MOLECULAR CLASSIFICATION OF ENDOMETRIAL CANCER AND POLE GENE SEQUENCING IN WALES

The latest WHO classification of female genital tumours (5th edition), endorses a molecular classification of endometrial cancer, where endometrial cancers are assigned to one of four molecular categories:

1. POLE ultramutated
2. MMR deficient
3. p53 mutant
4. No specific molecular profile (NSMP)

These molecular categories are of prognostic significance. Endometrial cancers will still be classified and graded using conventional histological criteria, as well as being assigned to a molecular category. New European guidelines (ESGO-ESTRO-ESP) for the management of patients with endometrial cancer acknowledge the importance of this molecular classification and provide guidance for the management of patients, both where the molecular classification is known and unknown.

Molecular classification of endometrial cancer requires immunohistochemistry for p53 and mismatch repair proteins, as well as genetic testing of tumour tissue, looking for pathogenic variants in the POLE gene. The ability to do POLE gene sequencing has only recently become available in Wales. POLE gene sequencing has been accepted onto the Test Directory for Genomics Hubs in England (from 1st April 2022), which means that funding is available for us to start doing this in Wales.

It is proposed that cases are selected for POLE gene sequencing based on the assessment of clinicopathological risk factors. Histopathologists reporting endometrial cancer in endometrial biopsy specimens, will have an important role in deciding which cases should be sent for POLE gene sequencing. Immunohistochemistry (IHC) for ER, p53 and mismatch repair proteins will be performed on all cases of endometrial cancer. This is already the current practice in most pathology departments in Wales. If an endometrial cancer is of endometrioid type, low grade (FIGO grade 1 or 2), ER+, p53 wild-type and shows retained staining for all 4 mismatch repair proteins (MMR proficient), then the endometrial cancer would be regarded as being low risk and POLE gene sequencing would not be requested. If an endometrial cancer shows ANY of the following features, then tissue would be sent for POLE gene sequencing:

1. Non-endometrioid sub-type (e.g. serous carcinoma, clear cell carcinoma, carcinosarcoma, undifferentiated carcinoma or de-differentiated carcinoma).
2. FIGO grade 3.
3. ER negative or very focal
4. Aberrant/mutation-type staining for p53.
5. Loss of staining for any of the MMR proteins (MMR deficient) - This includes cases which show loss of MLH1/PMS2 with MLH1 promoter methylation.

ER staining is typically strong and diffuse in endometrioid carcinomas. Universal ER IHC may help to identify rare subtypes of EC which may mimic endometrioid carcinoma, such as clear cell, mesonephric-like and gastro-intestinal-type mucinous EC (which are negative for ER). Knowledge of ER status may also help to inform decisions regarding hormonal therapy. The presence of completely negative or very focal ER expression should alert the pathologist to the possibility of a non-endometrioid sub-type of endometrial carcinoma, or the potential for aggressive behaviour in an endometrioid carcinoma.

p53 IHC should be reported as normal/wild-type or abnormal/aberrant/mutation-type. The terms "positive" and "negative" should be avoided. Abnormal patterns of staining should be described. These are strong and diffuse staining/over-expression, complete absence/null-type, cytoplasmic and subclonal. If p53 IHC staining is difficult to interpret (i.e. difficult to decide if it represents wild-type or aberrant/mutation-type staining), then tissue should be sent for both POLE and TP53 gene sequencing.

MMR IHC should be reported in accordance with the recommendations for Lynch syndrome testing in endometrial carcinoma.

The results of genetic testing will be returned to the pathologist, as well as going on to the Welsh Clinical Portal (WCP). The pathologist should then determine the molecular category of the endometrial tumour, using the diagnostic algorithm (fig 3) and issue a supplementary report, which would include the final molecular category. These results should be documented at the local and regional MDT meetings.

11 pathogenic POLE gene variants are recognised, with the variants in bold accounting for the majority of cases: **P286R (c.857C>G), V411L (c.1231G>T/C, S297F (c.890C>T), S459F (c.1376C>T), A456P (c.1366G>C)**, F367S (c.1100T>C), L424I (c.1270C>A), M295R (c.884T>G), P436R (c.1307C>G), M444K (c.1331T>A) and D368Y (c.1102G>T).

