Interpretation and Reporting Terminology for Mismatch Repair Protein Immunohistochemistry in Endometrial Cancer

Authors: Naveena Singh1, Richard Wong2, Nairi Tchrakian1, Shara-Gaye Allen3, Blaise Clarke3, C Blake Gilks4
1Barts Health NHS Trust, London, UK; 2Pamela Youde Nethersole Eastern Hospital, Hong Kong; 3University Hospital Network, Toronto, Canada; 4Vancouver General Hospital, Vancouver, Canada

Background

Biology of mismatch repair1-3

- Before a cell divides it needs to replicate its DNA, producing an identical copy for each daughter cell.
- Accurate DNA replication is essential for preservation of the genome, and three highly conserved biological processes govern its accuracy:
  i. accurate selection of the correct DNA base at each position, carried out by the polymerase portion of DNA polymerases (ε and δ)
  ii. proof-reading of the newly synthesized strand, carried out by the exonuclease domains of DNA polymerases (ε and δ)
  iii. post-replication correction of DNA mismatches, carried out by the mismatch repair (MMR) system
- These DNA mismatches are of two types: base-base mismatches and insertion-deletion (indel) errors at repetitive sequences.
- Segments of repetitive bases within the DNA strand are known as short tandem repeats or microsatellites; these are present throughout the genome and indel errors in such strands are particularly resistant to detection by the proof-reading function of DNA polymerases.
- The mismatch repair proteins, MLH1, PMS2, MSH2 and MSH6, recognise mismatches and target them for excision, accurate resynthesis and ligation.
- In the presence of a defective mismatch repair system, the cell becomes prone to acquiring large numbers of mutations and the development of cancer.
- Although all mismatches accumulate in the presence of a mismatch repair defect (MMRd), microsatellites are particularly susceptible to errors, resulting in a state described as microsatellite instability (MSI), or the widespread occurrence of indel errors in microsatellites.
- MSI is thus the result of MMRd, and the 2 terms are often used interchangeably.
Mismatch repair defects in endometrial carcinoma

- About 25-30% of endometrial carcinomas (EC) are characterized by defective MMR.
- This occurs sporadically within the endometrium in the majority of cases, most commonly as a result of methylation of the MLH1 promoter region and resultant epigenetic silencing of MLH1.
- A minority of MMRd EC (accounting for 3-5% of all EC) occur as a result of Lynch Syndrome (LS), a genetically inherited cancer susceptibility syndrome.
- In these cases, the patient inherits one defective allele from a parent and loss of function of the second allele occurs in the target tissue, most commonly colorectal or endometrial, resulting in cancer at that site.

Testing for a mismatch repair defect

- Detection of MMRd can be carried out through immunohistochemistry (IHC) for the MMR proteins or through MSI testing.
- The two methods have comparable sensitivity and show approximately 96% concordance.
- MMR IHC simply detects the presence or absence of the 4 MMR proteins within cancer cells.
- MMR IHC has the advantages of being cheaper, easily accessible to pathologists, amenable to IHC external quality assurance schemes, allowing correlation with morphology and enabling identification of the defective protein, thereby guiding downstream testing.
- MSI testing detects differences in the lengths of a selection of microsatellites, resulting from indel errors, between normal cells and tumour cells.
- Several approved commercial platforms are available for MSI testing; this test is currently more expensive than IHC.
- MSI testing is carried out on DNA extracted from tumour tissue.
- MSI test interpretation requires a high level of expertise as the changes seen in EC are reported to be more subtle than those in colonic cancer.

Reasons for MMR testing in endometrial (and ovarian) carcinoma

- The indications for MMR IHC in EC (and endometrioid and clear cell ovarian carcinomas) are all of the following:
  i. screening for LS: it is estimated that one in 250-300 people are affected by LS and that >95% of these individuals are unaware of their cancer-susceptibility risk. EC is often the first or sentinel cancer in a pedigree, and therefore provides an opportunity for detection of LS in a family. An EC patient with LS may go on to develop other cancers and EC precedes
subsequent cancers such as colorectal carcinoma by approximately a decade. The diagnosis of LS in a family allows surveillance and preventative measures that significantly reduce the mortality from subsequent LS-related cancers.

ii. molecular diagnosis of EC: the TCGA classification requires MMR IHC or MSI testing of all cases for identification of the hypermutated MMRd/MSI category of EC, with important management implications; such tumours are unlikely to respond to conservative treatment with progesterone, show a high likelihood of lymphovascular space invasion justifying a sentinel or other nodal procedure, and chemotherapy results in no significant survival benefit in these tumours, which on the other hand, respond well to radiotherapy.

iii. predictive testing: MMRd tumours of all sites are eligible for targeted treatment with immune checkpoint inhibitors.

