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Full length article

Application of the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) to patients conservatively treated: Outcomes from an institutional series

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ABSTRACT

Objective: To test the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) and determine the frequency of specific/prognostic molecular alterations within a cohort of endometrial cancer (EC) women conservatively treated by combined hysteroscopic resection and progestin therapy.
Study design: We used blocks of formalin-fixed paraffin-embedded tissue from the primary tumors of patients enrolled into the ECCo trial (EudraCT 2010-018581-23) between 2007 and 2016. In order to assign EC resectoscopic specimens to one of four ProMisE subgroups, testing involved sequential assessment of i) immunohistochemistry (IHC) for mismatch repair (MMR) proteins MLH1, MSH2, MSH6 and PMS2; ii) sequencing for POLE/POLD1 exonuclease domain mutations (EDMs); iii) p53 IHC.
Results: Molecular analysis methods were used in 25 patients (stage IA, G1-2 endometrioid EC), of whom 15 (60%) represented fully evaluable cases. Seven cases (46.7%) had abnormal MMR IHC, POLE/POLD1 EDMs were found in 3 cases (20%), and abnormal p53 IHC in 1 case (6.6%). Three patients (20%) had more than one molecular feature. Among 10 (40%) 'unclassifiable' patients, six failures in achieving complete molecular categorization were due to the low tumor volume. Molecular classification of the 15 fully evaluable cases yielded the following ProMisE subtypes: 7 (46.7%) MMR IHC abnormal, 1 (6.6%) POLE EDM, 0 (0%) p53 IHC abnormal, 7 (46.7%) p53 IHC wild-type.
Conclusions: Although larger series are needed to further assess the feasibility of a molecular categorization in a fertility-sparing setting, data presented are promising. In women with early stage low-volume disease, operative hysteroscopy could be advantageous to provide samples allowing complete genetic risk assessment.

Introduction

Although the primary treatment of endometrial cancer (EC) is usually hysterectomy, continuous progestin-based therapy may be offered as a temporizing measure for highly selected women wishing to preserve their fertility [1,2]. To date, candidates for conservative management are generally considered women of childbearing age with pathologic features indicative of excellent prognosis, such as intramucous, well-differentiated (G1), endometrioid EC [1,2]. In fact, it is acknowledged that: i) grade and histotype assignment is subject to imperfect concordance between diagnostic (biopsy or curetting) versus hysterectomy specimens; ii) tumor stage can only be assigned after definitive surgery, including hysterectomy and loss of reproductive potential; iii) women who are diagnosed with EC before age 50 years have a heightened risk for hereditary cancer syndrome. Therefore, decision making process with respect to a fertility-sparing management must take into consideration the inherent oncologic risk of an inadequately categorized/treated disease and the potential risk of an inherited genetic cancer.

Recent advances have expanded our understanding of the genomic features of ECs, leading to the identification of molecular signatures predictive of individual tumor behaviour. The Cancer

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Genome Atlas (TCGA) project stratified ECs into four genomically defined prognostic subgroups [3]. Hence, using low cost and simple assays broadly available in clinical practice, two research teams have shown that these four subgroups can be easily determined by their surrogate markers [4–8]. In particular, the *Pro*active *M*olecular R*isk* Classifier for *E*ndometrial cancers (ProMisE) is based on three key components: immunohistochemistry (IHC) for the presence of mismatch repair (MMR) proteins, sequencing for the presence of POLE exonuclease domain mutations (EDMs) and IHC for p53 [4–6]. It has been reported that this molecular classifier model works successfully on endometrial biopsies or curettages with high concordance with the hysterectomy specimens [9]. In a fertility-sparing setting, such a model could be a pragmatic option for an integrated (molecular and clinicopathological) classification system allowing a more reliable EC prognostic evaluation and early hereditary cancer risk assessment. ProMisE, however, has not been yet tested on a cohort of EC patients conservatively treated.

We designed the *E*ndometrial *C*ancer *Co*nservative management (ECCo) trial as a prospective institutional study of fertility preservation in early EC. Results presented here refer to an unplanned molecular analysis, aimed to classify all enrolled patients into the ProMisE clusters.

Materials and methods

We used blocks of formalin-fixed paraffin-embedded (FFPE) tissue from the primary tumors of EC patients enrolled into the ECCo trial between January 2007 and December 2016 to perform a molecular analysis aimed to test the ProMisE classifier and determine the frequency of specific/prognostic alterations within a cohort of highly selected low-risk EC women. ECCo (EudraCT number: 2010-018581-23) enrollment criteria as well as treatment and follow-up modalities are summarized in the Table 1 and further detailed in our previous paper [10].

