GYNAECOLOGICAL PATHOLOGY

Molecular insights into the classification of high-grade endometrial carcinoma

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Summary

Endometrial carcinoma, which is associated with a mortality rate of approximately 20%, is the most common gynecological malignancy in the Western world. It is a heterogeneous disease, with multiple histotypes, each constituting a different disease entity. However, interobserver diagnostic agreement is suboptimal, particularly among the most lethal histotypes. Most recent data also indicate that histotype assignment is not independently associated with survival, while in contrast, clinicopathological risk stratification and genomic classification are significantly prognostic. Recent work has shown that there are four molecular subgroups of endometrioid carcinomas instead of the two types proposed by Bokhman in the 1970s. Carcinomas with *polymerase E (POLE)* exonuclease domain hotspot mutations are highly prognostically favourable; those with copy-number alterations and *TP53* mutations are highly aggressive; and microsatellite unstable and 'copy-number low' endometrioid carcinomas are associated with intermediate prognoses. This review summarises the genetic foundations of the various histotypes of endometrial carcinoma and synthesises this information in the form of algorithms, or classifiers, that recapitulate genomic classification that is not only prognostic, but also potentially diagnostic and therapeutically predictive. A review of Lynch syndrome and Lynch-like syndrome is also provided.

Key words: Endometrial carcinoma; high-grade carcinoma; TCGA; molecular classification; *POLE*; microsatellite instability.

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INTRODUCTION

Endometrial carcinoma is the most common gynecological malignancy in the Western world and accounts for approximately 74,000 deaths per year worldwide.^{1,2} Although the majority of endometrial carcinomas are generally low grade and low stage, with favourable prognoses, the high-grade group accounts for a disproportionate number of endometrial cancer deaths.^{3,4}

From the pathogenetic point of view, Bokhman proposed that endometrial cancer can be classified into two types (type I and II) based on epidemiological, clinical, and endocrine characteristics.⁵ Type I tumours are low-grade and oestrogen-related endometrioid carcinomas, and are associated with

obesity, endometrial hyperplasia, and favourable outcomes. Type II tumours are oestrogen-independent, of non-endometrioid histology (mainly serous and clear cell carcinoma), are seen in post-menopausal women, and are associated with atrophic endometrium, and poor outcomes. While this dualistic model is useful and provided the framework for many studies that contributed to our current understanding of endometrial carcinomas, including their molecular alterations, it is imperfect and difficult to apply in clinical practice due to the significant overlap between type I and II tumours and substantial heterogeneity within each type. Many high-grade endometrial carcinomas, particularly International Federation of Gynecology and Obstetrics (FIGO) grade 3 endometrioid carcinomas and clear cell carcinomas, cannot be placed into either group.

Histologically, endometrial carcinomas are classified into the following subtypes based on the 2014 World Health Organization (WHO) classification system of gynecologic tumours: endometrioid carcinoma (FIGO grade 1, 2, and 3), serous carcinoma, clear cell carcinoma, undifferentiated/dedifferentiated carcinoma, carcinosarcoma (formerly malignant mixed Müllerian tumour), neuroendocrine tumours, and mixed type.⁶ The traditional stratification of endometrial carcinoma by patient age, tumour grade, and clinical stage has been important in assessing prognosis and guiding surgical treatment and subsequent use of adjuvant therapy.⁷ Early-stage endometrioid cancers are generally treated by surgery, with or without adjuvant brachytherapy. Advanced-stage carcinomas are treated with upfront surgery followed by chemotherapy, with or without radiation therapy. Histotyping, however, only has a profound influence on therapy when the carcinoma is low stage, as patients with serous carcinomas and carcinosarcomas typically undergo chemotherapy almost regardless of stage. Some data suggest that histotype may not be independently associated with clinical outcomes,^{8–10} as some histotypes, particularly serous carcinomas and carcinosarcomas, are intrinsically high grade, occur in older patients, and tend to present at an advanced stage. Other histotypes are highly heterogeneous from a morphological and genomic perspective, complicating efforts to link endometrioid or clear cell histotypes to specific clinical outcomes.

