Endometrial Cancer Molecular Testing

Recommendations Report

November 2024

Executive Summary

Summary of Recommendations:

1) Reflex immunohistochemistry testing for mismatch repair, p53 and estrogen receptor should be performed on the first diagnostic pathologic specimen. If this workup has not been performed prior to surgery, then it should be performed on tumour in the hysterectomy specimen.
2) Reflex POLE mutation testing is recommended on the first diagnostic specimen for the following:
    a. MMR deficient,
    b. p53 abnormal,
    c. ER-negative,
    d. Grade 2 endometrioid,
    e. Grade 3 endometrioid,
    f. High grade histologies.
3) The following patients should be referred to a gynecologic oncologist at a Gynecologic Oncology Centre (GOC) for management:
    a. MMR deficient,
    b. p53 abnormal,
    c. ER-negative,
    d. Grade 2 endometrioid,
    e. Grade 3 endometrioid,
    f. High grade histologies,
    g. Advanced stage (stage II – IV),
    h. Grade 1 endometrioid with myometrial invasion on pre-operative imaging, if done.
4) Grade 1 endometrioid adenocarcinoma that are estrogen receptor positive, mismatch repair intact and p53 normal, can be surgically managed by a general gynecologist at an affiliate or a GOC, if there are no concerns of myometrial invasion or advanced disease.

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Background

Endometrial cancer (EC) is the fourth most common cancer in women in Canada, with rising incidence and mortality over the last 20 years[1]. There has been a rapid evolution in the molecular characterization of EC and this guidance document was created to help physicians appropriately triage patients with EC to gynecologic oncology centres (GOC) and to facilitate their management. Topics covered in this guidance document include: 1. recommendations for reflex testing on endometrial biopsies and/or hysterectomy specimens, 2. appropriate and recommended language in reporting, and 3. criteria for referral to GOC based on reflex molecular testing. This guidance document does not contain details regarding adjuvant treatment algorithms or management.

The need for applying a consistent approach to molecular testing in EC was identified by the Ontario Health (Cancer Care Ontario) (OH-CCO) Gynaecological Cancers Advisory Committee. A multidisciplinary working group was formed by circulating a call for Expressions of Interest to streamline molecular testing for EC in Ontario. Working group members represented healthcare professionals who care for patients with EC, including pathologists, gynecologic oncologists, radiation oncologists, medical oncologists, general gynecologists, and a genetic scientist.

Through a review of the literature, two documents were identified as potential guidance to adapt to the Ontario health care context. The working group co-chairs reviewed both and selected the British Association of Gynaecological Pathologists (BAGP) and British Gynaecological Cancer Society (BGCS) guidance on *POLE* next-generation sequencing (NGS) testing in endometrial carcinoma[2] as an outline to create an Ontario-focused endometrial molecular testing guidance document. The working group developed a testing algorithm and associated recommendations, adapted from the BAGP/BGCS guidance, and based on the working group's expertise and evidence. The document was reviewed by the working group, Ontario Health (Cancer Care Ontario) disease site groups (e.g., the Ontario Gynecologic Cancers Advisory Committee), relevant program leadership and representatives, and additional clinical experts from across Ontario and Canada (see Appendix).

Studies indicate that the overall inter-observer agreement between pathologists for grade and histotype assignment in EC is only moderate (kappa range 0.40-0.67)[3,4], with major disagreement in histotype occurring in about one third of high-grade ECs[4–6]. As part of an effort to reduce inconsistencies, The Cancer Genome Atlas (TCGA) study used a complex and comprehensive molecular testing approach to identify four molecular subgroups in EC with distinct clinical outcomes, from the most to least favourable: *POLE* ultramutated, copy-number low, microsatellite instability (MSI) hypermutated, and copy-number high[7].

Based on the TCGA study, two research teams independently developed and validated a clinically applicable pragmatic classification tool[8–12]. Using immunohistochemistry (IHC) for mismatch repair (MMR) proteins and p53, and sequencing to identify clinically significant variations in the exonuclease domain of DNA polymerase epsilon (*POLE*), four molecular subtypes can be consistently identified from formalin-fixed paraffin embedded material, recapitulating the TCGA prognostic groups. Importantly, this pragmatic classification tool is applicable to all ECs, regardless of histotype. The correct order of segregation of ECs has been established: first identifying ECs with clinically significant

POLE variants (*POLE*mut)[13], then identifying patients whose tumour exhibits immunohistochemical MMR deficiency (MMRd) and finally, tumours with abnormal p53 expression on IHC (p53abn or p53 mutant). Throughout this document we will use p53abn to denote ECs with abnormal p53 IHC expression. ECs without any of these defining features are termed “no specific molecular profile” (NSMP) (Figure 1). The small percentage (~3%) of ECs with more than one molecular feature (i.e., multiple classifier EC) are classified using the same order of segregation[14]. *POLE*mut-p53abn EC are classified as *POLE*mut, and MMRd-p53abn EC are classified as MMRd.

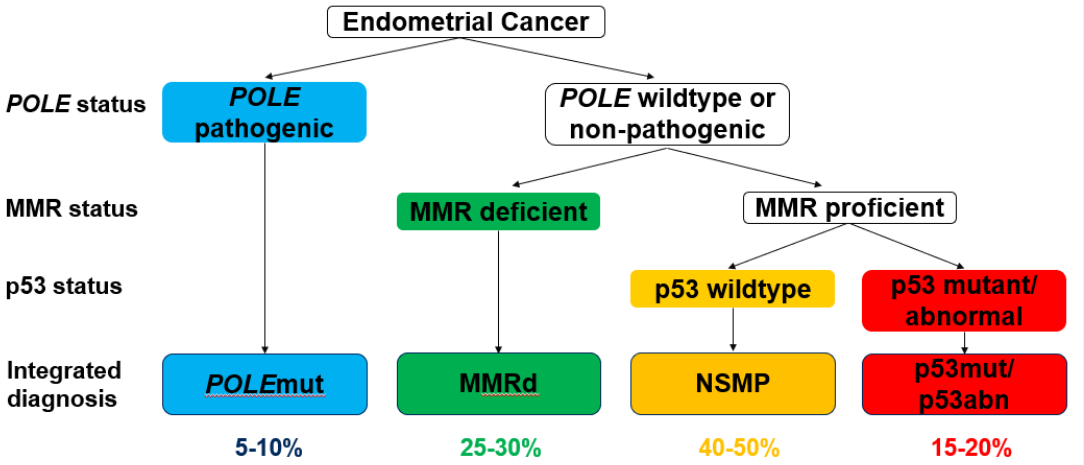

Figure 1. World Health Organization (WHO)-endorsed algorithm for molecular classification of EC[15].