In cases where POLE gene sequencing was not requested by the pathologist at the time of reporting the biopsy (based on the pathological features in the endometrial biopsy specimen), the MDT can ask the pathologist to request POLE gene sequencing in certain cases:

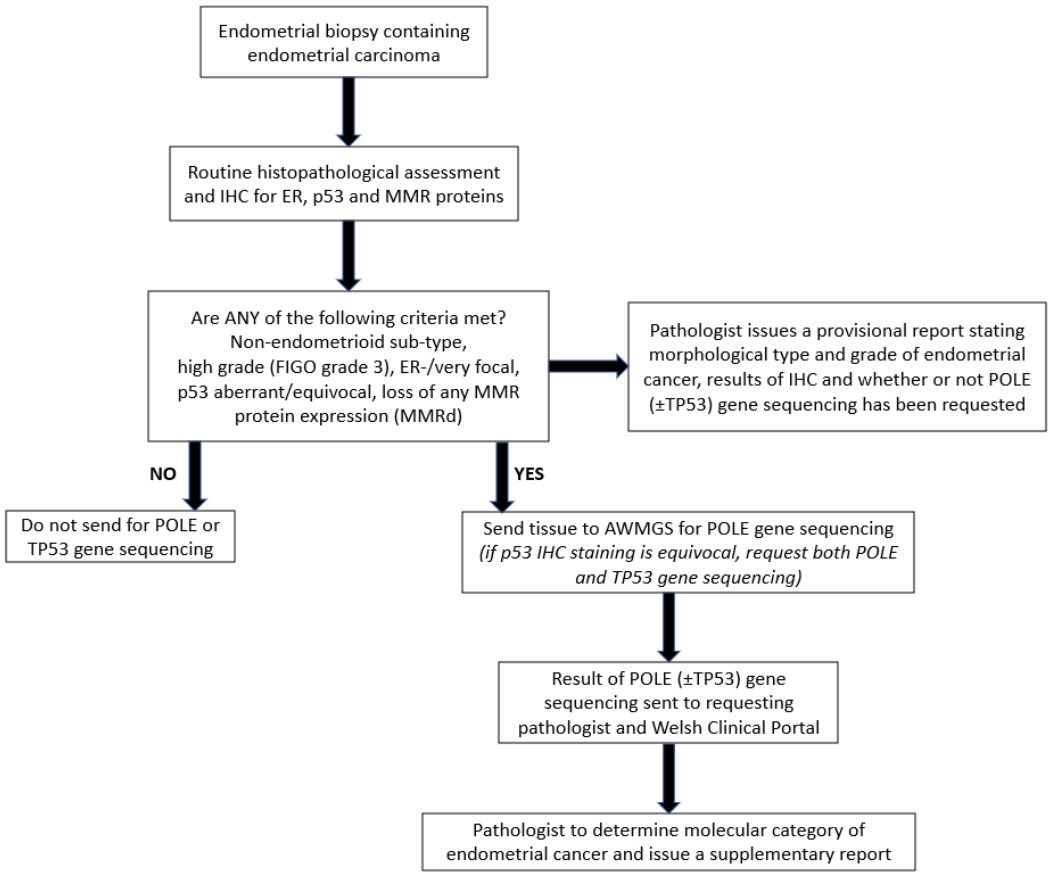
1. When the endometrial cancer appears to be FIGO (2009) stage IB or II on imaging.
2. Pathological examination of the hysterectomy specimen reveals any of the following:
    a. The tumour (or a component) is of non-endometrioid type or grade 3.
    b. >1 focus of lymphovascular space invasion (LVSI) is seen (more than focal LVSI).
    c. The tumour is FIGO (2009) stage IB or II.

POLE gene sequencing is not requested in cases of FIGO (2009) stage III or IV EC, unless this is recommended by the gynae-oncology MDT.