The Institutional Ethics Committee approved this study; all patients included in the present analysis gave written consent to data collection and to the use of personal records and biospecimens for health research. Data were retrieved on: patient- (age; body mass index), disease- (tumor diameter and grade), and treatment-related characteristics (type and duration of hormonal therapy). Complete regression was defined as no evidence of residual EC or atypical hyperplasia (AH) at follow-up endometrial sampling. Time until complete regression was measured from the progestin start date. Partial regression was defined as the presence of AH during follow-up endometrial sampling, persistent disease if no evidence of disease regression was observed within 6 months from progestin initiation, and progressive disease if higher than stage IA (according to 1988 FIGO staging system) and/or moderately (G2)/poorly differentiated (G3) EC was diagnosed during follow-up. Recurrence was defined as the presence of EC or AH during follow-up after an endometrial sample showing disease regression. Time to recurrence was measured from the date of complete regression. Patient follow-up data were gathered until the end of 2018.

Pathology was reviewed for all cases to identify suitable blocks for molecular analysis. The following patients were considered non-eligible for study inclusion: i) inadequate quantity/quality of tumor tissue/DNA; ii) hysteroscopic resection after December 1, 2016, ensuring minimum 2-year follow-up.

The ProMisE molecular classification scheme was used to assign EC resectoscopic specimens to one of four molecular subgroups using methodologies described in previous publications [4–6]. Testing involved sequential assessment of i) IHC for MMR proteins MLH1, MSH2, MSH6 and PMS2; ii) sequencing for POLE and POLD1 EDMs; iii) p53 IHC. Additional microsatellite instability (MSI) testing was planned to be performed only in patients showing concomitant MMR and POLE/POLD1 abnormalities or POLE/POLD1 EDM alone.

Table 1
*E*ndometrial *C*ancer *C*onservative management (ECCo) trial.

Inclusion criteria	• 18–40 years; • Pathological diagnosis of G1 EEC with PR ≥50% positivity at IHC; • No radiologic (TVS; abdomen–pelvis MR; CXR) evidence of • myometrial/cervical invasion, • retroperitoneal lymph node involvement, • ovarian tumors, • distant metastasis; • CA125 serum levels <35 IU/mL; • No contraindication for progestin treatment; • Strong desire to preserve fertility; • Written acceptance of an informed consent including availability for completing the follow-up program and definitive surgery after complete childbearing.
Exclusion criteria	• History of previous/concomitant cancer (except for adequately treated skin basal cell or in situ cervical cancer) • Breast/ovarian/colorectal cancer family history suggesting the presence of deleterious BRCA and/or MMR mutation (or known mutation) • Multifocal tumor
Interventions	Hysteroscopic resection of the i) tumor lesion, ii) endometrium adjacent to the tumor, iii) myometrium underlying the tumor. A LNG-IUD, releasing 20 μg of levonorgestrel daily, is inserted at the end of the hysteroscopic resection and planned to be in situ for at least 6 months.
Follow-up	3-monthly for 2 years*, then 6-monthly†: - general and gynecologic examinations; - TVS; - serum CA125; - diagnostic hysteroscopy with endometrial biopsy.

CA 125: cancer antigen 125; CXR: chest-X-ray; EEC: endometrioid endometrial cancer; G1: well-differentiated according to WHO criteria [31]; IHC: immunohistochemistry; LNG-IUD: levonorgestrel intrauterine device; MMR: mismatch repair; PR: progesterone receptors; TVS: transvaginal ultrasonography; MR: magnetic resonance.

* Abdomen-pelvis computed tomography (CT) is scheduled every 6 months.

† For patients still in complete response and wishing to maintain their reproductive potential.

MMR and p53 IHC

A representative FFPE block per case was cut at 4 μm thickness and transferred onto Superfrost + glass slides.

Expression of MMR and p53 proteins was investigated using the BOND-III (Leica Biosystems) automated IHC stainer according to the manufacturer's instructions.

