Testing for Lynch syndrome in people with endometrial cancer
Clinical guidance document

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Contents

1. Foreword

The aim of this document is to provide clinical and pathology staff with guidance on testing for Lynch syndrome in people with endometrial cancer.

2. Background

2.1 Lynch syndrome and endometrial cancer

Lynch syndrome is an inherited cancer susceptibility syndrome with an *autosomal dominant* mode of inheritance. It increases the risk of certain types of cancer, including colorectal cancer, endometrial cancer and certain types of ovarian cancer (endometrioid carcinoma and clear cell carcinoma). It is usually due to a *germline pathogenic gene variant* involving one of the mismatch repair (MMR) genes. If a person has Lynch syndrome, the pathogenic gene variant is present in every cell of their body. Endometrial cancer is often the first cancer that people with Lynch syndrome will have. Approximately 3% of patients with endometrial cancer have Lynch syndrome.

Identifying Lynch syndrome at the point of endometrial cancer diagnosis could allow patient surveillance, risk-reducing interventions (e.g. surgery) and early detection of Lynch syndrome-associated cancers in affected individuals and their family members. It should be noted that family history alone has a poor predictive value for Lynch syndrome in people with endometrial cancer. Lynch syndrome due to de novo germline pathogenic gene variants is well described. Endometrial cancers arising in the lower uterine segment are rare in the general population, but are more common in people with Lynch syndrome. However, the histological features of endometrial cancers in people with Lynch syndrome are non-specific. Furthermore, *sporadic cancers* can also occur in people with Lynch syndrome. It is therefore important to perform tumour testing to better identify potential cases of Lynch syndrome rather than relying on the presence of physical traits or observable characteristics (phenotype).

2.2 Pathogenesis of Lynch syndrome

MMR proteins form complexes which detect and correct mistakes made during DNA replication. There are four main MMR genes/proteins which we can assess (MLH1, PMS2, MSH2 and MSH6). Every cell contains two copies of each of these MMR genes. If all four MMR proteins show normal expression/function, a tumour is said to be mismatch repair proficient (MMRp). MMR function is lost when both copies of an MMR gene are inactivated, which may be due to a pathogenic gene variant (*somatic* or germline) or *promoter methylation*, which usually affects the MLH1 gene. If there is a loss of MMR protein expression/function, a tumour is said to be mismatch repair deficient (MMRd). In the presence of an MMR defect, mismatches can occur anywhere in the DNA, but they are prone to occur in repetitive stretches of DNA called microsatellites, resulting in a state described as microsatellite instability (MSI). In the presence of a defective MMR system, the cells will become prone to acquiring large numbers of mutations which go uncorrected and can cause cancer to develop.

Identification of an MMRd tumour does not necessarily mean that the patient has Lynch syndrome (although they may do). 25-30% of endometrial cancers have an MMR deficiency, but only 3% of people with endometrial cancer have Lynch syndrome. Most MMRd

endometrial cancers are the result of sporadic, somatic, hypermethylation of the promoter region of the MLH1 gene, which results in silencing of MLH1 gene transcription. This would result in loss of MLH1 protein expression. If MLH1 promoter methylation is present, the tumour is most likely to be sporadic (rather than Lynch syndrome associated). An exception to this is constitutional MLH1 promoter methylation, where every cell in an affected person's body shows MLH1 promoter methylation. Constitutional MLH1 promoter methylation also increases the risk of developing MMRd tumours. A small proportion of cases of constitutional MLH1 promoter methylation are inherited.

In some sporadic endometrial cancers, MMR deficiency results from two, somatic, MMR pathogenic gene variants or rarely through other genetic mechanisms. Both copies of an MMR gene have to be non-functioning in order to lose expression of that particular MMR protein. In people with Lynch syndrome there is usually a germline pathogenic gene variant involving one copy of an MMR gene. A separate, somatic, pathogenic gene variant affecting the other copy of the MMR gene can then lead to loss of expression of that MMR protein and the development of an MMRd cancer.