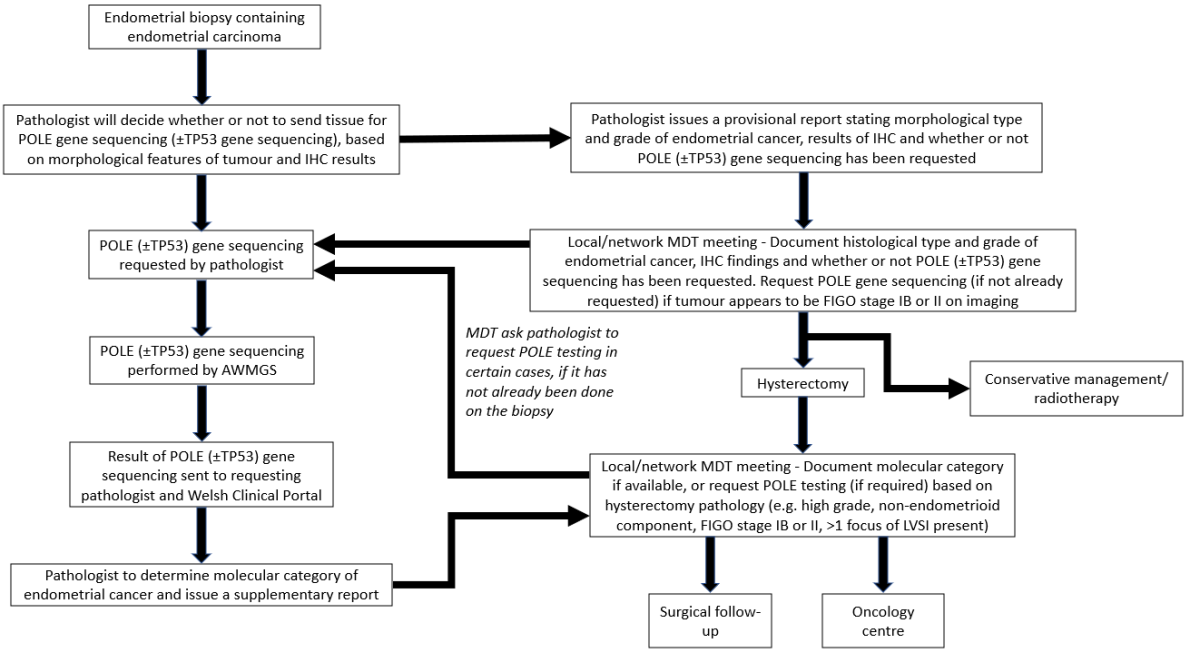
The diagnostic algorithm (fig 3) should be used to determine the molecular category of an endometrial carcinoma. Please note that an endometrial carcinoma can only be assigned to a molecular category following POLE gene sequencing and IHC (for p53 and MMR proteins). The final report on an endometrial biopsy for endometrial carcinoma should include the conventional histological type/grade and the molecular category in cases where this has been established. The molecular category should be given as POLEmut, MMRd, NSMP or p53mut.

An overview of Lynch syndrome testing in endometrial cancer and molecular classification of endometrial cancer is shown in fig 4.

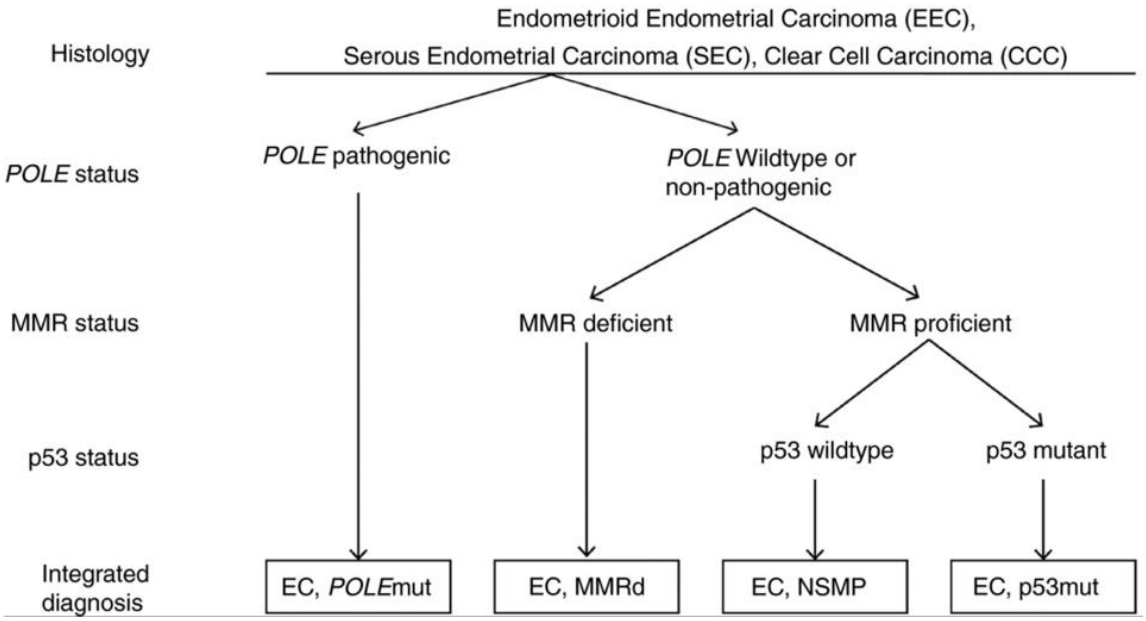
PATHOLOGY PATHWAY (fig 1)