The prognostic value of the molecular classification has been demonstrated in several studies[8–12,16–20]. The predictive value of molecular classification has also been shown in many trials, for response to radiotherapy[21], chemotherapy[20], and immunotherapy[22,23]. In the PORTEC-1 and PORTEC-2 trials, omitting radiotherapy seemed to be safe in *POLE*mut EC, external beam radiotherapy yielded a significantly better locoregional recurrence-free survival than vaginal brachytherapy or no adjuvant therapy in p53abn EC, and vaginal brachytherapy was as effective as external beam radiotherapy in NSMP EC[21]. In PORTEC-3, patients with p53abn EC had a highly significant benefit from chemotherapy with an absolute benefit of 22.4% and 23.1% for 5-year RFS and OS, respectively[20]. In both NRG GY-018 and RUBY trials[22-24], patients with MMRd advanced/recurrent EC derived substantial PFS benefit from immune checkpoint inhibitor.

The World Health Organization (WHO) recommends integration of molecular features into pathologic reporting[25]. Several treatment guidelines also incorporate molecular classification, including the 2020 ESGO/ESTRO/ESP guidelines[26], ESMO guidelines[27], and National Comprehensive Cancer Network guidelines[28]. Molecular classification is also part of the new 2023 FIGO staging system[29]. Molecular testing in EC has been funded by the Ontario Ministry of Health since 2021.

When describing molecular findings in tumours, the term "pathogenic" (or "likely pathogenic") is often used in the literature, including in several of the papers referenced in this report. The 2019 Somatic Cancer Panel Reporting in Ontario guideline[30] recommends using the term "clinically significant" or "clinically actionable" when reporting somatic molecular changes. Following this reporting guideline, the term “clinically significant” is used in place of "pathogenic" throughout this document.

The guidance in this document reflects **minimum care** (i.e. minimum recommended testing) for every patient with EC. Some centres may perform comprehensive molecular testing of all ECs to simplify workflow and/or improve timely access for patient participation in clinical trials.

Endometrial Cancer Molecular Testing Algorithm and Recommendations

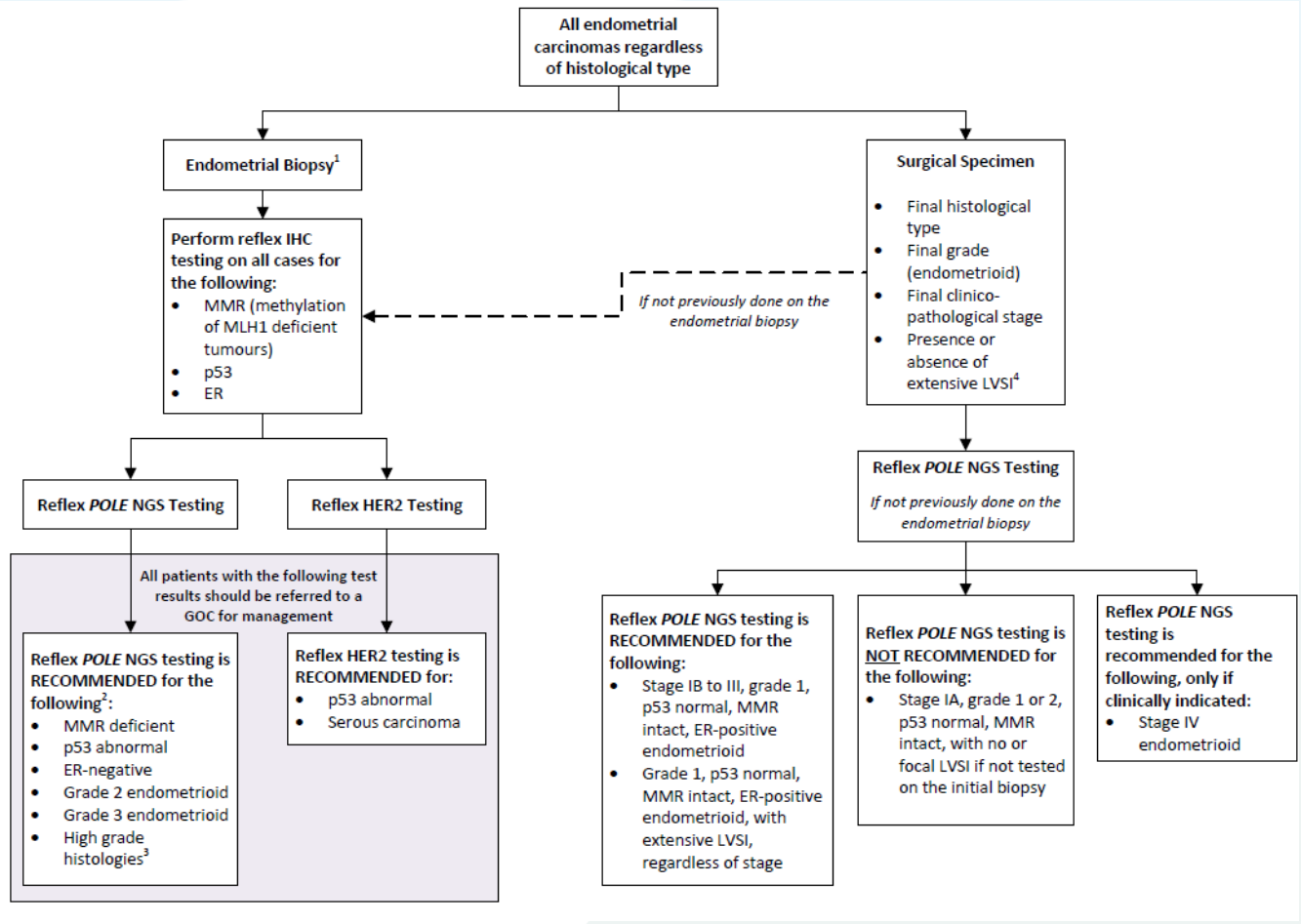

Figure 2. Ontario endometrial cancer molecular testing algorithm.

[1]While the endometrial biopsy is typically the first diagnostic pathologic specimen, different specimens (e.g., omentum for metastatic cases) may be used depending on the clinical situation.
[2]Centres may choose to perform reflex *POLE* NGS testing on low-risk, grade 1, p53 normal, MMR intact, ER-positive (NSMP) endometrioid due to workflow, participation in clinical trials, etc.
[3]High grade histologies include serous, clear cell, carcinosarcoma, mesonephric-like, gastrointestinal mucinous, mixed, undifferentiated, and poorly differentiated adenocarcinomas (all endometrial carcinomas except grade 1).
[4]According to the College of American Pathologists (CAP) Protocol for the Examination of Specimens from Patients with Carcinoma and Carcinosarcoma of the Endometrium, version 5.0.0.0, extensive LVSI is defined as greater than or equal to 5-vessel involvement, which aligns with the WHO and FIGO 2023 definitions. Due to different definitions of "extensive LVSI", if there is less than 5-vessel involvement, it is preferred for the exact number to be specified. Please ensure the status of LVSI of

"none", "focal" or "extensive", etc. is documented. For additional details, please see the CAP endometrial checklist.