2.3 Testing for Lynch syndrome

Several types of tests can be done in different orders and combinations to see if a cancer is likely to have been caused by Lynch syndrome. The initial screening investigation is usually either microsatellite instability (MSI) testing (which is performed in a Medical Genetics laboratory) or MMR immunohistochemistry (IHC) to see whether or not each of the MMR proteins is expressed by the tumour cells (which is performed in a Cellular Pathology laboratory). Further details on these testing strategies are given below.

MMR IHC can be performed for all four MMR proteins (MLH1, PMS2, MSH2 and MSH6) upfront or by initially testing for two MMR proteins (PMS2 and MSH6), with MLH1 and MSH2 testing only being performed if PMS2 or MSH6 show an abnormality. This is no specific recommendation as to which of these approaches should be used and it is really down to departmental preference.

If a tumour sample shows either microsatellite instability or loss of expression of MLH1 on IHC then MLH1 promoter methylation testing (and BRAF mutation testing in the case of colorectal cancer) would be performed next to identify if the tumour is likely to be either a sporadic case of cancer or a tumour which is associated with Lynch syndrome.

As MSI testing, MMR IHC and MLH1 promoter methylation testing are performed on tumour samples, patient consent is not required. These tests are used to screen for patients who are more likely to have Lynch syndrome. If a patient is felt to be at risk of having Lynch syndrome based on these tests, then further genetic testing of non-tumour DNA (germline testing) to make a definite diagnosis of Lynch syndrome is needed; this does require patient consent following adequate support, information and counselling.

2.4 NICE recommendation

The National Institute for Health and Care Excellence (NICE) published "Testing strategies for Lynch syndrome in people with endometrial cancer" in October 2020[1]. The NICE recommendation is that testing for Lynch syndrome should be offered to all people who are

diagnosed with endometrial cancer and that MMR IHC should be used as the first line investigation to identify MMRd tumours. This should be followed by MLH1 promoter methylation testing in cases which show loss of staining for MLH1 (which is usually accompanied by loss of staining for PMS2). MMR IHC may detect more people with Lynch syndrome, as MSI testing may miss tumours with pathogenic variants involving the MSH6 gene. There is a relatively high prevalence of MSH6 mutations in Lynch syndrome associated endometrial cancer. IHC has the advantages of being cheaper and quicker than MSI testing. It permits correlation with the morphology and also shows which MMR gene is likely to contain a pathogenic gene variant.

3. Pathology

3.1 Tissue selection and sample preparation

MMR IHC should be performed on all endometrial biopsies (and other samples such as curettings and polypectomy specimens) which contain endometrial carcinoma (all subtypes of endometrial carcinoma, including carcinosarcoma). MMR IHC should not be performed when the changes amount to atypical hyperplasia alone. However, if the endometrial sample is considered to show at least atypical hyperplasia with changes suspicious for endometrial carcinoma, then it may be appropriate to perform MMR IHC. This will need to be decided on an individual basis, based on the degree of suspicion and whether or not further endometrial sampling is going to be attempted.

Good fixation is important for obtaining reliable and reproducible patterns of MMR expression by IHC. For this reason, MMR IHC should be performed on endometrial biopsies, wherever possible, rather than on hysterectomy specimens. In some cases, MMR IHC may have to be performed on a hysterectomy specimen, such as when endometrial carcinoma is found incidentally in a hysterectomy specimen. Hysterectomy specimens for endometrial cancer should be sliced and put into formalin as soon as possible following surgery. MMR IHC should be reported only in the presence of positive internal control cells, such as stromal cells or lymphoid cells, that are present immediately adjacent to the tumour cells being assessed.

If MMR IHC has been performed on an endometrial biopsy (with conclusive results) it should not be repeated on the subsequent hysterectomy specimen.

3.2 Interpretation and reporting of MMR IHC

For a more detailed description please refer to the British Association of Gynaecological Pathologists (BAGP) document: Interpretation and Reporting Terminology for Mismatch Repair Protein Immunohistochemistry in Endometrial Cancer[2]. For a summary of the recommended terminology for reporting MMR IHC +/- MLH1 promoter methylation results, see appendix 1.