High-grade endometrial carcinoma constitutes a biologically, morphologically, genetically, and clinically heterogeneous group of tumours. Histologically, high-grade endometrial carcinomas are diagnostically recognisable in prototypic examples on review of haematoxylin and eosin (H&E) slides, with or without immunohistochemistry;

however, as many as 30% of high-grade endometrial carcinomas are not prototypic.⁹ Several studies have shown sub-optimal interobserver reproducibility in the pathological diagnosis of high-grade endometrial carcinoma, even with the use of immunohistochemistry,^{9–11} limiting the ability to develop individualised treatments and calling for the need to improve and refine the current classification schemes. Over the past decade, many studies have assessed genetic alterations in endometrial carcinoma associated with each histological type. The recent application of next-generation sequencing technologies has led to a rapid and substantial shift toward understanding the molecular alterations in endometrial carcinoma. In 2013, The Cancer Genome Atlas Research (TCGA) network performed a comprehensive genomic and transcriptomic analysis of endometrioid and serous carcinomas and classified them into four molecular subgroups, including *polymerase E (POLE)*-ultramutated, microsatellite unstable/hypermethylated, copy-number low/microsatellite stable, and copy-number high (serous and serous-like) tumours.¹² The TCGA genomic classification system provided significant prognostic and potential therapeutic information; *POLE*-ultramutated tumours were associated with the best disease-free survival (DFS), copy-number high tumours were associated with the worst DFS, and the other two groups were associated with intermediate DFS. Subsequently, several investigators developed more practical methods to identify distinct subgroups with a prognostic signature, consistent with the molecular subgroups identified by TCGA,^{13,14} and attempted to apply this construct to other high-grade endometrial carcinomas, such as clear cell carcinoma¹⁵ and undifferentiated and dedifferentiated carcinomas.¹⁶

In this review, we discuss the various histological types of high-grade endometrial carcinoma and the molecular aberrations and immunophenotype of each type. We also discuss the TCGA molecular classification of endometrial carcinomas, including the characteristics of each subgroup. Finally, we highlight the role of integrating molecular pathology into the current pathological classification scheme.

HISTOLOGICAL CLASSIFICATION OF HIGH-GRADE ENDOMETRIAL CARCINOMA, IMMUNOPHENOTYPE AND GENOTYPE

FIGO grade 3 endometrioid carcinoma

FIGO grade 3 endometrioid carcinoma demonstrates solid, trabecular or nested growth and may resemble poorly differentiated, non-keratinising squamous cell carcinoma (Fig. 1). Endometrioid glandular differentiation is usually focally present with or without other features that support endometrioid differentiation, including squamous and mucinous differentiation.^{6,17} At the molecular level, *PTEN* (a tumour suppressor gene), *PIK3CA*, and *ARID1A* (a chromatin remodelling gene) are mutated in >60% of FIGO grade 3 endometrioid carcinomas.^{12,18,19} *KRAS* and *CTNNB1* (β -catenin) are mutated in 40%,^{12,18–20} while high somatic copy-number alterations and abnormal DNA ploidy are seen in 24% and 37% of such tumours, respectively.^{12,21} Overall, approximately 20–30% of endometrioid carcinomas are microsatellite instability-high (MSI-H),^{12,18–20,22,23} in most cases due to *MLH1* promoter methylation. *TP53* mutations have been reported in 20–30% of FIGO grade 3

Fig. 1 International Federation of Gynecology and Obstetrics (FIGO) grade 3 endometrioid carcinoma with a solid growth pattern and nuclear atypia.

endometrioid carcinomas.^{12,19,24} Immunohistochemically, a large percentage of FIGO grade 3 endometrioid carcinomas show loss of *PTEN*, *ARID1A* (BAF250a), and/or DNA mismatch repair protein expression, and at least focal expression of oestrogen and progesterone receptors (ER and PR, respectively).^{25,26} However, *PTEN* staining can be difficult to interpret, as the antibody can be technically challenging to work with.²⁶ Aberrant p53 and p16 expression is seen in a subset of FIGO grade 3 endometrioid carcinomas.²⁵ Aberrant p53 is usually in the form of over-expression (>60–75% strong uniform staining) corresponding to a missense mutation, or in a minority of cases, there will be complete loss of p53 staining in tumour cells in the presence of positive (wild-type positivity) internal control. This ‘null-pattern’ staining corresponds to a nonsense mutation, leading to the formation of a truncated protein that is not detected by commercially available p53 antibodies.^{27,28} A recent report on *TP53*-mutated ovarian carcinomas described two additional very uncommon staining patterns that result from *TP53* mutation: diffuse cytoplasmic staining and patchy, low-level expression that cannot be distinguished from the ‘wild-type’ staining pattern seen in tumours lacking *TP53* mutation.²⁹ To our knowledge, this has not yet been studied in the endometrium.