Abbreviations: IHC = immunohistochemistry, MMR = mismatch repair, ER = estrogen receptor, NGS = next-generation sequencing, NSMP = no specific molecular profile, LVSI = lymph-vascular space invasion

Routine immunohistochemical and molecular work-up of endometrial cancer

The recommended testing algorithm is shown in Figure 2.

Reflex testing on all endometrial cancers at the time of diagnosis

Reflex testing on the first diagnostic pathologic specimen (endometrial biopsy or first biopsy, e.g., omentum, if metastatic) is recommended and should be performed regardless of tumour histotype or grade. Most commonly, this first specimen is endometrial biopsy material however, in patients with advanced disease, this may be from an extra-uterine site (e.g., omentum). The following should be tested using IHC:

1) mismatch repair (MMR) proteins (MLH1, MSH2, MSH6 and PMS2);
2) p53; and,
3) estrogen receptor (ER).

See below for pathology explanatory notes. When loss of MLH1/PMS2 immunoexpression is identified, it is recommended that reflex methylation testing be initiated, as per OH-CCO recommendations. Those ECs that are deficient in expression of in MLH1/PMS2 (without promoter methylation identified), PMS2 only, MSH2/MSH6 or MSH6 only should be referred to clinical genetics for germline testing for Lynch syndrome.

It is recommended to perform initial testing on endometrial biopsy (not hysterectomy specimen) since it mitigates issues related to tissue fixation, has high concordance with final hysterectomy specimen and will prevent delays in management[31-33]. Having timely access to molecular testing on the initial diagnostic biopsy will allow appropriate triage and immediate referral of patients to GOC, identify individuals at risk for Lynch syndrome and need for genetic testing, determine surgical and adjuvant therapies based on molecular subtype and prevent delays in treatment. However, if reflex testing was not able to be performed on initial biopsy (e.g., due to limited/minimal tumour material), these IHC tests should be done on the final hysterectomy specimen.

Reflex testing for Her2/neu on all serous or p53abn endometrial cancers at the time of diagnosis

Treatment options for p53abn or serous EC are limited. These patients often present with advanced disease and the majority will recur and die of their cancer. Approximately 30% of p53abn or serous EC show Her2/neu overexpression by IHC (score of 3+) and for those with advanced disease (stage III/IV), the addition of trastuzumab to standard chemotherapy improves progression-free and overall survival[34]. Therefore, it is essential to have timely assessment for Her2/neu status so that this targeted therapy can be added to chemotherapy in those with stage III/IV Her2/neu positive p53abn or serous EC. See below for pathology explanatory notes.

Reflex *POLE* next generation sequencing (NGS) at the time of diagnosis, on endometrial biopsy

Reflex *POLE* NGS should be completed at the time of diagnosis, on endometrial biopsy (or first biopsy (e.g., omentum, if metastatic)), for all the following:

- Tumours showing loss of MMR protein expression (MMRd);
- Tumours showing an abnormal p53 expression pattern (p53abn);
- ER negative tumours;
- Grade 2 and 3 endometrioid histology; and,
- All non-endometrioid high grade histologies, including mixed histotypes.

Tumours with one of the currently recognized 11 clinically significant *POLE* variants are classified as *POLE*mut EC[12]. These ECs are often histologically high grade however, they have an excellent prognosis[15]. There is growing evidence that patients with *POLE*mut tumours would benefit from de-escalation of radiation therapy[20,35]. *POLE*mut tumours often demonstrate abnormal patterns of p53 expression and loss of MMR protein expression[15] and in such situations (*POLE*mut-p53abn or *POLE*mut-MMRd), the EC should be considered as *POLE*mut and not be classified into the p53mut or MMRd subgroups. This paradigm has significant implications for adjuvant therapy recommendations, since NGS is a DNA based sequencing test that may take several weeks to get the results of *POLE* mutation status. To make timely decisions regarding adjuvant therapy, it is essential to perform this testing on the initial endometrial biopsy material, as opposed to the final surgical specimen to prevent further delays. If the patient has undergone surgery and NGS for *POLE* has not been done on the diagnostic endometrial biopsy, then it should be performed on the hysterectomy specimen, when warranted. Some centres may choose to perform NGS for *POLE* on all EC at initial diagnosis, including grade 1 endometrioid, p53 normal, MMR intact, ER positive (NSMP), due to workflow and timely assessment for participation in clinical trials.

Which patients should be sent to a GOC for management?

With the transition to incorporating molecular features into EC classification, we can more accurately identify patients who will benefit from management at a GOC and those that can be managed safely in the community under the care of general gynecology.

High-grade histology and advanced stage

As per the Organizational Guideline for Gynecologic Oncology Services in Ontario, all patients with grade 2 and grade 3 endometrioid carcinoma, serous carcinoma, clear cell carcinoma, carcinosarcoma, mixed adenocarcinoma, undifferentiated or de-differentiated carcinoma, and other rare types regardless of molecular subtype should be referred to a GOC for surgical management (lymph node assessment and omentectomy) due to high rate of occult advanced disease and need for adjuvant therapy. In addition, all patients with suspected advanced stage EC (involvement of cervix, vagina, lymph nodes, peritoneum, or distant sites) should be referred to a GOC, regardless of histologic subtype. Patients with grade 1 endometrioid EC and evidence of myometrial invasion on

pre-operative imaging should be referred to GOC for discussion of surgical staging, regardless of molecular subtype.

p53 abnormal EC (p53abn)

Any tumour that has an abnormal p53 IHC expression, including subclonal abnormal expression, should be referred to a GOC for management. The majority of theses cases will fall into the p53 abnormal (p53abn) molecular subgroup. Approximately 10-15% of EC patients will demonstrate an abnormal p53 immunoexpression pattern[36]. Though less common, this molecular subgroup of EC makes up the majority of deaths due to EC. These patients should be referred to GOC for surgical management and adjuvant therapy regardless of histotype. Of note, approximately 5% of histologically low-grade endometrioid endometrial cancers have an abnormal p53 immunoexpression pattern and require surgical management (surgical staging including lymph node assessment and omentectomy) and adjuvant therapy at a GOC[19]. This group of patients have a survival benefit with adjuvant chemotherapy independent of the histotype[18]. As well, these patients may benefit from immunotherapy in the recurrent setting after failing chemotherapy[37].