MMR IHC panel was comprised of four primary antibodies: i) anti-hMLH1 (ES05, 1:50, Leica Biosystems, Nussloch, Germany); ii) anti-hMSH2 (25D12, 1:60, Leica Biosystems, Nussloch, Germany); iii) anti-hMSH6 (PU21, 1:200, Leica Biosystems, Nussloch, Germany); iv) anti-hPMS2 (M0R4G, 1:100, Leica Biosystems, Nussloch, Germany); used according to the manufacturer's protocol. Tumor was considered aberrant if tumor cells showed complete absence of nuclear staining with positive non-neoplastic internal control, and intact if tumor cells show nuclear positivity. Equivocal and uninterpretable cases (e.g., no tumor tissue) had FFPE blocks requisitioned for repeat staining of the MMR protein or proteins needed. Results were ultimately

binarized: i) MMR absent = 0 (one or more than one of four MMR proteins missing); ii) MMR intact = 1 (all four proteins present). Tumors showing loss of MLH1 expression were planned to be tested for methylation status of the 5' regulatory region of MLH1, using methylation-specific PCR.

For p53 immunostaining, the primary antibody was DO-7 anti-hp53 (1:50, Dako), used according to the manufacturer's protocol. Appropriate on-slide positive and negative controls were used. Immunostaining for p53 was considered abnormal when there was no staining of tumour cell nuclei strong and diffuse staining (absent p53 protein or aberrant increased protein accumulation, respectively), while intermediate levels of expression were considered to be wild-type.

For immunohistochemical analyses, all slides were evaluated by a dedicated gynecologic pathologist (NL), blinded for patient characteristics and outcomes.

DNA extraction, targeted sequencing and analysis

DNA from FFPE tumor blocks was extracted using the Qiagen FFPE tissue Kit and quantified with the dsDNA HS assay kit on the Qubit 2.0 Fluorometer (Invitrogen, Monza, Italy). A targeted re-sequencing NGS custom panel was used to analyse hotspot and regions of the exonuclease domain, exons 9–13 and 7–12, of POLE and POLD1 genes, respectively. The panel is composed of two primer pools multiplexing 25 amplicons (amplicon range 125–175 bp). Libraries were prepared starting from 10 ng of genomic DNA for each pool, according to the manufacturer's instructions. One hundred pM of each library were multiplexed and clonally amplified on Ion sphere particles (ISPs) by emulsion PCR performed on the Ion One Touch 2 instrument with the Hi-Q Ion PGM template OT2 200 kit v2 (Life Technologies, Monza, Italy) according to the manufacturer's instructions. Finally, the template ISPs were enriched, loaded on an Ion 316 chip and sequenced on a PGM sequencer with the Hi-Q Ion PGM™ sequencing 200 kit v2 according to the manufacturer's instructions. The raw data were analyzed using the torrent suite software v5.04 (Life Technologies, Monza, Italy) with a pre-established workflow. Mutations were detected using the variant caller v 5.04 with low stringency settings. The limit of detection (LOD) was set at 2%. In the variant list obtained, each mutation was verified in the integrative genome viewer (IGV) from the Broad Institute (http://www.broadinstitute.org/igv/).

MSI analysis

The microsatellite analysis was performed directly from FFPE cancer tissue using the Idylla™ MSI Assay, according to the manufacturer's instructions. The cartridge allowed a complete automated workflow including sample preparation, DNA amplification followed by melting curve analysis and qualitative detection of mutations in 7 novel MSI loci (ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, and SULF2). An integrated software system for interpretation and reporting automatically classified the tumor as microsatellite stable (MSS) or MSI-high (MSI-H).

Results

The study flow chart is detailed in Fig. 1, showing 15 fully evaluable cases. Patient exclusions were as follows: missing written consent to the use of personal bio-specimens for health research (2), insufficient tumour tissue to enable MMR IHC status determination (3), failed sequencing or no DNA available for POLE/POLD1 status evaluation (7), hysteroscopic resection after December 2016 (3).

All patients but one had endometrioid, well-differentiated, intramucous EC at pathological examination of resectoscopic specimens (Table 2). Molecular features according to ProMisE

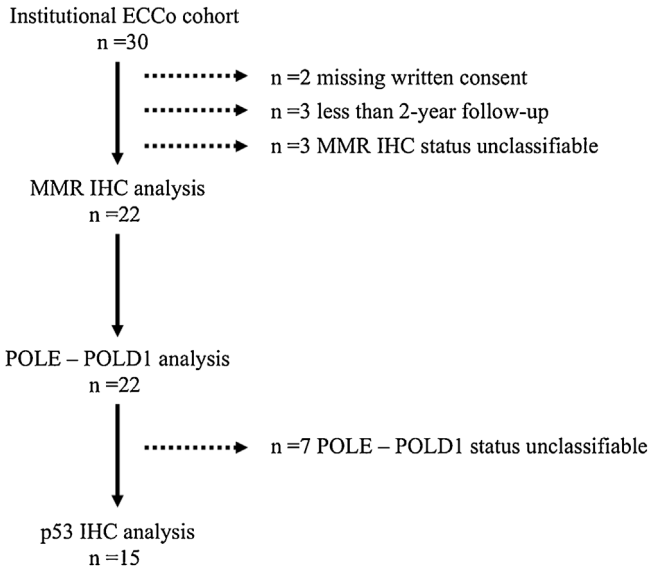

Fig. 1. Flow chart of sample analyses.

