Performance Characteristics of Screening Strategies to Identify Lynch Syndrome in Women With Ovarian Cancer

Soyoun Rachel Kim, MD1,2; Alicia Tone, PhD3; Raymond H. Kim, MD, PhD4,5; Matthew Cesari, MD6; Blaise A. Clarke, MD6; Lua Eiriksson, MD, PhD4,7; Tae Hart, PhD4,8; Melyssa Aronson, MS4; Spring Holter, MS4; Alix Lytwyn, MD9; Manjula Maganti, MSc10; Leslie Oldfield, MSc11; Steven Gallinger, MD12; Marcus Q. Bernardini, MD1,2; Amit M. Oza, MD5; Bojana Djordjevic, MD6; Jordan Lerner-Ellis, PhD6; Emily Van de Laar, MSc4; Danielle Vicus, MD, MD2,13; Trevor J. Pugh, PhD1,3,14,15; Aaron Pollett, MD6,16; and Sarah E. Ferguson, MD1,2,4

BACKGROUND: For women with ovarian cancer (OC), the optimal screening strategy to identify Lynch syndrome (LS) has not been determined. In the current study, the authors compared the performance characteristics of various strategies combining mismatch repair (MMR) immunohistochemistry (IHC), microsatellite instability testing (MSI), and family history for the detection of LS. METHODS: Women with nonserous and/or nonmucinous ovarian cancer were recruited prospectively from 3 cancer centers in Ontario, Canada. All underwent germline testing for LS and completed a family history assessment. Tumors were assessed using MMR IHC and MSI. The sensitivity, specificity, and positive and negative predictive values of screening strategies were compared with the gold standard of a germline result. RESULTS: Of 215 women, germline data were available for 189 (88%); 13 women (7%) had pathogenic germline variants with 7 women with mutS homolog 6 (MSH6); 3 women with mutL homolog 1 (MLH1); 2 women with PMS1 homolog 2, mismatch repair system component (PMS2); and 1 woman with mutS homolog 2 (MSH2). A total of 28 women had MMR-deficient tumors (13%); of these, 11 had pathogenic variants (39%). Sequential IHC (with MLH1 promoter methylation analysis on MLH1-deficient tumors) followed by MSI for nonmethylated and/or MMR-intact patients was the most sensitive (92.3%; 95% confidence interval, 64%-99.8%) and specific (97.7%; 95% confidence interval, 94.2%-99.4%) approach, missing 1 case of LS. IHC with MLH1 promoter methylation analysis missed 2 patients of LS. Family history was found to have the lowest sensitivity at 55%. CONCLUSIONS: Sequential IHC (with MLH1 promoter methylation analysis) followed by MSI was found to be most sensitive. However, IHC with MLH1 promoter methylation analysis also performed well and is likely more cost-effective and efficient in the clinical setting. The pretest probability of LS is high in patients with MMR deficiency and warrants universal screening for LS. Cancer 2020;126:4886-4894. © 2020 The Authors. Cancer published by Wiley Periodicals LLC on behalf of American Cancer Society This is an open access article under the terms of the Creative Commons Attribution NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

KEYWORDS: Lynch syndrome, ovarian cancer, screening, tumor testing.

INTRODUCTION
Lynch syndrome (LS) is an autosomal dominant inherited cancer susceptibility syndrome caused by germline mutations in DNA mismatch repair (MMR) genes, primarily mutL homolog 1 (MLH1); mutS homolog 2 (MSH2); mutS homolog 6 (MSH6); PMS1 homolog 2, mismatch repair system component (PMS2); and epithelial cell adhesion molecule (EPCAM) [1,2]. LS is associated with increased lifetime risks of colorectal cancer (CRC; 40%-80%), endometrial cancer (EC; 33%-61%), and ovarian cancer (OC; 9%-12%). [3,4]. In women with LS, gynecologic tumors usually present as the sentinel malignancy with a significant lead time of up to 10 years, thereby creating an opportunity to identify and treat premalignant or early-stage cancers at other sites. In addition, identifying first-degree relatives through cascade testing

Additional supporting information may be found in the online version of this article.

DOI: 10.1002/cncr.33144, Received: June 11, 2020; Revised: July 17, 2020; Accepted: July 20, 2020, Published online August 18, 2020 in Wiley Online Library (wileyonlinelibrary.com)
offers an opportunity for cancer prevention through cancer screening and risk reduction strategies.