MMR deficient (MMRd) EC

Any tumour that has an MMR deficient (MMRd) IHC expression, including subclonal loss pattern, should be referred to a GOC for management. The majority of theses cases will fall into the MMRd molecular subgroup. Approximately 25-30% of all endometrial cancers are MMRd and the majority are low grade endometrioid histotype[38]. These patients have an intermediate prognosis with up to 25% risk of recurrence[19]. As well, they are often found to have adverse features on hysterectomy such as deep myometrial invasion and lymphovascular space invasion (LVSI)[39]. There is now evidence of improved survival with the addition of immunotherapy to chemotherapy for those with advanced disease at presentation and at recurrence[21,22]. These patients should be referred to a GOC for surgical staging, surveillance, and appropriate adjuvant therapy.

ER negative EC

ER negative tumours are those which show 10% or less of tumour nuclei with ER IHC expression. ER negative tumours span all four molecular subgroups, but the prognostic significance is most well appreciated in the NSMP molecular subgroup. These patients have a poor prognosis, and the tumours can show a variety of histological appearances[40,41]. Patients with these tumours require referral to GOC for surgical staging (lymph node assessment), surveillance, and possible adjuvant therapy.

Which patients can be surgically managed by general gynecology?

Grade 1 endometrioid/ER positive (NSMP ER positive)

A large proportion (>40%) of patients with EC have grade 1 endometrioid tumours that are ER positive, MMR intact and p53 normal on IHC and are classified as no specific molecular profile (NSMP) subgroup [40]. These patients overall have an excellent prognosis and if there is no evidence of

advanced disease on clinical assessment, these patients can be managed either in the community with general gynecology or at a GOC by gynecologic oncology.

Pathologic Interpretation

Clinically significant *POLE* variants

1) Interpretation of the significance of *POLE* mutation results should be based on published evidence. Currently, 11 mutations (Table 1, most common starred) have been established as being clinically significant (i.e., unequivocally leading to an ultramutated phenotype that confers better prognosis)[13]. When one of the listed mutations is identified, the tumour should be classified within the *POLE*mut category.
2) *POLE*mut tumours do demonstrate some relatively characteristic morphological and immunohistochemical changes but overall, they cannot be reliably histologically identified[42].

Clinically significant *POLE* variants associated with favourable prognosis (adapted from León-Castillo et al.[13])

Clinically significant *POLE* variants
P286R* (c.857C>G, p.(Pro286Arg))
V411L* (c.1231G>T/C, p.(Val411Leu))
S297F* (c.890C>T, p.(Ser297Phe))
S459F* (c.1376C>T, p.(Ser459Phe))
A456P*(c.1366G>C, p.(Ala456Pro))
F367S (c. 1100T>C, p.(Phe367Ser))
L424I (c.1270C>A, p.(Leu424Ile))
M295R (c.884T>G, p.(Met295Arg))
P436R (c.1307 C>G, p.(Pro436Arg))
M444K (c.1331T>A, p.(Met444Lys)
D368Y (c.1102 G>T, p.(Asp368Tyr))

Interpretation of MMR and p53 immunohistochemistry

MMR

1) In Ontario, most if not all centres utilize a 4-stain approach (i.e., evaluation of expression of MLH1, PMS2, MSH2 and MSH6). The four MMR proteins which are evaluated exist as heterodimers where MLH1 and MSH2 are the dominant partners and PMS2 and MSH6 can only maintain stability (existence) in each cell in the presence of their dominant partner.
2) MMR protein expression is normally nuclear in location (in both non-neoplastic and neoplastic tissues). In tumours, the staining intensity is typically strong and uniform, but it may vary. Our understanding of abnormal expression patterns has evolved, and it is now recognized that the spectrum of abnormal expression extends beyond complete absence of nuclear expression[43]. Some examples of MMR expression patterns are shown in Figure 5.

3) Clear terminology should be utilized when reporting MMR immunohistochemical results and, if possible, a synoptic template should be used, which includes standard/canned comments regarding recommendations for referral to clinical genetics services. “Normal” or “intact” should be used to indicate the expected nuclear expression in non-deficient tumours, while “loss” or “deficient” should be used to indicate an abnormal pattern. An “equivocal” or “cannot be determined” designation may be used where interpretation is compromised by fixation artifact or other reasons. The terms “positive” and “negative” should be categorically avoided, as these are confusing and can lead to wrongful clinical interpretation of the reported results.