**MMR IHC interpretation**

**Normal MMR protein expression**

- MMR protein expression in normal tissues is seen as nuclear staining (Figure 1).
- The staining is generally uniform but may be of variable intensity, with more rapidly proliferating cells showing more intense staining.
- In cancer cells, generally characterized by higher proliferation rates relative to normal tissue, the staining intensity is typically strong, and generally higher than that seen in the background stroma, normal glands or inflammatory cells that serve as an internal control (Figure 2).

Figure 1a. MMR protein expression in normal inactive endometrium (PMS2).

Figure 1b. MMR protein expression in secretory endometrium (MSH2).
Abnormal MMR expression

- In the presence of a mismatch repair defect, there is loss of expression of one or more of the MMR proteins (Figure 3).
- The 4 major MMR proteins occur as heterodimers, MLH1 pairing with PMS2 and MSH2 with MSH6.
- While MLH1 and MSH2 can stabilize in the cell by forming heterodimers with other proteins, PMS2 and MSH6 can only exist stably in the cell in the presence of MLH1 and MSH2 respectively.
- This has two important consequences:
  1. There are therefore 4 typical abnormal MMR IHC patterns:
     i. loss of both MLH1 and PMS2; this occurs in MLH1 deficiency
     ii. loss of both MSH2 and MSH6; this occurs in MSH2 deficiency
     iii. isolated loss of MSH6; this occurs in MSH6 deficiency
     iv. isolated loss of PMS2; this occurs in PMS2 deficiency
  2. The second consequence is that testing for just 2 proteins, PMS2 and MSH6, can be used to screen for MMRd with equivalent accuracy to testing for all 4 proteins\textsuperscript{5,11,12}, provided there is due regard to the pitfalls discussed below.
Figure 3a. Loss of MSH6 expression in an endometrial carcinoma. The presence of nuclear staining in internal controls (as shown by stromal cells) is essential for the interpretation of abnormal MMR expression.

Figure 3b. Loss of MSH6 expression in another case of endometrial carcinoma. While the stromal cells exhibit minimal staining in this area, the presence of weak nuclear staining in non-neoplastic endometrial glands (upper left) allows this interpretation to be made.

Figure 3c. Loss of MSH6 expression in an endometrial carcinoma (right), in contrast with the variable nuclear staining in the non-neoplastic epithelium, stromal cells and lymphocytes (left).

Figure 3d. Histology of the endometrial carcinoma from the same area as Figure 3c.
Problems and pitfalls in MMR IHC interpretation$^{9,10}$

- **MMR IHC is fixation-sensitive.** Poor fixation is a common problem in pathological reporting of EC. It is vital that well-fixed areas are examined when reporting MMR IHC, to avoid erroneous interpretation of one or more stains as loss of expression (Figure 4). For this reason MMR IHC should be carried out
on biopsies, with the added advantage that this vital information is available at the time of diagnosis of EC.

Figure 4a. Patchy expression of MSH6 in an endometrial carcinoma. Note the lack of nuclear staining in stromal cells in this area, which would signify the possibility of suboptimal fixation. A gradation of staining intensity may be observed in such areas.

Figure 4b. Patchy expression of PMS2 in the same case of endometrial carcinoma as Figure 4a. While the appearance may resemble subclonal loss of expression, this area is not interpretable due to the lack of valid internal controls.

Figure 4c. In this area (PMS2 for same case of endometrial carcinoma as Figure 4a), there is complete absence of staining for PMS2 in both tumour cells and stromal cells. This area is non-interpretable for the lack of valid internal controls, likely representing a poorly fixed area with antigen degradation.

Figure 4d. In another area (PMS2 for same case of endometrial carcinoma as Figure 4a), there is weak to moderate nuclear staining in the tumour cells, together with scanty faint staining in the stromal cells as internal controls. This is interpreted as retained expression. The patchy staining in this case probably represents an artifact related to suboptimal fixation instead of genuine subclonal expression.
- **Very weak or very focal expression may be seen in the presence of MMRd.**
  
  Very weak or very focal MMR expression can occur in the presence of a defective MMR protein and similarly weak or focal MSH6 expression may be seen in the presence of MSH2 mutation (Figures 5 & 6). As already stated the expression of MMR proteins is generally strong and diffuse relative to the internal control and any deviation from this, including very weak/focal expression, should be noted and reported either as defective or equivocal. Repeating the staining on a different section or a biopsy rather than the hysterectomy specimen can solve some of these issues.

