Genetic analysis of Endometrial Cancer

Overview

Endometrial cancer is the 4th most common cancer among women in the UK (1) with 8,000 cases diagnosed each year (2).

Genetic testing of endometrial cancer is performed for two reasons:

1. As part of the molecular classification of an endometrial cancer.
2. To help establish whether the cancer might be associated with Lynch syndrome.

Molecular classification of endometrial cancer

In 2013, the Cancer Genome Atlas Research Network (TCGA) published a genomic characterisation of endometrial cancer by combining data generated from various genomic techniques (3). The WHO classification of female genital tumours (5th edition) incorporates these molecular classifications into the clinical diagnosis of endometrial cancer patients. Each molecular classification is prognostically distinct and exhibits a characteristic response to therapy (4).

Table 1: The different molecular classifications of endometrial cancer

Molecular classification	Percentage of endometrial cancers (5).
POLE ultramutated	9%
Mismatch repair deficient	28%
Non-specific molecular profile	50%
TP53 deficient	12%

POLE Ultramutated: Endometrial cancers which harbour pathogenic variants in the extranuclease domain (exons 9-14) of the enzyme DNA polymerase *epsilon* (*POLE*). These tumours are ultramutated with an exceptionally high tumour mutational burden. These endometrial cancers are associated with an excellent prognosis (5).
Mismatch Repair Deficient: Endometrial cancers without a pathogenic variant in *POLE* but showing a mismatch repair (MMR) defect, most commonly due to MLH1 promoter methylation. These tumours are hypermutated, show high tumour mutational burdens and have a favourable prognosis (5).
TP53 deficient: Endometrial cancer without a pathogenic variant in *POLE* and MMR proficient but TP53 deficient. These tumours are associated with a poor prognosis (5).
Nonspecific molecular profile (NSMP): These endometrial tumours without a *POLE* variant and without deficiency in MMR or TP53 have a good prognosis (5).

Molecular analysis by the AWGL alongside histopathological evaluation by pathologists will enable molecular classification of endometrial tumours which will provide insight on the prognosis of the cancer and inform personalised treatment options. All resection specimens will be prioritised for testing to ensure prompt treatment management options after surgery.

Lynch syndrome

The frontline test for Lynch syndrome in endometrial cancers is immunohistochemistry for the MMR proteins, performed in the histopathology laboratory. Patients with tumours with MMR loss not including MLH1 are referred directly to clinical genetics. Tumours with MMR loss that includes loss of MLH1 are sent to the All-Wales Genomics Laboratory (AWGL) for *MLH1* promoter methylation testing. If the *MLH1* promoter is not methylated this is consistent with the tumour being Lynch-associated and the patient should be offered referral to clinical genetics. If *MLH1* promoter hypermethylation is detected then the tumour is most likely sporadic (not Lynch-associated) and the patient should only be referred to genetics if there is a strong suggestion of Lynch syndrome due to: endometrial cancer occurring under the age of 50 OR a first degree relative with bowel or endometrial cancer at any age OR the patient also has bowel cancer; as there is a small possibility of Lynch syndrome due to germline *MLH1* promoter methylation.

Test Information

The All-Wales Genomics Laboratory utilises the Illumina TruSight Oncology 500 High Throughput DNA assay for next generation sequencing using the Illumina NovaSeq 6000™ to identify nucleotide variants in the *POLE* (and *TP53*) genes in patients with endometrial cancer.

Table 2: Endometrial cancer DNA Gene Panel for *POLE* (OMIM 174762) and *TP53* (OMIM 191170)

Gene	Regions covered
POLE	Exons 9-14
TP53	Whole gene

Note: *POLE* (exons 9-14) will be sequenced for **ALL** referred endometrial carcinoma specimens. *TP53* (full gene) will **only** be sequenced when requested by the pathologist due to P53 immunohistochemistry giving equivocal results.

The All-Wales Genomics Laboratory uses the PyroMark Q48 Autoprep for MLH1 promoter methylation analysis by pyrosequencing.