Serous carcinoma

Serous carcinoma typically shows irregular papillae with micropapillae and irregular luminal borders, slit-like spaces and tufted dyshesive cells exhibiting marked nuclear atypia and pleomorphism with smudgy chromatin or macronucleoli (Fig. 2). Occasionally, serous carcinoma shows predominantly glandular and/or solid architecture, which can mimic FIGO grade 3 endometrioid carcinoma. Cytoplasmic clearing might mimic clear cell carcinoma. Atrophic endometrium and/or an atrophic polyp is usually seen in the background.^{6,17} At the genetic level, serous carcinoma is characterised by frequent mutations in *TP53* (>90%), *PPP2R1A*, *FBXW7*, *Her2* amplification and high copy-number alterations, whereas mutations involving *PTEN*, *ARID1A*, DNA mismatch repair genes, and *KRAS* are rare.^{12,18–20,30} Abnormal DNA ploidy is seen in the majority of serous

Fig. 2 Serous carcinoma exhibiting papillary and micropapillary patterns and diffusely atypical nuclei.

carcinomas.²³ In challenging cases, a panel of immunohistochemical stains, including p53, p16, PTEN, ARID1A and DNA mismatch repair proteins, may provide important ancillary information in the distinction between serous carcinoma and FIGO grade 3 endometrioid carcinoma. Typical serous carcinomas demonstrate aberrant p53 and p16 immunohistochemical stains, whereas ARID1A, PTEN and DNA mismatch repair proteins are usually retained.^{25,26,31–33} Up to 25% of serous and serous-like tumours show Her2 overexpression.¹²

Clear cell carcinoma

Clear cell carcinoma is a rare type of endometrial carcinoma. It should be diagnosed predominantly based on cytoarchitectural features rather than cytoplasmic clearing, which can be occasionally seen in FIGO grade 3 endometrioid carcinoma or serous carcinoma. The papillae tend to be round with hyalinised cores, lined by one or two cell layers with uniform but high-grade nuclei (Fig. 3).^{6,17} *TP53*, *PIK3CA*, *PPP2R1A*, *FBX7*, and *ARID1A* are the most commonly mutated genes in endometrial clear cell carcinoma.^{15,34–36} Approximately 20%

Fig. 3 Clear cell carcinoma with a predominantly papillary architecture.

of endometrial clear cell carcinomas are MSI-H, and approximately 5% have a *POLE* mutation.¹⁵ The remaining endometrial clear cell carcinomas are *TP53* mutated (~35%), have mutations usually associated with endometrioid carcinoma or a non-specific genotype.¹⁵ Immunophenotypically, clear cell carcinoma frequently shows expression of hepatocyte nuclear factor-1 β , Napsin-A and racemase, while ARID1A, ER and PR are frequently lost.³³ A substantial minority shows overexpression of p53 and p16.^{26,35}

Undifferentiated/dedifferentiated carcinoma

Undifferentiated and dedifferentiated endometrial carcinomas are part of an uncommon but highly aggressive family of tumours related to endometrioid carcinomas,³⁷ and were first described by Silva and colleagues.³⁸ Undifferentiated carcinoma, often mistaken for FIGO grade 3 endometrioid carcinoma, high-grade endometrial stromal sarcoma or lymphoma, is characterised by the presence of sheets of monotonous, ovoid to round cells with moderate-to-large nuclei, without convincing histological evidence of epithelial differentiation, such as glands, nests, trabeculae, or papillae (Fig. 4). Dedifferentiated carcinomas show foci of differentiated endometrioid carcinoma, usually low grade, juxtaposed against undifferentiated carcinoma without evident transitions.³⁹ Undifferentiated carcinoma lacks diffuse expression of epithelial markers, such as pancytokeratin and E-cadherin, and it is frequently microsatellite unstable.³⁷ Except for the very rare *TP53*-mutated examples, the tumours are negative for PAX8, ER, and PR.⁴⁰ Recently, it has been shown that these carcinomas also show frequent loss of ARID1A, SMARCB1, and SMARCA4 by immunohistochemistry, suggesting a role for the SWI/SNF chromatin remodelling complex in the pathogenesis of this aggressive tumour.^{41,42} Rosa-Rosa *et al.* recently showed that undifferentiated endometrial carcinomas are genetically heterogeneous and may share molecular alterations with the four molecular subgroups of endometrial carcinoma described by TCGA, akin to FIGO grade 3 endometrioid carcinoma.¹⁶

Undifferentiated carcinomas are part of a family of carcinomas, including carcinosarcomas, thought to arise via epithelial-to-mesenchymal transition (EMT). EMT is a process by which epithelial cells lose polarity and intercellular adhesion, while acquiring properties of mesenchymal cells. EMT in uterine tumours typically involves upregulation of the miRNA 200 series, with consequent upregulation of established EMT-related proteins such as ZEB-1, SNAIL and TWIST, and downregulation of E-cadherin.⁴³

Carcinosarcoma

Uterine carcinosarcomas are biphasic neoplasms composed of morphologically malignant epithelial and stromal elements (Fig. 5). The epithelial component is typically serous, FIGO grade 3 endometrioid carcinoma, or too poorly differentiated to subclassify. The stromal component is histologically high grade, featuring obvious nuclear atypia, pleomorphism, and mitotic activity.^{6,17} Heterologous elements, most commonly rhabdomyoblastic and chondrosarcomatous, are found in approximately half of cases. The sarcomatous component is thought to arise from metaplastic conversion due to strong EMT involving upregulation of the miRNA 200 series, as in undifferentiated and dedifferentiated carcinomas.⁴³ From the molecular point of view, a subset of carcinosarcomas (~20%)

