%) at six months. There was no difference in estrogen receptor or progesterone receptor expression between the two subgroups. MMR protein status could be used as a predictive biomarker for selecting patients who could benefit from hormone therapy.

Immunotherapy

Immunotherapy provides a new treatment option for patients with endometrial cancer. One of the main immunosuppressive pathways is the programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) interaction taking place between T-cell PD-1 receptor and PD-L1 located on various types of cells, including immune cells and carcinoma cells.[50] Based on preliminary results from the phase 1 GARNET trial,[51] European Medicines Agency granted anti–PD-1 antibody dostarlimab a conditional authorization for the treatment of MMRd/microsatellite instability-high recurrent or advanced endometrial cancer that has progressed on or following prior treatment with a platinum-containing regimen.

POLE ultramutated and microsatellite unstable hypermutated endometrial carcinomas contain large numbers of neoantigens and activated cytotoxic tumor infiltrating lymphocytes that often express PD-1 and PD-L1.[52] In agreement with these findings, *POLE*mut and MMRd tumors more frequently than NSMP and p53 abn show PD-L1 expressing immune cells, combined positive score of PD-L1 expression in immune cells and carcinoma cells, and abundant intratumoral T-cell infiltrates ($P < 0.001$).[53] Clinical trials are needed to elucidate the applicability of immunotherapy in different molecular subgroups of endometrial carcinoma.

Poly (ADP-ribose) polymerase inhibitors

Homologous recombination deficiency (HRD) testing is useful for predicting the likely magnitude of benefit from poly (ADP-ribose) polymerase (PARP) inhibitors in ovarian cancer. de Jonge *et al.* assessed the prevalence of HRD in endometrial carcinomas that were classified into molecular subgroups.[54] The cohort was enriched for high-grade endometrioid and nonendometrioid carcinomas. HRD was observed in six out of 12 p53 abn tumors, but in none of the 11 NSMP/MMRd/*POLE*mut tumors. In another study, high HRD score was associated with worse disease-free survival in endometrial carcinoma.[55] These findings support prospective trials investigating PARP inhibitors to target HRD in endometrial cancer.

Implementation into clinical practice

Updated guidelines for endometrial carcinoma by ESGO, ESTRO and ESP were published in January 2021.[45] Assessment of prognosis and adjuvant therapy decisions are based on classification of endometrial carcinomas into five risk groups with specific clinicopathologic features. Integration of molecular classification is encouraged for a more personalized risk-assessment when molecular tools are available. ESGO-ESTRO-ESP guidelines propose treatment intensification in early-stage p53 abn carcinomas, and treatment de-escalation in early-stage *POLE*mut carcinomas, regardless of traditional clinicopathologic risk factors.

To assess the frequency of shift between risk groups with integration of molecular classification, we comprehensively classified 515 endometrial carcinomas into ESGO-ESTRO-ESP clinicopathologic and molecular integrated risk groups.[56] Molecular classification caused a risk group shift in 38 patients (7.4%). Of them, 27 were upshifted and 11 downshifted. Shifts mostly occurred in the high-intermediate risk group.

We also compared risk group outcomes with and without molecular knowledge and confirmed distinct outcomes for the five risk groups with both approaches.[56] With NSMP as the reference subgroup, p53 abn was associated with poor disease-specific survival within clinicopathologic low risk carcinomas (hazard ratio 9.1, 95% confidence interval 2.0–41; P = 0.004). In contrast, MMRd was associated with poor survival within clinicopathologic high-intermediate risk carcinomas (hazard ratio 3.5, 95% confidence interval 1.2–10; P = 0.024). Thus, clinicopathologic risk factors may differently modify the prognostic impact of molecular subgroups. This emphasizes the need for adjuvant therapy trials where patients are randomized to treatment arms separately within each molecular subgroup.

Our current practice for molecular classification is outlined in Figure 3. *POLE* sequencing, the most laborious component of the analyses, may alter risk-assessment of clinicopathologic low-risk carcinomas only when p53 staining is abnormal, which is a rare finding (<5% of cases). Thus, it seems reasonable to perform *POLE* sequencing in these tumors only when p53 is abnormally expressed, whereby the low-risk *POLE*mut–p53 abn double classifiers can be identified. *POLE* mutational testing can be further reduced by omitting it in advanced (stage III–

IV) carcinomas in which adjuvant therapy decisions are not altered by molecular classification.[45] By this approach, *POLE* sequencing can be restricted to 40% of endometrial carcinomas.[56]

Conclusion

Breakthroughs in molecular diagnostics have provided tools for personalized medicine in endometrial carcinoma. Molecular subgroups first gained interest as independent prognostic factors but were subsequently found to be potentially important in modifying the effect of traditional prognostic factors and predicting the response to adjuvant therapies.

