Breast and Gynecologic Tumors

5

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List of Frequently Asked Questions

I **Breast tumor**

1. What are the roles of molecular testing in breast pathology?
2. What are the most common hereditary mutations associated with breast cancer?
3. What are the main pathologic features of breast cancer associated with hereditary disease?
4. When should we consider recommending breast cancer patients for genetic testing for hereditary breast cancer?
5. What is the significance of identifying breast cancer patients with hereditary mutations?
6. Which genes should be included in genetic testing for hereditary breast cancer?
7. What are the testing methods for *BRCA* mutations?
8. What are the limitations of the genetic testing for hereditary breast cancer?
9. What is the molecular subclassification of breast cancer? What are the clinicopathologic features of breast cancers by molecular subclassification?
10. What is the relationship between triple-negative and basal-like subtype breast cancers?
11. When should the biomarkers in breast cancers be assessed?
12. Why does the ASCO/CAP recommend testing PR status in breast cancers although there is no targeted therapy?
13. When should we consider repeating biomarker testing in breast cancers on the resection specimen?
14. Should the biomarker status be repeated in the breast specimen status/post neoadjuvant therapy?
15. What are the key points of ASCO/CAP recommendations for pre-analytic variables for biomarker assessment in breast cancers? What are the common pre-analytic, analytic, and post-analytic factors that could affect the biomarker testing in breast cancer?
16. How to handle a bone specimen if it is suspicious for metastatic breast cancer?
17. Do the ASCO/CAP guidelines for biomarker tests in breast cancer exclude testing of cytology specimens (fluids and aspirates) that have been fixed in 95% ethanol rather than formalin?
18. How should we interpret the ER/PR staining results in breast cancers?
19. What are the semiquantitative scoring methods for immunohistochemical assessment of ER and PR in breast cancers?
20. What is the significance of the ER low positivity in breast cancer?
21. Are other ER expression assays acceptable for identifying patients likely to benefit from endocrine therapy?
22. What are the possible causes for the discordant ER status by IHC and RT-PCR (Oncotype Dx result)?
23. Which method is better for HER2 testing in breast cancers, immunohistochemistry (IHC), or in situ hybridization (ISH)?
24. When should the breast cancer case be considered for HER2 ISH testing if using IHC as the primary test?
25. What are single-probe and dual-probe assays in HER2 in situ hybridization assays? Which assay is better?
26. What are the HER2 ISH groups in breast cancers on dual-probe assays? What are the unusual HER2 groups?
27. What are the possible causes for the discordant HER2 results between IHC and ISH analyses?
28. What are the common discordances of biomarker status with histology or other clinicopathologic findings?

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What are the possible causes for these discordances? How to solve these discordances?

29. What are the possible causes of discordant biomarker status between primary and metastatic breast tumors?
30. What are gene signature and molecular profiling of cancers? What are the roles of molecular profiling tests in early stage ER+ breast cancers? What are the commercially available molecular profiling tests for prognostication in early stage ER+ breast cancers?
31. When should we raise the suspicion for a major discordance between pathologic findings and Oncotype recurrence risk result? What shall we do if there is major discordance between the pathology result and Oncotype DX recurrence score (RS) result?
32. What is the role of testing the *PIK3CA* mutation in breast cancer?
33. How can molecular testing help in the diagnosis of breast tumors?

II **Gynecologic tumors**

1. What are the roles of molecular testing in gynecologic pathology?
2. Why testing *BRCA1* and *BRCA2* genomic status is important in patients with ovarian carcinomas?
3. What is homologous recombination deficiency (HRD)?
4. How to diagnose HRD?
5. In which individual should risk evaluation, counseling, and genomic testing for germline and somatic tumor alterations in ovarian cancer be performed?
6. What is the best approach for testing *BRCA* mutations in patients with ovarian/tubal/primary peritoneal carcinomas?
7. What is the significance of detecting deficient mismatch repair (dMMR) in patients with ovarian carcinomas?
8. What are the pathologic features of Lynch syndrome-associated endometrial cancer?
9. Why testing for mismatch repair protein is important in patients diagnosed with endometrial carcinomas?
10. Who should be screened for Lynch syndrome on tissue when diagnosed with endometrial cancer?
11. What are the common testing methods for screening Lynch syndrome?
12. How to interpret the immunohistochemical stains for MMR proteins? What are the pitfalls in the interpretation of MMR on IHC? How to resolve these pitfalls?
13. Should the mismatch repair protein testing be repeated in cases of tumor recurrence?
14. What is microsatellite instability (MSI)? What are the MSI testing methods? What are MSI-high, MSI-low, and MSI stable?
15. What is *MLH1* promoter methylation? How to test *MLH1* promoter methylation?
16. What are the possible causes for normal tissue testing result in patients with known MMR gene mutations? What are the possible causes for abnormal MSI/IHC results with non-detectable MMR gene mutations?
17. What are the advantages and disadvantages of IHC method, PCR-based MSI testing, and NGS-based MSI testing for Lynch syndrome screening?
18. What is the TCGA molecular classification of endometrial carcinomas?
19. What are the roles of molecular testing in endometrial stromal tumors?
20. What are the biomarker tests in endometrial carcinoma? How should these biomarkers be reported?
21. What is the role of HER2 testing in uterine serous carcinoma?
22. What is molecular human papillomavirus (HPV) testing in the cervical cytology specimen?
23. When should p16 immunostaining be performed in the lower anogenital squamous lesions?
24. What are the roles of molecular testing in sex cord stromal tumors of the ovary?
25. What are the roles of molecular testing in the diagnosis of gestational trophoblast disease?

