Prevalence and Prognosis of Lynch Syndrome and Sporadic Mismatch Repair Deficiency in Endometrial Cancer

Cathalijne C. B. Post, Ellen Stelloo, PhD, Vincent T. H. B. M. Smit, MD, PhD, Dina Ruano, PhD, Carli M. Tops, PhD, Lisa Vermij, MD, Tessa A. Rutten, BSc, Ina M. Jürgenliemk-Schulz, MD, PhD, Ludy C. H. W. Lutgens, MD, PhD, Jan J. Jobsen, MD, PhD, Remi A. Nout, MD, PhD, Emma J. Crosbie, MD, Melanie E. Powell, MD, PhD, Linda Mileskhn, MD, PhD, Alexandra Leary, MD, PhD, Paul Bessette, MD, PhD, Hein Putter, PhD, Stephanie M. de Boer, MD, PhD, Nanda Horeweg, MD, PhD, Hein Putter, PhD, Stephanie M. de Boer, MD, PhD, Nanda Horeweg, MD, PhD, Cathalijne C. B. Post, MD, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, the Netherlands (e-mail: c.c.b.post@lumc.nl).

1Department of Radiation Oncology, Leiden University Medical Center, Leiden, the Netherlands; 2Department of Pathology, Leiden University Medical Center, Leiden, the Netherlands; 3Department of Clinical Genetics, Leiden University Medical Center, Leiden, the Netherlands; 4Department of Radiation Oncology, University Medical Center Utrecht, Utrecht, the Netherlands; 5Department of Radiation Oncology, MAASTRO Clinic, Maastricht, the Netherlands; 6Department of Radiation Oncology, Medical Spectrum Twente, Enschede, the Netherlands; 7Division of Cancer Sciences, University of Manchester, St Mary's Hospital, Manchester, UK; 8Department of Obstetrics and Gynaecology, Manchester University NHS Foundation Trust, Manchester, UK; 9Department of Clinical Oncology, Barts Health NHS Trust, London, UK; 10Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, Australia; 11Department of Medical Oncology, Gustave Roussy Cancer Center—INSERM U981, Université Paris Saclay, Villejuif, France; 12Department of Obstetrics and Gynecology, University of Sherbrooke, Sherbrooke, Quebec, Canada; and 13Department of Biostatistics, Leiden University Medical Center, Leiden, the Netherlands

Abstract

Background: Standard screening of endometrial cancer (EC) for Lynch syndrome (LS) is gaining traction; however, the prognostic impact of an underlying hereditary etiology is unknown. We established the prevalence, prognosis, and subsequent primary cancer incidence of patients with LS-associated EC in relation to sporadic mismatch repair deficient (MMRd)-EC.

Methods: After MMR-immunohistochemistry, MLH1-promoter methylation testing, and next-generation sequencing, tumors were classified into 3 groups according to the molecular cause of their MMRd-EC: 1) MLH1-hypermethylated MMRd-ECs; 2) PMS2-hypermethylated MMRd-ECs; 3) MutS homologue 6 (MSH6) or PMS2 MMRd-ECs; 4) MLH1-hypermethylated non-MMRd-ECs; 5) other MMRd-ECs; and 6) non-MMRd-ECs.

Results: Among the 1336 ECs, 410 (30.7%) were MMRd. A total of 380 (92.7%) were fully triaged: 275 (72.4%) were MLH1-hypermethylated MMRd-ECs; 36 (9.5%) LS MMRd-ECs, and 69 (18.2%) MMRd-ECs due to other causes. Limiting screening of EC patients to 60 years or younger or to 70 years or younger would have resulted in missing 18 (50.0%) and 6 (16.7%) LS diagnoses, respectively. Five-year recurrence-free survival was 91.7% (95% confidence interval [CI] = 83.1% to 100%; hazard ratio = 0.45, 95% CI = 0.16 to 1.24, P = 0.12) for LS, 95.5% (95% CI = 90.7% to 100%; hazard ratio = 0.17, 95% CI = 0.05 to 0.55, P = 0.003) for “other” vs 78.6% (95% CI = 73.8% to 83.7%) for MLH1-hypermethylated MMRd-EC. The probability of subsequent LS-associated cancer at 10 years was 11.6% (95% CI = 0.0% to 24.7%), 1.5% (95% CI = 0.0% to 4.3%), and 7.0% (95% CI = 3.0% to 10.9%) within the LS, “other,” and MLH1-hypermethylated MMRd-EC groups, respectively.

