Molecular pathology of endometrial cancer: recent advances in classification, prognostication, and management

Mona Alfaraidi
Jutta Huvila
C Blake Gilks

Abstract

Endometrial carcinoma is the most common gynaecological malignancy in the UK and its incidence is increasing worldwide. The classification of endometrial carcinoma (EC) has been based on cell type (histotype) for decades, and this, together with grade, lymphovascular space invasion (LVSI) and stage of the tumor was used for risk assessment, guiding decisions about the extent of surgery and the need for post-surgical adjuvant treatment. There has been, and remains, considerable variation in clinical practice worldwide, with respect to both the extent of surgery (lymph node dissection, omentectomy, pelvic washings) and use of adjuvant therapy (radiation, chemotherapy, or both) for patients with identical risk factors but treated at different centers. Furthermore, EC has tended to be treated as a single disease, irrespective of histotype. In the past five years there has been a significant move to more personalized risk assessment and treatment with the introduction of routine molecular assessment of EC, and diagnosis of molecular subtype. The four EC molecular subtypes differ with respect to molecular pathology, genetic and environmental risk factors, precursor lesions, prognosis and response to specific treatments. This review will discuss the assessment of EC molecular subtype in clinical practice and how this information impacts on risk assessment and treatment.

Keywords Endometrial carcinoma; MMR; molecular subtype; p53; POLE

Mona Alfaraidi *MBBS Gyn Fellow, Department of Pathology, Vancouver General Hospital and University of British Columbia, Vancouver BC Canada. Conflicts of interest: none declared.*

Jutta Huvila *MD PhD Consultant Pathologist, Department of Pathology, University of Turku, Turku University Hospital, Turku, Finland. Conflicts of interest: none declared.*

C Blake Gilks *MD Consultant Pathologist, Department of Pathology, Vancouver General Hospital and University of British Columbia, Vancouver BC Canada. Conflicts of interest: none declared.*

Introduction

This review will discuss the development of surrogate molecular markers for subclassification of EC into one of four molecular subtypes, emphasizing the main characteristics of each molecular subtype, including prognosis and opportunities for subtype-specific treatment.

In order to understand current practice, with routine assessment of molecular markers and diagnosis of endometrial carcinoma (EC) molecular subtype, we will first briefly review the history of endometrial carcinoma subclassification (Goebbels review). Endometrial carcinoma was divided into two major pathogenic types by Bokhman in 1983.[1] According to Bokhman's dualistic classification, type 1 endometrial carcinoma is associated with unopposed estrogen stimulation, prototypically is of low grade endometrioid histotype and is associated with an excellent prognosis, while type 2 endometrial carcinoma is unrelated to estrogen stimulation and is of serous histotype with high-grade cytological features and aggressive behavior. This framework was very useful for illustrating the diversity of endometrial carcinoma biology but never entered clinical practice. There were far too many cases that could not be readily classified as type 1 or type 2. EC has therefore been subclassified in practice based on tumour cell type (histotype) and FIGO grade rather than as type 1/type 2, but the assessment of both of these aspects of tumour morphology is subject to considerable inter-observer variability, even among expert gynaecological pathologists. Both histotype and grade, as diagnosed on the biopsy specimen, can change significantly based on examination of the hysterectomy specimen. This lack of diagnostic reproducibility accounts for some of the variation in EC treatment between different centers, and has held back clinical trials (for example, if a trial is to enroll patients with serous endometrial carcinoma, will that be based on central or local histotype diagnosis, and will it be based on the biopsy or hysterectomy specimen?).

This approach to EC subclassification started to change in 2013 when The Cancer Genome Atlas (TCGA) recognized four distinct molecular subgroups based on genomic architecture (somatic copy number alterations, microsatellite instability, and tumor mutational burden).[2] These four prognostically significant molecular subtypes were: Polymerase Epsilon (*POLE*) ultra-mutated, microsatellite instability (MSI) hypermutated, copy number low and copy number high endometrial cancer. The latter two groups do not have *POLE* mutations or MSI and are characterized by low numbers versus high numbers of somatic copy number alterations, respectively. This was an exciting insight into the complexity of endometrial carcinoma i.e. that EC can be assigned to one of four categories based on genomic architecture, but the approach used by TCGA to classify individual tumors was not applicable in routine clinical practice, as expensive and highly specialized analyses were undertaken for this study, using snap-frozen tumour samples, with poorly defined boundaries between the groups. In order for a tumour subclassification system to be of sufficient value to warrant it being used in practice it must be: 1. Clinically relevant, providing information that can meaningly inform patient care, 2: Reproducible, such that results of classification performed on different samples (biopsy versus hysterectomy) or in different centers are highly concordant, 3: Able to consistently

classify tumours into a single category, with few or no indeterminate or equivocal results, 4: Accessible and affordable (acknowledging that these aspects vary considerably in differently resourced settings worldwide). With regards to clinical relevance it is necessary that this aspect be consistently demonstrable, across different patient populations, to ensure the generalizability of results.

Surrogate markers for molecular classification of endometrial carcinoma in clinical practice

Two research groups independently and simultaneously developed straightforward and reproducible algorithmic approaches to identify the four molecular subtypes of EC.[3–9] The Vancouver group used population-based cohorts from Canada and Germany to develop the ProMisE (Proactive Molecular Risk Classifier for Endometrial Cancer) classifier while the TransPORTEC group used clinical trials cases to arrive at the same classifier algorithm (Figure 1). These different cohorts used the by the two groups proved to be complementary and has provided a robust validation of the clinical significance of molecular subtype diagnosis. Three molecular features are assessed in order to assign molecular subtype: pathogenic mutations in the exonuclease domain of POLE, mismatch repair (MMR) protein expression by immunostaining, and p53 immunostaining for wild type versus mutational expression patterns. These are then interpreted in a stepwise approach to determine molecular subtype (Figure 1). The TCGA initially only included EC of endometrioid and serous histology, however the Leiden/TransPORTEC and Vancouver/ProMisE validated this approach to molecular classification in EC of rare histotypes such as clear cell, carcinosarcoma and undifferentiated carcinoma. This pragmatic approach to molecular subtype diagnosis has been applied in many thousands of cases and it is important to note that it is this approach that is validated against treatment and patient outcome in multiple cohorts, not that of the original TCGA study. Although the TCGA first identified the four molecular subtypes, subsequent studies have used surrogate markers of the molecular subtypes, not the original TCGA methodology. It is these subsequent studies that have demonstrated that molecular subtype diagnosis fulfills the requirements of a classification system suitable for routine use in practice i.e. it is clinically relevant, reproducible both between biopsy and hysterectomy, and when different laboratories analyze the same tumour, allows a molecular subtype to be diagnosed in almost every case (see discussion below about tumours with more than one molecular classifying feature, so called "multiple classifier" ECs, and it is relatively inexpensive and (increasingly) widely available.