classifier are detailed, case by case, in Table 3. In total, 7 of 15 fully evaluable cases (46.7%) had abnormal MMR IHC; POLE/POLD1 EDMs were found in 3 cases (20%); abnormal p53 IHC was detected in one case (6.6%). Three cases (20%) had more than one molecular feature. The assessment of MMR IHC is the first step in ProMisE classifier model. Therefore, three cases showing abnormal MMR IHC were classified as 'MMR IHC abnormal' despite a POLE/POLD1 mutation and abnormal p53 IHC were detected in two and one cases, respectively. In summary, molecular classification of the 15 fully evaluable cases yielded the following ProMisE subtypes: 7 (46.7%) MMR IHC abnormal, 1 (6.6%) POLE EDM, 0 (0%) p53 IHC abnormal, and 7 (46.7%) p53 IHC wild-type. The spectrum of POLE/POLD1 EDMs identified is detailed in Table 3.

The median follow-up time from the hysteroscopic resection was 106 months (range, 24 to 134 months).

The planned adjuvant hormonal therapy was given to all patients according to the protocol. Complete regression was achieved in 80% and 86.6% of the patients after 6 and 9 months from the progestin start date, respectively. Patients no. 3 and no. 7 presented with persistent disease at 6 months and progressive disease at 3 months, respectively. Both these patients underwent definitive surgery and the final pathology showed a stage IA (intramucous) G1 endometrioid EC for the former, and a stage IA (with myometrial invasion) G3 endometrioid EC for the latter.

Patient no. 5, 41 months from complete response, was found to have an ovarian mass and treated by definitive surgery, showing a stage IA G1 endometrioid ovarian cancer (OC) and a synchronous asymptomatic endometrioid G1 intramucous EC. Patient no. 13, 8 months from complete response to re-treatment of persistent disease at 6 months, was found to have bilateral ovarian masses and underwent definitive surgery with a diagnosis of stage IIB G1 endometrioid OC and endometrial AH. Patient no. 14 recurred 11 months from complete regression, after 2 months from the first oocyte retrieval cycle during assisted reproduction technology; this patient was diagnosed with AH and successfully re-treated with hysteroscopic resection and LNG-IUD.

Patient no. 4, after 102 months from complete regression, was diagnosed with poorly differentiated right-sided colon cancer (T4b N2 M1b, according to AJCC 8th Edition). She resulted to be a carrier of germline mutation in the MMR gene PMS2.

Patient no. 10 showed metachronous breast cancer (ductal carcinoma in situ) 81 months from complete regression. This

Table 2
Demographics, clinicopathologic characteristics, and oncologic outcomes of endometrial cancer patients conservatively treated.

Case #	Age (years)	BMI (kg/m^2)	Tumor diameter (cm)/grade	Adjuvant HT (months)	Oncologic outcome at 6 months	Relapse (months)	Second cancer (months)	Follow-up (months)	Current status
1	39	26.3	≤2/G1	60	CR	–	–	134	NED
2	37	23.5	≤2/G1	30	CR	–	–	130	NED
3	28	53.5	≤2/G1	6	Persistence	–	–	125	NED
4	39	48	≤2/G1	60	CR	–	Intestinal (105)	124	DOD*
5	40	24.2	>2/G1	24	CR	41	Ovarian (41)	120	NED
6	25	24.5	≤2/G1	24	CR	–	–	108	NED
7	39	24.3	>2/G2	3	Progression†	–	–	107	NED
8	39	25	≤2/G1	60	CR	–	–	106	NED
9	36	28.3	≤2/G1	24	CR	–	–	86	NED
10	38	26.3	≤2/G1	6	CR	–	Breast (84)	85	AWD‡
11	37	31	≤2/G1	18	CR	–	–	79	NED
12	38	30.1	≤2/G1	12	CR	–	–	66	NED
13	35	23.2	≤2/G1	18	CR§	8	Ovarian (8)	61	NED
14	35	29.1	≤2/G1	6	CR	11	–	24	NED
15	33	22.7	≤2/G1	24	CR	–	–	24	NED

AWD: alive with disease; BMI: body mass index; CR: complete regression; DOD: died of disease; HT: hormonal therapy; NED: no evidence of disease.
* Right-sided colon cancer.
† Definitive surgery at 3 months.
‡ Breast cancer.
§ After re-treatment of persistent disease at 6 months.