Table 3: Pyrosequencing testing for *MLH1* (OMIM 120436).

Gene	Test
MLH1	Bisulfite conversion and pyrosequencing to establish whether the *MLH1* promoter is methylated. *MLH1* promoter methylation is correlated with deficient MMR (loss of MLH1/PMS2).

Note: *MLH1* promoter methylation analysis will **only** be requested if immunological assessment of the endometrial carcinoma specimen shows loss of MLH1 and PMS2.

Sample requirements for testing

- 1 x ~5 micron H&E stained slide with area of neoplastic cell content highlighted and the neoplastic cell proportion estimated.
- 6 x 10 micron air dried unstained sections mounted on slides
- Pathology report
- A neoplastic cell proportion ≥20% is required for *MLH1* methylation analysis.
- A neoplastic cell proportion of ≥10% is required for *POLE* (and *TP53*) analysis.

To request testing use the '**Solid Tumour Request Form: Endometrial Cancer'** and complete all fields.

Reports

For *POLE* (and *TP53*) a non-interpretative NGS report is issued by the AWGL for endometrial cancer. This will provide only genetic variant information with no diagnostic, prognostic or treatment implications. Genetic variant information provided by the AWGL is for incorporation in a histopathology report to give the overall molecular classification of the tumour.

Twelve *POLE* exonuclease domain variants have been classified as pathogenic based on characteristic genomic alterations associated with an ultramutational subtype (6,7) (Table 4). The AWGL will only report these *POLE* exonuclease domain variants as pathogenic. Any other *POLE* exonuclease domain variant will be reported as a variant of uncertain significance.

Table 4: A list of established *POLE* exonuclease domain pathogenic variants.

POLE pathogenic variant	Exon	% of cases
c.857C>G p.(Pro286Arg)	9	43%
c.1231G>T/C p.(Val411Leu)	13	27%
c.890C>T p.(Ser297Phe)	9	6%
c.1376C>T p.(Ser459Phe)	14	4%
c.1366G>C p.(Ala456Pro)	14	4%
c.1100T>C p.(Phe367Ser)	11	4%
c.1270C>A p.(Leu424Ile)	13	4%
c.884T>G p.(Met295Arg)	9	2%
c.1307C>G p.(Pro436Arg)	13	2%
c.1331T>A p.(Met444Lys)	13	2%
c.1102G>T p.(Asp368Tyr)	11	2%

Variants identified in the *TP53* gene will be classified using a combination of the CanVIG-UK TP53 Gene Specific Guidance and the ClinGen TP53 Expert Panel Specifications to the ACMG/AMP variant interpretation guidelines.