In the presence of an MMR deficiency, there is loss of staining for one or more of the MMR proteins. The four mismatch repair proteins occur as heterodimers, which means that MLH1 pairs with PMS2 and MSH2 pairs with MSH6. MLH1 and MSH2 can stabilise in the cell by forming heterodimers with other proteins, whilst PMS2 and MSH6 can only exist stably in the cell in the presence of MLH1 and MSH2 respectively. Loss of the “dominant” partner (either

MLH1 or MSH2) leads to relative instability and loss of the other partner (PMS2 or MSH6 respectively). Loss of MLH1 expression results in loss of staining for MLH1 and PMS2, whilst loss of PMS2 expression results in loss of staining for PMS2 only. Loss of MSH2 expression results in loss of staining for MSH2 and MSH6, whilst loss of MSH6 expression results in loss of staining for MSH6 only.

3.3 Retained staining for all of the MMR proteins

If the tumour cell nuclei show retained staining for all of the MMR proteins tested, the case can be reported as showing no immunohistochemical evidence of a mismatch repair deficiency. It is unlikely that the patient's endometrial cancer is due to Lynch syndrome. However, if the person has a personal or family history which is suggestive of Lynch syndrome, then they should be referred to Clinical Genetics, as MMR IHC may miss some cases of Lynch syndrome.

3.4 Loss of staining for MLH1 and PMS2 (or MLH1 alone)

If the tumour cell nuclei show loss of staining for MLH1 and PMS2 (or MLH1 alone), this indicates a mismatch repair deficiency due to an MLH1 abnormality (MLH1 promoter methylation or an MLH1 pathogenic gene variant, which could be either somatic or germline). This would be reported by the pathologist (in the pathology report on the endometrial biopsy) as showing a mismatch repair deficiency which may be either sporadic or due to Lynch or related syndromes. The pathologist needs to request MLH1 promoter methylation testing to clarify the cause of the MLH1 abnormality. The pathologist must complete the appropriate request form and send it with tissue sections to the All Wales Medical Genetics Laboratory (Cardiff), where this investigation is undertaken. The MLH1 promoter methylation testing request form (which can be obtained using the following link) states the tissue requirements for this investigation:
LF-GEN-BRAFMLH1FUForm.pdf (medicalgenomicswales.co.uk)