*POLE*mut: polymerase epsilon ultra-mutated endometrial carcinoma

Polymerase epsilon (*POLE*) is a gene involved in DNA replication with a proofreading activity that can correct DNA synthesis errors and help protect against genomic instability. Somatic mutations in the proofreading exonuclease domain of *POLE* leads to tumors with very high tumor mutation burdens (ultramutated). *POLE*-mutated EC have one of the highest somatic mutation frequencies of any tumor, accumulating more than 100 genetic variants per megabase (Mb). The mutations associated with pathogenic *POLE* mutations are very characteristic: >20% C>A substitutions, >4% T>G substitutions, <0.6% C>G substitutions, <5% indels, and >100 mut/Mb. It is important to note that *not all mutations in POLE are pathogenic*. Mutations outside the exonuclease domain are never pathogenic and are relatively commonly encountered in mismatch repair deficient endometrial carcinoma. The *in silico* tools for prediction of mutation effect must be used with caution in the interpretation of mutations in *POLE*. The pathogenic mutations are consistently in the exonuclease domain and are associated with a change in function, rather than loss of function. Truncating mutations, for example, that are predicted to result in loss of function, are not pathogenic, and do not give rise to the characteristic genomic alterations of *POLE*mut endometrial carcinoma.

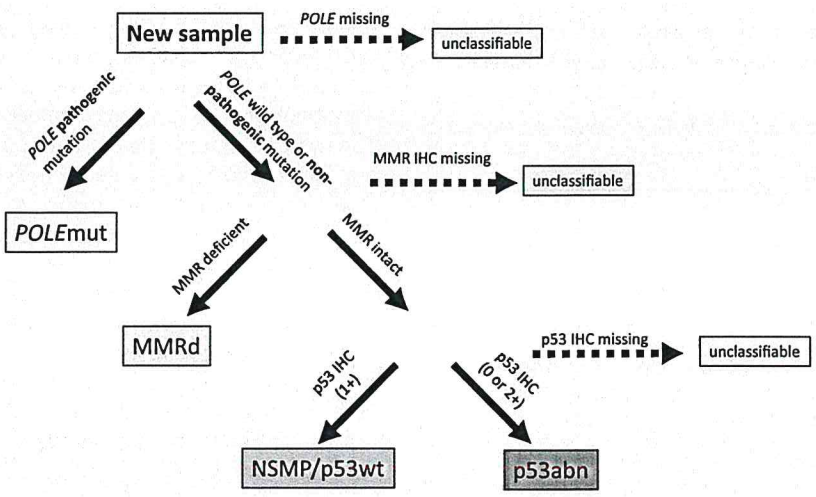

Figure 1 ProMisE algorithm. Classification begins with identifying ECs with pathogenic POLE mutations, next mismatch repair deficiency (loss of MMR proteins on immunostaining), and finally identifying aberrant vs. wild type p53 immunostaining. Approximately 3% of ECs harbour more than one molecular classifying feature ('multiple-classifier' ECs) with the shown order of segregation appropriately defining their predominant tumor biology and clinical behaviour.

At the time of writing this review there are only 11 confirmed pathogenic mutations in *POLE*;[9] this number will undoubtedly grow over time, as new mutations are characterized, but it is important to only diagnose *POLE*mut molecular subtype based on confirmed pathogenic mutations, given the implications of such a diagnosis, with possible de-escalation of treatment (discussed below). The P286R and V411L mutations account for 75–80% of all pathogenic *POLE* mutations. The detection of pathogenic *POLE* mutations can be done by sequencing the exonuclease domain, or use of PCR based assays to detect specific hot-spot mutations. To date, there is no immunohistochemical surrogate marker to identify *POLE*mut EC. Pathogenic *POLE* mutations are detected in 7–10% of endometrial cancers. *POLE*mut EC occur in younger women, present at an early stage (confined to the uterine corpus in almost every case) and have an exceptionally good prognosis despite frequently showing high-grade morphology and/or extensive LVI.[10] The morphology of *POLE*mut EC varies but they are typically endometrioid with prominent intratumoral lymphocytic infiltration and scattered tumor giant cells (Figure 2). Morphological heterogeneity within the tumour can be prominent, and serous, clear cell, and mixed histotypes have all been reported. There are no morphological features of clinical significance in *POLE*mut EC; neither FIGO grade nor histotype shows an association with prognosis. This favorable prognosis of *POLE*mut raises the possibility of safely de-escalating adjuvant therapy in these patients; a study conducted by McAlpine et al. showed that patients with pathogenic POLE mutations appear to not benefit from adjuvant therapy.[10] Given their high immunogenicity, these tumours are regarded as potential candidates for immune checkpoint inhibitors in recurrent and advanced disease.

MMRd: microsatellite instability hypermutated endometrial carcinoma

MMR proteins are responsible for repairing DNA mismatch errors during DNA replication, introducing errors in microsatellites, which are short tandem repeats found in the human genome. Microsatellite instability (MSI) is the genomic phenotype that is seen as a result of mismatch repair deficiency caused by inactivation of MMR genes: *MLH1*, *MSH2*, *MSH6*, and *PMS2*. Mutations in these genes, either germline mutations as in Lynch syndrome, or somatic mutations, result in MMRd EC. The majority of cases of MMRd EC are unrelated to mutations in the MMR genes but are caused by epigenetic silencing of *MLH1*. MMRd EC have a high a mutational burden, but not as high as in *POLE*mut tumours, and account for approximately 25–30% of EC.[11] Three methods for testing MSI/MMR are clinically available: PCR, sequencing, and immunohistochemistry. PCR based methods classify MSI status into high (MSI-H) when there is more than 1 abnormality in the 5 DNA microsatellites assessed, low (MSI-L) when one abnormality is present, and microsatellite stable (MSS) when no abnormality is detected. Sequencing of microsatellites can be done as part of next generation sequencing panels, but the bioinformatics for determination of MSI-H versus MSI-L or indeterminate versus MSS must be validated against an assay with known test performance characteristics. It is important to reiterate that most MMRd EC do not have a somatic or germline mutation in the MMR genes, so simply sequencing these genes as

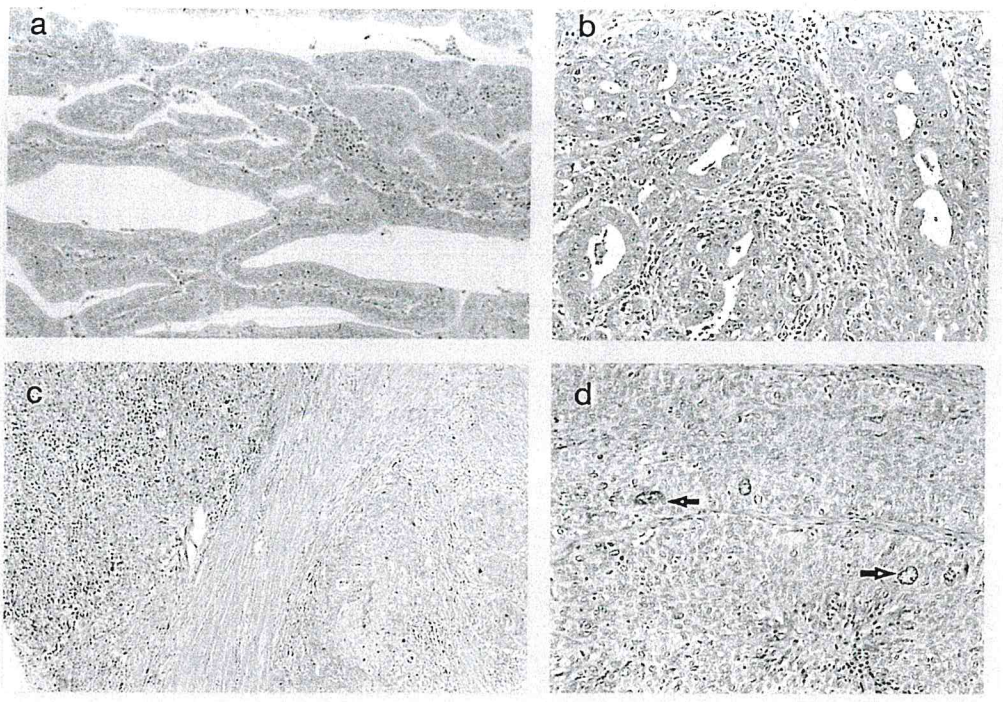

Figure 2 The morphological diversity of *POLE*mut endometrial carcinoma. Four examples of tumours containing a pathogenic mutation in POLE. **(a)**. Low-grade endometrioid morphology, with no unusual features. **(b)**. Glandular architecture with high-grade nuclear features (serous morphology). **(c)**. High-grade tumour with intra-tumoral heterogeneity. There is high-grade carcinoma in the lower right while the tumor at the left shows solid and spindle growth, with numerous intra-tumoral lymphocytes. **(d)**. Solid high-grade carcinoma with large bizarre nuclei (arrows).

part of an NGS panel will not allow accurate diagnosis of MMRd molecular subtype.