Table 3
MMR, POLE/POLD1 and p53 results across the cohort (MSI assay and germline LS genetic testing, as planned).

Case #	Pathology	MMR IHC abn	POLE – POLD-1 mutation; AF (%)	p53 IHC	MSI assay	Germline LS genetic testing
1	G1 EEC	–	–	wt	n/p	negative
2	G1 EEC	–	–	wt	n/p	negative
3	G1 EEC	MSH6; PMS2	–	wt	n/p	n/a
4	G1 EEC	PMS2	–	abn	n/p	PMS2 mut
5	G1 EEC	–	–	wt	n/p	negative
6	G1 EEC	–	–	wt	n/p	negative
7	G2 EEC	MSH2; MSH6	POLE: p.A341 T (c.1021 G > A); 13.5	wt	MSI-H	n/a
8	G1 EEC	MSH2; MSH6	–	wt	n/p	n/a
9	G1 EEC	–	POLE: p.V411 L (c.1231 G > T); 30.7	wt	MSS	negative
10	G1 EEC	MSH6	POLD-1: p.D402 N (c.1204 G > A); 35.4	wt	MSS	MSH6 mut
11	G1 EEC	MSH2; MSH6	–	wt	n/p	n/a
12	G1 EEC	–	–	wt	n/p	negative
13	G1 EEC	–	–	wt	n/p	negative
14	G1 EEC	MSH2	–	wt	n/p	negative
15	G1 EEC	–	–	wt	n/p	negative

Abn: abnormal; AF: allelic frequency; G1: well differentiated; G2: moderately differentiated; EEC: endometrioid endometrial cancer; IHC: immunohistochemistry; LS: Lynch syndrome; MMR: mismatch repair; MSI: microsatellite instability; MSI-H: microsatellite instability-high; MSS: microsatellite stable; mut: mutation; n/a: not available; n/p: not planned; wt: wild-type.

patient, showing no BRCA mutation, had already presented with and received treatment for rectal adenomas with high-grade dysplasia, and resulted to be carrier of MSH6 germline mutation.

Overall, at the end of the observation period, 13 patients were alive with no evidence of disease, 1 experienced relapse and died from colon cancer, and 1 patient was alive with breast cancer (Table 2).

Discussion

During the last decade, it has become increasingly clear that histological classification alone is inadequate to encompass the peculiar heterogeneity of endometrial neoplasms [11]. The TCGA has reported a comprehensive genomic and transcriptomic analysis of ECs, identifying four molecularly defined prognostic subgroups [3] which can be more easily determined by their surrogate markers [4–8]. In particular, ProMisE represents the first validated classifier with the potential for immediate testing in the clinical setting, showing high concordance between diagnostic and hysterectomy specimens [4,9]. Our study, although based on a limited number of patients, represents the first series published so far testing a validated molecular classification system on a cohort of EC patients conservatively treated. Our results show a considerable molecular heterogeneity within a group of intramucous, G1 ECs which is currently thought to be relatively homogeneous with regard to survival outcomes, and suggest that an adequate and standardized pre-treatment genetic risk assessment should be provided.

The decision making process in case of EC fertility-sparing management is currently based on familial (5–10% risk of MMR germline mutation), and individual disease risk evaluated using clinical-pathological features [12–19]. Therefore, patients eligible for conservative management are generally considered those younger than 40 years with well-differentiated, endometrioid EC clinically limited to the endometrium, and without a family history suggesting a Lynch syndrome.

In this scenario, an integrated classification system incorporating molecular and clinical-pathological features seems to best respond to the clinical requirements aimed to i) more reliable prognostic information; ii) genetic risk stratification.

Next-generation sequencing, analysis of DNA methylation, reverse-phase protein array and microsatellite instability, however, require long turnaround time, are complex, costly and not available at all centres, resulting, therefore, unsuitable for a wide clinical application. Hence, using relatively low-cost and

simple assays broadly available in the clinical practice, two research teams have shown that these four subgroups can be more easily determined by their surrogate markers [4–8]. In particular, ProMisE represents the first validated classifier according to the Institute of Medicine guidelines for the development of omics-based tests [4–6].