Historically, LS has been identified through analysis of family histories that met Amsterdam II clinical criteria. However, due to the low sensitivity, multiple bodies now recommend universal tumor testing with immunohistochemistry (IHC) for MMR protein expression or microsatellite instability (MSI) testing at the time of diagnosis of EC and/or CRC.⁵,⁷ Due to defects in MMR genes, LS-associated tumors characteristically demonstrate loss of MMR protein expression on IHC (MMR deficient [MMRd]) or MSI.⁸ However, because the majority of patients of MMRd EC and/or CRC are sporadic, with MLH1/PMS2 deficiency from epigenetic MLH1 promoter methylation,¹⁹ patients with MMRd tumors without MLH1 promoter methylation are referred for further genetic counseling and germline testing for LS. In keeping with these recommendations to identify individuals at risk of developing LS, the province of Ontario, Canada, has implemented IHC followed by MLH1 promoter methylation analysis of all EC and CRC specimens in patients aged <70 years.⁶,¹⁰,¹¹ Compared with MSI testing, IHC is less expensive, can direct genetic testing, and is easier to operationalize with superior performance characteristics.¹⁰

Although universal screening is becoming routine in patients with EC and CRC to identify LS, patients with OC appear to be completely neglected by current recommendations.⁶,⁷ OC is the third most common LS-associated cancer in women, with carrier-specific risks of 5% to 20% in MLH1, 10% to 38% in MSH2, 1% to 11% in MSH6, and an indeterminate risk in PMS2.³ Most commonly, LS-associated OCs are nonserous and nonmucinous, with an enrichment in endometrioid histology (>50%).¹² Retrospective studies have estimated that 2% to 29% of nonserous OCs are MMRd,¹³,¹⁵ but to our knowledge it is unknown how many of these patients are LS carriers. Furthermore, although there is some evidence that supports the need for histotype-specific screening to identify LS in patients with OC,¹⁶ to our knowledge there is no comparative study of various screening approaches. Therefore, the primary objective of the current study was to compare the performance characteristics of various screening strategies to identify LS in a prospective cohort of nonserous and/or nonmucinous OC. The secondary objective was to establish the incidence of LS in this cohort.

**MATERIALS AND METHODS**

**Participants**
Participants were recruited prospectively from 3 Ontario gynecology oncology centers between September 2015 and June 2019. Institutional research ethics board approval and written informed consent were obtained. Eligibility criteria included histologically confirmed nonserous and/or nonmucinous invasive epithelial OC of all histologic grades and International Federation of Gynecology and Obstetrics (FIGO) stages. All participants were asked to complete family history questionnaires (FHQs), underwent testing of their ovarian tumors with IHC and MSI, and provided blood samples for germline mutation testing using a next-generation sequencing (NGS) gene panel of MMR genes. Clinicodemographic information was extracted from the electronic patient records.

**Family History**
Participants were asked to complete an extended FHQ (eFHQ), which was developed to create a 3-generation pedigree as previously described.¹⁷ Details from the eFHQ then were used to determine whether the patients met the criteria for referral for genetic assessment based on Amsterdam II clinical criteria, Society of Gynecologic Oncology (SGO) 20% to 25%, or Ontario Ministry of Health (OMOH) family history criteria.¹⁸ For the purposes of the current study, OMOH family history criteria (see Supporting Table 1) was chosen as the family history variable of interest to calculate the performance characteristics because it encompasses the Amsterdam II clinical criteria and SGO 20% to 25% criteria and is the current family history criteria used in Ontario for LS genetic testing.¹¹

**Tumor Testing: IHC and MSI**
All OC specimens were reviewed by a pathologist experienced with ovarian pathology and MMR IHC and who was blinded to the germline results. IHC was used to test for expression of MLH1, MSH2, MSH6, and PMS2 proteins on 4-µm paraffin sections of all tumors as described previously (see Supporting Information).¹¹ The tumors were considered to be MMRd if there was an absence of staining in the tumor cell nuclei compared with adjacent normal tissue.¹¹ For MSI testing, DNA was extracted from tumor and normal tissue and amplified using polymerase chain reaction as previously described (see Supporting Information).¹¹ Tumors were considered MSI-high (MSI-H) if ≥2 of 5 markers were unstable, and were considered to be microsatellite stable (MSS) if <2 markers were unstable. Tumors were considered equivocal if <3 loci could be amplified unless ≥2 markers demonstrated stability.¹⁹ For the purpose of sensitivity and specificity calculations, tumors with focal or heterogeneous loss of MMR protein expression
by IHC were considered to be MMRd\(^2\) and any equivocal case on IHC or the MSI test was considered to be MMR intact and/or MSS.