3.5 Results of MLH1 promoter methylation testing

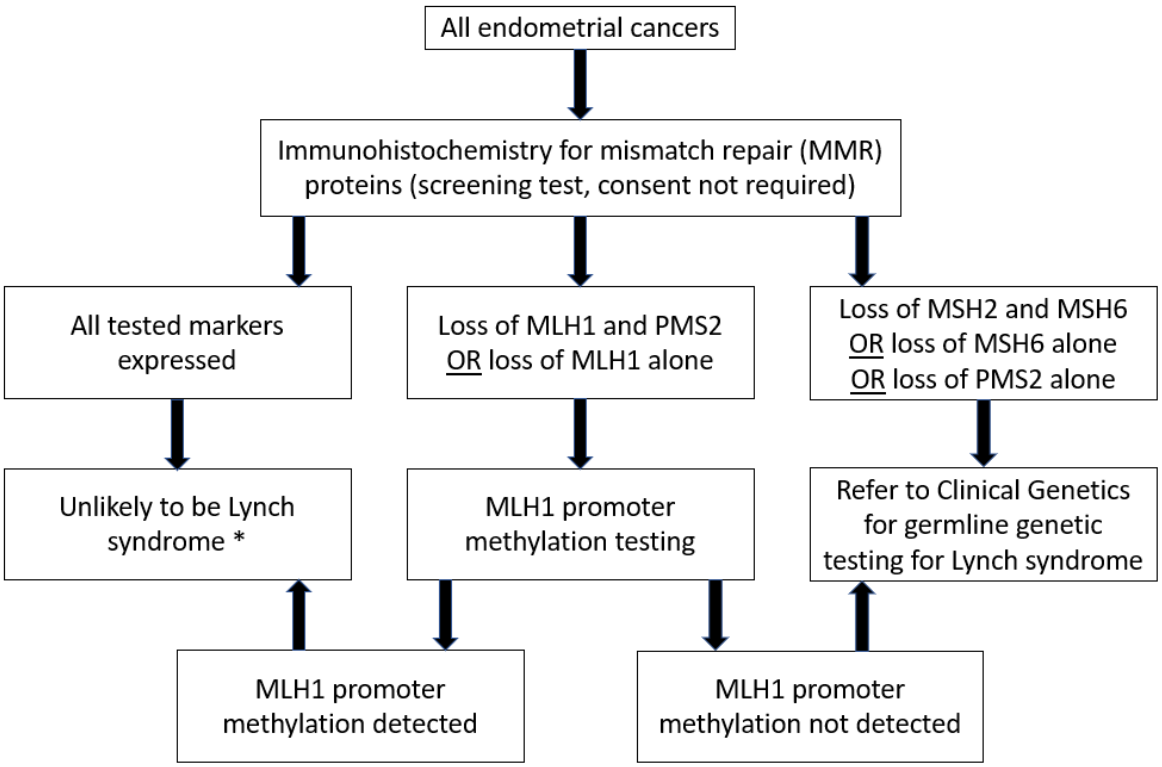
The results of MLH1 promoter methylation testing are provided in a report, which is issued by the All Wales Medical Genetics Laboratory. This report is uploaded to the Welsh Clinical Portal (WCP) and is also sent by e-mail and paper copy to the requesting pathologist. Upon receipt of the sample, the All Wales Genetics Laboratory aims to complete MLH1 promoter methylation testing in 14 calendar days. These results should be integrated into the original pathology report (as a supplementary report). The pathologist should document the significance of the results and state if the patient should be referred to the Clinical Genetics service. If MLH1 promoter methylation is absent, the report would state that whilst this mismatch repair deficiency could be sporadic, it is probable that the mismatch repair deficiency is due to Lynch or related syndromes and the patient should be referred to Clinical Genetics. If MLH1 promoter methylation is present, then the tumour is most likely to be sporadic, rather than Lynch syndrome associated. However, tumours with MLH1 promoter methylation are occasionally seen in patients with Lynch syndrome due to constitutional MLH1 promoter methylation, which in some cases can be inherited. If the person has a personal or family history which is suggestive of Lynch syndrome, then they should be referred to Clinical Genetics.

3.6 Other patterns of MMR protein loss

If the tumour cells show loss of staining for MSH2 and MSH6 OR loss of MSH6 alone OR loss of PMS2 alone, the pathologist would state (in the pathology report on the endometrial biopsy) that this mismatch repair deficiency is associated with Lynch and related syndromes and the patient should be referred to Clinical Genetics.

Subclonal loss may occur in a minority of cases. This is defined as a focal loss of expression; in order to identify this and distinguish it from variable expression as a result of a fixation artefact, normal staining must be seen in the internal control in the area showing loss of expression in tumour cells. An arbitrary cut-off of 10% is suggested to avoid reporting this pattern in cases where it is extremely focal and of unlikely clinical significance. For more information, please see reference 2.

3.7 Pathology pathway



(*) Unlikely to be Lynch syndrome, but if there is a personal or family history which is suggestive of Lynch syndrome, the patient should be referred to Clinical Genetics for further investigation.

4.Clinical Pathway

Whenever a new diagnosis of endometrial cancer is made on a tissue specimen (all subtypes of carcinoma, including carcinosarcoma), the reporting pathologist will request MMR IHC, followed by MLH1 promoter methylation testing, in cases where there is loss of staining for MLH1 (which is usually accompanied by loss of staining for PMS2). The MMR IHC will be reported by the pathologist using terminology recommended by the BAGP[2]. The pathology report will be discussed at the local gynae-oncology MDT meeting, where the results of MMR

investigations should be documented. The patient may also be referred for discussion at the regional gynae-oncology MDT meeting; the results of MMR investigations should be documented at both the local and regional MDT meetings (if referred). The results of MMR investigations should be available for the patient's first MDT discussion in most cases. However, it is possible that the results might not be available, particularly if MLH1 promoter methylation testing is being undertaken; this should be documented in the MDT notes. Each MDT must have a process in place to ensure outstanding MMR investigations/MLH1 promoter methylation testing results are reviewed and documented, with onward referral to Clinical Genetics if required.