We have applied for the first time ProMisE in a fertility-sparing setting, looking at a possible model able to adequately stratify recurrence risk, predict/reduce the occurrence of synchronous/metachronous ovarian/colorectal cancer, and, eventually, to direct personal and family cancer screening modalities. In our series, molecular analysis methods according to ProMisE classifier were used in 25 EC patients with long-term follow-up, of whom 15 (60%) were fully evaluable. It has to be taken into account that among 10 (40%) 'unclassifiable' patients, the three failures in achieving MMR status were due to the very low tumor volume (<4 mm), and three of the seven cases which were unclassifiable according to POLE/POLD1 status presented with no residual tumor after MMR IHC. All women included in the present analysis were conservatively treated by combined operative hysteroscopy and progestin therapy. It is reasonable to think that the rate of 'unclassifiable' patients could be even higher after progestin treatment following an endometrial biopsy only. Moreover, hysteroscopic resection could allow a better identification of possible intratumoral genetic heterogeneity [7,11].

Molecular analyses have been shown to work successfully on pre-operative endometrial biopsy or curettages with high concordance with the hysterectomy specimens [9,20–22]. Data concerning the applicability of diagnostic samples are very limited, however, and do not allow to draw definitive conclusions on the feasibility of molecular classification in the pre-treatment workup. The only retrospective study on the application of ProMisE to pre-staging samples from 23 endometrioid G1 ECs was published by Talhouk et al., and showed a complete molecular categorization in 98% of diagnostic samples [9]. This high rate, in such retrospective non-conservative setting, can be explained by the fact that only FFPE blocks with large tumor load were selected for analysis. The findings from that study (26% MMR IHC abnormal, 17.4% POLE EDM, 8.7% p53 IHC abnormal, 47.8% p53 IHC wild-type) are different from those observed in our series (46.7% MMR IHC abnormal, 6.6% POLE EDM, 0% p53 IHC abnormal, and 46.7% p53 IHC wild-type), likely due to the differences between the study populations.

In our series of low-risk EC selected for conservative treatment, there has been evidence for about 50% MMR defects at IHC analysis. The presence of such mutations correlates with the clinical outcome with 4 out of 7 mutated patients showing EC persistence/progression or metachronous Lynch syndrome-associated tumors (Tables 2 and 3). In particular, a double molecular profiling (PMS2 IHC abnormal and p53 mutation; MSH6 IHC abnormal and POLED1 mutation) was detected in the two patients developing metachronous Lynch syndrome-associated tumors.

Two of the three patients presenting with BMI $\geq$ 30 kg/m^2 were also MMR mutated, making the influence of obesity hardly evaluable.

On the contrary, only 2 out of the 8 patients with no (or POLE in one case) mutations showed unfavourable events (long-term local relapse associated with early stage OC). Both these patients underwent BRCA germline testing with evidence for a mutation of uncertain significance (S104 N) in one.

Previous studies reported the presence of more than one molecular feature in about 3% of EC cases [4,5,9]. Two patients of our series presented with combined POLE/POLD1 and MMR defects (Table 3). Similarly, Talhouk et al. identified two patients who had concomitant POLE and MMR abnormalities [5,9], confuting the previous observations of apparent mutual exclusivity between POLE EDM and MMR deficiency [23,24]. The role of somatic POLE/POLD1 EDMs in MMR deficient tumors, however, has not been yet defined. In particular, also due to the lower frequency of POLD1 vs. POLE EDMs in ECs (3.7% and 8.1%, respectively) [25], data on the co-occurrence of POLD1 and MMR abnormalities are even more limited than those on the concomitance of POLE and MMR defects [26,27]. Interestingly, in our cohort, the only POLD1 mutated MMR-deficient tumor occurred in a MSH6 germline mutation carrier and presented with loss of MSH6 expression and MSS (Table 3). This finding is consistent with that previously reported on the loss of MSH6 function, which causes no or only weak repeat instability [28,29]. Although waiting for more mature data to assess the prognostic impact of 'double positive status', preliminary reports do suggest that MMR deficiency has the most important value in patients showing concomitant MMR and POLE/p53 abnormalities [6,7].