Conclusions: The LS prevalence in the Post Operative Radiation Therapy in Endometrial Carcinoma trial population was 2.8% and among MMRd-ECs was 9.5%. Patients with LS-associated ECs showed a trend towards better recurrence-free survival and higher risk for second cancers compared with patients with MLH1-hypermethylated MMRd-EC.

The diagnosis of Lynch syndrome (LS) in endometrial cancer (EC) is crucial for counseling and cancer surveillance of patients and their relatives. LS is a highly penetrant, hereditary, cancer-prone syndrome caused by germline variants in the DNA mismatch repair (MMR) genes: mutL homologue 1 (MLH1), mutS homologue 2 (MSH2), mutS homologue 6 (MSH6), or postmeiotic segregation increased 2 (PM2). The cancer risk varies per gene and is substantially lower for PMS2 (1,2). EC is often the first
malignancy affecting women with LS (3), and their risk of metachronous cancer is approximately 24% at 10 years (4).

LS-associated cancers arise following MMR deficiency (MMRd) due to the somatic inactivation of the remaining wild-type MMR allele. MMRd leads to the accumulation of mismatches, insertions, and deletions in repeated sequences also known as microsatellite instability (MSI). MMRd is not an exclusive feature of LS; the vast majority (about 70%) of MMRd-ECs present with somatic inactivation of the MLH1 gene via hypermethylation of the promoter region (5,6). Most of the cases that are neither MLH1 hypermethylated nor harbor a MMR germline variant are considered sporadic due to biallelic somatic MMR gene inactivation; few are caused by an undetectable hereditary syndrome (frequently referred to as Lynch-like syndrome) (7-9). MMRd-ECs are known to have an intermediate prognosis within the molecular classification with a good response to immunotherapy (10-13). The diagnosis of LS may allow clinicians to tailor treatment and patient information; LS-associated tumors may have a more favorable outcome (14), although there are no previous studies available on the prognostic impact of LS among MMRd-ECs.

Tumor triage by MMR-immunohistochemistry (IHC) and/or MSI analysis in combination with targeted MLH1-methylation testing can identify patients with LS. The Proportion of Endometrial Tumours Associated Lynch Syndrome study showed that IHC-based triage is most accurate, whereas clinical selection based on age and family history were imprecise predictors (15). Overall, an estimated 3% of EC cases are associated with LS (15-17), which is similar in colorectal cancer (CRC) (18). However, these estimations were mostly based on small trials with methodological heterogeneity, often selecting their test population by age and/or family history, and incomplete testing (16).

Given its relative rarity, the prevalence and prognosis of LS should be investigated in a large population, such as the well-documented combined cohort of the Post Operative Radiation Therapy in Endometrial Carcinoma (PORTEC)-1,-2, and -3 trials. These randomized controlled trials have had a major impact on guidelines for treatment in ECs (19-21). Together they included 1336 evaluable patients comprising all risk groups with long and complete follow-up information and collected tumor blocks. The aim of our study was to investigate the prevalence and prognosis of LS-associated EC in relation to MLH1 hypermethylated MMRd-EC. Secondary objectives were to evaluate currently used age criteria for IHC-based tumor triage and the probability of developing a subsequent primary LS-associated cancer.

**Methods**

**Study Population**

In total, 1336 of 1801 ECs from the PORTEC-1,-2, and -3 clinical trials were eligible for analysis based on availability of formalin-fixed paraffin-embedded (FFPE) slides. In the PORTEC-1 trial (1990-1997), 714 patients with stage I low-intermediate and high-intermediate risk endometrioid EC were randomly assigned to receive pelvic radiotherapy or no additional treatment (19). In the PORTEC-2 trial (2002-2006), 427 endometrioid EC patients with high-intermediate risk features were randomly assigned to receive pelvic radiotherapy or vaginal brachytherapy (if stage I: 0-60 years) (20). In the international PORTEC-3 trial (2006-2013), 660 EC patients with high-risk features were randomly assigned to receive pelvic radiotherapy or chemoradiotherapy (21). In all trials, patients with a history of invasive cancer (for PORTEC-3 within the last 10 years), except for non-melanoma skin cancer, were excluded. Full details and results of these trials have been published previously (19-21). The study protocols were approved by the Dutch Cancer Society and the medical ethics committees at participating centers. All patients provided informed consent for participation in the trial, and for use of their tumor block for subsequent translational research.