Molecular classification has become a standard in endometrial cancer care and will remain a necessity for further research in the field. We recognize two main areas to be explored. First, the role of potential prognostic factors should be examined separately for each molecular subgroup. Improved risk-assessment is especially important for NSMP and MMRd whose outcomes are more indeterminate compared with *POLE*mut and p53 abn. Second, the efficacy of various oncological therapies, whether standard or more novel such as immunotherapy and PARP inhibition, should ideally be investigated in clinical trials where randomization takes into account the molecular subgroup.

Contributors All authors contributed equally to the development of this review.

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Competing interests None declared.

Patient consent for publication Participant consent was waived because of the retrospective design of our research. Approvals were obtained from the Institutional Review Board of the Helsinki University Hospital (journal number 135/13/03/03/2013, date 29 May 2013) and the National Supervisory Authority for Welfare and Health (journal number 753/06.01.03.01/2016, date 9 February 2016).

Table 1. Comparison of prognostic studies of molecular subgroups in endometrial carcinoma.

Study	N	Histology	Stage	Outcome	Confounders
Talhouk 2015[5]	143	Endometrioid, serous/mixed	All stages	Overall survival, disease-specific survival, recurrence-free survival	European Society for Medical Oncology 2013 clinical risk group
Stelloo 2016[6]	834	Endometrioid	Stage I	Overall survival, locoregional recurrence, distant recurrence	Age, grade, myometrial invasion, lymphovascular space invasion, L1 cell adhesion molecule, adjuvant therapy
Talhouk 2017[7]	319	Endometrioid, nonendometrioid	All stages	Overall survival, disease-specific survival, progression-free survival	Age, body mass index, histology, grade, adjuvant therapy
Bosse 2018[27]	381	Grade 3 endometrioid	All stages	Overall survival, recurrence-free survival	Age, stage
Cosgrove 2018[13]	982	Endometrioid	All stages	Overall survival, disease-specific survival, progression - free survival	Age, stage, grade, lymphovascular space invasion, adjuvant therapy
Kommoss 2018[8]	452	Endometrioid, nonendometrioid	All stages	Overall survival, disease-specific survival, progression - free survival	Age, body mass index, histology, grade

Table 2. Multivariable Cox regression overall survival analyses.

	Stage I (n = 340) n cancer-related deaths = 77 Median follow-up time 83 months (range 1–132)			All stages (n = 478) n cancer-related deaths = 147 Median follow-up time 80 months (range 1–136)		
	N	HR (95% CI)	P	N	HR (95% CI)	P
Molecular subgroup (Leiden)			0.068			0.003
No specific molecular profile	151	1		199	1	
Mismatch repair deficient	117	2.0 (1.2–3.4)	0.012	175	1.9 (1.3–2.8)	0.002
Polymerase-ϵ mutant	33	0.90 (0.26–3.1)	0.863	36	0.43 (0.13–1.4)	0.164
p53 abnormal	39	1.4 (0.66–3.0)	0.376	68	1.3 (0.75–2.2)	0.370
Age >65 years	190	3.9 (2.0–7.3)	<0.001	280	1.8 (1.2–2.7)	0.003
Stage II-IV	N/A			138	0.91 (0.53–1.6)	0.740
Histology			0.332			0.063
Grade 1–2 endometrioid	281	1		345	1	
Grade 3 endometrioid	35	1.2 (0.49–3.1)	0.656	70	1.4 (0.88–2.4)	0.149
Nonendometrioid	24	2.0 (0.80–5.2)	0.137	63	1.9 (1.1–3.2)	0.021
Myometrial invasion ≥50%	97	2.0 (1.1–3.5)	0.025	199	1.7 (1.1–2.5)	0.018
Tumor size ≥5 cm	52	3.1 (1.8–5.2)	<0.001	124	2.2 (1.5–3.2)	<0.001
Lymphovascular space invasion	61	1.8 (0.98–3.2)	0.057	134	1.6 (1.1–2.4)	0.010
Positive peritoneal cytology	7	11 (2.9–40)	<0.001	35	3.7 (2.2–6.5)	<0.001
Adjuvant therapy			0.014			0.001
None	53	1		63	1	
Vaginal brachytherapy (VBT)	219	0.40 (0.21–0.77)	0.006	219	0.44 (0.25–0.77)	0.004
Whole pelvic radiotherapy (WPRT)	41	0.30 (0.10–0.90)	0.032	76	0.31 (0.16–0.59)	<0.001
Chemotherapy	4	2.2 (0.48–9.7)	0.319	20	0.94 (0.42–2.1)	0.873
Chemotherapy and VBT/WPRT	23	0.28 (0.078–1.0)	0.053	100	0.39 (0.21–0.72)	0.003