International guidelines [NCCN] have recently introduced the MSI and/or MMR IHC screening in individual EC diagnosed before the age of 50, with approximately 90% concordance between germline analysis and IHC [NCCN]. In our experience, germline mutation was detected in two/third of cases identified by IHC. At this point, the question arising is what the ProMisE could add to the universal age-based MSI/MMR IHC screening in EC fertility-sparing setting. Based on present knowledge and on our preliminary findings, it is not possible to provide a definitive answer. Additional POLE/POLD1 and p53 analysis could potentially refine the prognostic profiling, although this was not the case in our limited series.

ProMisE, performed on resectoscopic specimens of EC patients candidate for conservative treatment, could be a pragmatic model for stratifying genetic risk of EC women, feasible in a very short time in 60% of cases in our series. Genetic high risk patients could be identified earlier, further counselled about the conservative management, and considered for preventative measures.

The limited sample size and retrospective setting represent the main limitations of our preliminary study. In contrast, the use of whole sections for immunostaining rather than tissue microarray could have minimized confounders in MMR status assessment and it has to be considered a strength.

In conclusion, although the biological effects and clinical relevance of mutations tested in ProMisE are to be better understood in a context of conservatively treated EC, data presented seem to be promising and such a molecular classifier potentially useful.

Larger series are needed to further assess the feasibility and utility of ProMisE in a fertility-sparing setting. In this respect, it is to be mentioned that a Gynecologic Cancer Inter-Group (GCIG) project is ongoing with the aim of prospectively registering conservatively treated EC cases [30], and, in this framework, data from a pooled molecular analysis will be available.

References

[1] National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology. Uterine neoplasms, Version 2. Available from. 2019. https://www.nccn.org/professionals/physician_gls/pdf/uterine.pdf.
[2] Colombo N, Creutzberg C, Amant F, Bosse T, González-Martín A, Ledermann J, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: diagnosis, treatment and follow-up. Ann Oncol 2016;27(1):16–41.
[3] Cancer Genome Atlas Research Network, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. Nature 2013;497(7447):67–73.
[4] Kommoss S, McConechy MK, Kommoss F, Leung S, Bunz A, Magrill J, et al. Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series. Ann Oncol 2018;29(5):1180–8.
[5] Talhouk A, McConechy MK, Leung S, Yang W, Lum A, Senz J, et al. Confirmation of ProMisE: a simple, genomics-based clinical classifier for endometrial cancer. Cancer 2017;123(5):802–13.
[6] Talhouk A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, et al. A clinically applicable molecular-based classification for endometrial cancers. Br J Cancer 2015;113(2):299–310.
[7] Stelloo E, Nout RA, Osse EM, Jürgenliemk-Schulz IJ, Jobsen JJ, Lutgens LC, 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 2016;22(16):4215–24.