The interpretation of PMS2, MLH1, MSH6 and MSH2 immunostaining has evolved significantly over the last decade and readers are referred to the British Association of Gynaecological Pathologists website (https://www.thebagp.org/) for a detailed guidance document on interpretation of these very important molecular markers. For example, it is no longer true that there must be complete absence of immunostaining to consider a tumor MMR deficient; MSH6, in particular, can show much reduced expression, less than in the normal cells that serve as an internal control, and this results in MMR deficiency (Figure 3). Immunostaining for the four MMR proteins (MLH1, MSH2 MSH6 and PMS2) is highly concordant with MSI status as determined by PCR,[12] but occasional false negative results can occur with either approach. While loss of nuclear expression of MSH2, MSH6 and PMS2 are more likely secondary to a germline mutation in these genes, intact expression does not exclude Lynch syndrome. In the case of MSH6 loss, in particular, the PCR or sequencing-based microsatellite assays may show false negative results i.e. microsatellite stability. Given that Lynch Syndrome associated with a mutation in *MSH6* is more likely to present with EC rather than a GI malignancy, this can be a more problematic issue for EC than in Lynch syndrome screening of gastrointestinal cancers.

Absent MLH1 immunostaining is commonly secondary to a sporadic *MLH1* promoter methylation,[13] as noted previously, and there can be subclonal loss of MLH1 expression. At this time the threshold for considering a tumor to be MMRd is not evidence based but in practice any degree of MLH1 loss of expression is noted in the final diagnostic pathology report, and subclonal loss in more than 10% of tumour cells will typically lead to a diagnosis of MMRd molecular subtype.

Because both immunostaining and DNA-based assessment of MSI can give occasional false negative results, either loss of nuclear immunostaining in any of the four MMR proteins on immunostaining or MSI as determined by PCR or sequencing will result in a tumor being diagnosed as MMRd. There are histological similarities between MMRd EC and POLE-mutated EC; they both frequently are high grade and endometrioid histotype with abundance of tumor infiltrating lymphocytes and extensive lymphovascular invasion (Figure 4).

MMRd EC carry an intermediate prognosis and when associated with Lynch syndrome the prognosis is more favorable than

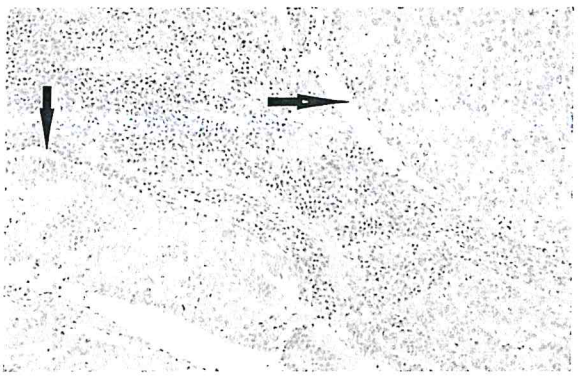

Figure 4 Mismatch repair deficient (MMRd) endometrial carcinoma showing intratumoral heterogeneity, with both low-grade (vertical arrow) and high-grade (horizontal arrow) components, and numerous intra-tumoral lymphocytes.

that of sporadic MMRd EC.[11] There are predictive implications in identifying MMRd EC as these tumors benefit from FDA-approved check point inhibitors and demonstrate an increased response to radiotherapy even in the absence of adjuvant chemotherapy.[14] The MMRd endometrial carcinomas associated with MLH1 methylation occur at an older age, on average, than those associated with Lynch syndrome and, as noted previously, have a worse prognosis. The only morphological features of prognostic significance in MMRd EC are the presence of extensive LVI or the presence of an un/dedifferentiated component. FIGO grade or histotype are not of prognostic significance with the exception of un/dedifferentiated EC, half of which have been shown to be MMRd, and these will be discussed in more detail below.

p53abn: copy number high/*TP53* mutated endometrial carcinoma

After the identification of *POLE*mut and MMRd molecular subtypes, the remaining cases of EC are subclassified based on p53 immunostaining. It is important to first identify the *POLE*mut and MMRd molecular subtypes as correct interpretation of p53 immunostaining/*TP53* sequencing is only possible in *POLE* wild type and MMR proficient endometrial carcinomas. This will be discussed in detail, below, in the section on Multiple Classifiers i.e. endometrial carcinomas with more than one molecular

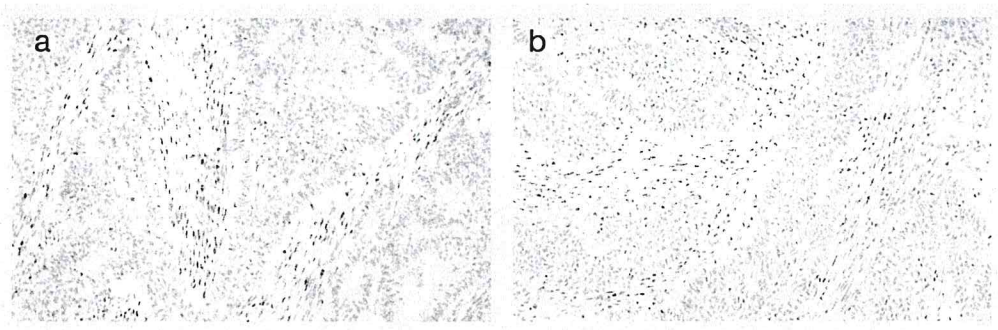

Figure 3 There is markedly reduced expression of MSH6 expression, relative to the internal control of lymphocytes and benign stromal cells (a), and this is associated with complete loss of MSH2 expression (b) in this mismatch repair deficient (MMRd) endometrial carcinoma. Although most MMRd endometrial carcinomas show complete loss of expression of one or more MMR proteins on immunostaining, there are also occasional tumours where there is abnormal MMR expression that is greatly attenuated but not lost completely.