**IHC, MSI, Methylation Analysis, and Next-Generation Sequencing (NGS)**

Patients were included in the current analysis if they showed loss of expression of at least 1 of the 4 MMR proteins with positive internal control (including subclonal loss defined as abrupt and complete regional loss with intervening stromal positivity) or MSI-high status when MMR-IHC failed. Details on MMR-IHC and MSI testing and scoring were described previously (5,11,12,22). Cases with MMRd phenotype are referred to as MMRd-EC in this study irrespective of POLE mutation status.

MLH1 methylation testing was performed on MLH1-deficient and/or MSI-high tumors as described previously (23). All cases with loss of MLH1 or MSI-high status without MLH1 hypermethylation; loss of MSH2 and/or MSH6; or isolated loss of PMS2 were triaged as potential LS-associated MMRd-EC. DNA isolated from matched normal/tumor FFPE tissues of these cases was amplified using long-range polymerase chain reaction followed by targeted NGS for variants in the exonic regions of MLH1, MSH2, MSH6, PMS2, POLE, and POLD1 using the Ion Proton System or Ion SS System (Thermo Fisher Scientific) (24,25). Variants were annotated according to the following GenBank reference sequences: NM_000249.3 (MLH1), NM_000251.2 (MSH2), NM_000179.2 (MSH6), NM_000535.5 (PMS2), NM_006251.2 (POLE), and NM_001256849.1 (POLD1). All patients with germline variants (likely) affecting function (path_MMR) were verified by a clinical laboratory geneticist (C.M.T.) and considered to have LS.

**Statistical Analysis**

Following complete triage, cases were classified into 3 groups according to the molecular cause of their MMRd-EC: LS, methylated (including cases with MLH1 hypermethylation and subclonal MLH1 loss), and other causes (a mixed group having alternative causes of MMRd; see the Supplementary Methods and Supplementary Figure 1 for full definitions, available online). χ² Statistics or Fisher’s exact test for categorical variables and 1-way analysis of variance or Kruskal-Wallis test for continuous variables were used to compare characteristics.

The sample size ensured sufficient power to detect an LS prevalence of 3.0% with a precision of 0.009 (95% confidence
Among the 1336 evaluable ECs, 410 (30.7%) were MMRd and eligible for further analysis. Median age of MMRd-EC patients was 65 years (interquartile range = 59-73 years). Most MMRd-ECs were early-stage tumors (74.2%) of low-grade endometrioid subtype (66.8%) and were treated with pelvic radiotherapy (51.7%). All characteristics of MMRd-ECs differed between the 3 PORTEC trials, in line with the inclusion criteria (Table 1).

### MMR Causes and Variant Analysis

Complete triage was accomplished for 380 (92.7%) of the MMRd-ECs (Figure 1; insufficient material in 27 cases for MLH1 methylation assay and 3 for NGS). Thirty-six path/MMR variant carriers were identified, giving a 2.8% LS prevalence in the overall population and a 9.5% LS prevalence within the MMRd group. There were 18 path_MSH6, 10 path_PMS2, 6 path_MSH2, and 2 path_MLH1 variant carriers. An overview of the LS cases is displayed in Table 2. In total, 275 (72.4%) cases were classified as methylated. The remaining 69 (18.2%) MMRd cases were neither LS nor MLH1 hypermethylated and were therefore classified as “other.”

LS patients were younger, with a median age of 60 years (interquartile range = 54-67 years) and more often had p53 aberrant staining (20.0%) and serous (13.9%) or clear cell (8.3%) histology compared with the patients with methylated MMRd-EC (Table 3). Limiting screening of EC patients to age 50 years or younger, 60 years or younger, and 70 years or younger would have missed 31 (86.1%), 18 (50.0%), and 6 (16.7%) LS diagnoses, respectively. Figure 2 displays the distribution of the involved MMR proteins; all LS cases identified by the 4-panel approach would also have been identified by a 2-panel approach including only PMS2- and MSH6-IHC. No germline POLE/POLD1 variants affecting function were identified. LS patients with path_MSH6 and path_PMS2 variants were older than those with path_MLH1 and path_MSH2 variants (median age = 63, 62, 50, and 50 years, respectively, P = .01; Supplementary Table 1, available online).