Fig. 4 Undifferentiated carcinoma. (A) Typical appearance: sheets of monomorphous tumour cells lacking any evident epithelial differentiation. (B) Undifferentiated carcinoma composed of rhabdoid cells floating in myxoid stroma.

shows mutations in *PTEN*, *KRAS*, *ARID1A*, DNA mismatch repair genes, and *POLE* (i.e., endometrioid-like), while the majority share common molecular aberrations with ovarian and endometrial serous carcinoma, such as *TP53* and *PPP2R1A* mutations, suggesting that while some carcinosarcomas arise from an endometrioid lineage, the majority transdifferentiate from a serous precursor.^{19,44}

Mixed epithelial carcinoma

Mixed carcinoma is a heterogeneous group of tumours composed of two or more distinct histological types (each must comprise at least 5% of tumour).⁶ Mixed carcinomas are difficult to classify and have poor interobserver reproducibility.⁴⁵ Recent data reported by Köbel *et al.* showed shared clonality in both components of most mixed epithelial carcinomas studied.⁴⁶ Sixteen of 18 mixed carcinomas contained elements with shared molecular alterations, despite showing different histological components. This suggests that most mixed carcinomas represent one tumour type with one component that morphologically mimics another type.^{46,47} True mixed epithelial carcinomas are rare; the prototype is a microsatellite unstable mixed endometrioid and clear cell carcinoma.⁴⁶

Fig. 5 Carcinosarcoma with a high-grade epithelial component (carcinoma, not otherwise specified, with squamous differentiation) and malignant chondroid component.

TCGA GENOMIC CLASSIFICATION OF ENDOMETRIAL CARCINOMA

In 2013, the multi-institutional TCGA project, sponsored by the National Cancer Institute and National Human Genome Research Institute, presented a molecular classification scheme for endometrial carcinoma.¹² TCGA performed an integrated genomic, transcriptomic, and proteomic analysis of 373 endometrial carcinomas using massively parallel sequencing and array-based technologies in combination with DNA methylation, reverse phase protein array, and MSI analyses. The study focused on endometrioid ($n = 307$), serous ($n = 53$), and mixed endometrioid and serous ($n = 13$) carcinomas. Based on the integration of the somatic gene mutations, MSI and somatic copy-number alterations results, endometrial carcinomas were categorised into four genomic groups, as follows.¹²

1. *POLE*-ultramutated: An ultramutated group harbouring mutations in the exonuclease domain of *POLE* gene (a subunit of DNA polymerase epsilon that has a role in DNA replication) and characterised by very high mutation rates, few copy-number aberrations, increased frequency of C → A transversions, mutations in *PTEN*, *PIK3CA*, and *KRAS*, and highly favourable clinical outcomes.¹²
2. MSI-H/hypermuted: A hypermutated group of endometrial carcinomas, mostly of endometrioid histology, MSI-H due to *MLH1* promoter methylation, and characterised by high mutation rates, few copy-number alterations, and frequent *KRAS* and *PTEN* mutations.¹² Lynch syndrome-associated endometrial carcinomas constitute a subset of the MSI-H tumours, which are discussed in detail below.
3. Copy-number low/microsatellite stable: A group of endometrioid carcinomas composed mostly of microsatellite-stable grade 1 and grade 2 tumours with low mutation rates, and characterised by frequent *PTEN*, *CTNNB1*, *KRAS* and *ARID1A* mutations.¹²
4. Copy-number high (serous and serous-like): A group of tumours with extensive copy-number alterations (i.e., amplifications and deletions), low mutation rates and recurrent *TP53*, *FBXW7*, and *PPP2R1A* mutations, infrequent *PTEN* and *KRAS* mutations, and composed of serous carcinomas and 25% of FIGO grade 3 endometrioid carcinomas.¹²

TCGA data provided a completely novel insight into the genetic and clinical heterogeneity of FIGO grade 3

endometrioid carcinomas. Unlike serous carcinomas, FIGO grade 3 endometrioid carcinomas did not segregate into a single molecular cluster, but were represented in all four TCGA subgroups.