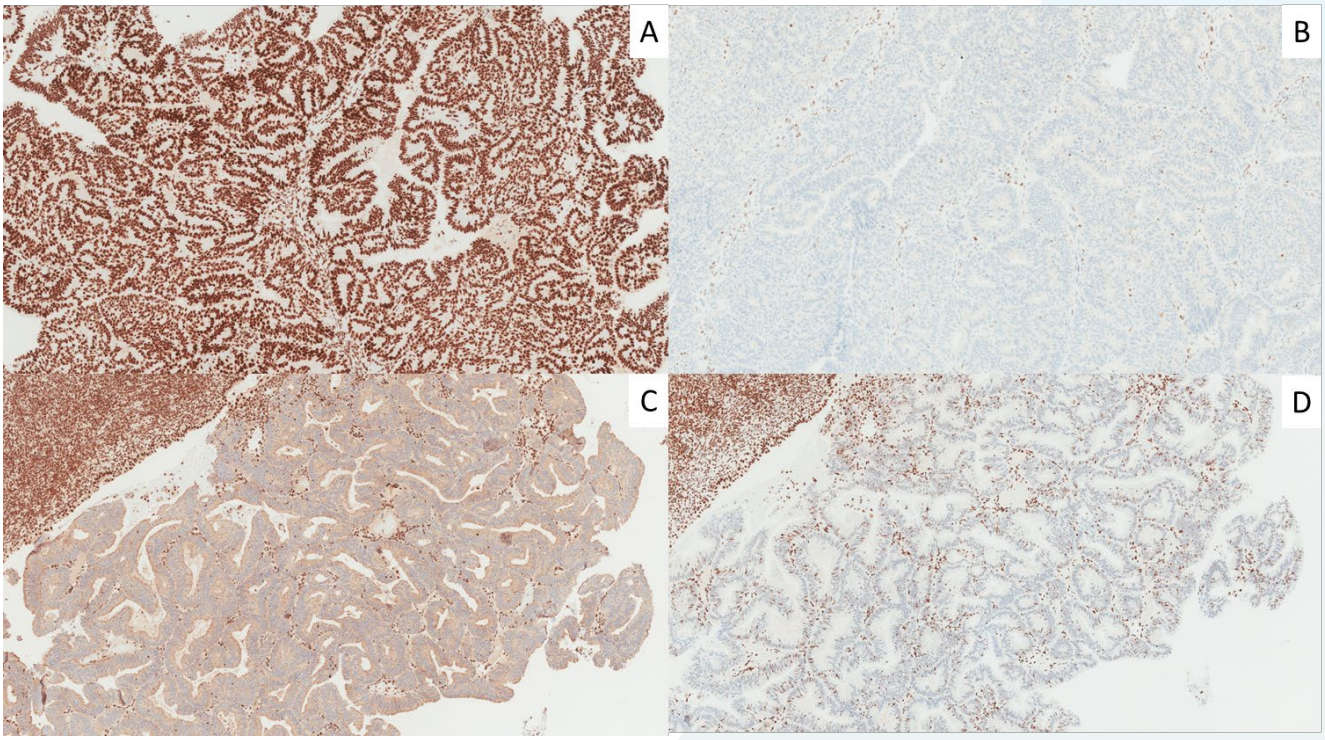

Figure 5. MMR immunohistochemical expression patterns in EC. A. Intact nuclear expression is typically strong and uniform, as in this case, but fixation may alter this. B. Loss of nuclear expression; note the retained expression in interspersed stromal cells. C. Cytoplasmic expression may be misinterpreted as a normal/intact pattern. There is a lack of nuclear staining, and this pattern may possibly be attributable to technical factors. Note the normal nuclear expression in interspersed stromal cells and in an intact polyp fragment in the top left corner. D. Weak/patchy nuclear expression may be misinterpreted as a normal/intact pattern. Compared to the tumour cells, note the normal nuclear expression in interspersed stromal cell staining and in an intact polyp fragment in the top left corner. Other unusual patterns which may be mistaken for a normal/intact pattern include punctate/dot-like nuclear and membranous staining.

4) Loss of MMR expression (MMR deficiency) is usually diffuse within a tumour (i.e., the abnormal or mutant pattern is seen in all the neoplastic cells) however, a subclonal pattern of abnormal expression (i.e. the clustered loss of expression is seen in only a proportion of the neoplastic cells) may also be seen in some cases. This phenomenon most commonly occurs in the setting of MLH1 promoter hypermethylation or in the context of an underlying clinically significant *POLE* variant. True subclonal loss of expression should be distinguished from fixation-related loss or other

artifacts and internal control expression must be maintained. In general, application of a minimum cut-off of 10% is suggested, to avoid reporting a focal abnormality that may be clinically insignificant. Repeat assessment on another tumour-containing biopsy block (if available) or on hysterectomy material should be considered, to further determine the extent of the subclonal pattern. An example of subclonal loss of MMR expression is shown in Figure 6.

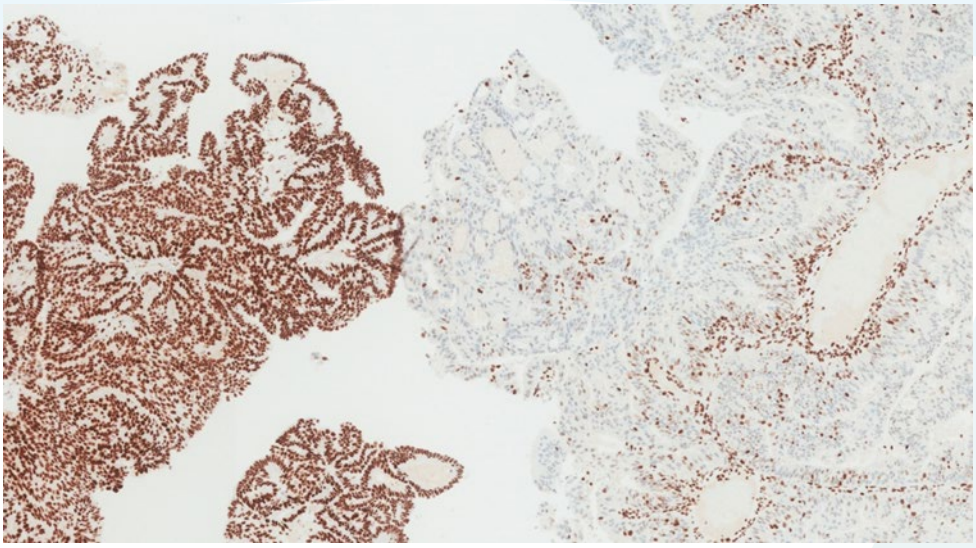

Figure 6. Subclonal loss of MMR expression in EC. Loss of nuclear expression is seen on the right side of the image; patchy/weak areas are seen but there is a much stronger internal control signal. The left side of the image shows an immediately adjacent tumour fragment with intact nuclear expression.

p53

1) In general, mutant pattern p53 immunohistochemical expression is an excellent surrogate for TP53 mutation status[44].
2) Clear terminology should be utilized when reporting p53 immunohistochemical results and, if possible, a synoptic template should be used. “Normal” vs. “abnormal” or alternatively, “aberrant/mutation type” vs. “wild type” are accepted terms. The terms “positive” and “negative” should be categorically avoided, as these are confusing and are not biologically relevant.
3) The normal or wild type pattern of p53 expression shows a spectrum but in general, scattered nuclear staining of varying intensity will be observed (Figure 7). The abnormal patterns are designated as “overexpressed”, “null” and “cytoplasmic”, and these are reflective of/caused by different types and locations of mutations within the TP53 gene. According to the WHO, aberrant p53 immunostaining is defined as strong nuclear expression in >80% of tumour nuclei (overexpressed), complete absence of expression in tumor cell nuclei with retained internal control (null) or unequivocal cytoplasmic expression (cytoplasmic)[23]. Examples of a normal pattern of expression and the three recognized abnormal patterns of expression are shown in Figure 8. Either mutant pattern p53 expression by IHC or a clinically actionable TP53 mutation by NGS is sufficient to classify a tumor as being of p53abn molecular subtype.