**Germline Targeted Panel Sequencing**

All participants were offered germline testing using a NGS panel we developed with hybrid capture probes tiling: 1) all exons, introns, and flanking regions of MMR genes \(MLH1\), \(MSH2\), \(MSH6\), and \(PMS2\); 2) all exons of \(EPCAM\); and 3) the intergenic region between \(EPCAM\) and \(MSH2\).\(^2\)

The panel has been validated to recapitulate the results of clinical testing.\(^2\) Description of the panel (see Supporting Table 2), laboratory workflow, bioinformatics workflow, and variant interpretation are available in the Supporting Information. Briefly, normal DNA was extracted from either blood buffy coat or adjacent normal tissue that was macrodisseected from formalin-fixed, paraffin-embedded tumor slides. After extraction, DNA was sheared and target-enriched genomic libraries were prepared on each sample. Samples were sequenced on an Illumina NextSeq 500 device with the resulting reads aligned to the human reference genome (UCSC Genome Browser hg38). Our bioinformatics pipeline queries the MMR genes for germline single-nucleotide variants, insertions and deletions, copy number alterations, and structural rearrangements (see Supporting Fig. 1). Germline variant filtration and interpretation were performed according to American College of Medical Genetics and Genomics guidelines, blinded to tumor testing results.\(^2\) For all MLH1-deficient patients, \(MLH1\) promoter methylation analysis was performed using our panel as described in the Supporting Information. For the calculation of performance characteristics, patients with pathogenic or likely pathogenic variants were considered to have LS, whereas those with a variant of unknown significance (VUS) were considered to have a negative germline result.

**Screening Strategies**

We compared the performance characteristics (sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV]) of the following strategies compared with the germline test as the gold standard: 1) IHC only (any case that is IHC deficient is considered as testing positive); 2) IHC with \(MLH1\) promoter methylation analysis of MLH1-deficient patients (any case that is IHC deficient without \(MLH1\) promoter hypermethylation is considered as testing positive); 3) MSI only (any case that is MSI-H is considered as testing positive); 4) family history (any case that meets OMOH criteria is considered as testing positive); 5) IHC plus MSI (sequential testing with IHC followed by the MSI test on any IHC-intact patients; any case that is IHC deficient and/or MSI-H is considered as testing positive); and 6) IHC with \(MLH1\) promoter methylation analysis plus MSI (sequential testing with IHC [with \(MLH1\) promoter methylation analysis for all \(MLH1\)-deficient patients] followed by MSI testing on any nonmethylated and/or IHC-intact case; any case that is IHC deficient without \(MLH1\) promoter hypermethylation and/or MSI-H is considered as testing positive).

**Statistical Analysis**

Categorical variables were summarized using counts and percentages whereas continuous variables were summarized using medians and ranges. Groups were compared using the Fisher exact test or Wilcoxon rank sum test. The sensitivity, specificity, PPV, and NPV of various screening strategies were calculated using germline testing as the gold standard. Exact binomial confidence intervals were calculated with 95% confidence intervals (95% CIs) for estimation of proportions and the McNemar test was used to compare sensitivities and specificities. All analyses were performed using SAS statistical software (version 9.4), and statistical significance was set at \(P = 0.05\).

**RESULTS**

**Baseline Demographics**

In total, 278 consecutive patients were approached for the study, of whom 215 with nonserous and/or nonmucinous OCs provided consent (Fig. 1):\(^1\) 185 had OC alone (86.1%) and 30 had synchronous OC and EC (13.9%) (Table 1). The median age at the time of diagnosis was 53 years (range, 21-71 years). The most common histology was endometrioid (48.8%), followed by clear cell (40.9%). The majority of patients had stage I disease (66.5%). Women with MMRd and/or MSI-H OCs had more patients of synchronous OC and EC (37.9% vs 10.2%; \(P < 0.001\)) and endometrioid histology (65.5% vs 46.2%; \(P = 0.011\)) when compared with those with MMR-intact and/or MSS tumors.

Germline results were available for 189 of 215 patients (87.9%) (Table 1). There were 17 patients who declined germline testing and 9 patients without any blood or normal tissue available for testing. Overall, 13 of these 189 patients (6.9%) were found to have a pathogenic and/or likely pathogenic variant in 1 of the MMR
genes, including 7 with a pathogenic variant in MSH6 (3.7%), 3 with a pathogenic variant in MLH1 (1.6%), 2 with a pathogenic variant in PMS2 (1.1%), and 1 with a pathogenic variant in MSH2 (0.5%). Thirty-one of the 189 patients (16.4%) had a VUS in an MMR gene (see Supporting Table 3), whereas 145 patients (76.7%) had negative germline results.