Frequently Asked Questions

I **Breast tumor**

1. **What are the roles of molecular testing in breast pathology?**
   - Molecular testing for genetic and genomic variations has become an integral part of breast cancer management. The applications of molecular testing in breast pathology include the following:
     – Testing for hereditary DNA mutation in patients with breast cancer to define hereditary cancer syndrome and identify patients for selected therapy
     – Subclassifying molecular tumor types
     – Identifying biomarkers that can predict the response to treatment
     – Testing for genomic signatures in early stage estrogen receptor (ER) positive breast cancers for prognostication of cancers and predicating benefit from adjuvant chemotherapy
     – Testing for tumor genomic mutations to identify mutations for targeted therapy in metastatic breast cancer or other experimental therapy for precision medicine purpose

  - Testing for specific translocations/markers to facilitate the diagnosis of certain breast cancers

2. **What are the most common hereditary mutations associated with breast cancer?**
   - Approximately 5–10% of breast cancers are linked to a specific inherited high penetrance germline mutation in a breast cancer susceptibility gene [1].
   - The common hereditary mutations in breast cancers include *BRCA1/2, PALB2*, Li-Fraumeni syndrome (*TP53* pathologic variant), Cowden syndrome (*PTEN* pathologic variant), hereditary diffuse gastric cancer syndrome (*CDH1* pathologic variant), and Peutz-Jeghers syndrome (*STK11* pathologic variant). Among them, more than 50% of these pathologic germline variants are mutations of *BRCA1* and *BRCA2* genes, and women have a 57–60% and 49–55% lifetime risk of developing breast cancer if they carry a *BRCA1* or *BRCA2* mutation, respectively [2–5].

3. **What are the main pathologic features of breast cancer associated with hereditary disease?**
   - The majority (~75%) of *BRCA1/2* associated breast cancer is ductal carcinoma.
   - When compared to the sporadic breast cancer, a significant higher frequency of *BRCA1/2*-associated tumors are present in younger patients, having higher histologic grade with medullary pathologic features (circumscribed border, high histologic grade, brisk host immune cell response) and somatic *TP53* mutations. The tumor is usually ER negative/progesterone receptor (PR) negative/human epidermal growth factor receptor 2 (HER2) negative/epidermal growth factor receptor (EGFR) positive in *BRCA1*-associated cancers (see Fig. 5.1), while in the *BRCA2*-associated breast cancers, the biomarker expressions are similar to those in the sporadic breast cancers [5, 6].