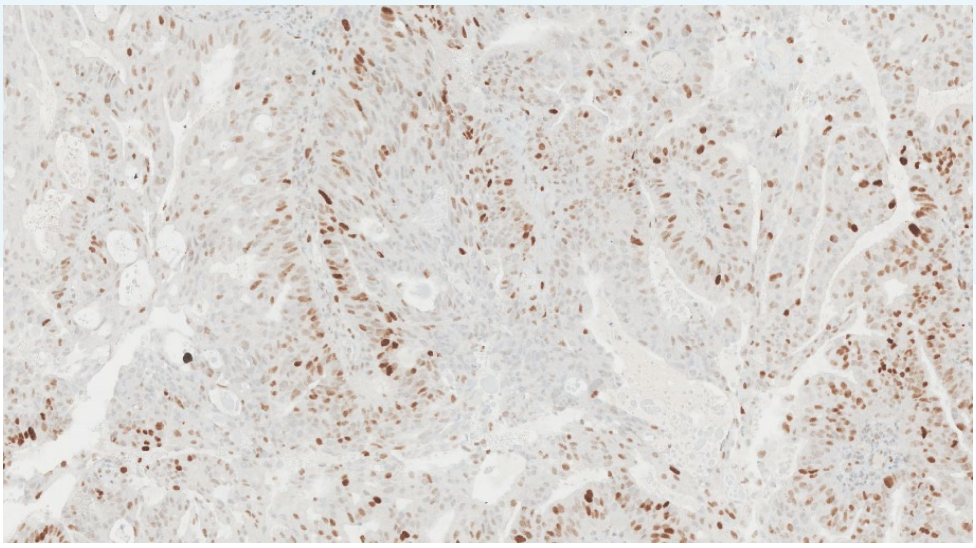

Figure 7. Wild type/normal expression of p53 in EC. Staining of variable intensity is the hallmark of the normal expression pattern. The proportion of tumour nuclei with expression can range from minimal to the majority.

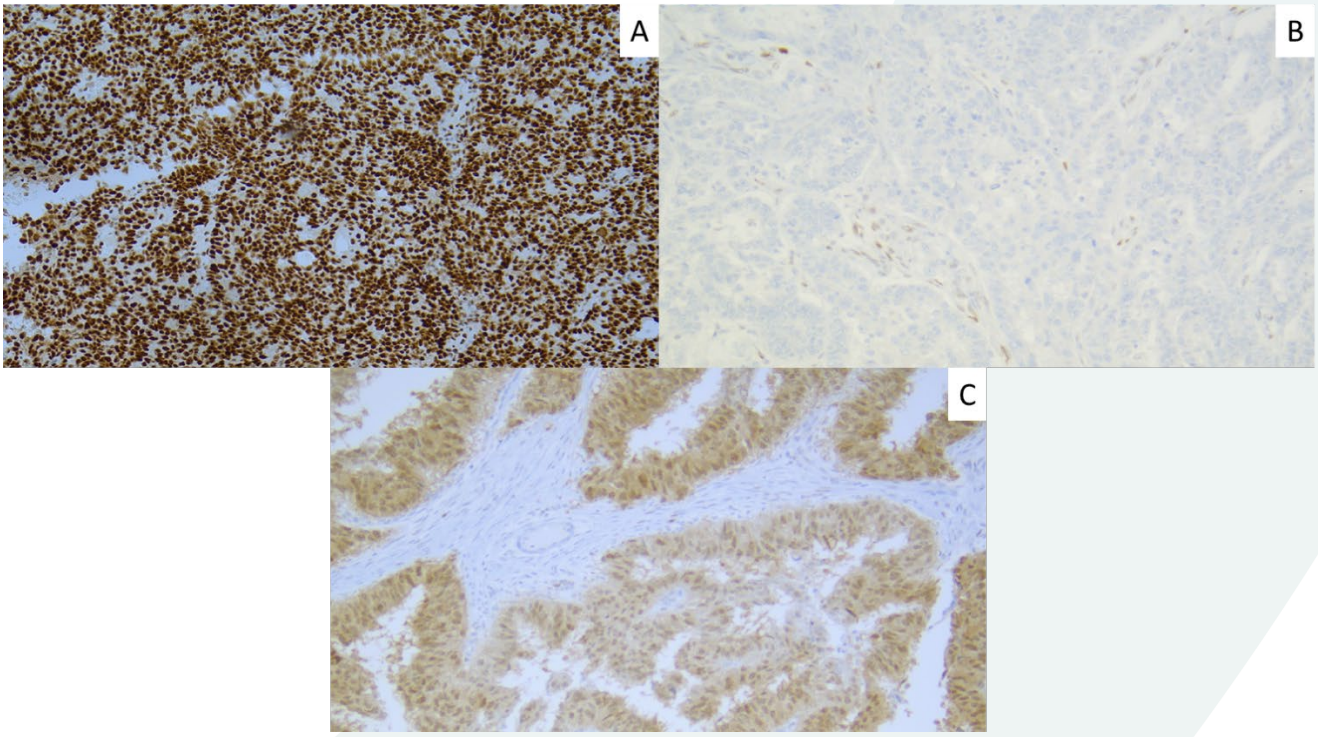

Figure 8. Mutant/abnormal p53 immunohistochemical expression patterns in EC. A. Overexpressed. B. Null (note the internal control). C. Cytoplasmic.

4) Abnormal expression of p53 (p53abn) is usually diffuse within a tumour (i.e., the abnormal or mutant pattern is seen in all the neoplastic cells) however, a subclonal pattern of abnormal expression (i.e., the abnormal or mutant pattern is seen in only a proportion of the neoplastic cells) may also be seen in some cases. A subclonal pattern typically reflects a *TP53* mutation that

has developed in part of the tumour, and this most commonly occurs in the context of an underlying clinically significant *POLE* variant or mismatch repair deficiency, but rarely, it may also occur in the absence of those findings. A minimum cut off of 10% has been suggested, for clinical significance purposes[45]. Examples of a subclonal abnormal pattern of p53 expression is shown in Figure 9.

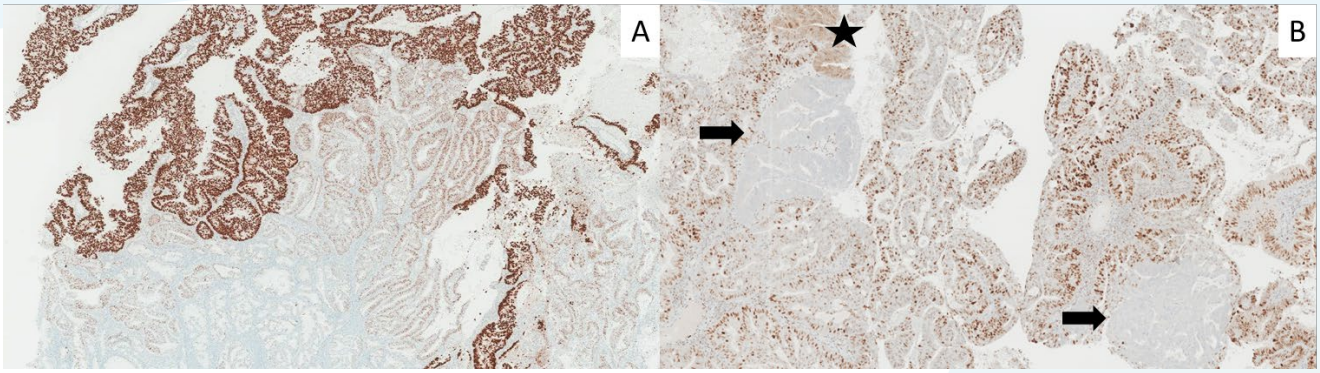

Figure 9. Examples of subclonal p53 immunohistochemical expression in EC. A. This fragment of tumour shows aberrant overexpression towards the top of the image, immediately juxtaposed beside wild type/normal areas towards the bottom of the image. This tumour was MMR-deficient, with underlying MLH1 promoter methylation. B. In this example, the majority of the tumour demonstrates a wild type/normal pattern of expression however, interspersed abrupt foci of null pattern are noted (black arrows), as is a small adjacent focus showing a cytoplasmic pattern (star). This tumour was found to have an underlying clinically significant *POLE* variant.