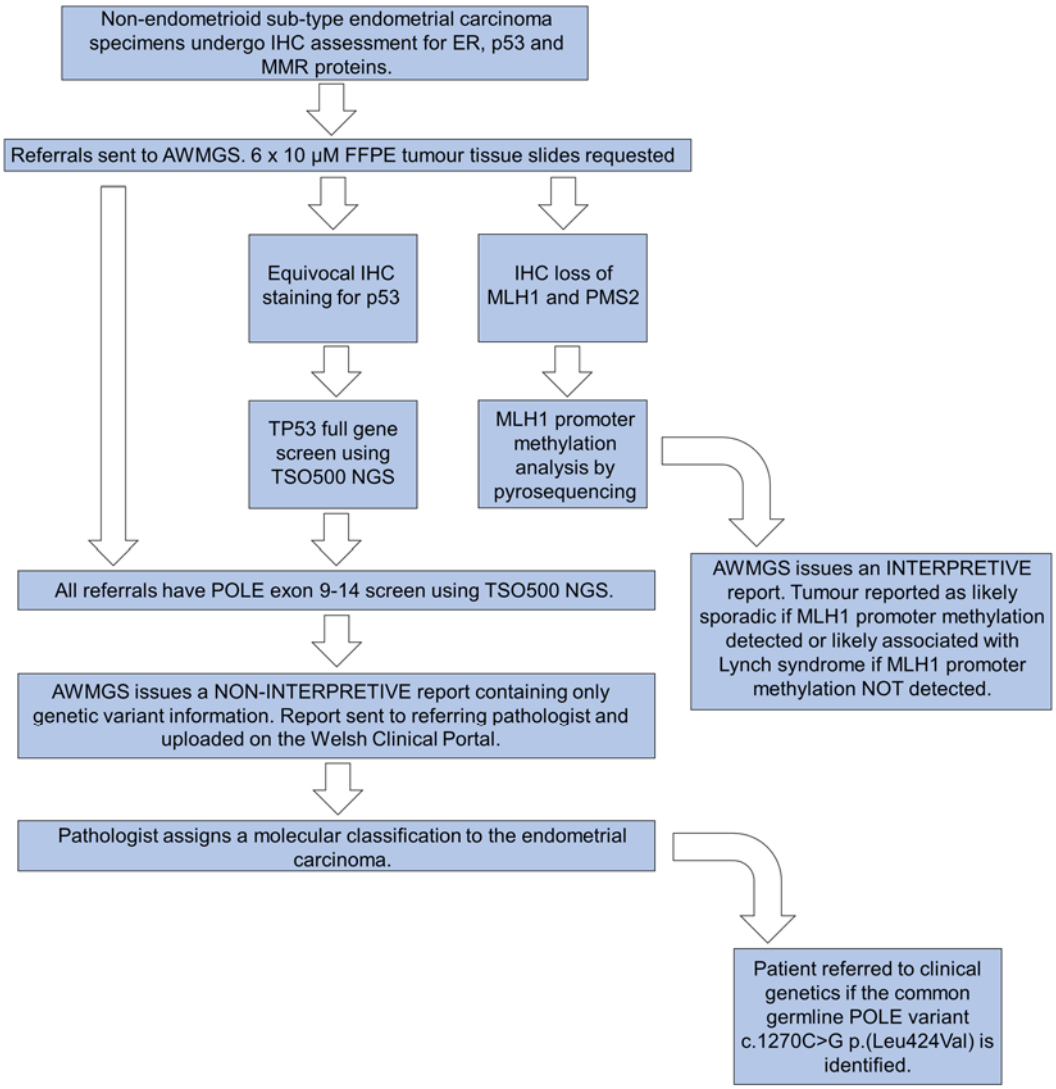
The test is performed to detect variants in the *POLE* and *TP53* genes within endometrial cancer specimens and cannot determine whether a variant is germline or somatic. The germline *POLE* c.1270C>G p.(Leu424Val) pathogenic variant has been identified in 0.5-2% of patients with intestinal polyposis (8). Referral to clinical genetics will be recommended if this variant is detected in an endometrial carcinoma specimen.

The AWGL will issue an interpretative report for MLH1 promoter methylation based on the pathology pathway (section 3.7) of the "testing for Lynch syndrome in people with endometrial cancer – clinical guidance document" (9). The tumour will be reported as likely sporadic if MLH1 promoter methylation is detected. However, germline MLH1 methylation is also occasionally detected in patients with Lynch syndrome and a referral to clinical genetics will be recommended if the following criteria is applicable:

(1) Patient is less than 50 years of age
(2) A first or second degree relative has a personal history of bowel or endometrial cancer at any age
(3) The patient also has a personal history of bowel cancer.

If MLH1 promoter methylation is not detected in the endometrial tumour then the report will recommend that the patient is referred to clinical genetics for germline genetic testing for Lynch syndrome.

Fig 1: An outline of the endometrial cancer service delivered by the AWGL.



Links for further information

Orphanet: www.orpha.net

OMIM: www.omim.org
Genetic Test Directory: https://www.england.nhs.uk/publication/national-genomic-test-directories/

Endometrial cancer links

https://www.cancerresearchuk.org/about-cancer/womb-cancer https://patient.info/doctor/endometrial-cancer

All-Wales Genomics Laboratory (AWGL)

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Email: lab.genetics@wales.nhs.uk Website: http://www.wales.nhs.uk/AWMGS

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