Of the 215 patients with IHC results, 28 (13%) were MMRd, with 15 patients deficient in MLH1/PMS2 (53.6%), 7 patients deficient in MSH6 (25%), 5 patients deficient in MSH2/MSH6 (17.9%), and 1 patient deficient in PMS2 (3.6%) (Table 2). Of these 28 MMRd patients, 11 had pathogenic germline variants (39%) (Fig. 1). Of 215 patients, 162 MSI results were available (75.3%), 19 of which were MSI-H (11.7%); of these 19 MSI-H tumors (of which 18 patients also were found to be MMRd on IHC), 9 had pathogenic germline variants (47%) (Fig. 1). Overall, 29 of the 215 patients demonstrated MMRd and/or MSI-H (13.5%) with concordance of IHC and MSI, with the exception of 1 case that was MSI-H but MMR intact; of these 29 patients, 12 had a pathogenic germline variant (41%). For the assessment of family history, 163 patients had eFHQ results available (75.8%), with 17 having results that met OMOH family history criteria (10.4%), 9 that met SGO 20% to 25% criteria (5.5%), and 2 that met Amsterdam II clinical criteria (1.2%). Of the 17 eFHQ results that met OMOH family history criteria for genetic testing, 6 patients had germline mutations (35%).

**MMR Protein-Deficient Patients**

Of the 15 patients with tumors that were MLH1/PMS2 deficient, 2 had MLH1 pathogenic germline variants (13.3%) (Table 2). Of the 13 patients with either negative or VUS germline results in MLH1, 12 had somatic MLH1 promoter hypermethylation in their tumors and 1 case had biallelic somatic MLH1 copy number deletion. All 7 patients with MSH6 deficiency (100%) and 1 of 5 patients (20%) with MSH2/MSH6 deficiency had pathogenic germline variants (Table 2). Of 4 patients with negative germline results in MSH2, 1 patient had biallelic single-nucleotide variants in the tumor, whereas the other 3 patients declined tumor sequencing. One patient with PMS2 deficiency in the tumor had a confirmed pathogenic germline variant.
Comparison of Screening Strategies

The performance characteristics of the various screening strategies are presented in Table 3. Compared with other strategies, IHC with MLH1 promoter methylation plus MSI was found to be the most sensitive (92.3%; 95% CI, 64.0%-99.8% \(P\) = not significant) and specific (97.7%; 95% CI, 94.2%-99.4% \(P\) < .05), with a PPV of 75.0% (95% CI, 47.6%-92.7%) and a NPV of 99.4% (95% CI, 96.8%-99.9%), and missing 1 case of LS with a germline PMS2 pathogenic variant (study ID 8 in Table 4).24 IHC with MLH1 promoter methylation analysis also performed well, with a sensitivity of 84.6% (95% CI, 54.6%-98.1%) and a specificity of 97.7% (95% CI, 94.3%-99.4%), and missing 2 patients of LS. One case was study ID 8 and the other case was study ID 11 (Table 4) with a MLH1 c.306G>T pathogenic variant with an IHC-intact tumor. Family history assessment alone was found to have the lowest sensitivity at 54.5% (95% CI, 23.4%-83.3%).

Clinicopathologic Characteristics of Patients With LS

There were 13 LS carriers who were significantly younger than those without LS (aged 50 years vs 53 years).
years; \( P = .042 \) (Table 4). The subgroup of women with MMRd tumors without LS had a median age of 54 years (range, 43-62 years). The rate of LS was 17% in women with synchronous OC and EC (30 women). Women with LS were more likely to have a higher stage of disease (62% vs 31%; \( P = .042 \)). After excluding patients with synchronous OC and EC, women with LS still demonstrated a trend toward higher stage disease (2stage III: 40% vs 16%; \( P = .196 \)). Eleven women (84.6%) had a gynecologic malignancy as their sentinel cancers. Four women (30.8%) with LS did not meet Amsterdam II clinical criteria, SGO 20% to 25% criteria, or OMOH family history criteria for genetic assessment and were identified only through IHC or MSI testing.