Interpretation of ER immunohistochemistry

1) Assessment of ER expression in EC serves several purposes, including identification of subtle high-grade tumours (ex. clear cell carcinoma, mesonephric-like carcinoma among others) which may be confused with low-grade endometrioid carcinoma and morphologically low-grade tumours that will likely behave in an aggressive fashion.
2) ER is expressed in the nucleus and the extent (% staining tumour nuclei) and strength of expression (weak, moderate, intense) should be reported. Examples of the tumours showing different levels of ER expression are shown in Figure 10.
3) There is ongoing interest and study into the role ER expression may play in identifying low grade endometrioid carcinomas in the NSMP category which may be suitable for de-escalation of adjuvant radiotherapy. In the clinical trial environment, the current threshold for de-escalation of therapy is much higher than 10% (currently greater than or equal to 67% with moderate to strong intensity, or equivalent to an Allred score of 7 or 8)[46,47].

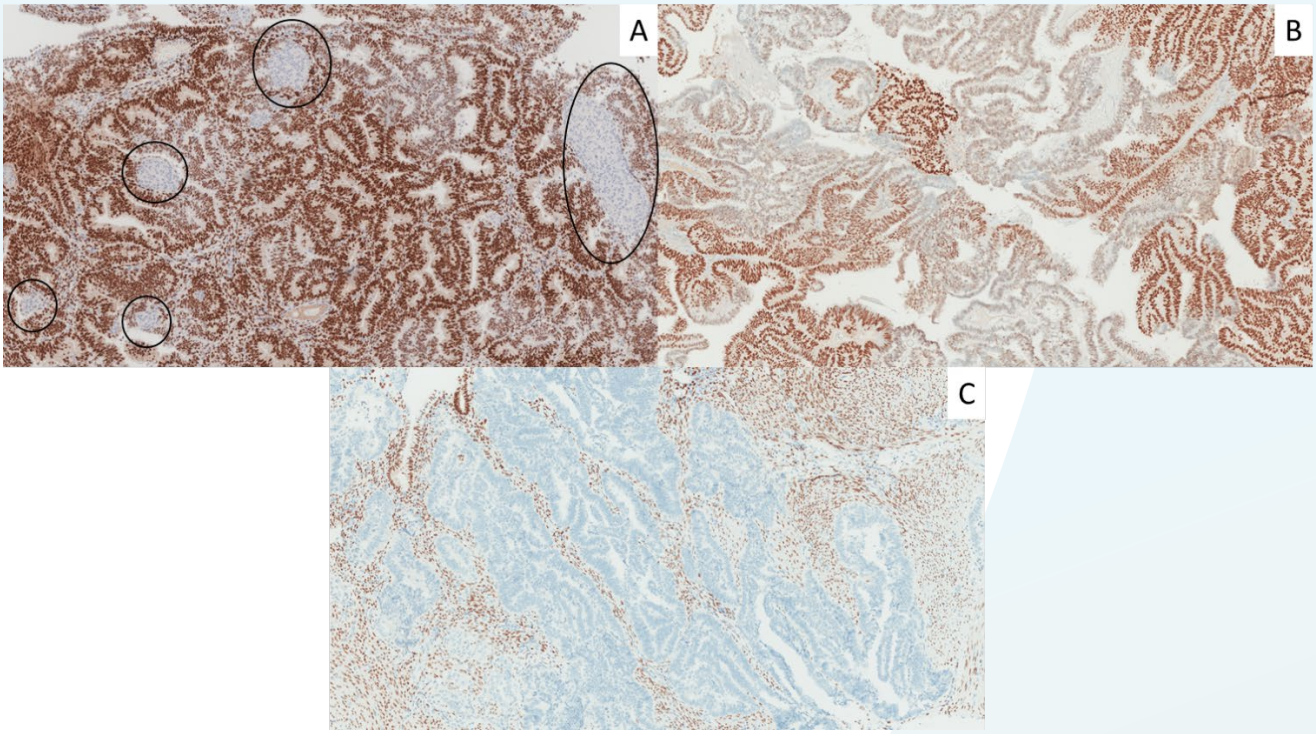

Figure 10. ER expression in EC. A. This example shows strong and relatively diffuse expression but note, areas of morular metaplasia are negative (circles). B. This tumour shows diffuse expression but there is variability in the staining intensity. C. The example shows a complete lack of expression; note the background internal control staining.

Interpretation of HER2/neu immunohistochemistry

1) Evaluation of Her2/neu immunoexpression in EC, like in other carcinomas from different organs sites, is scored according to a 0-3+ scoring scheme where 0 and 1+ are considered negative, 2+ is equivocal and 3+ is positive[48]. Membranous expression is evaluated, and it may be complete or lateral/basolateral in location. A number of scoring schemes exist and ongoing work in this field is being undertaken but, to ensure consistency, scoring for both Her2/neu IHC and FISH should be performed according to the current College of American Pathologists (CAP) gynecologic molecular protocols[49]. Examples of Her2/neu IHC expression in EC are shown in Figure 11.
2) Her2/neu expression is known to be highly heterogeneous (Figure 12). As such, there should be, in general, a low threshold for repeat testing. This phenomenon, in addition to the question regarding the optimal specimen or specimens for testing, are ongoing areas of research[50].