![Figure 5a](image1.jpg) Focal weak staining for MSH6 in tumour nuclei is observed in this endometrial carcinoma. MSH2 is lost in this case (not shown).

![Figure 5b](image2.jpg) At low power magnification (same case as Figure 5a), the majority of tumour nuclei are negative for MSH6, in contrast to the stronger nuclear staining in stromal cells and non-neoplastic epithelium. This case should be interpreted as abnormal/loss of MSH6 expression despite focal weak staining in some tumour nuclei.
Figure 6a. Focal weak nuclear staining for MLH1 is observed in the tumour cells of this endometrial carcinoma, with stronger staining present in the stromal cells.

Figure 6b. Another area (same case as Figure 6a) demonstrates that most tumour nuclei are negative while the positively stained stromal cells have an overall stronger intensity, supporting the interpretation of abnormal MMR expression. This case was confirmed as having a germline MLH1 mutation.

- **Subclonal expression may occur in a minority of cases.** Subclonal expression 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.

Subclonal expression is believed to occur as an acquired defect, as when MLH1 promoter methylation occurs during tumour progression (Figures 7 & 8). An acquired or secondary subclonal MSH6 loss is also described and occurs secondary to any mechanism of MMRd (Figure 9). This is due to the occurrence of a mutation in a microsatellite within the MSH6 gene, a so-called “passenger mutation” in MSH6, as a result of the underlying MMRd. This pattern may also occur in the presence of pathogenic POLE mutations. When subclonal MSH6 loss occurs with another defect, the reporting terminology should be as for the underlying defect. When subclonal MSH6 loss occurs as an isolated abnormality, this should be reported as abnormal as it may indicate an underlying germline abnormality, most likely in a gene other than MSH6.
Subclonal MSH6 loss is sometimes accompanied by variable expression of MSH2.

The diagnostic, prognostic and therapeutic implications of this pattern remain incompletely understood. The rationale for reporting these cases as ‘abnormal’ is that (i) a low proportion of such cases may harbour an underlying germline defect, and (ii) the behaviour of these cases with regard to their molecular category and/or responses to treatment, including immune modulation, is unknown.

Figure 7a. Subclonal expression of MLH1 in part of this endometrial carcinoma (right) with loss of staining in other foci (left). Note that stromal cells show positive staining throughout the entire area. This pattern may occur in endometrial carcinomas with MLH1 promoter methylation.

Figure 7b. High power view of subclonal MLH1 expression from the same tumour area as Figure 7a.
Figure 7c. Subclonal expression of PMS2 in the same case as Figure 7a, demonstrating similar pattern as that observed with MLH1.

Figure 7d. High power view of subclonal PMS2 expression from the tumour area in Figure 7c.

Figure 7e. Normal expression of MSH6 in the same case as Figure 7a.

Figure 7f. Histology of the same tumour area as Figure 7a.
Figure 8a. Subclonal expression of PMS2 in part of the tumour (upper) with absence of nuclear staining in other areas (lower). Note the positive staining of stromal cells in both areas which enables its distinction from artifactual weak staining.

Figure 8b. Histology of the same tumour area as Figure 8a.

Figure 9a. Subclonal expression of MSH6 in part of this endometrial carcinoma (left) coexisting with an area of negative staining (right). Note the positive staining of stromal cells among the negative tumour cells.

Figure 9b. Expression of MSH2 in the same tumour area as Figure 9a.
A low proportion of MLH1 loss cases can show punctate nuclear expression that may be erroneously interpreted as retained/normal expression. This pattern has been described in previous publications and should be reported as loss of expression (Figures 10 & 11). To the best of our knowledge this does not occur with any other protein, and is thought to be a technical artifact, seen with the MLH1 (M1) clone (Roche Diagnostics).
pattern of staining has been recognized as an artifact which may be specific to certain antibody clones. This case should be reported as abnormal MMR expression.

Figure 11a. Punctate nuclear staining for MLH1 may closely resemble intact expression at low power, as shown in this endometrial carcinoma.