**DISCUSSION**

The results of the current study established the incidence of LS as 7% in a prospective cohort of women with nonserous and/or nonmucinous OC. This rate is much higher than what has been reported in the literature (range, 3%-4%), although previous studies were limited by small numbers and their retrospective nature, as well as a lack of central pathology review.16 The rate of MMRd or MSI-H in the current study was 13% and 11.7%, respectively, and given a tumor demonstrating MMRd or MSI-H, the pretest probability of LS was 41%, which is higher than what is reported for EC and CRC.25,26 Prospective IHC studies have shown that approximately 25% of unselected ECs are MMRd, with 20% of MMRd patients testing positive for LS.25 Similarly, 15% to 20% of CRCs are MMRd, with 10% to 15% of all MMRd/MSI-H patients representing LS.26 Given the high pretest probability of LS in this cohort of patients with nonserous and/or nonmucinous OC, reflex tumor testing for MMR defects should be done routinely as standard of care. We advocate for screening with IHC with MLH1 promoter methylation analysis because this approach was found to be highly sensitive and specific and is likely to be easiest to implement in the clinical setting.

Various strategies to identify LS have been considered for EC and CRC, with no testing strategy proven to be perfect.7,27 For example, the current screening algorithm in patients with EC using IHC and MLH1 promoter methylation analysis leads to a complex cascade of additional tests for the confirmation of LS, and is time-consuming for patients and clinicians.6,7 Comprehensive NGS approaches may be simpler and more efficient, and are becoming the standard of care in other cancer types such as high-grade serous OC for BRCA1/2 mutations.28 Likewise, in patients with CRC, a recent study has established that upfront tumor sequencing with an NGS panel can replace the current sequential tests for LS29; based on this study, the National Comprehensive Cancer Network guidelines now list upfront tumor sequencing as a possible testing strategy in patients with CRC.29 In a similar manner, upfront tumor sequencing should be explored further in patients with EC and nonserous and/or nonmucinous OC because this approach will simplify the current cascade of tumor testing and provide tumor-specific information to open doors to new therapeutics such as pembrolizumab for patients with recurrent MMRd cancers after chemotherapy.30

With comprehensive tumor NGS panels on the horizon, the costs of the various screening approaches...
**TABLE 4.** Clinical Characteristics of Women with Mismatch Repair Germline Pathogenic Variants (Lynch Syndrome) and Newly Diagnosed Nonserous and/or Nonmucinous Ovarian Cancer

| Study ID | Age, Years | Germline Pathogenic Variant | MMR IHC | MSI Testing | Reason for Referral to Genetic Services | Met FH Criteria | History of LS Cancer | FIGO Stage |
|----------|------------|-----------------------------|---------|-------------|----------------------------------------|----------------|----------------------|------------|
| 1        | 38         | MSH6, c.3939_3957dup p.(Ala1320Serfs*5) | MSH6 deficient | MSS | IHC | None | None | Mixed ovary | IC         |
| 2        | 56.8       | PMS2, c.(803+1_804-1)_ (903+1_904-1)del | PMS2 deficient | MSI-H | IHC/MSI | None | None | Endometrioid grade 3 | IIIC       |
| 3        | 53.2       | MSH6, c.1304T>C | OC: MSH6 deficient | EC: MSH6 deficient | OC: MSH6 deficient | FH and IHC | SGO 20%-25% | None | OC: Clear cell | EC: Endometrioid grade 3 | IIIC       |
| 4        | 49.6       | MSH6, c.766_767delAG p.(Ser256?) | OC: MSH6 deficient | EC: MSH6 deficient | OC: MSH6 deficient | FH and IHC | SGO 20%-25% | None | OC: Undifferentiated | EC: Endometrioid grade 2 | II         |
| 5        | 48.2       | MSH6, c.508C>T | MSH6 deficient | MSI-H | IHC/MSI | Missing | None | Endometrioid grade 3 | IIB        |
| 6        | 52.3       | MSH6, c.400T+1G>C | MSH6 deficient | MSI-H | IHC/MSI | None | None | Endometrioid grade 2 | IIB        |
| 7        | 44.1       | MLH1, c.1731G>A p.Ser577Ser | OC: MLH1/PMS2 deficient | EC: MLH1/PMS2 deficient | EC: MLH1/PMS2 deficient | FH and IHC | AMSII | OC: Ampullary cancer | OC: IIA     |
| 8        | 56.3       | MLH1, c.137G>T p.Ser46Ile | OC: intact | EC: intact | OC: intact | Based on GC discretion | None | None | EC: Endometrioid grade 1 | OC: I1C     |
| 9        | 35         | MLH1, c.546-7_677+7del p.Tyr183_Arg226del | MLH1/PMS2 deficient | Missing | FH and IHC | SGO 20%-25% | None | Clear cell | IIA        |
| 10       | 33.6       | MSH6, c.161TdupT p.Tyr538Leufs*4 | MSH6 deficient | MSI-H | FH and IHC/MSI | SGO 20%-25% | None | Endometrioid grade 2 | IIA        |
| 11       | 44.4       | MLH1, c.306G>T r.? | Intact | MSI-H | MSI | Missing | Colon | Endometrioid grade 1 | IIA        |
| 12       | 52.0       | MSH6, c.308C>A p.Ser1028* | MSH6 deficient | Missing | IHC | None | None | Endometrioid grade 2 | IIB        |
| 13       | 56.3       | MSH6, c.3416dup p.Lys1140Glnfs*24 | OC: Equivocal | EC: MSH6 deficient | OC: MSH6 deficient | FH and IHC/MSI | AMSII | SGO 20%-25% | None | OC: Endometrioid grade 1 | OC: I3C     |