TCGA GENOMIC CLASSIFICATION OF ENDOMETRIAL CARCINOMA, CLINICAL CORRELATIONS

TCGA also conveyed crucial prognostic information. The genomic classification of endometrioid carcinomas stratified these tumours into clinically low-risk (*POLE*-ultramutated), intermediate-risk (copy-number low and MSI-H groups) and high-risk (copy-number high/serous-like) categories. With the exception of variable clinical outcomes based on clinical risk stratification, influenced heavily by stage, there is little apparent clinical heterogeneity within the *POLE*-ultramutated and copy-number high/serous-like groups. In contrast, there is obvious heterogeneity within the copy-number low group and some variability in the MSI-H group. In the copy-number low group, the presence of chromosome 1q amplification, a *TP53* or *CTNNB1* mutation predicts an increased risk for recurrence in a subset of low-grade, low-stage endometrioid tumours.⁴⁸ With regard to the MSI-H group, the clinical variability observed might be due to differences across centres with respect to staging adequacy and delivery of adjuvant therapy. In other words, withholding adjuvant therapy from patients with occult/un-discovered high-stage disease may be a particularly important negative prognostic factor in this group, as these tumours appear to be highly sensitive to adjuvant therapy.^{49–51} Finally, it is currently unclear whether there are important clinical differences between copy-number high (serous-like) endometrioid carcinomas and serous carcinomas.

For several years, there has been evidence showing that clear cell carcinomas with a *TP53* mutation are prognostically unfavourable.^{34,52} DeLair and colleagues recently published their comprehensive clinicopathological, immunophenotypic, and genomic evaluation of endometrial clear cell carcinomas.¹⁵ Even though four genomic clusters were recognised, similar to the TCGA results for endometrioid carcinomas, two clinical clusters emerged: prognostically highly favourable, represented by *POLE*-ultramutated and hypermutated (MSI-H) carcinomas, and prognostically highly unfavourable, represented by *TP53*-mutated carcinomas and those lacking *TP53* mutation, ultramutation, and hypermutation.¹⁵

Although both carcinosarcomas⁴⁴ and un/de-differentiated carcinomas⁵³ contain *POLE*-ultramutated and MSI-H subgroups, there is currently insufficient information to determine their clinical relevance. As will be discussed below, it is possible that *POLE*-ultramutated and MSI-H tumours share unique features, irrespective of histotype, that may have prognostic and therapeutic relevance.

ENDOMETRIAL CARCINOMAS WITH HIGH MUTATION BURDEN AND RICH IMMUNE MICROENVIRONMENT (*POLE*-ULTRAMUTATED AND MSI-H TUMOURS)

These tumours have many unique biological, pathological, and clinical properties.

Fig. 6 *POLE*-mutated endometrioid carcinoma. *POLE* carcinomas may have eosinophilic tumour cells with giant, bizarre nuclei and a noticeable lymphoid infiltrate.

POLE-ultramutated tumours

POLE encodes the catalytic subunit of DNA polymerase epsilon. The resulting enzyme is involved in DNA repair and chromosomal DNA replication.

Detecting this subgroup of endometrial carcinomas was exciting, because of the discovery of very favourable clinical outcomes, even among high-grade tumours. Our group assessed the clinicopathological features of *POLE*-ultramutated endometrial carcinomas in the TCGA series, as well as eight additional *POLE*-ultramutated tumours from an independent series of 99 high-grade endometrial carcinomas from the University of Calgary.^{54,55} The vast majority of *POLE*-ultramutated tumours presented at stage I. Most of them were FIGO grade 3 endometrioid carcinomas, rich in tumour-infiltrating lymphocytes and/or peritumoural (Crohn's-like) lymphocytes, and many of them showed marked intratumoural heterogeneity, with morphological ambiguity, including features mimicking serous carcinoma (Fig. 6).⁵⁵ Approximately 8% of endometrial carcinomas overall and 15–20% of FIGO grade 3 endometrioid carcinomas show *POLE* exonuclease domain mutation and an ultramutated genotype.^{12,56–59} Multiple studies have demonstrated that patients with *POLE*-ultramutated endometrial carcinoma have excellent prognoses, irrespective of other clinicopathological variables such as high histological grade and advanced stage.^{12,56–59} The reasons for such a favourable outcome are not completely understood; however, Meng *et al.* had previously speculated that this may be a result of the general or specific mutation burden conferred by the ultramutated genotype and the dramatic increase in base substitutions, which could be incompatible with tumour cell growth and survival.⁵⁹ We had previously suggested an immune-mediated role, given the rich tumour-infiltrating and peritumoural lymphocytes in these tumours.⁵⁵ More recently, many researchers have investigated the role of tumour-infiltrating lymphocytes in *POLE*-ultramutated tumours.^{60–62} Howitt *et al.* showed that *POLE*-ultramutated endometrial carcinoma is associated with high neoantigens and elevated CD8+ tumour infiltrating lymphocytes, which is counterbalanced by overexpression of program death-ligand