feature. The emergence of a single simple immunostain as a surrogate marker for the genomic architecture (high numbers of somatic copy number abnormalities a.k.a. copy number high) seen in the most aggressive molecular subtype of EC started with the TCGA data, where the copy number high group almost all had mutations in *TP53* (especially when taking into consideration conservative calls of mutations in that study, such that some mutations may have been missed), while only a single tumour in the copy number low group had a *TP53* mutation (Figure 5). Based on this, p53 immunostaining, TP53 sequencing, and FISH for copy number alterations were assessed as surrogate markers of the copy number high molecular subtype, with p53 immunostaining being taken forward.[6] p53 immunostaining and *TP53* mutational analysis show high concordant rates in endometrial cancer, and p53 IHC can be used as a surrogate marker for the detection of p53abn EC.[15,16] Tumours of this molecular subtype are characterized by a low mutation rate but show the highest number of somatic copy number alterations. Mutant pattern p53 staining can be diffuse nuclear overexpression, complete absence (null), or cytoplasmic with variable nuclear staining (Figure 6). These mutant staining patterns, in general, reflect the underlying type of *TP53* mutations; nuclear overexpression occurs as a result of *TP53* missense mutations, null staining is secondary to loss of function mutation (deletion or nonsense mutations), and cytoplasmic staining secondary to mutations in the nuclear localization domain of the p53 protein. For cases with nuclear overexpression, the International Society of Gynecological Pathologist recommend that at least 80% of tumor cells show strong nuclear staining.[17]

p53abn EC accounts for 15 % of EC and presents in older postmenopausal women. They mostly show a serous or serous-like histology but also include high-grade endometrioid carcinomas, carcinosarcomas, and carcinomas with mixed histology. Less than 5% of low-grade endometrioid carcinoma show mutant pattern p53 staining.[18]

Occasionally there is subclonal mutant pattern p53 expression (Figure 6d).[16] In some such cases there is an underlying *TP53* mutation present in both the areas with mutant pattern expression and wild type expression, recognized by the presence of a TP53 mutation at a high allelic frequency, while in an approximately equal number of cases only the area of tumour with subclonal mutant pattern p53 immunostaining pattern has a *TP53* mutation, and the wild type staining correlates with wild type *TP53* on sequencing. In this latter situation it is assumed that the p53abn component has arisen through tumor progression from a p53 wild type/*TP53* wild type NSMP EC, provided *POLE* is wild type and MMR is intact/normal. In the former situation the tumors are best classified as p53abn EC while in the latter situation it is suggested to report the extent of subclonal p53 mutant expression and, although no standardized cut off is agreed upon, a 10% cut off identified patients with more aggressive disease, supporting its use to separate p53abn EC from NSMP EC when there is a small subclonal population of tumour cells with mutant pattern p53 expression.[16]

Endometrial carcinomas of this molecular subgroup are associated with aggressive behavior and carry the worst prognosis among all molecular subgroups regardless of histotype, grade and stage at presentation. There are no differences in outcome between p53abn serous, high-grade endometrioid and carcinosarcomas.[19] Even p53abn low-grade endometrioid carcinomas have a significantly increased likelihood of recurrence and death due to disease, compared to NSMP low-grade endometrioid carcinomas.[18] p53abn EC accounts for the majority of EC associated mortality. Patients with p53abn EC have significantly improved outcomes when treated with combined adjuvant chemotherapy and radiotherapy rather than radiotherapy alone.[20] A proportion of this molecular subtype have homologous recombination deficiency (HRD) suggesting the use of poly (ADP-ribose) polymerase inhibitors (PARPi) as a potential therapeutic agent.[21] Balestra et al. demonstrated that overexpression of HER2 was present in 31.4% of p53abn endometrial carcinomas and that among these, 36.3% showed *HER2/neu* gene amplification.[22] Patients with p53abn EC and *HER2/neu* amplification, benefit from trastuzumab, a monoclonal antibody against *HER2* receptor, in combination with chemotherapy as indicated by results of a phase II

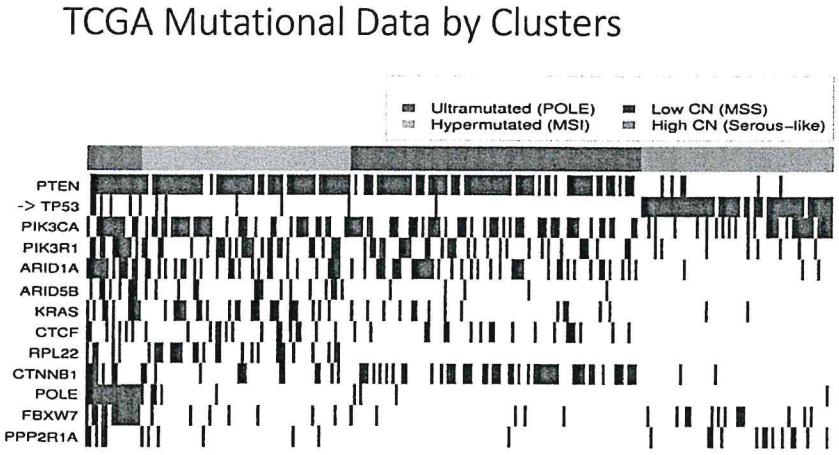

Figure 5 Data from the original case series studied by The Cancer Genome Atlas, with genes listed at the left, and the four molecular subtypes indicated at the top. The presence of a green vertical bar indicates a single tumour with a mutation present, while a white bar indicates wild type sequence. Note that *TP53* mutations are present in all but 5 of the copy number high (high CN) tumors, while *TP53* is wild type in all but one of the copy number low (Low CN) tumours.