Most patients will undergo hysterectomy for their endometrial cancer, although a small proportion of patients will have conservative management or radiotherapy. MMR IHC can be performed on a hysterectomy specimen in cases where there is no pre-operative biopsy, or there is insufficient material in the biopsy, or where the changes in the pre-operative biopsy were not considered to be diagnostic of endometrial carcinoma.

Ideally, the pathology report on the hysterectomy specimen should note the results of MMR testing performed on the previous endometrial sample and provide a reference to the previous pathology specimen number. This is particularly useful where the endometrial samples and hysterectomy specimens are reported in different hospitals.

If MMR testing shows a mismatch repair deficiency which is associated with Lynch and related syndromes, the patient should be referred to Clinical Genetics for germline testing for Lynch syndrome. The referral to Clinical Genetics (All Wales Medical Genomics Service) should be made by the operating surgeon / gynae-oncologist responsible for the care of the patient (see Referral to Clinical Genetics section below).

If there is retained IHC staining for all of the MMR proteins tested, the endometrial cancer is most likely to be sporadic (not associated with Lynch or related syndromes). However, if the patient has a personal or family history which is suggestive of Lynch syndrome, they should be referred to Clinical Genetics, as MMR IHC is a screening investigation which may miss some cases of Lynch syndrome.

If the tumour shows loss of staining for MLH1 (±PMS2) AND MLH1 promoter methylation IS DETECTED, the endometrial cancer is most likely to be sporadic (not associated with Lynch or related syndromes). However, if the patient has a personal or family history which is suggestive of Lynch syndrome, the patient should be referred to the All Wales Medical Genomics Service (AWMGS) to investigate the possibility of constitutional MLH1 promoter methylation, which is a rare cause of Lynch syndrome. Testing of a germline DNA sample would be required to see if this is constitutional MLH1 promoter methylation

5. Referral to Clinical Genetics

Patients should be referred to Clinical Genetics (AWMGS) using the following referral form found under Endometrial Cancer Referrals at AWMGS - ARE YOU REFERRING A PATIENT? (medicalgenomicswales.co.uk).

Patients should be provided with an information sheet, explaining why they are being referred to Clinical Genetics found under Endometrial Cancer Referrals at AWMGS - ARE YOU REFERRING A PATIENT? (medicalgenomicswales.co.uk) .

Please refer to the AWMGS referral guidelines, when deciding if a patient should be referred to Clinical Genetics based on their personal or family history, even if there is no IHC evidence of an MMR deficiency.

5.1 For patients with a personal history of cancer:

https://medicalgenomicswales.co.uk/images//refer-a-patient-forms/Ref_Guidelines_affected_v10.pdf

5.2 For patients with a family history of cancer:

https://medicalgenomicswales.co.uk/images//refer-a-patient-forms/Ref_Guidelines_fh_1_v10.pdf

5.3 For patients where MLH1 promoter methylation is detected in the tumour:

If there is loss of MLH1 staining (±PMS2) on IHC and MLH1 promoter methylation is detected, the following patients should be referred to Clinical Genetics to be tested for constitutional MLH1 promoter methylation:

1. Any woman <50 years of age, diagnosed with an endometrial cancer which shows MLH1 promoter methylation.

2. A woman of any age with MLH1 promoter methylation in their endometrial tumour, who also has a first or second degree relative with bowel or endometrial cancer at any age.