1 (PD-L1; an immune checkpoint), suggesting that such tumours might be excellent candidates for anti-PD-L1 targeted therapies.⁶⁰ Immune checkpoints other than PD-L1, such as cytotoxic T-lymphocyte antigen-4 (CTLA-4), lymphocyte activation gene-3 (LAG-3), and indoleamine 2,3-dioxygenase (IDO), may also be upregulated in *POLE*-ultramutated tumours, which is known as 'adaptive immune resistance'.^{60,63–65} Although the discussion to this point was focused on somatic *POLE* hotspot mutations, *germline* mutations in *POLE* and *polymerase D1 (POLD1)* have also been reported to predispose to endometrial and colorectal carcinoma.^{56,66–68}

MSI-H endometrial carcinomas

These tumours are typified by a microsatellite unstable phenotype that arises from defects in the post-replicative DNA mismatch repair system. MSI-H endometrial cancers are characterised by high mutation rates, few copy-number aberrations, recurrent *KRAS* and *PTEN* mutations, and few *TP53*, *PPP2R1A*, and *FBXW7* mutations. Approximately 20–30% of endometrial carcinomas show MSI or mismatch repair protein abnormalities;^{12,18–21,23,64,69} this is most commonly due to sporadic events such as *MLH1* methylation.

MSI-H endometrial carcinomas are characterised by several morphological and topographical features (Fig. 7) including tumour-infiltrating lymphocytes, peritumoural lymphocytes, tumour heterogeneity, undifferentiated/dedifferentiated carcinoma, or non-endometrioid histology in young patients, particularly clear cell and mixed endometrioid and clear cell carcinomas, tumours originating in the lower uterine segment, and endometrial carcinoma associated with synchronous ovarian clear cell carcinoma.^{70–73} The prognostic impact of DNA mismatch repair status in endometrial carcinomas is unclear. Some studies have demonstrated that endometrial carcinomas associated with DNA mismatch repair deficiency and MSI are associated with poor prognostic indicators, including higher histological grade, presence of lymphovascular invasion and higher clinical stage,^{74,75} whereas others found no association with prognosis⁷⁶ or have shown improved clinical outcomes.²² As

Fig. 7 Microsatellite-unstable endometrioid carcinoma showing a peritumoural Crohn's-like infiltrate and tumour infiltrating lymphocytes, a feature that is sometimes shared with *POLE* carcinomas.

mentioned previously, the conflicting conclusions of various studies may reflect institutional differences in staging and delivery of adjuvant therapy. Similar to *POLE*-ultramutated endometrial cancers, the presence of high mutation load, along with the rich immune microenvironment, make these tumours interesting potential candidates for immune checkpoint inhibitors such as anti-PD-L1 therapy.^{60,63–65}

While most of the MSI-H tumours are sporadic, approximately 20–25% of MSI-H endometrial carcinomas are related to Lynch syndrome.

Lynch syndrome

This is an autosomal dominant cancer-susceptibility syndrome responsible for most heritable endometrial carcinomas and <5% of ovarian carcinomas. Three general mechanisms, each of which impairs the DNA mismatch repair system, are responsible for the development of Lynch syndrome: (1) *germline* mutation in one of the DNA mismatch repair genes *MLH1*, *MSH2*, *MSH6*, or *PMS2*; (2) abnormalities in genes that regulate mismatch repair gene expression, such as *EPCAM*; or (3) constitutive epimutation.^{64,69} *Germline* mutation of one of the DNA mismatch repair genes is by far the most common and best understood mechanism underlying the development of Lynch syndrome. *Germline EPCAM* mutation, on the other hand, leads to *MSH2* promoter methylation, with consequent *MSH2* inactivation and acquisition of a MSI-H phenotype. Even though this scenario is considered part of the spectrum of Lynch syndrome, it has been reported that *EPCAM*-mutant Lynch syndrome families have more in common with Lynch-like syndrome (LLS) families (discussed below), meaning that the penetrance associated with the development of carcinoma may be muted with this genotype.^{77,78} Another rare mechanism underpinning the development of Lynch syndrome is the presence of intragenic insertions, deletions, and structural variations in the DNA mismatch repair genes, which are not always detectable using conventional assays. Next-generation deep DNA sequencing is more likely to identify these types of abnormalities when compared to Sanger DNA sequencing.⁷⁹ Yet another rare example is DNA mosaicism in DNA derived from lymphocytes, usually used for *germline* DNA sequencing in Lynch syndrome diagnosis.⁷⁹ This type of DNA mosaicism tends to be absent in the target organ, which in some cases harbours the pathogenic Lynch syndrome-defining *germline* DNA mismatch repair mutations. All of these scenarios are difficult to identify and may explain clinical manifestations of Lynch syndrome when conventional *germline* gene mutation analysis appears negative (i.e., false negative). The use of next-generation sequencing has also uncovered novel DNA mismatch repair mutations, some of which have not been confirmed to be pathogenic. These are termed 'variants of uncertain (or unknown) significance' (VUS). Most of these cases previously would have appeared to be false-negative assays due to the inability of Sanger sequencing to detect the abnormality. Whether these cases constitute Lynch syndrome is uncertain.