Figure 11b. At high power (same case as Figure 11a), the dot-like staining in the tumour nuclei can be distinguished from the homogeneous nuclear staining in stromal cells.

- **Cytoplasmic/membranous staining does not constitute normal expression and should be reported as abnormal.** The MMR proteins are localised to the nucleus. In some cases, possibly related to technical reasons, there is relatively conspicuous cytoplasmic or membranous staining in the absence of nuclear staining; such cases should be reported as abnormal (Figure 12).
In addition to the typical patterns listed above, a range of other patterns/problems may occur, such as loss of 3 or more proteins, discordance between MMR IHC and MSI or between MMR IHC and genetic testing. A superadded somatic defect, usually $MLH1$ promoter methylation, can be seen in any MMRd, resulting in unusual MMR IHC patterns. For example, cases with germline $MSH6$ mutation can show MSH2 loss on IHC (Figure 13), due to somatic MSH3 loss.\textsuperscript{18} Mutations that result in a functionally defective but antigenically preserved protein can give rise to MMRd with normal IHC. It is important to note that MMR IHC loss with absence of $MLH1$ promoter methylation does not equate to LS, and only about half of the cases will be proven to have an inherited defect; this is reflected in the recommended terminology. The majority of these occur due to biallelic somatic loss of an MMR protein.
Figure 13a. Loss of MSH6 expression in this endometrial carcinoma associated with LS. This case illustrates discordance between MMR IHC and genetic testing, as there is loss of both MSH2 and MSH6 with known germline MSH6 mutation. It has been postulated that somatic MSH3 loss (and subsequent lack of stabilizing partner for MSH2) might account for the unexpected MSH2 loss in such cases.

Figure 13b. MSH6 loss in another area from the same case as Figure 13a.

Figure 13c. Loss of expression of MSH2 from the same area as Figure 13a. This is an unusual finding given the known germline MSH6 mutation in this case.

Figure 13d. Loss of MSH2 expression from the same area as Figure 13b.
Reporting terminology for MMR IHC

- It is important to adhere to standard reporting terminology for MMR IHC reporting to ensure correct clinical management (Table 1).

Table 1. Recommended terminology for reporting mismatch repair protein immunohistochemistry (MMR IHC) +/- MLH1 promoter methylation results

<table>
<thead>
<tr>
<th>MMR result</th>
<th>Recommended report</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, MLH1, PMS2, MSH2 and MSH6 tested</td>
<td>MMR IHC Normal:</td>
</tr>
<tr>
<td></td>
<td>The tumour cells show normal nuclear staining for MLH1,</td>
</tr>
<tr>
<td></td>
<td>PMS2, MSH2 and MSH6.</td>
</tr>
<tr>
<td></td>
<td>Conclusion: There is no immunohistochemical evidence of a</td>
</tr>
<tr>
<td></td>
<td>mismatch repair deficiency*.</td>
</tr>
<tr>
<td>Normal, only MSH6 and PMS2 tested</td>
<td>MMR IHC Normal:</td>
</tr>
<tr>
<td></td>
<td>The tumour cells show normal nuclear staining for PMS2</td>
</tr>
<tr>
<td></td>
<td>and MSH6.</td>
</tr>
<tr>
<td></td>
<td>Conclusion: There is no immunohistochemical evidence of a</td>
</tr>
<tr>
<td></td>
<td>mismatch repair deficiency*.</td>
</tr>
<tr>
<td>Abnormal, MSH6 loss</td>
<td>MMR IHC Abnormal, MSH6 loss:</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td></td>
<td>The tumour cells show loss of expression of the mismatch repair protein MSH6 (with normal nuclear staining for MLH1, MSH2 and PMS2).</td>
</tr>
<tr>
<td></td>
<td>Conclusion: This mismatch repair deficiency is associated with Lynch and related syndromes.</td>
</tr>
<tr>
<td></td>
<td><strong>This patient should be referred to Clinical Genetics services.</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Abnormal, PMS2 loss</th>
<th>MMR IHC Abnormal, PMS2 loss:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The tumour cells show loss of expression of the mismatch repair protein PMS2 (with normal nuclear staining for MLH1, MSH2 and MSH6).</td>
</tr>
<tr>
<td></td>
<td>Conclusion: This mismatch repair deficiency is associated with Lynch and related syndromes.</td>
</tr>
<tr>
<td></td>
<td><strong>This patient should be referred to Clinical Genetics services.</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Abnormal, MSH2 and MSH6 loss</th>
<th>MMR IHC Abnormal, MSH2 loss:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The tumour cells show loss of expression of the mismatch repair proteins MSH2 and MSH6 (with normal nuclear staining for MLH1 and PMS2).</td>
</tr>
<tr>
<td></td>
<td>Conclusion: This mismatch repair deficiency is associated with Lynch and related syndromes.</td>
</tr>
<tr>
<td></td>
<td><strong>This patient should be referred to Clinical Genetics services.</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Abnormal, MLH1 and PMS2 loss, <em>MLH1</em> promoter hypermethylation absent</th>
<th>MMR abnormality, MLH1 loss and <em>MLH1</em> Promoter hypermethylation absent:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The tumour cells show loss of expression of the mismatch repair proteins MLH1 and PMS2 (with normal nuclear staining for MSH2 and MSH6). <em>MLH1</em> promoter hypermethylation is not present.</td>
</tr>
<tr>
<td></td>
<td>Conclusion: While this mismatch repair deficiency could be sporadic, it is probable that this mismatch repair deficiency is due to Lynch or related syndromes.</td>
</tr>
<tr>
<td></td>
<td><strong>This patient should be referred to Clinical Genetics services.</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Abnormal, MLH1 and PMS2 loss, <em>MLH1</em> promoter hypermethylation</th>
<th>MMR abnormality, MLH1 loss and <em>MLH1</em> Promoter hypermethylation present:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The tumour cells show loss of expression of the mismatch repair proteins MLH1 and PMS2 (with normal nuclear staining for MSH2 and MSH6). <em>MLH1</em> promoter hypermethylation is present.</td>
</tr>
<tr>
<td></td>
<td>Conclusion: While this mismatch repair deficiency could be sporadic, it is probable that this mismatch repair deficiency is due to Lynch or related syndromes.</td>
</tr>
<tr>
<td></td>
<td><strong>This patient should be referred to Clinical Genetics services.</strong></td>
</tr>
</tbody>
</table>
present staining for MSH2 and MSH6). The *MLH1* promoter shows hypermethylation is present in the tumour.