### Results

**Study Population**

Among the 1336 evaluable ECs, 410 (30.7%) were MMRd and eligible for further analysis. Median age of MMRd-EC patients was 65 years (interquartile range = 59-73 years). Most MMRd-ECs were early-stage tumors (74.2%) of low-grade endometrioid subtype (66.8%) and were treated with pelvic radiotherapy (51.7%). All characteristics of MMRd-ECs differed between the 3 PORTEC trials, in line with the inclusion criteria (Table 1).

### Table 1: Patient, tumor, and treatment characteristics

| Characteristic | All MMRd-EC | PORTEC-1 | PORTEC-2 | PORTEC-3 | P* |
|---------------|-------------|----------|----------|----------|----|
| Total, No. (%) | 410 (100.0) | 145 (35.6) | 114 (27.8) | 151 (36.8) | <.001 |
| Age at random assignment | | | | | |
| Median (IQR), y | 65 (59-73) | 67 (61-73) | 70 (65-77) | 60 (56-66) | <.001 |
| FIGO 2009 stage, No. (%) | | | | | |
| IA | 104 (25.4) | 62 (42.8) | 25 (21.9) | 17 (11.3) | <.001 |
| IB | 200 (48.8) | 83 (57.2) | 87 (76.3) | 30 (19.9) | <.001 |
| II | 36 (8.8) | 0 (0.0) | 1 (0.9) | 35 (23.2) | <.001 |
| III | 70 (17.1) | 0 (0.0) | 1 (0.9) | 69 (45.7) | <.001 |
| Histological grade and type, No. (%) | | | | | |
| EEC grade 1 or 2 | 274 (66.8) | 122 (84.1) | 91 (79.8) | 61 (40.4) | <.001 |
| EEC grade 3 | 99 (24.1) | 22 (15.2) | 21 (18.4) | 56 (37.1) | <.001 |
| Serous | 11 (2.7) | 1 (0.7) | 2 (1.8) | 8 (5.3) | <.001 |
| Clear cell | 12 (2.9) | 0 (0.0) | 0 (0.0) | 12 (7.9) | <.001 |
| Other | 14 (3.4) | 0 (0.0) | 0 (0.0) | 14 (9.3) | <.001 |
| Myometrial invasion, No. (%) | | | | | |
| ≥50% | 274 (66.8) | 83 (57.2) | 90 (78.9) | 101 (66.9) | <.001 |
| Lymphovascular space invasion, No. (%) | | | | | |
| Present | 131 (32.0) | 13 (9.0) | 16 (14.0) | 102 (67.5) | <.001 |
| Received adjuvant treatment, No. (%) | | | | | |
| No treatment | 73 (17.8) | 71 (49.0) | 2 (1.8) | 0 (0.0) | <.001 |
| External beam radiotherapy | 212 (51.7) | 74 (51.0) | 58 (50.9) | 80 (53.0) | <.001 |
| Vaginal brachytherapy | 54 (13.2) | 0 (0.0) | 54 (47.4) | 0 (0.0) | <.001 |
| Chemoradiotherapy | 71 (17.3) | 0 (0.0) | 0 (0.0) | 71 (47.0) | <.001 |

*P values reflect y statistics or Fisher’s exact test for categorical variables and Kruskal-Wallis test for age. EC = endometrial cancer; EEC = endometrioid endometrial cancer; FIGO = International Federation of Gynecology and Obstetrics; IQR = interquartile range; MMRd = mismatch repair deficient; PORTEC = Post Operative Radiation Therapy in Endometrial Carcinoma.*
Survival

The estimated RFS for the MMRd population at 5 years was 83.7% (95% CI = 80.1% to 87.4%): 91.7% (95% CI = 83.1% to 100%) for patients with LS-associated MMRd-EC, 78.6% (95% CI = 73.8% to 83.7%) for patients with methylated MMRd-EC, and 95.5% (95% CI = 90.7% to 100%) for patients with other causes of MMRd-EC (P = .001; Figure 3, A; LS vs methylated: HR = 0.45, 95% CI = 0.16 to 1.24, P = .12; other vs methylated: HR = 0.17, 95% CI = 0.05 to 0.55, P = .003).