Impairment of DNA mismatch repair leads to MSI and a predisposition for developing multiple malignancies, including colon and endometrial cancers, among others.⁶⁴ While colon cancer has been the most commonly studied cancer in this disorder, women with Lynch syndrome have a significant lifetime risk of developing endometrial carcinoma

and/or ovarian carcinoma that exceeds that of colon cancer, and in greater than 50% of cases, endometrial carcinoma is the first, or 'sentinel', malignancy.^{64,80,81} The identification of Lynch syndrome in endometrial carcinoma patients is important, as it provides the patient with the opportunity to be screened for synchronous and metachronous Lynch syndrome-associated tumours, particularly colon cancer.⁸⁰ Furthermore, it allows their close family members to undergo screening for Lynch syndrome⁸² and then prophylactic hysterectomy and salpingo-oophorectomy, if appropriate. Germline DNA mismatch repair protein gene defects have been reported in 1.8–5.9% of unselected patients with endometrial carcinoma.^{79,83–86} The optimal strategy for identifying patients with Lynch syndrome among newly diagnosed endometrial carcinoma patients, however, remains controversial. While age and family history are useful but rough screening criteria, their sensitivity has been shown to be significantly suboptimal in detection of Lynch syndrome-associated endometrial carcinoma.^{74,87} Testing tumours for DNA mismatch repair protein expression using immunohistochemistry with or without MSI assays has been shown to be a more sensitive and specific method for identifying patients.⁷⁴ In many institutions, these screening tests are performed on tumours from all patients diagnosed with endometrial carcinoma. Universal screening has been recently recommended by the American Society of Gynecologic Oncology,⁸⁸ with the following rationale: (1) the incidence of Lynch syndrome-associated carcinomas increases with age and is by no means confined to younger patients; (2) Lynch syndrome screening identifies endometrial carcinoma patients at risk of developing another Lynch syndrome-associated carcinoma, such as colorectal carcinoma, which has medicolegal implications and, more importantly, profound clinical importance; and (3) Lynch syndrome screening of an index patient affords generations of Lynch syndrome family members the opportunity to be screened, allowing early detection of carcinoma and its prevention. Figure 8 shows an algorithm for the detection of endometrial carcinoma patients who are at high risk for having Lynch syndrome.

The importance of DNA mismatch repair immunohistochemistry is not only crucial for Lynch syndrome screening,

but also for therapeutic prediction (i.e., sensitivity to immune checkpoint inhibitors, as discussed above), prognostication (i.e., assignment to a clinically relevant TCGA subgroup, as discussed above), and differential diagnosis, as discussed in following portions of this review.

Lynch-like syndrome (LLS)

LLS is diagnosed in the presence of an MSI-H and/or DNA mismatch repair-deficient carcinoma, lacking MLH1 promoter methylation, without an identifiable DNA mismatch repair germline mutation. Most of the work on this topic has been performed in the setting of colorectal carcinoma, and it is assumed that many of the underlying mechanisms that account for LLS in endometrial and ovarian carcinomas are likely similar, but other details need confirmation. LLS patients present at approximately the same age as Lynch syndrome patients (approximately 10–20 years younger than patients with sporadic carcinomas), have significantly lower rates of developing colorectal carcinoma, and less frequently have a family or personal history of Lynch syndrome-associated carcinomas.^{79,89–93} However, LLS patients and their first-degree relatives are thought to be at higher risk of developing colorectal carcinoma as compared to patients with sporadic endometrial carcinoma, irrespective of MSI status. LLS carcinomas constitute 30–60% of all MSI-H carcinomas lacking DNA mismatch repair germline mutation and MLH1 promoter methylation. The presence of rare, germline abnormalities, discussed previously, should be excluded before considering LLS.

The literature has emphasised the importance of bi-allelic somatic mutations that affect the DNA mismatch repair genes as a widespread mechanism for the development of an LLS-associated carcinoma.^{94–98} It has been postulated that the first hit might occur in a cancer precursor lesion, while the second hit occurs with the development of carcinoma. By virtue of the presence of somatic mutations and the absence of germline mutations, these patients lack a heritable cancer syndrome and, thus, it is not necessarily recommended that they undergo close surveillance to detect early colorectal carcinoma. A somatic mutation in *polymerase E* or *polymerase D* (*POLE* or *POLD*, respectively) with secondary somatic mutations in one or more of the DNA mismatch repair genes⁹⁹ has been reported as an uncommon mechanism for the development of an LSS phenotype.