**Conclusion:** This combination indicates that this mismatch repair deficiency is almost certainly sporadic rather than due to Lynch Syndrome.

*This patient does not require referral to Clinical Genetics services*.  

| Abnormal, MLH1 and PMS2 loss, *MLH1* promoter hypermethylation not tested | **MMR abnormality, MLH1 loss and MLH1 Promoter hypermethylation not tested:** The tumour cells show loss of expression of the mismatch repair proteins MLH1 and PMS2 (with normal nuclear staining for MSH2 and MSH6). *MLH1* promoter hypermethylation has not been tested.  

**Conclusion:** 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 hypermethylation is recommended OR this patient may be referred to Clinical Genetics services.* |

| Abnormal, MLH1 and PMS2 loss, *MLH1* promoter hypermethylation pending | **MMR abnormality, MLH1 loss and MLH1 Promoter Hypermethylation testing results pending:** The tumour cells show loss of expression of the mismatch repair proteins MLH1 and PMS2 (with normal nuclear staining for MSH2 and MSH6). *MLH1* promoter hypermethylation testing in the tumour has been requested.  

**Conclusion:** This pattern of mismatch repair deficiency may be either sporadic or due to Lynch or related syndromes – the result of testing for *MLH1* promoter hypermethylation will provide further information. A supplementary report will be issued when these results become available. |

| Abnormal, subclonal loss of MLH1 and PMS2 | **MMR abnormality, subclonal MLH1 loss:** The tumour cells show subclonal loss of expression of the mismatch repair proteins MLH1 and PMS2 (with normal nuclear staining for MSH2 and MSH6).  

**Conclusion:** 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 hypermethylation is recommended.**

<table>
<thead>
<tr>
<th>Abnormal, MLH1 and PMS2 loss with subclonal loss of MSH6</th>
<th>Report as above for other cases of MLH1 loss.</th>
</tr>
</thead>
</table>
| Abnormal, subclonal loss of MSH6 | MMR IHC Abnormal, subclonal MSH6 loss: The tumour cells show loss of expression of the mismatch repair protein MSH6 (with normal nuclear staining for MLH1, MSH2 and PMS2).

Conclusion: This mismatch repair deficiency may be associated with Lynch and related syndromes.

This patient should be referred to Clinical Genetics services.

*Referral to Clinical Genetics services should be considered despite this result in the presence of a strong family/clinical history.*

**References**