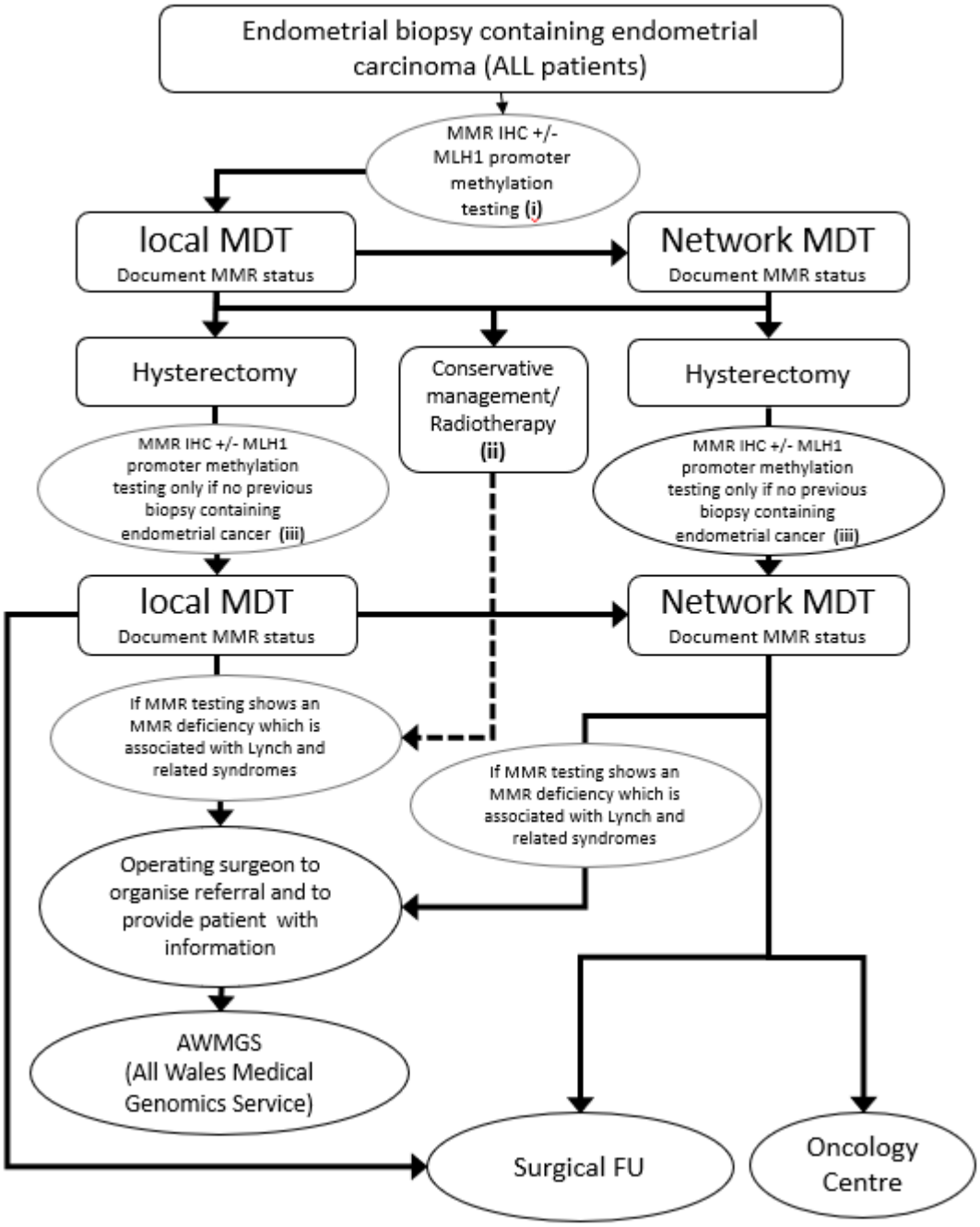
3. A woman of any age with MLH1 promoter methylation in their tumour, who has had both bowel and endometrial cancer.

Genetic counselling and informed consent are required for germline genetic testing for Lynch syndrome (including investigation of possible constitutional MLH1 promoter methylation). This will be undertaken by Clinical Genetics (AWMGS) and not by the referring gynaecologist.

6. Lynch Syndrome Testing in Ovarian Cancer

It is considered to be good practice to perform MMR IHC +/- MLH1 promoter methylation testing on endometrioid and clear cell carcinomas of the ovary. However, this is not currently a NICE recommendation.