Abbreviations: AMSII, Amsterdam II clinical criteria; EC, endometrial cancer; FH, family history; FIGO, International Federation of Gynecology and Obstetrics; GC, genetic counsellor; IHC, immunohistochemistry; LS, Lynch syndrome; MLH1, mutL homolog 1; MMR, mismatch repair; MSH2, mutS homolog 2; MSH6, mutS homolog 6; MSI, microsatellite instability; MSH-H, microsatellite instability-high; MSS, microsatellite stable; OC, ovarian cancer; OMOH, Ontario Ministry of Health; PMS2, PMS1 homolog 2, mismatch repair system component; SGO, Society of Gynecologic Oncology.

Variant nomenclature follows Human Genome Variation Society (HGVS) format.²⁴
need to be taken into consideration. A recent microcosting study in patients with EC compared combinations of MSI, IHC, MLH1 methylation analysis, and NGS and found that initial tumor triage with IHC was the least expensive approach. In the CRC literature, it is well accepted that IHC is approximately 3-fold less expensive than MSI, with the added benefit of IHC being easier to operationalize, with superior sensitivity and the ability to direct germline testing for the affected MMR gene. In the current study, although the sequential approach of IHC (with reflex MLH1 promoter methylation analysis) plus MSI was found to have the best sensitivity, it detected only one additional case of LS when compared with IHC (with reflex MLH1 promoter methylation analysis). In the long term, tumor triage with IHC is likely to be the most cost-effective approach and will be easier to implement. Until the cost of tumor NGS panels decrease, tumor triage with IHC will need to be the standard of care in the majority of institutions.

Although small in number, the 13 patients with confirmed LS in the current study were similar in age to what has been reported in the literature (mean age, 47.6 years vs 45.3 years). A previous review examined the clinical characteristics of 747 women with LS-associated OC; the most frequent mutation identified was MSH2 (47%) and the most frequent histology was endometrioid and/or clear cell subtype. Unlike that review, the most common germline mutation in the cohort in the current study was found in MSH6, with all MSH6-deficient patients found to have a pathogenic variant. Previous studies have shown that the majority of patients with LS-associated OCs present at an early stage (>80% at stage I/II) with excellent survival outcomes, although the LS cohort in the current study had higher stages of disease with poor prognostic features.

One of the limitations of the current study was the incomplete information regarding MSI due to the lack of normal adjacent tissue on the formalin-fixed, paraffin-embedded slides. In the clinical setting, MSI testing would occur reflexively at the time of review of the hysterectomy specimen; therefore, pathologists would have access to normal tissue to proceed with MSI testing. Another limitation was the incomplete germline information; given that all 26 patients with missing germline information had MSS-intact and/or MMR-intact tumors, our estimate of the incidence of LS in the current study cohort may be slightly higher than the true incidence. Furthermore, given that the study institutions were specialized cancer centers, there may have been inherent referral bias. However, all gynecologic cancer care in Ontario is regionalized, and the current study is reflective of the real-world scenario. Furthermore, for the purpose of the current analysis, patients found to have focal MMRd on IHC (4 patients) were considered to be MMRd, and 1 case with an equivocal MMR result was considered to be MMR intact. There is evidence that patients with focal MMR deficiency and/or heterogenous loss of MMR protein expression harbor unique molecular aberrations, and these patients should be tested further for germline mutations.

The results of the current study demonstrated that sequential IHC (with reflexive MLH1 promoter methylation analysis) plus MSI is the most sensitive and specific screening strategy with which to identify LS in women with nonserous and/or nonmucinous OC. Considering the cost of real-world implementation, IHC with reflexive MLH1 promoter methylation analysis can be a suitable strategy with excellent performance characteristics. Strong consideration should be given to making reflex tumor testing the standard of care for all patients who are newly diagnosed with nonserous and/or nonmucinous OC, with all MMRd and/or nonmethylated patients undergoing confirmatory testing for LS.