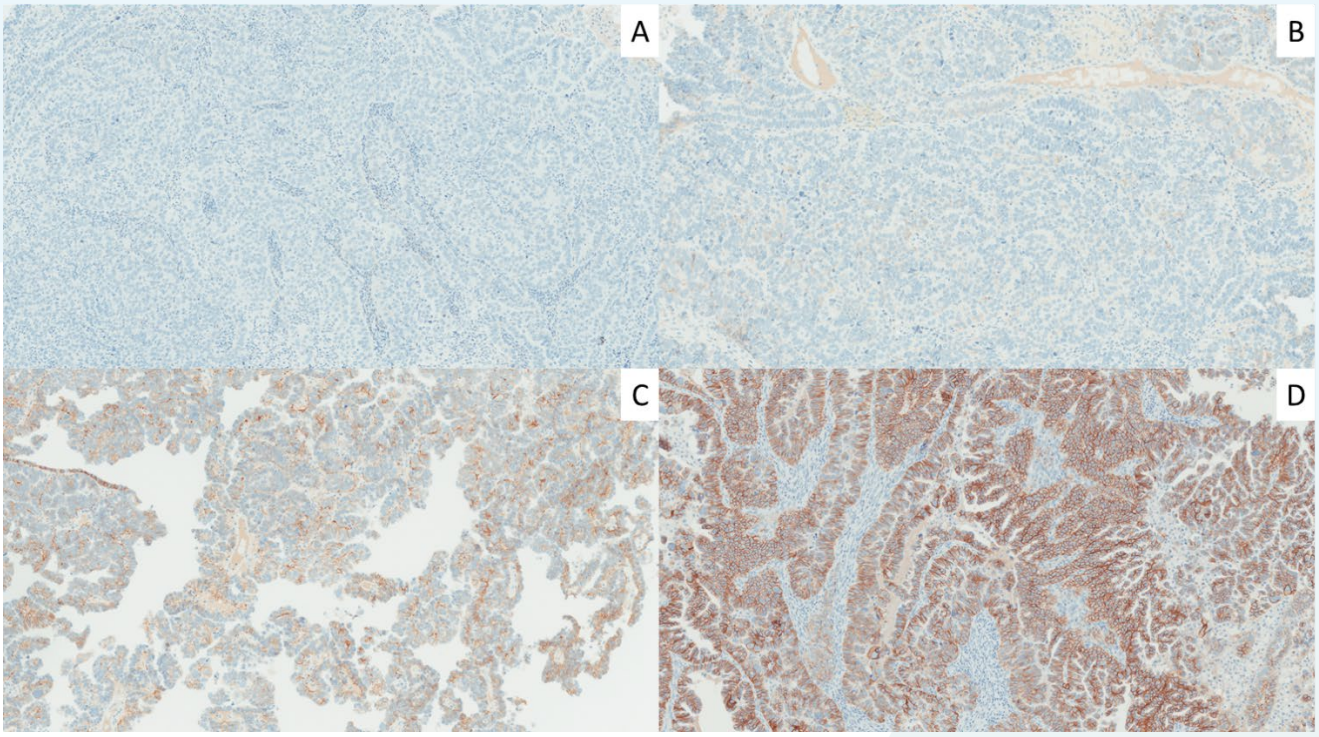

Figure 11. Examples of HER2 immunohistochemical expression in EC. Assessment should be undertaken in serous carcinoma and other tumours falling into the p53-driven molecular subgroup. A. Score of 0 – no expression. B. Score of 1+ – this example shows weak/barely perceptible expression. C. Score of 2+ – this example shows weak to moderate expression in >10% of tumour cells. HER2 FISH should be initiated when a score of 2+ is assigned. D. Score of 3+ – this example shows intense complete and lateral/basolateral membranous expression in >30% of tumour cells.

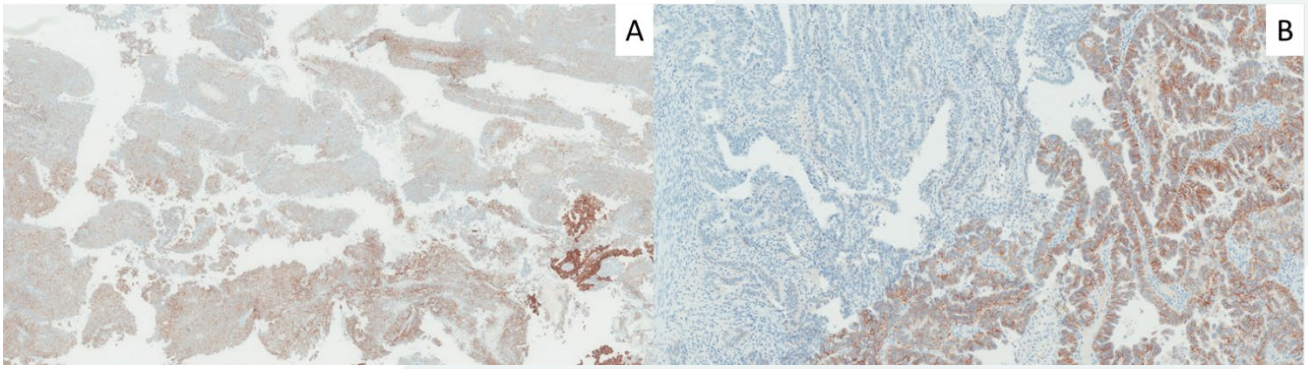

Figure 12. Heterogeneity in HER2 immunohistochemical expression in EC. A. This image shows fragments of tumour with varying intensity of expression, appreciable at low power magnification. B. In this example, there is an evident abrupt juxtaposition between the portion of the tumour in the top left of the image (weak/barely perceptible expression) and the bottom right of the image (moderate to strong complete and lateral/basolateral membranous expression).

Other Biomarkers

The Ontario Health (Cancer Care Ontario) Comprehensive Cancer Biomarker Testing Program supports patient management by making sure patients have access to standardized, comprehensive, evidence-based molecular testing for EC. Additional details can be found in the latest version of the Comprehensive Cancer Biomarker Testing Program guidance.

MMR, p53, ER and HER2 immunohistochemistry and *POLE* mutation testing are discussed in the sections above.

Progesterone receptor (PR) immunohistochemistry, while not part of the molecular classification and prognostication algorithm, still has some utility from a diagnostic perspective and also provides information in the fertility-sparing and recurrent/metastatic disease contexts.

In Ontario, most, if not all, centers performing NGS will utilize a panel approach (i.e., sequencing of additional genes beyond *POLE*). Evaluation of alterations in *TP53* and clinically significant variants should be reported if the information is available. Mutant pattern p53 IHC expression and *TP53* mutation status are concordant approximately 90 - 95% of the time[15,44] and while p53 IHC results allow for quick reporting and triage of patients to a GOC for management, *TP53* mutation testing also allows for the identification of the small proportion of cases with a clinically significant *TP53* variant and normal p53 IHC expression. Reporting of variants in *KRAS*, *CTNNB1*, *PIK3CA* and *PTEN* is encouraged for diagnostic and research purposes but is optional.

Sample Language for Management Guidance in Pathology Reports

Endometrial cancer with ANY of the following characteristics should be referred to a gynecologic oncology centre (GOC):

1) Grade 2 and 3 endometrioid and all high-grade carcinomas
2) Any abnormal p53 expression (including subclonal pattern) regardless of histologic type or grade
3) MMR deficient (MMRd) tumours (including subclonal pattern), regardless of histologic type of grade
4) ER negative carcinomas (10% or less nuclear staining by immunohistochemistry)

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