7. Summary of Clinical Pathway



- Majority of mismatch repair (MMR) testing will take place at this point in the pathway.
- A small proportion of patients will have conservative management/radiotherapy with no further biopsies.
- A small proportion of cases will undergo MMR testing following hysterectomy (e.g. no previous endometrial biopsy or no/insufficient endometrial cancer in biopsy to permit MMR testing. MMR IHC is best done on optimally fixed biopsies

References

1. Testing strategies for Lynch syndrome in people with endometrial cancer. Diagnostic guidance. Published: 28 October 2020. www.nice.org.uk/guidance/dg42

2. Interpretation and reporting terminology for mismatch repair protein immunohistochemistry in endometrial cancer. https://www.thebagp.org/resources/?wpdmc=bagp-guidance-documents

Glossary

Autosomal dominant: One of the ways in which a genetic trait or condition can be inherited. In autosomal dominant inheritance, a genetic condition occurs when a pathogenic variant is present in only one allele (copy) of a given gene.

Germline pathogenic gene variant: A gene change in a body's germ cell (egg or sperm) that becomes incorporated into the DNA of every cell in the body of the offspring. These pathogenic gene variants are passed from parents to offspring.

Lynch-like syndrome: Where a person has an MMR deficient cancer and a personal or family history which is suggestive of Lynch syndrome, but where no germline pathogenic variants are found in one of the MMR genes.

Promoter methylation: The binding of methyl groups to the promoter region of a gene, which suppresses transcription of that gene and results in gene silencing (i.e. it switches the gene off).

Somatic pathogenic gene variant: Alterations in DNA which occur in any of the cells of the body except germ cells (egg or sperm) and are therefore not passed from parents to offspring.

Sporadic cancers: Cancers which are not due to inherited pathogenic gene variants.

Appendix 1

Summary of recommended terminology for reporting MMR IHC +/- MLH1 promoter methylation results[2].

MMR Result	Conclusion
MLH1: retention PMS2: retention MSH2: retention MSH6: retention	MMR IHC normal: There is no immunohistochemical evidence of a mismatch repair deficiency. Referral to Clinical Genetics is not indicated unless the patient has a personal or family history which is suggestive of Lynch syndrome.
MLH1: retention PMS2: retention MSH2: retention MSH6: loss	MMR IHC abnormal, MSH6 loss: This mismatch repair deficiency is associated with Lynch and related syndromes. This patient should be referred to Clinical Genetics.
MLH1: retention PMS2: loss MSH2: retention MSH6: retention	MMR IHC abnormal, PMS2 loss: This mismatch repair deficiency is associated with Lynch and related syndromes. This patient should be referred to Clinical Genetics.
MLH1: retention PMS2: retention MSH2: loss MSH6: loss	MMR IHC abnormal, MSH2 and MSH6 loss: This mismatch repair deficiency is associated with Lynch and related syndromes. This patient should be referred to Clinical Genetics.
MLH1: loss PMS2: loss MSH2: retention MSH6: retention	MMR IHC abnormal, MLH1 and PMS2 loss: This pattern of mismatch repair deficiency may be either sporadic or due to Lynch or related syndromes. The result of MLH1 promoter methylation testing will provide further information. A supplementary report will be issued when the results are available. IF MLH1 PROMOTER METHYLATION IS ABSENT While this mismatch repair deficiency could be sporadic, it is probable that this mismatch repair deficiency is due to Lynch or related syndromes. This patient should be referred to Clinical Genetics.

IF MLH1 PROMOTER METHYLATION IS PRESENT

The results indicate that this mismatch repair deficiency is almost certainly sporadic rather than due to Lynch syndrome. Referral to Clinical Genetics is not indicated unless the patient has a personal or family history which is suggestive of Lynch syndrome.

MLH1: subclonal loss PMS2: subclonal loss MSH2: retention MSH6: retention	MMR IHC abnormal, subclonal loss of MLH1 and PMS2: This pattern is likely to be sporadic, although it is possible that this mismatch repair deficiency is due to Lynch or related syndromes. Testing for MLH1 promoter methylation is recommended.
MLH1: loss PMS2: loss MSH2: retention MSH6: subclonal loss	MMR IHC abnormal, MLH1 and PMS2 loss with subclonal loss of MSH6: Report as for other cases of MLH1 loss.
MLH1: retention PMS2: retention MSH2: retention MSH6: subclonal loss	MMR IHC abnormal, subclonal loss of MSH6: This mismatch repair deficiency may be associated with Lynch and related syndromes. This patient should be referred to Clinical Genetics.