[8] Stelloo E, Bosse T, Nout RA, MacKay HJ, Church DN, Nijman HW, et al. Refining prognosis and identifying targetable pathways for high-risk endometrial cancer; a TransPORTEC initiative. Mod Pathol 2015;28(6):836–44.
[9] Talhouk A, Hoang LN, McConechy MK, Nakonechny Q, Leo J, Cheng A, et al. Molecular classification of endometrial carcinoma on diagnostic specimens is highly concordant with final hysterectomy: earlier prognostic information to guide treatment. Gynecol Oncol 2016;143(1):46–53.
[10] Falcone F, Laurelli G, Losito S, Di Napoli M, Granata V, Greggi S. Fertility preserving treatment with hysteroscopic resection followed by progestin therapy in young women with early endometrial cancer. J Gynecol Oncol 2017;28(1):e2.
[11] Murali R, Soslow RA, Weigelt B. Classification of endometrial carcinoma: more than two types. Lancet Oncol 2014;15(7):e268–78.
[12] Antonsen SL, Jensen LN, Loft A, Berthelsen AK, Costa J, Tabor A, et al. MRI, PET/CT and ultrasound in the preoperative staging of endometrial cancer – a multicenter prospective comparative study. Gynecol Oncol 2013;128(2):300–8.
[13] Kisu I, Banno K, Lin LY, Ueno A, Abe T, Kouyama K, et al. Preoperative and intraoperative assessment of myometrial invasion in endometrial cancer: comparison of magnetic resonance imaging and frozen sections. Acta Obstet Gynecol Scand 2013;92(5):525–35.
[14] Wu LM, Xu JR, Gu HY, Hua J, Haacke EM, Hu J. Predictive value of T2-weighted imaging and contrast-enhanced MR imaging in assessing myometrial invasion in endometrial cancer: a pooled analysis of prospective studies. Eur Radiol 2013;23(2):435–49.
[15] Savelli L, Testa AC, Mabrouk M, Zannoni L, Ludovisi M, Seracchioli R, et al. A prospective blinded comparison of the accuracy of transvaginal sonography and frozen section in the assessment of myometrial invasion in endometrial cancer. Gynecol Oncol 2012;124(3):549–52.
[16] Helpman L, Kupets R, Covens A, Saad RS, Khalifa MA, Ismiil N, et al. Assessment of endometrial sampling as a predictor of final surgical pathology in endometrial cancer. Br J Cancer 2014;110(3):609–15.
[17] Francis JA, Weir MM, Ettler HC, Qiu F, Kwon JS. Should preoperative pathology be used to select patients for surgical staging in endometrial cancer? Int J Gynecol Cancer 2009;19(3):380–4.
[18] National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology. Genetic/familial high-risk assessment: colorectal. Version 1. Available from. 2018. https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf.
[19] Lu KH, Schorge JO, Rodabaugh KJ, Daniels MS, Sun CC, Soliman PT, et al. Prospective determination of prevalence of lynch syndrome in young women with endometrial cancer. J Clin Oncol 2007;25(33):5158–64.
[20] Stelloo E, Nout RA, Naves LC, Ter Haar NT, Creutzberg CL, Smit VT, et al. High concordance of molecular tumor alterations between pre-operative curettage and hysterectomy specimens in patients with endometrial carcinoma. Gynecol Oncol 2014;133(2):197–204.
[21] Church DN, Stelloo E, Nout RA, Valtcheva N, Depreeuw J, ter Haar N, et al. Prognostic significance of POLE proofreading mutations in endometrial cancer. J Natl Cancer Inst 2014;107(1):402.
[22] Bosse T, Nout RA, Stelloo E, Dreef E, Nijman HW, Jürgenliemk-Schulz IM, et al. L1 cell adhesion molecule is a strong predictor for distant recurrence and overall survival in early stage endometrial cancer: pooled PORTEC trial results. Eur J Cancer 2014;50(15):2602–10.
[23] Domingo E, Freeman-Mills L, Rayner E, Glaire M, Briggs S, Vermeulen L, et al. Somatic POLE proofreading domain mutation, immune response, and prognosis in colorectal cancer: a retrospective, pooled biomarker study. Lancet Gastroenterol Hepatol 2016;1(3):207–16.
[24] Church DN, Briggs SE, Palles C, Domingo E, Kearsey SJ, Grimes JM, et al. DNA polymerase ε and δ exonuclease domain mutations in endometrial cancer. Hum Mol Genet 2013;22(14):2820–8.
[25] Availablefrom: https://www.cbioportal.org.
[26] Jansen AM, van Wezel T, van den Akker BE, Ventayol Garcia M, Ruano D, Tops CM, et al. Combined mismatch repair and POLE/POLD1 defects explain unresolved suspected Lynch syndrome cancers. Eur J Hum Genet 2016;24(7):1089–92.
[27] Bellido F, Pineda M, Aiza G, Valdés-Mas R, Navarro M, Puente DA, et al. POLE and POLD1 mutations in 529 kindred with familial colorectal cancer and/or polyposis: review of reported cases and recommendations for genetic testing and surveillance. Genet Med 2016;18(4):325–32.
[28] Berends MJ, Wu Y, Sijmons RH, Mensink RG, van der Sluis T, Hordijk-Hos JM, et al. Molecular and clinical characteristics of MSH6 variants: an analysis of 25 index carriers of a germline variant. Am J Hum Genet 2002;70(1):26–37.
[29] Wu Y, Berends MJ, Mensink RG, Kempinga C, Sijmons RH, van Der Zee AG, et al. Association of hereditary nonpolyposis colorectal cancer-related tumors displaying low microsatellite instability with MSH6 germline mutations. Am J Hum Genet 1999;65(5):1291–8.
[30] Creutzberg CL, Kitchener HC, Birrer MJ, Landoni F, Lu KH, Powell M, et al. Gynecologic Cancer InterGroup (GCIG) Endometrial Cancer Clinical Trials Planning Meeting: taking endometrial cancer trials into the translational era. Int J Gynecol Cancer 2013;23(8):1528–34.
[31] Kurman RJ, Carcangiu ML, Herrington CS, Young RH. WHO classification of tumours of female reproductive organs. fourth edition 2014.