FUNDING SUPPORT
Supported by funding from the Canadian Cancer Society (grant 704038).

CONFLICT OF INTEREST DISCLOSURES
The authors made no disclosures.

AUTHOR CONTRIBUTIONS
Soyoun Rachel Kim: Data curation, formal analysis, project administration, visualization, writing—original draft, and writing—review and editing. Alicia Tone: Conceptualization, data curation, investigation, methodology, and writing—review and editing. Raymond H. Kim: Investigation and writing—review and editing. Matthew Cesari: Investigation and writing—review and editing. Blaise A. Clarke: Investigation and writing—review and editing. Lua Eiriksson: Investigation, resources, and writing—review and editing. Tae Hart: Resources and writing—review and editing. Melyssa Aronson: Resources and writing—review and editing. Spring Holter: Resources and writing—review and editing. Alice Lytwyn: Investigation and writing—review and editing. Manjula Maganty: Formal analysis and writing—review and editing. Leslie Oldfield: Formal analysis and writing—review and editing. Steven Gallinger: Conceptualization and writing—review and editing. Marcus Q. Bernardini: Conceptualization and writing—review and editing. Amit M. Oza: Conceptualization and writing—review and editing. Bojana Djordjevic: Writing—review and editing. Jordan Lerner-Ellis: Investigation and writing—review and editing. Emily Van de Laar: Data curation, project administration, and writing—review and editing. Danielle Vicus: Investigation, resources, and writing—review and editing. Trevor J. Pugh: Formal analysis, investigation, and writing—review and editing. Aaron Pollett: Investigation and writing—review and editing. Sarah E. Ferguson: Conceptualization, formal analysis, funding acquisition, project administration, investigation, methodology, supervision, and writing—review and editing.
REFERENCES

1. Lynch HT, Lynch PM, Lanspa SJ, Snyder CL, Lynch JF, Boland CR. Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. Clin Genet. 2000;76:1-18.

2. Barrow E, Hill J, Evans DG. Cancer risk in Lynch syndrome. Fam Cancer. 2013;12:229-240.

3. Bonadona V, Bonaiti B, Olschwang S, et al. Cancer risks associated with germine mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. JAMA. 2011;305:2304-2310.

4. Helder-Woolderink JM, Blok EA, Vasen HF, Hollema H, Mourits MJ, De Boek GH. Ovarian cancer in Lynch syndrome: a systematic review. Eur J Cancer. 2016;55:65-73.

5. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. Genet Med. 2009;11:35-41.

6. Pollett A, Brown J, Aronson M, Clark B, Baxter E, Tomiak E. Molecular Oncology Advisory Committee. Screening for Lynch Syndrome by Immunohistochemistry, BRAF Mutations Analysis, and MLH1 Promoter Methylation Analysis for Patients in Ontario with Colorectal or Endometrial Cancers. Toronto (ON): Cancer Care Ontario; 2015 September 28. Program in Evidence-Based Care Evidence Summary MOAC-3, available on the CCO website.

7. National Comprehensive Cancer Network. Genetic/familial high-risk assessment: colorectal (Version 1. 2020). Accessed May 10, 2020. https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf

8. Li GM. Mechanisms and functions of DNA mismatch repair. Cell Res. 2008;18:85-98.

9. Bruegl AS, Djordjevic B, Urbauer DL, et al. Utility of MLH1 methylation analysis in the clinical evaluation of Lynch Syndrome in women with endometrial cancer. Curr Pharm Des. 2014;20:1655-1663.

10. Ferguson SE, Aronson M, Pollett A, et al. Performance characteristics of screening strategies for Lynch syndrome in unselected women with newly diagnosed endometrial cancer who have undergone universal germline mutation testing. Cancer. 2014;120:3932-3939.

11. Wang M, AlDubayan S, Connor AA, et al. Genetic testing for Lynch syndrome in the province of Ontario. Cancer. 2016;122:1672-1679.

12. Ryan NAJ, Evans DG, Green K, Crosbie EJ. Pathological features and clinical behavior of Lynch syndrome–associated ovarian cancer. Gynecol Oncol. 2017;144:491-495.

13. Xiao X, Melton DW, Gourley C. Mismatch repair deficiency in ovarian cancer—molecular characteristics and clinical implications. Gynecol Oncol. 2014;132:506-512.

14. Murphy MA, Wentszens N. Frequency of mismatch repair deficiency in ovarian cancer: a systematic review. Int J Cancer. 2011;129:1914-1922.