Lynch syndrome can be distinguished from LSS when well-understood abnormalities reported in the setting of LS can be documented. This typically requires next-generation sequencing. A somatic mutation of *POLE*, *POLD* and/or bi-allelic somatic mutation of one or more of the DNA mismatch repair genes effectively precludes a diagnosis of Lynch syndrome, meaning that these patients do not need the close surveillance required for Lynch syndrome patients. Furthermore, there is a spectrum of poorly understood abnormalities, such as the presence of a VUS, which may place the patient and her relatives at increased risk for the development of Lynch syndrome-associated carcinomas. Affected patients should consult with a clinical geneticist in collaboration with her treating physician to determine whether close clinical surveillance, such as that offered to known Lynch syndrome patients, is indicated.

Testing scheme

Fig. 8 Flow chart for Lynch syndrome triage using universal DNA mismatch repair immunohistochemistry as a starting point.

Fig. 9 (A–D) Endometrial carcinoma genotyping using POLE sequence and immunohistochemistry for the DNA mismatch repair proteins and p53. (A) POLE sequence (figure courtesy of Dr Britta Weigelt); (B) loss of expression of MSH6 in tumour cell nuclei, with an intact internal control; (C) aberrant p53 immunostaining (overexpression); (D) aberrant p53 immunostaining (no expression in tumour cell nuclei with an intact internal control).

PRACTICAL APPLICATIONS OF TCGA DATA

Many researchers have assessed the usefulness of incorporating next-generation sequencing into the differential diagnosis of high-grade endometrial carcinomas.^{12–14,61,88} This might be of particular interest in histologically ambiguous tumours that are difficult to classify and in tumours with non-informative immunohistochemical results. Several groups have proposed surrogate TCGA molecular classification systems.^{13,14,61} According to work from the Vancouver group, endometrioid and serous endometrial carcinomas can be assigned to one of the four TCGA groups without the extensive assays that originally generated the genomic clusters by combining *POLE* mutational analysis with p53 and DNA mismatch repair protein (MLH1, PMS2, MSH2 and MSH6) immunohistochemistry¹⁴ (Fig. 9)—the so-called ‘PROMISE’ classifier (Proactive Molecular Risk Classifier for Endometrial Cancer). An adaptation of the PROMISE classifier can be found in Fig. 10. Application of the classifier to 143 endometrial carcinomas resulted in the following distributions: *POLE*-ultramutated 9%, microsatellite instable 32%, copy-number low 18%, and copy-number high (serous and serous-like) 39%. In a multivariate analysis, both PROMISE classification and clinicopathologic risk group assignment [European Society for Medical Oncology (ESMO) guidelines] were significantly associated with clinical outcomes, whereas histotype was not. This model provides independent prognostic information beyond traditional clinicopathological variables and can be used in clinical practice. Other investigators have reported similar data.¹³

The integration of molecular testing has the potential to provide additional prognostic and predictive information to the traditional morphological, immunohistochemical, and clinical risk group assignment. Although the PROMISE classifier and related classifiers are feasible in resource-rich environments, there are obvious restrictions in other settings. That being said, there is little concern among pathologists and clinicians that resources be allocated to extensive testing of pulmonary and colorectal carcinomas, where targeted genetic profiling and immune checkpoint

Fig. 10 Flow chart for endometrial carcinoma genotyping using available ancillary tests CN-H, copy number high (serous and serous-like); CN-L, copy number low; DNA MMR, DNA mismatch repair proteins; EMC, endometrioid carcinoma; MSI-H, microsatellite unstable; POLE, *polymerase epsilon*. *Applies mostly to endometrioid carcinoma, but also to undifferentiated and clear cell carcinomas. **Applies mostly to FIGO grade 3 endometrioid carcinoma, but also to clear cell carcinomas. Carcinosarcomas are found predominantly but not exclusively in the CN-H group..

immunohistochemistry is standard in the former and DNA mismatch repair immunohistochemistry with frequent genetic profiling is performed in the latter. In the near future, there will be increasing interest, if not pressure, from educated clinicians for the molecular profiling of endometrial carcinoma as well. At present, this is best accomplished as outlined in the preceding text. Going forward, it is conceivable that both somatic and germline genetic testing of endometrial carcinomas and endometrial carcinoma patients will be performed using a multi-gene next-generation sequencing platform that will allow detection of somatic *POLE* and *Tp53* mutations, as well as an MSI score. With patient consent, such technology will be able to identify germline *POLE*, *POLD*,^{56,66–68} and *DNA mismatch repair* mutations as well.

CONCLUSION

In this review, we have discussed the traditional pathological classification and the recent molecular classification of endometrial carcinoma. The integration of molecular features into the current pathological classification may provide additional prognostic and predictive information that can better define biologically and clinically relevant subsets of endometrial cancer.

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