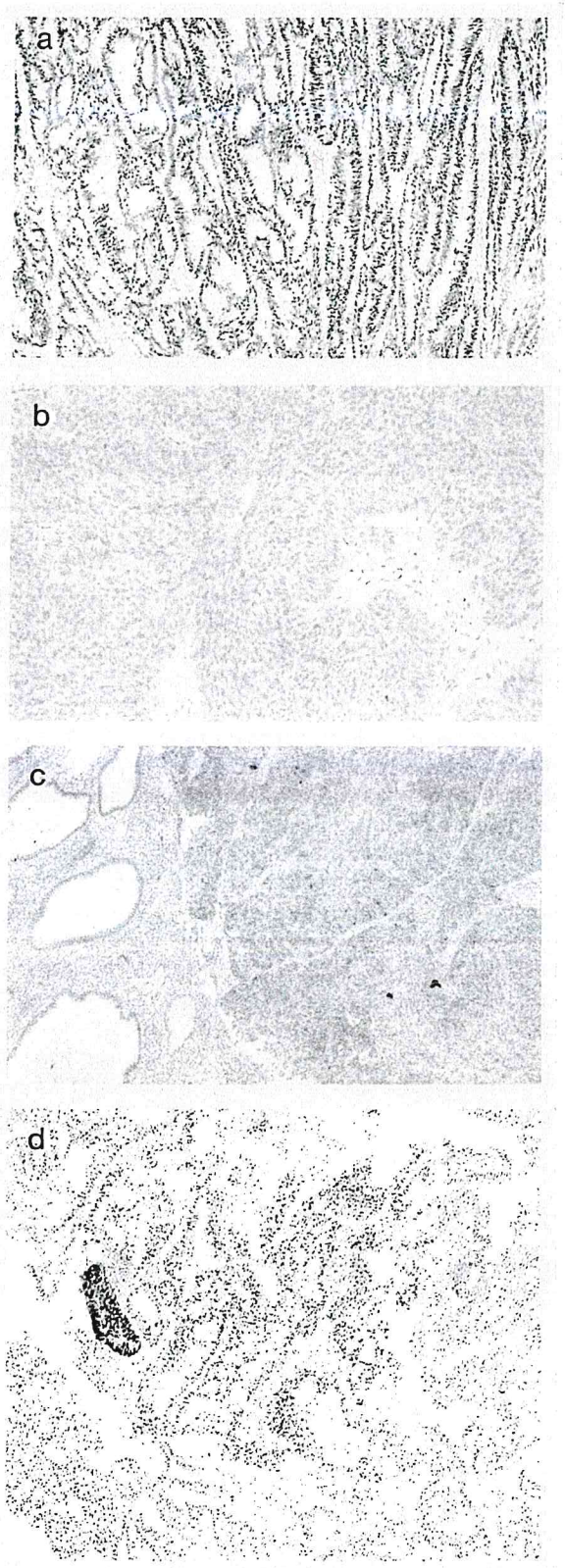

Figure 6 p53 immunostaining indicative of an underlying *TP53* mutation: **(a)**. Overexpression, the most common pattern encountered in practice, **(b)**. Null (complete absence of staining in tumour cells), **(c)**. Cytoplasmic, the least common mutation-associated staining pattern, and **(d)**. Subclonal mutant pattern p53 staining, with overexpression in a single gland (in this case less than 10% of the tumor cells show p53 overexpression); this very focal mutant pattern expression is not sufficient to warrant classification as p53abn molecular subtype.

clinical trial.[23] The ability to target HER2 in EC is evolving rapidly and introduction of a novel anti-body drug conjugate, trastuzumab deruxtecan, means there are likely to be changes in the use of HER-targeted therapy for EC, especially for the p53abn molecular subtype, where new and effective treatments are most needed.

NSMP: endometrial carcinomas of no specific molecular profile

NSMP ECs are defined by absence of any of the three molecular features (*POLE* mutation, MMR deficiency, *TP53* mutation) that are used to define molecular subtype. As a group they are characterized by few mutations and low numbers of somatic copy number abnormalities. They represent the most common subgroup (approximately 50% of all EC) and have an intermediate prognosis between the favorable *POLE*mut ECs and the aggressive p53abn EC. It is critical to recognize, however, that NSMP EC are clinically, pathologically and genetically heterogenous tumors. Approximately 80% of NSMP EC are "low-risk", defined as being low-grade (FIGO grade 1 or 2) endometrioid carcinomas with estrogen receptor (ER) expression.[23,24] They have a 1.6% 5 year disease specific death rate for all stages and 1.4% for stage I.[23] This subset, identifiable on biopsy or curetting specimens by performing p53, MMR and ER immunostains, has a prognosis similar to that of *POLE*mut EC. Because of this very favorable prognosis, the possibility of de-escalation of therapy can be considered for these low-risk NSMP EC. Further validation work is required, but the ability to identify low-risk EC based on a combination of histotype, grade and molecular features has the potential to dramatically change EC management, by identifying, based on the first diagnostic specimen, approximately half of patients with EC whose risk of recurrence/metastasis is so low that simple hysterectomy is adequate therapy. Furthermore, hormonal therapy to allow preservation of the uterus is an option in selected cases, an approach to treatment identical to that used for atypical endometrial hyperplasia/EIN. Because these low-risk NSMP EC arise as a result of prolonged estrogenic stimulation of the endometrium they often present in patients with significant co-morbidities, such as obesity and diabetes, where avoiding potential treatment-related complications is critical. The remaining 20% of NSMP EC are highly variable, consisting of FIGO grade 3 endometrioid carcinomas as well as other aggressive histotypes such as clear cell, mesonephric-like, gastric type and undifferentiated/dedifferentiated. These latter four histotypes have little in common with the rest of NSMP EC and will be considered separately below. A theme of this review and an increasingly important aspect of all areas of surgical pathology practice is the accurate subclassification of carcinomas; it would be a step backwards to suggest that high-risk NSMP EC, that fall outside the homogeneous low-risk group, can be considered as a uniform group of intermediate risk NSMP EC.[23,24] While the high ER expression in low-risk NSMP EC may indicate the possible benefits of endocrine therapy, there is no role for endocrine therapy in the rare histotypes of NSMP EC, which express little or no ER. With respect to a cut-off for separating low-risk NSMP and high-risk NSMP EC, there

remains work to be done. A large majority of low-grade endometrioid carcinomas, however, show intermediate to strong intensity of ER staining in a diffuse distribution. In contrast, ER expression in clear cell, gastric-type or mesonephric-like carcinomas are ER negative or show weak/focal staining. Given that a diagnosis of low-risk NSMP could result in treatment de-escalation it is important that the approach to diagnosis of this subset of EC be conservative, so as to minimize the chances of undertreatment, and a reasonable working cut-off for identification of an NSMP EC as being low-risk would be FIGO grade 1 or 2 AND intermediate to strong and diffuse ER positivity.

Rare endometrial carcinoma histotypes of NSMP molecular subtype

Clear cell carcinoma (CCC)

CCC of the endometrium are uncommon. As with CCC at other sites in the female genital tract, distinction between CCC and other histotypes with clear cells can be difficult. Clear cells can be seen in p53abn or MMRd EC, and these are best considered to be equivalent to p53abn or MMRd EC of other histotypes, respectively, for treatment purposes.[25] The prototypical immunophenotype of CCC is ER negative and positive for NapsinA and/or HNF-1beta. Because it is so rare the molecular pathology of CCC of the endometrium is not well characterized. All CCC are considered high grade.

Mesonephric-like adenocarcinoma (MLA)

Mesonephric-like adenocarcinomas were first recognized based on their resemblance to the cervical mesonephric carcinomas that arise out of mesonephric remnants. Unlike the cervical tumours, though, MLA of the endometrium are never associated with mesonephric remnants and may be admixed with conventional EC histotypes. These tumors were presumably diagnosed as endometrioid carcinoma in the past but are now recognized as a distinct entity in the WHO Classification of Female Genital Tumours. They show a wide range of architectural features, including glandular, papillary and solid architecture, and may have prominent spindle cell growth (Figure 7). They consistently lack squamous differentiation. The nuclei are uniform and may show features similar to papillary carcinoma of the thyroid but this is not consistent. Although architecturally differentiated they are clinically aggressive and are associated with pulmonary metastases in a significant number of cases, which is unusual for EC. The use of molecular markers has greatly enhanced detection of MLA; as well as the morphological features they typically show expression of GATA3 and TTF1, negativity for ER and *KRAS* mutations. At this time it is not clear which of these features are most important in diagnosis and how to handle those cases with some but not all the diagnostic features of MLA.

Gastric-type adenocarcinoma

Gastric-type adenocarcinoma of the endometrium was first recognized as a distinct entity relatively recently. It is identical, morphologically, to gastric-type carcinoma of the cervix, the most common of the HPV-independent cervical adenocarcinomas. Like CCC and MLA it is typically ER negative, although focal weak ER immunostaining may be observed (but less than is seen in low-grade endometrioid carcinoma). Although it can have bland cytological features (Figure 8) it is associated with aggressive behaviour. It is not possible based on immunophenotype to determine primary site of gastric-type adenocarcinoma that is present as detached fragments of tumour in a biopsy or curetting specimen. This is also true of CCC. Unlike CCC though, most gastric-like carcinomas arise in the cervix rather than endometrium.