15. Lu FJ, Gilks CB, Mulligan AM, et al. Prevalence of loss of expression of DNA mismatch repair proteins in primary epithelial ovarian tumors. Int J Gynecol Pathol. 2012;31:524-531.

16. Vierkoefter KR, Ayabe AR, VanDrunen M, Ahn HJ, Shimizu DM, Terada KY. Lynch syndrome in patients with clear cell and endometrioid cancers of the ovary. Gynecol Oncol. 2014;135:81-84.

17. Eriksson L, Aronson M, Clarke B, et al. Performance characteristics of a brief Family History Questionnaire to screen for Lynch syndrome in women with newly diagnosed endometrial cancer. Gynecol Oncol. 2015;136:311-316.

18. Lancaster JM, Powell CB, Chen LM, Richardson DL; SGO Clinical Practice Committee. Society of Gynecologic Oncology statement on risk assessment for inherited gynecologic cancer predispositions (published correction appears in Gynecol Oncol. 2015;138:765). Gynecol Oncol. 2015;136:3-7.

19. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res. 1998;58:5248-5257.

20. McCarthy AJ, Cape-Chichi JM, Spence T, et al. Heterogenous loss of mismatch repair (MMR) protein expression: a challenge for immunohistochemical interpretation and microsatellite instability (MSI) evaluation. J Pathol Clin Res. 2015;5:115-129.

21. Oldfield L, Li T, Danesh A, et al. An integrative panel to accurately detect Lynch Syndrome and somatic mismatch repair deficiency. Presented at the American College of Medical Genetics and Genomics Annual Clinical Genetics Meeting; March 21-March 25, 2017; Phoenix, AZ.

22. Oldfield L, Li T, Tone A, et al. An integrative DNA sequencing and methylation panel to assess mismatch repair deficiency. Genet Med (In press). 2020.

23. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-424.

24. den Dunnen JT, Dalgleish R, Maglott DR, et al. HGVS recommendations for the description of sequence variants: 2016 update. Hum Mutat. 2016;37:564-569.

25. Buchanan DD, Tan YY, Walsh MD, et al. Tumor mismatch repair immunohistochemistry and DNA MLH1 methylation testing of patients with endometrial cancer diagnosed at age younger than 60 years optimizes triage for population-level germline mismatch repair gene mutation testing. J Clin Oncol. 2014;32:90-100.

26. Kawakami H, Zaanan A, Sincrope FA. Microsatellite instability testing and its role in the management of colorectal cancer. Curr Treat Options Oncol. 2015;16:30.

27. Hogle M, Ferber M, Mao R, et al. ACMG technical standards and guidelines for genetic testing for inherited colorectal cancer (Lynch syndrome, familial adenomatous polyposis, and MYH-associated polyposis). Genet Med. 2014;16:101-116.

28. McCuaig JM, Stockley TL, Shaw P, et al. Evolution of genetic assessment for BRCA-associated gynecologic malignancies: a Canadian multisociety roadmap. J Med Genet. 2018;55:571-577.

29. Hampel H, Pearlman R, Beigtol M, et al. Assessment of tumor sequencing as a replacement for Lynch syndrome screening and current molecular tests for patients with colorectal cancer [published correction appears in JAMA Oncol. 2018;4:891]. JAMA Oncol. 2018;4:806-813.

30. US Food and Drug Administration. FDA approves first cancer treatment for any solid tumor with a specific genetic feature. Accessed April 2, 2020. https://www.fda.gov/news-events/press-announcements/fda-approves-first-cancer-treatment-any-solid-tumor-specific-genetic-feature

31. Ryan NAJ, Davison NJ, Payne K, Cole A, Evans DG, Crosbie EJ. A micro-costing study of screening for Lynch syndrome-associated pathogenic variants in an unselected endometrial cancer population: cheap as NGS chips? Front Oncol. 2019;9:81.

32. Debnath T, Kurzawski G, Gorski B, Kladny J, Domagala W, Lubinski J. Value of pedigree/clincial data, immunohistochemistry and microsatellite instability analyses in reducing the cost of determining hMLH1 and hMSH2 gene mutations in patients with colorectal cancer. Eur J Cancer. 2000;36:49-54.

33. Woolderink JM, De Boek GH, de Hullu JA, et al. Characteristics of Lynch syndrome associated ovarian cancer. Gynecol Oncol. 2018;150:324-330.

34. Forbes L, Durocher-Allen LD, Vu K, et al. Regional models of care for systemic treatment: standards for the organization and delivery of systemic treatment. Toronto (ON): Ontario Cancer Care; 2019 July 5. Program in Evidence-Based Care Guideline No.: 12-10 Version 2.