The estimated OS for the MMRd population at 5 years was 82.8% (95% CI = 79.2% to 86.5%); 88.5% (95% CI = 78.5% to 99.8%) for patients with LS-associated MMRd-EC, 78.5% (95% CI = 73.7% to 83.5%) for patients with methylated MMRd-EC, and 97.0% (95% CI = 93.0% to 100%) for patients with other causes of MMRd-EC (P < .001; Figure 3, B; LS vs methylated: HR = 0.50, 95% CI = 0.24 to 1.02, P = .06; other vs methylated: HR = 0.27, 95% CI = 0.13 to 0.55, P < .001). After adjustment for age, the trend for better OS in the LS group was no longer observed (vs methylated MMRd-EC: HR = 0.73, 95% CI = 0.35 to 1.52, P = .40), whereas age and having another cause of MMRd were statistically significant prognostic factors (HR = 1.07, 95% CI = 1.04 to 1.09, P < .001; other vs methylated MMRd-EC: HR = 0.41, 95% CI = 0.20 to 0.85, P = .02).

Second Primary Cancers

At 10 years, the cumulative incidence of developing a second LS-associated tumor was 11.6% (95% CI = 0.0% to 24.7%) among EC patients with LS, 1.5% (95% CI = 0.0% to 4.3%) among patients with other MMRd-EC, and 7.0% (95% CI = 3.0% to 10.9%) among patients with methylated MMRd-EC (Supplementary Figure 2, available online). Three of the 4 LS-patients who developed a second primary LS-associated cancer had colon cancer (after 3.8, 4.8, and 14.9 years) and 1 had ureteral cancer (after 8.0 years; Supplementary Table 2, available online, shows cancer type distribution). The cause-specific hazard ratio for developing an LS-associated second cancer was 1.9 (95% CI = 0.63 to 5.7, P = .26) for patients with LS vs patients with methylated MMRd-EC.

Discussion

After complete IHC-based tumor triage, we found a 2.8% prevalence of LS in 1 of the largest EC trial populations worldwide, comprising all risk groups with long and complete follow-up. The prevalence of LS among patients with MMRd-EC was 9.5%. Patients with LS were relatively young, but restricted testing to...
| No. | Study     | Age, y | Tumor histology | Grade | Molecular class | Affected MMR proteins | MLH1 promoter methylation | Coding DNA alteration | TCGA surrogate | Classification |
|-----|-----------|--------|-----------------|-------|-----------------|------------------------|--------------------------|-----------------------|---------------|---------------|
| 1   | PORTEC-1  | 47     | Endometrioid    | IB    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 2   | PORTEC-1  | 47     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 3   | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 4   | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 5   | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 6   | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 7   | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 8   | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 9   | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 10  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 11  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 12  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 13  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 14  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 15  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 16  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 17  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 18  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 19  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 20  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 21  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 22  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 23  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 24  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 25  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 26  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 27  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 28  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 29  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 30  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 31  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 32  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 33  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 34  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 35  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |
| 36  | PORTEC-1  | 52     | Endometrioid    | IA    |                 | MMRd                   | NA                       | c.1351C>T              | MSH2         |                |

*Classification according to the 5-tiered InSiGHT rules: class 5 is pathogenic, class 4 is likely pathogenic, G = grade, IHC = immunohistochemistry.
women who are 60 years or younger would have missed one-half of the cases. Patients with LS tend to have a better RFS and a higher risk of developing second primary cancers compared with patients with methylated MMRd-ECs. No trend for more favorable OS was found after adjustment for age.