Undifferentiated/dedifferentiated carcinoma

An uncommon variant of EC is the coexistence of low-grade endometrioid carcinoma and undifferentiated component lacking any evidence of glandular or squamous differentiation, and not always recognizable as being epithelial based on H&E (Figure 9). The designation of dedifferentiated is used if there is a conventional low grade component and undifferentiated if no such component is present. The undifferentiated component shows weak expression of epithelial markers such as broad spectrum cytokeratin and typically shows loss of PAX8 and ER. Up to half of these tumors are MMRd and loss of one or more of the proteins that form the SWI/SNF complex (SMARCA4, SMARCB1, ARID1A, ARID1B) are a common feature but are not required for diagnosis. The diagnostic criteria for cases showing some but not all the features of undifferentiated carcinoma are not yet worked out.

Multiple classifier EC

A small but clinically relevant group of EC harbor two or, less commonly, all three of the molecular features used to diagnose EC molecular subtype: these are referred to, collectively, as multiple classifier EC. They account for approximately 3% of EC. Because multiple classifier EC are so uncommon there is limited evidence regarding these tumours at present. A multi-center

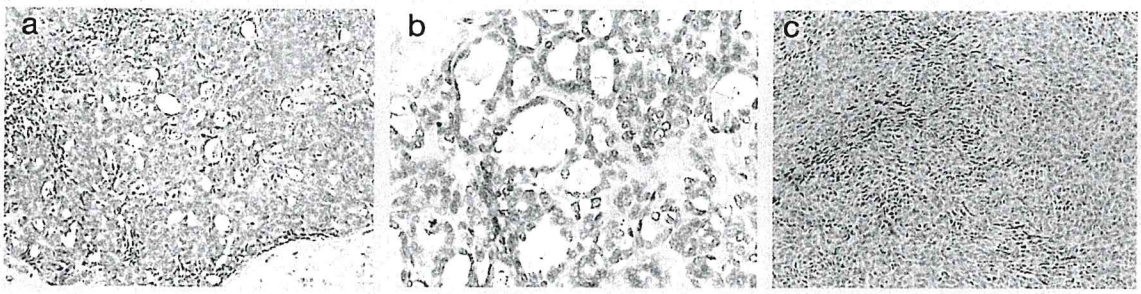

Figure 7 Mesonephric-like adenocarcinoma, with glandular differentiation (**a**), low grade nuclear features (**b**) and solid/spindled growth (**c**).

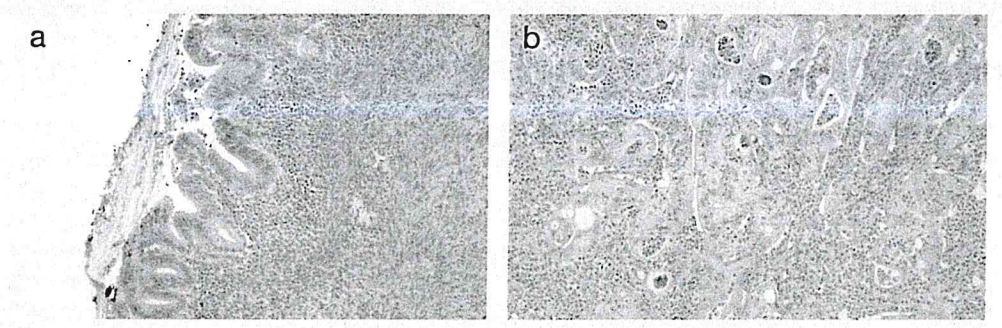

Figure 8 Gastric-type adenocarcinoma of the endometrium. Note the resemblance to low-grade endometrioid carcinoma (**a**). At the invasive front this tumour is less well differentiated (**b**).

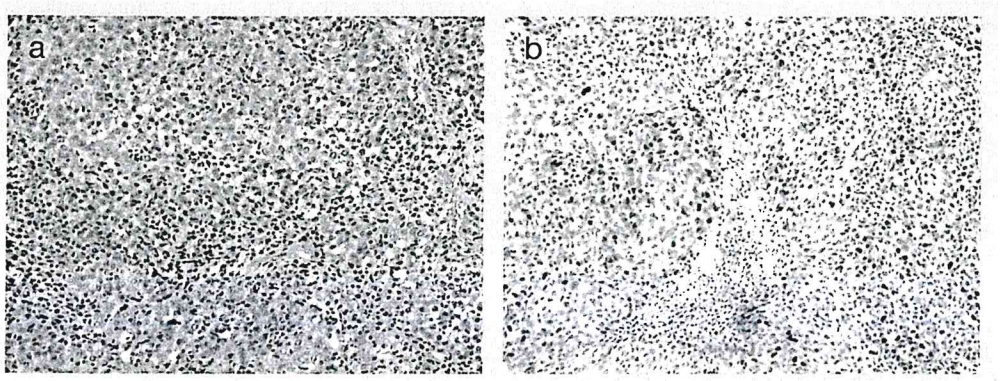

Figure 9 Undifferentiated carcinoma (**a**), with wild type p53 immunostaining (**b**). When a component of low-grade endometrioid carcinoma is present this would be diagnosed as "dedifferentiated carcinoma".

study by Leon-Castillo et al. drawing on more than 3000 cases of EC of known molecular subtype, identified 31 POLE/p53, 64 MMR/p53 and 13 POLE/MMR double classifier EC, and 12 triple classifier EC (i.e. POLE/MMR/p53 all showing abnormal results).[26] They showed that tumours with mutant pattern p53 expression and either MMR deficiency or *POLE* mutation have significantly better clinical outcomes than single classifier p53abn EC, with outcomes similar to that of single classifier *POLE*mut or MMRd EC, respectively. Subclonal p53 expression was frequently observed in these tumors suggesting that the *TP53* mutation occurs as a later event during progression of the tumor. Together these results suggest that the double classifier POLE/p53 and MMR/p53 EC can be considered to be *POLE*mut or MMRd, respectively, supporting the use of the classification algorithm (Figure 1).[26] De Vitis et al also found that *POLE*mut-p53abn EC share clinicopathological characteristics with *POLE*mut EC, however in contrast to Leon-Castillo' results, the clinicopathological characteristics of MMRd-p53abn EC appeared to be intermediate between MMRd EC and p53abn EC.[27] Regarding triple-classifier tumors (*POLE*-MMR-p53 EC) their biological behavior is uncertain, however the frequent presence of subclonal p53 staining in these tumors also suggest that *TP53* mutations are secondary events rather than driver mutations. There have also been too few MMR/*POLE* double classifier EC characterized to understand the biology and clinical significance of this particular multiple classifier. It is expected that with the spread of molecular profiling in routine clinical practice the number of patients diagnosed with multiple molecular classifying features will increase and additional studies aiming to further understand their biology are required. Note that the percentage of EC found to be multiple classifiers varies depending on the specific assays to assess molecular subtype, in particular whether p53 immunostaining or *TP53* sequencing is used. For example, mutations in *TP53* are picked up commonly in the ultramutated *POLE*mut EC (approximately 50% of tumours), but these mutations are often associated with wild type p53 expression (specifically in the *POLE*mut molecular subtype) so that a sequencing based approach to assessment of p53/*TP53* will identify more double classifiers than using p53 immunostaining.