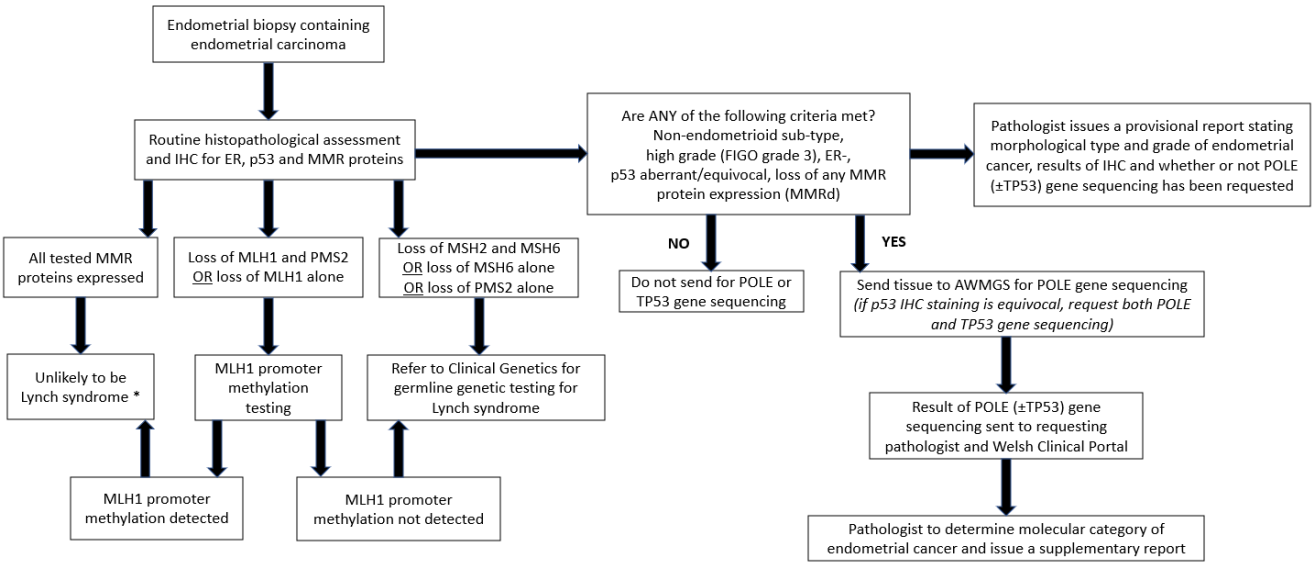
INTEGRATED CLINICAL/PATHOLOGY PATHWAY (fig 2)



DIAGNOSTIC ALGORITHM FOR ASSIGNING A MOLECULAR CATEGORY (fig 3)



SUMMARY OF LYNCH TESTING AND MOLECULAR CLASSIFICATION IN ENDOMETRIAL CANCER (fig 4)



GLOSSARY

EC	Endometrial cancer.
MMR	Mismatch repair. MMR deficient (MMRd) means that there is loss of expression/IHC staining for one or more of the MMR proteins. MMR proficient (MMRp) means that there is retained expression/IHC staining for all of the MMR proteins.
POLE	DNA polymerase epsilon. A member of the DNA polymerase family of enzymes. POLE pathogenic means that a pathogenic POLE variant has been identified on POLE gene sequencing. POLE wild-type or non-pathogenic means that either no POLE variants have been identified or a POLE variant has been identified which is not considered to be pathogenic.
p53	In this diagram (fig 3), p53 refers to the p53 protein, which is assessed by IHC staining. p53 IHC is reported as showing either wild-type or aberrant/mutation-type patterns of staining. If the pattern of p53 IHC staining is difficult to determine, then TP53 gene sequencing can be requested.
POLEmut MMRd NSMP p53mut	These are the molecular categories of endometrial carcinoma, which are determined following POLE gene sequencing and IHC for p53 and MMR proteins, using the diagnostic algorithm (fig 3). NSMP = No specific molecular profile.

CONTRIBUTORS

Adam Boyde, Consultant Histopathologist, University Hospital of Wales.

Rhian White, Consultant Clinical Scientist, All Wales Medical Genomics Service.

Louise Hanna, Consultant Oncologist, Velindre Cancer Centre.

Sheila Palmer-Smith, Clinical Scientist, All Wales Medical Genomics Service.