This is the first study to our knowledge investigating the prognostic value of LS within the MMRd-EC subgroup. Most of the recent research showed that MMRd-ECs, predominantly driven by the large number of MLH1 hypermethylated cases, have an intermediate prognosis within the molecular classification introduced by The Cancer Genome Atlas (10-12). Our survival analysis showed that EC patients with LS tend to have a better RFS than patients with methylated MMRd-EC (HR = 0.45, \( P = .12 \)), whereas LS had no statistically significant prognostic value for OS after adjustment for age (age-adjusted HR = 0.73, \( P = .40 \)). The favorable prognosis has been assumed to be induced by the active local immune response (14,27). Comparable survival analysis in CRC has been published. One study showing a better OS for 85 CRC patients with LS compared with 67 sporadic MMRd patients after adjustment for age, stage, and BRAF status (HR = 0.29, 95% CI = 0.09 to 0.95, \( P = .04 \)) (28). The other study also showing better OS in 37 CRC patients with LS compared with 106 methylated MMRd patients, although the difference was minimal after adjusting for age and stage (29).

The cumulative incidence for developing a second LS-associated cancer at 10 years was 11.6% (95% CI = 0.0% to 24.7%) for patients with LS vs 7.0% (95% CI = 3.0% to 10.9%) for patients with methylated MMRd-EC (HR = 1.90, 95% CI = 0.63 to 5.7, \( P = .26 \)). Our analysis was underpowered due to the small number of events in the LS group. Nevertheless, the elevated risk strengthens previous reports on subsequent cancers in EC or non-CRC LS patients (15-24%) (4,30) and is of importance for surveillance strategies.

The 2.8% prevalence of LS-EC is consistent with previous publications in which prevalences of 2.8%-3.2% were reported (15-17). This prevalence is likely a slight underestimation. Firstly, our NGS panel did not include EPCAM and could not detect large rearrangements. To detect large rearrangement in EPCAM or the MMR genes, Multiplex Ligation-dependent Probe Amplification is most commonly used but performs poorly on FFPE tissue. Secondly, the patient selection in our trial design may have affected the prevalence. Patients younger than 60 years with stage I ECs were excluded from the PORTEC-2 trial. Nevertheless, the total PORTEC population deviates minimally in the Proportion of Endometrial Tumours Associated Lynch Syndrome study, an unselected, prospective, cross-sectional study in the United Kingdom among 500 EC patients (15). Moreover, patients with a history of cancer were excluded from publications in which prevalences of 2.8%-3.2% were reported (15-17). This prevalence is likely a slight underestimation.

### Table 3. Patient, tumor, and treatment characteristics according to the molecular cause of their MMRd-EC

| Characteristic | All MMRd-EC | Methylated | Other | LS | \( P^a \) |
|---------------|------------|------------|-------|----|--------|
| Total, No. (%) | 410b       | 275 (72.4) | 69 (18.2) | 36 (9.5) | <.001  |
| Age at random assignment |            |            |       |    |        |
| Median (IQR), y | 65 (59-73) | 67 (62-74) | 59 (55-66) | 60 (54-67) | <.001  |
| Trial, No. (%) |            |            |       |    | .002   |
| PORTEC-1 | 145 (35.4) | 99 (36.0) | 22 (31.9) | 12 (33.3) |        |
| PORTEC-2 | 114 (27.6) | 87 (31.6) | 8 (11.6) | 9 (25.0) |        |
| PORTEC-3 | 151 (36.8) | 89 (32.4) | 39 (56.5) | 15 (41.7) |        |
| FIGO 2009 stage, No. (%) |            |            |       |    | .20    |
| IA | 104 (25.4) | 70 (25.5) | 17 (24.6) | 7 (19.4) |        |
| IB | 200 (48.8) | 137 (49.8) | 27 (39.1) | 21 (58.3) |        |
| II | 36 (8.8) | 22 (8.0) | 11 (15.9) | 1 (2.8) |        |
| III | 70 (17.1) | 46 (16.7) | 14 (20.3) | 7 (19.4) |        |
| Histological grade and type, No. (%) |            |            |       |    | <.001  |
| EEC grade 1 or 2 | 274 (66.8) | 197 (71.6) | 40 (58.0) | 19 (52.8) |        |
| EEC grade 3 | 99 (24.1) | 64 (23.3) | 18 (26.1) | 8 (22.2) |        |
| Serous | 11 (2.7) | 2 (0.7) | 4 (5.8) | 5 (13.9) |        |
| Clear cell | 12 (2.9) | 2 (0.7) | 6 (8.7) | 3 (8.3) |        |
| Other | 14 (3.4) | 10 (3.6) | 1 (1.4) | 1 (2.8) |        |
| Myometrial invasion, No. (%) |            |            |       |    | .41    |
| >50% | 274 (66.8) | 187 (68.0) | 43 (62.3) | 27 (75.0) |        |
| Lymphovascular space invasion, No. (%) |            |            |       |    | .96    |
| Present | 131 (32.0) | 90 (32.7) | 23 (33.3) | 11 (30.6) |        |
| POLEmut in tumor, No. (%) |            |            |       |    | .002   |
| Exonuclease domain mutations | 19 (4.7) | 8 (2.9) | 9 (13.4) | 2 (5.7) |        |
| p53 immunohistochromy, No. (%) |            |            |       |    | <.001  |
| Aberrant | 31 (7.7) | 7 (2.6) | 14 (20.9) | 7 (20.0) |        |
| Received adjuvant treatment, No. (%) |            |            |       |    | .10    |
| No treatment | 73 (17.8) | 47 (17.1) | 10 (14.5) | 9 (25.0) |        |
| External beam radiotherapy | 212 (51.7) | 145 (52.7) | 40 (58.0) | 13 (36.1) |        |
| Vaginal brachytherapy | 54 (13.2) | 39 (14.2) | 3 (4.3) | 6 (16.7) |        |
| Chemoradiotherapy | 71 (17.3) | 44 (16.0) | 16 (23.2) | 8 (22.2) |        |