The relationship between endometrial carcinoma histotype and molecular subtype

The question of how histotype relates to molecular subtype, and vice versa, is a critically important one, but this larger overarching perspective is often absent from discussions on molecular pathology of EC. As a starting point, it should be abundantly clear that molecular subtype and histotype are significantly correlated. For example, serous carcinomas and carcinosarcomas are almost always p53abn (Figure 10). It has been proposed that all endometrial adenocarcinomas with mutant pattern p53 expression, and wild type *POLE*/intact MMR be diagnosed as serous, but this means that tumors with squamous differentiation and glandular features i.e. clearly endometrioid based on H&E, would be diagnosed as "serous", making that morphological

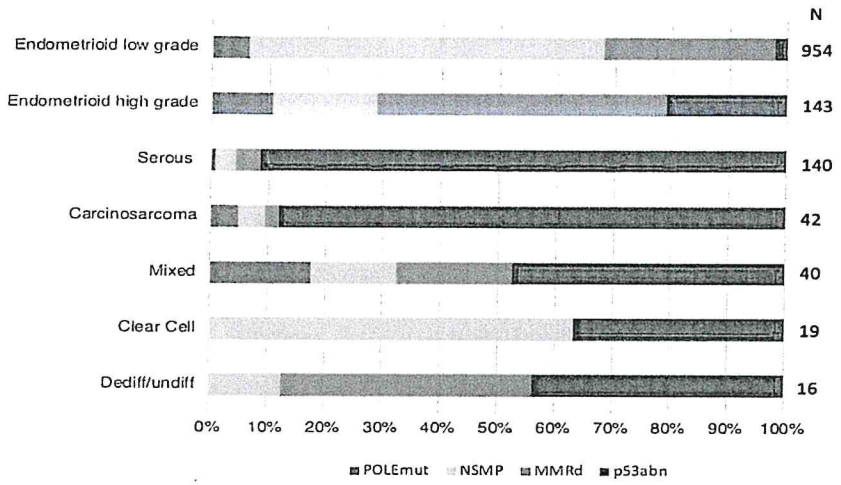

Figure 10 The number of cases of each molecular subtype, for different endometrial carcinoma histotypes, based on a series of >1000 cases from 2016. Reproduced from reference 28 with permission from Elsevier.

designation meaningless; it would carry no information about the tumor except for the results of p53 immunostaining. In considering the relationship between histotype and molecular subtype, it is important to acknowledge that while they are related there is not a perfect correlation (Figure 11), and histotype can only provide inaccurate insight into the molecular subtype. Over time, we have seen molecular markers influence histotype diagnosis (p53abn are more likely to be called serous, if morphology is ambiguous, while *POLE*mut and MMRd are more likely to be diagnosed as endometrioid, even when there are high-grade cytological features) but histotype never impacts on molecular subtype diagnosis, which is made independent of the histopathological features. The current state is that for p53abn there is no ability to subclassify meaningfully based on morphology. The same holds true for *POLE*mut. For MMRd it is important to recognize the more aggressive undifferentiated/dedifferentiated carcinomas, but otherwise histotype and grade are non-contributory. In contrast, for NSMP EC histotype is a critical variable and will remain so for the foreseeable future.

Molecular classification incorporated into risk assessment and FIGO staging

Molecular subclassification is of little value unless it impacts on patient treatment. In the case of EC molecular subtypes there was a relatively rapid move from identification of four molecular subtypes by TCGA to having those molecular subtypes incorporated into clinical practice guidelines today. There were multiple steps in this progression from a research finding to a clinical diagnosis, but some highlights include: rapid development of surrogate markers of molecular subtypes, with validation and verification of their prognostic significance in multiple cohorts (both clinical trials and population based case series), the wide availability of the surrogate markers in clinical practice, with robust quality assurance processes already in place, including

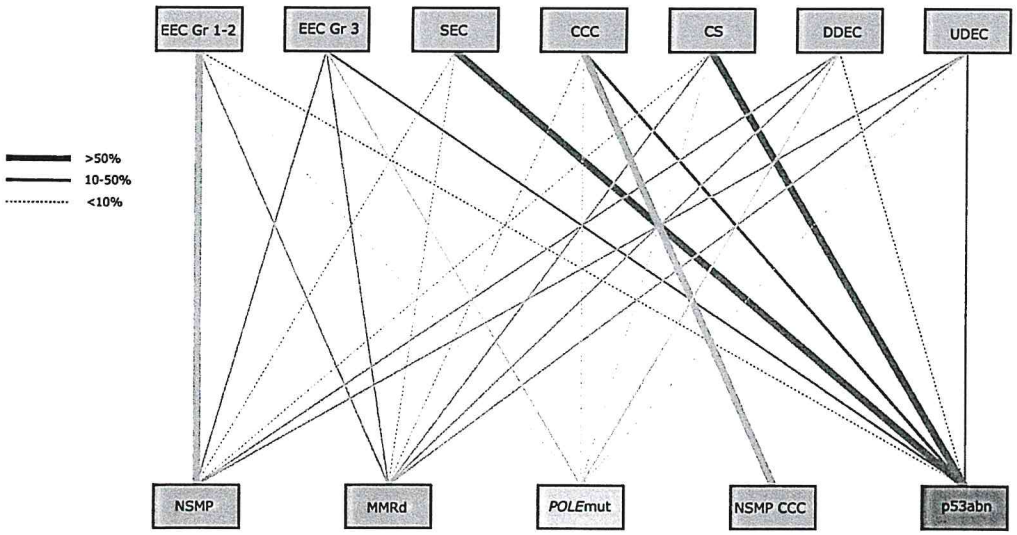

Figure 11 The relationship between histotype and molecular subtype depicted graphically. The thickness of the lines between histotype and molecular subtype indicate the percentage of the given histotype that are of that molecular subtype. Reproduced from reference 29 with permission from John Wiley and Sons.

external proficiency testing programs, demonstration of inter-observer and inter-laboratory concordance in molecular subtype diagnosis, and the very high degree of agreement between molecular subtype diagnosis based on biopsy and subsequent hysterectomy. The molecular subtype is uniform throughout with few exceptions (e.g. gain of *TP53* mutations during tumor progression) so that intratumoral heterogeneity has not been an insurmountable problem, and small samples can be analyzed with confidence that they reflect the properties of the tumour. To impact on patient management a molecular test can establish that the prognosis of a subset of patients is so favorable that they do not stand to benefit from a given treatment i.e. there is greater potential for harm than benefit if treatment is given. This is a very stringent cut off in clinical practice. For example, a biomarker might separate patients into those with a 40% versus 20% objective response rate to therapy, but the therapy will be given to both groups of patients, rendering testing irrelevant as it doesn't impact on the decision to treat or not treat. In the case of *POLE*mut EC and low-risk NSMP EC (low-grade endometrioid, ER+) the prognosis is so favorable that consideration can be given to offering no adjuvant treatment or even doing less extensive surgery. There is not level 1 evidence to support treatment de-escalation but clinical trials are on-going and until those results are available decisions about treatment must continue to be made. The wide variation we see in the extent of surgery and use of adjuvant therapy for EC reflects a paucity of level 1 evidence to inform more individualized treatment decisions. The biggest driver of molecular subtype entering clinical practice guidelines, however, was arguably the retrospective analysis of molecular subtype in the TransPORTEC clinical trials cases, and demonstration that the addition of chemotherapy to radiotherapy was associated with significantly improved outcomes for patients with p53abn EC but no improvement for patients with MMRd EC. This ability of molecular subtype to predict response to specific treatments must be confirmed in the setting of a prospective randomized clinical trial but until those results are available this data from an unbiased post-hoc analysis of a randomized clinical trial is sufficiently compelling evidence that the oncology community has chosen to incorporate it into treatment guidelines. The first were from ESGO/ESTRO/ECP,[30] with ESMO publishing similar guidelines shortly thereafter. In 2023 FIGO followed their lead and incorporated molecular subtype into staging.[31] As can be seen in Figure 12, the ESGO and FIGO categories, incorporating molecular subtype, are very similar. Whether staging should include tumour features or should only reflect the anatomic extent of disease remains controversial, and the British Gynaecological Cancer Society and British Association of Gynaecological Pathologists (and other groups) have recommended not adopting FIGO 2023. Whether FIGO 2023 is adopted or not, it remains true that molecular subtype diagnosis is widely used and all clinical practice guidelines recognize the importance of molecular subtype in the care of patients with EC.