Figure legends

Figure 1. Schemas for molecular classification of endometrial carcinomas by The Cancer Genome Atlas classifier (A), the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) (B), and Leiden classifier (C).

Figure 2. Kaplan-Meier disease-specific survival curves by the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) (n = 604) and the Leiden classifier (n = 515). Subgroup terms comply with those used in the original method descriptions. p53 wt and NSMP correspond to copy-number low; MMR IHC abn and MSI to microsatellite unstable hypermutated; *POLE* EDM and *POLE*-mutant to polymerase-ϵ ultramutated; and p53 abn and p53-mutant to copy-number high of The Cancer Genome Atlas classification system. Abbreviations: abn, abnormal; EDM, exonuclease domain mutation; IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability; NSMP, no specific molecular profile; POLE, polymerase-ϵ; wt, wild type.

Figure 3. A proposal for targeting of molecular classification in clinical practice. Percentages are based on our own research. p53 staining is abnormal in <5% of clinicopathologic low-risk carcinomas. Abbreviations: IHC, immunohistochemistry; LVSI –/+, lymphovascular space invasion negative or focal; MMR, mismatch repair; POLE, polymerase-ϵ.

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FIGURES

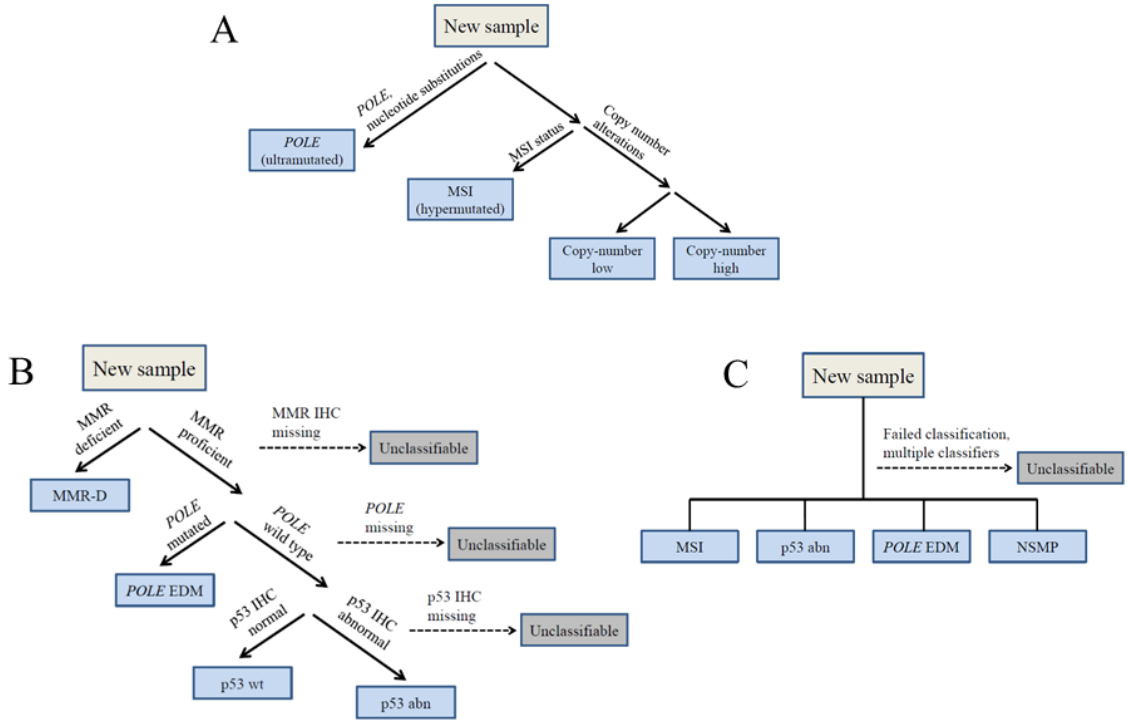

Figure 1

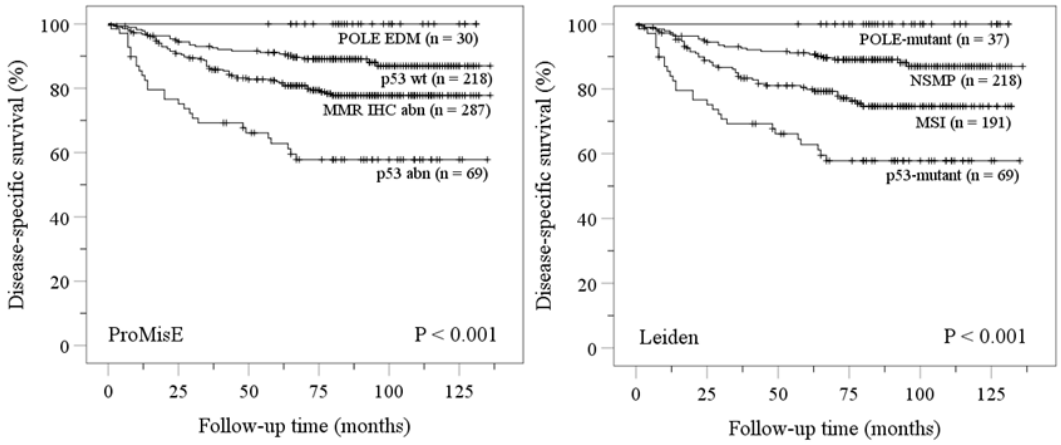
Disease-specific survival (%)
100
80
60
40
20
0
POLE EDM (n = 30)
p53 wt (n = 218)
MMR IHC abn (n = 287)
p53 abn (n = 69)
ProMisE
P < 0.001
0
25
50
75
100
125
Follow-up time (months)
Disease-specific survival (%)
POLE-mutant (n = 37)
NSMP (n = 218)
MSI (n = 191)
p53-mutant (n = 69)
Leiden
P < 0.001
Follow-up time (months)

Figure 2

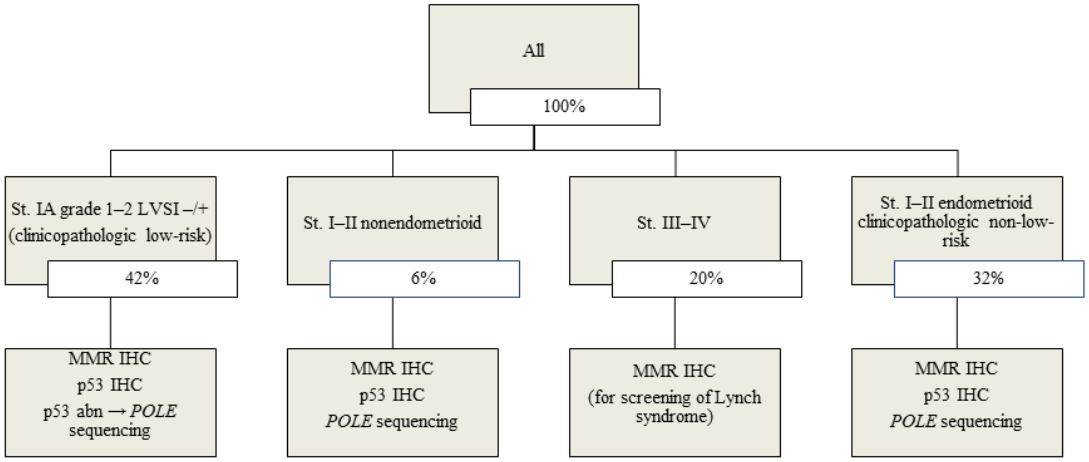
All
100%
St. IA grade 1–2 LVSI –/+ (clinicopathologic low-risk)
42%
St. I–II nonendometrioid
6%
St. III–IV
20%
St. I–II endometrioid clinicopathologic non-low-risk
32%
MMR IHC
p53 IHC
p53 abn → *POLE* sequencing
MMR IHC
p53 IHC
POLE sequencing
MMR IHC
(for screening of Lynch syndrome)
MMR IHC
p53 IHC
POLE sequencing

Figure 3