\(^a\) \( P \) values reflect \( z \) statistics or Fisher’s exact test for categorical variables and Kruskal-Wallis test for age. EC = endometrial cancer; EEC = endometrioid endometrial cancer; FIGO = International Federation of Gynecology and Obstetrics; IQR = interquartile range; MMRd = mismatch repair deficient; POLEmut = POLE-ultramutated; PORTEC = Post Operative Radiation Therapy in Endometrial Carcinoma.

\(^b\) All MMRd-ECs including those with insufficient material for MLH1-methylation assay (\( n = 27 \)) and normal tissue next-generation sequencing (\( n = 3 \)).
The determination of a sporadic explanation excludes potential hypermethylation nor a methylation–based triage in 2.8% of 1336 patients. It is hypothesized that the majority will be explained by a sporadic origin through biallelic somatic MMR inactivation (15,40).

Further research into the causes of the 63 cases with neither MLH1 hypermethylation nor a MMR germline variant is ongoing. The diagnosis of LS in EC is crucial for counseling and cancer surveillance even though these patients might be older than those presenting with CRC (18). Moreover, LS screening in incident ECs will have consequences for the patient’s family. Cascade testing of at-risk relatives can identify patients who can benefit from cancer surveillance and risk-reducing treatment (37,38). The clinical impact depends on the gene-specific cancer risk and is substantially lower for path_PMS2 carriers (1,2). Finally, LS identification may have consequences by allowing clinicians to better estimate and explain prognosis and to potentially tailor treatment in the upcoming immunotherapy era (14,27,39).

In conclusion, LS was identified using MMR-IHC with targeted MLH1 methylation testing, and as has been adopted widely for CRC, may be a more effective strategy to identify these LS families than age- and family history–based triage. An upper age screening limit would not be recommended, because limiting screening to EC patients who are aged 70 years or younger would have missed 6 (16.7%) LS diagnoses. We confirmed that a 2-antibody panel including MSH6- and PMS2-IHC, with MSH2- or MLH1-IHC only in case of inconclusive staining, is as sensitive as the full panel to detect LS (3). Moreover, LS screening in incident ECs will have consequences for the patient’s family.

Figure 2. Details on the mismatch repair (MMR) protein expression according to the molecular cause of their MMR-deficient endometrial cancer (MMRd-EC). MMR protein expression was scored as following complete loss (CL), retained (R), subclonal loss (SL), unknown/failed (UK). The concordance of these 2 columns shows that a 2-antibody (MSH6 and PMS2) panel is as sensitive as the full panel to detect Lynch syndrome (LS). All MMRd-ECs including those with insufficient material for MLH1 methylation assay (n = 27) and next-generation sequencing (n = 3).
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Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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