Endometrial carcinoma diagnosis when molecular testing is not available

Worldwide there is considerable variability in the availability of molecular markers (immunostains, PCR, sequencing etc.). How does practice differ when such testing is not available? We will briefly compare three different scenarios: 1. when there is no biomarker testing available, 2. when there is immunostaining (p53, MMR, ER) but no sequencing or PCR testing for *POLE* mutations, 3. When full biomarker testing is available. In Box 1

	ESGO/ESTRO/ECP		FIGO2023
Low-risk	Stage I-II POLE mut, no residual disease	IA	IAm$_{POLEmut}$ POLEmut endometrial carcinoma, confined to the uterine corpus or with cervical extension, regardless of the degree of LVSI or histological type
	Stage IA MMRd/NSMP EEC low-grade + LVSI neg		IA1 Non-aggressive histological type limited to an endometrial polyp OR confined to the endometrium IA2 Non-aggressive histological types involving less than half of the myometrium with no or focal LVSI
			IA3 Low-grade endometrioid carcinomas limited to the uterus and ovary
Intermediate risk	Stage IB MMRd/NSMP EEC Low-grade + LVSI neg	IB	Non-aggressive histological types with invasion of half or more of the myometrium, and with no or focal LVSI
	Stage IA p53abn and/or non-EEC without myometrial invasion	IC	Aggressive histological types limited to a polyp or confined to the endometrium
	Stage IA MMRd/NSMP EEC high-grade + LVSI neg	IIC	Aggressive histological types with any myometrial involvement (see later)
High-intermediate	Stage I MMRd/NSMP EEC with substantial LVSI	IIB	Substantial LVSI of non-aggressive histological types
	Stage II MMRd/NSMP EEC	IIA	Invasion of the cervical stroma of non-aggressive histological types
	Stage IB MMRd/NSMP high-grade EEC (LVSI +/-)	IIC	Aggressive histological types *(high-grade EECs (grade 3), serous, clear cell, undifferentiated, mixed, mesonephric-like, gastrointestinal mucinous type carcinomas, and carcinosarcomas)* with any myometrial involvement
High risk	Stage I MMRd/NSMP *serous, undiff, and carcinosarcoma* with no residual disease		
	Stage I-IVA p53abn with myometrial invasion	IICm	IICm$_{p53abn}$ p53abn endometrial carcinoma confined to the uterine corpus with any myometrial invasion, with or without cervical invasion, and regardless of the degree of LVSI or histological type
	Stage III-IVA MMRd/NSMP EEC with no residual disease	IIIA	IIIA1 Spread to ovary or fallopian tube (except when meeting stage IA3 criteria)
			IIIA2 Involvement of uterine subserosa or spread through the uterine serosa
	Stage II-IVA MMRd/NSMP *serous, undiff, and carcinosarcoma* with no residual disease	IIIB	IIIB1 Metastasis or direct spread to the vagina and/or the parametria
			IIIB2 Metastasis to the pelvic peritoneum
		IIIC	IIIC1 Metastasis to the pelvic lymph nodes i micrometastasis, ii macrometastasis
			IIIC2 Metastasis to para-aortic lymph nodes up to the renal vessels, with or without metastasis to the pelvic lymph nodes i micrometastasis, ii macrometastasis
		IVA	Invasion of the bladder mucosa and/or the intestinal/bowel mucosa
Advanced Metastatic	Stage III-IVA with residual disease Stage IVB of any molecular type	IVB	Abdominal peritoneal metastasis beyond the pelvis
		IVC	Distant metastasis, including metastasis to any extra- or intra-abdominal lymph nodes above the renal vessels, lungs, liver, brain, or bone

Figure 12 Comparison of risk assessment/treatment guidelines from ESGO/ESTRO/ESP and the proposed FIGO 2023 staging guidelines for endometrial carcinoma. The assessment of individual cases of endometrial carcinoma is identical using these two systems for a large majority of patients.

Reporting of three endometrial carcinomas when no biomarker testing is available

Endometrioid carcinoma, FIGO grade 3
Endometrioid carcinoma, FIGO grade 1
Serous carcinoma

Treatment decisions will be made based on grade and histotype.

Box 1

three different EC diagnoses are provided, as they would appear in a diagnostic report. In limited resource settings surgery is typically the only available treatment and this report provides enough information to guide surgical management. In Box 2 a diagnosis is provided for the same three EC, but this time with addition of immunostaining results. Note that molecular subtype cannot be determined without assessment of *POLE* mutations as mutant pattern p53 expression can be seen in POLEmut EC and does not constitute evidence of p53abn molecular subtype unless *POLE* wild type and MMR intact is established. Compared to full molecular subtype this approach would result in occasional patients being treated as if they had a more aggressive tumour because their *POLE* mutation status is not known. There are only a small number of such patients, estimated at 2–3%. Not all patients with a *POLE*mut EC would receive treatment if the *POLE* mutation were not recognized; for example a stage Ia low-grade endometrioid carcinoma with wild type p53 staining and intact MMR expression would typically receive no additional treatment, irrespective of the presence of a *POLE* mutation. Some centres do *POLE* testing only when it will potentially change treatment e.g. deep myometrial invasion, high grade), to save costs. Box 3 provides diagnoses for the same three EC but with full molecular subtype diagnosis provided.

Reporting of the same three endometrial carcinomas listed in Box 1, but with immunostaining results for p53 and MMR available

Endometrioid carcinoma, FIGO grade 3 (MMR abnormal with MLH1 loss, wild type p53)
Endometrioid carcinoma, FIGO grade 1 (MMR intact, wild type p53)
Serous carcinoma (MMR intact, mutant pattern p53)

Note that molecular subtype cannot be diagnosed as *POLE* mutation testing has not been done.

Box 2

Reporting of the same three endometrial carcinomas listed in Boxes 1 and 2, but this time with the availability of all markers necessary for diagnosis of molecular subtype

Endometrioid carcinoma, FIGO grade 3, MMRd molecular subtype
Endometrioid carcinoma, FIGO grade 1, *POLE*mut molecular subtype
Serous carcinoma, p53abn molecular subtype

Box 3

Conclusion

The four molecular subtypes of endometrial carcinoma (*POLE*mut, MMRd, p53abn, NSMP) have become vital determinants in planning patient management. The EC molecular subtypes differ with respect to molecular pathology, genetic and environmental risk factors, precursor lesions, prognosis and response to chemotherapy, including immunotherapy. As a result, reflex testing for molecular biomarkers has become the norm, with MMR and p53 immunostaining being the most commonly performed molecular markers. These can be accurately assessed based on a biopsy or curetting specimen. Access to *POLE* testing is not as widely available as the immunostains but is expanding as it is necessary to have this information for molecular subtype diagnosis. While histotype and grade are of limited or no relevance in *POLE*mut, MMRd and p53abn EC, they are critical in NSMP EC. ER has also emerged as an important biomarker in this molecular subtype. ◆

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