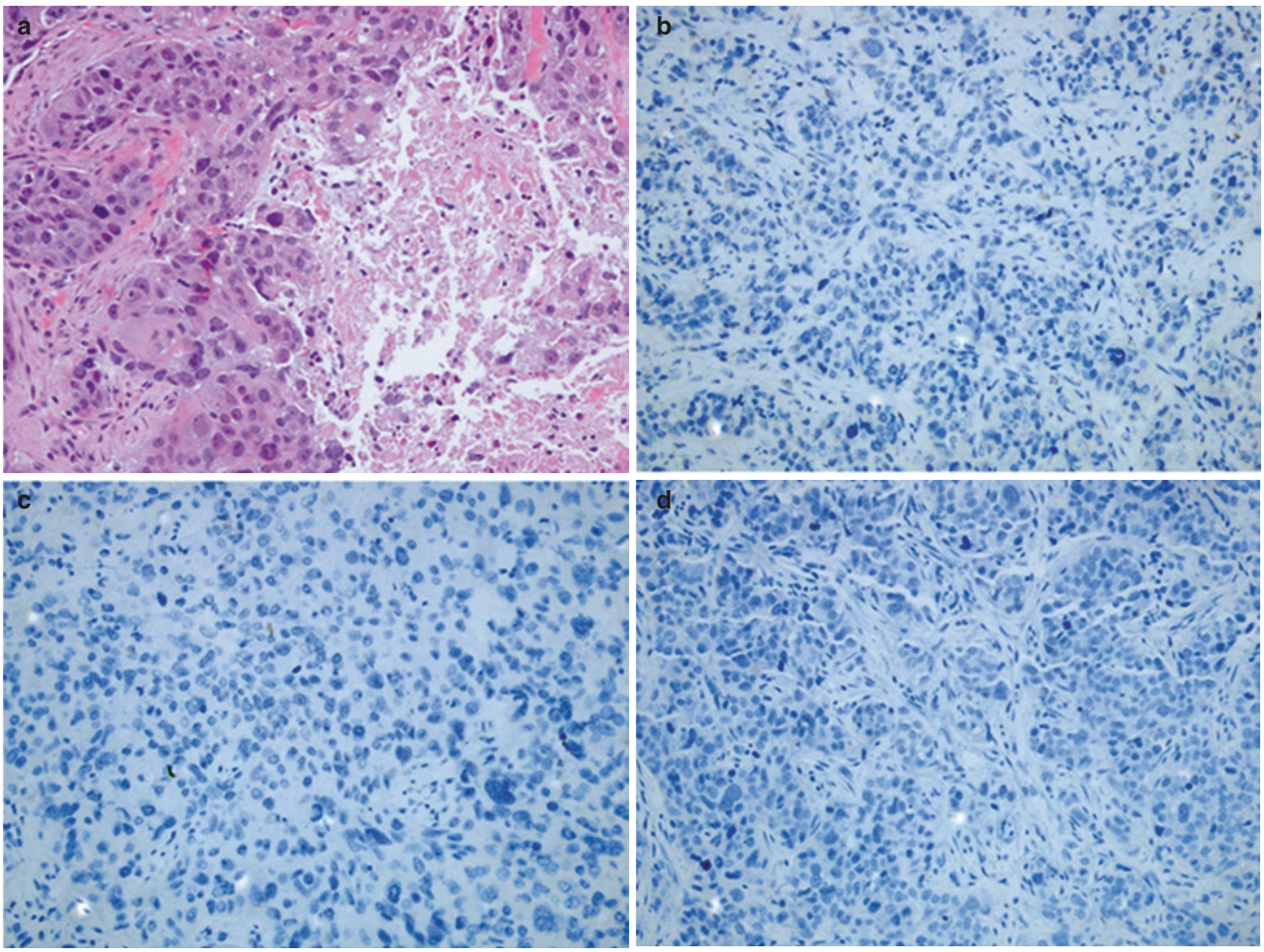

Fig. 5.1 Invasive ductal carcinoma in a 38-year-old woman with known *BRCA1* deleterious mutation. H&E section shows high-grade morphology with frequent mitotic figures and focal necrosis (**a**, ×200). The tumor cells are negative for ER (**b**, ×200), PR (**c**, ×200), and HER2 (**d**, ×200)

- The pathologic features of breast cancer associated with other pathologic germline mutations are less characterized.

4. **When should we consider recommending breast cancer patients for genetic testing for hereditary breast cancer?**
   - According to the National Comprehensive Cancer Network (NCCN) guidelines [7], clinicians should consider genetic testing for breast cancer patients in:
     - 50 years old or younger
     - Triple-negative breast cancer at an age younger than 60 years old
     - Male patients with breast cancer
     - Bilateral or a second primary breast cancer
     - Prior history of ovarian cancer
     - For individuals without the personal history and additional family history criteria must be met including:
   - At least one relative with breast cancer diagnosed at 50 years old or younger
   - One relative with ovarian cancer
   - More than two relatives with breast cancer, prostate cancer (Gleason score≥7 or metastatic disease), or pancreatic cancer.
   - The recent guidelines from the American Society of Breast Surgeons recommended that genetic testing should be offered to all breast cancer patients, both newly diagnosed and those with a previous personal history, or patients without breast cancer but who otherwise meet NCCN guidelines [8].
5. **What is the significance of identifying breast cancer patients with hereditary mutations?**
   - Identification of patients with pathologic variants of these hereditary mutations can impact patient management in terms of high-risk testing, surveillance, risk reduction, and therapeutic intervention related to surgery, radiation, and system therapy including the application of poly(ADP-ribose) polymerase (PAPR) inhibitors.
   - It also has potential impact on the patient's family member health and management.
6. **Which genes should be included in genetic testing for hereditary breast cancer?**
   - A wide variety of genetic testing panels are available with different genes on different panels.
   - There is lack of consensus among professional societies and experts regarding which genes should be tested in different clinical scenarios, but it is recommended that the panel should include at least *BRCA1/BRCA2* and *PALB2*, with other genes as appropriate for the clinical scenarios and family history.
   - A survey among clinicians and clinical scientists in UK recommended gene panel with majority agreement (>75%) for breast cancer should include *BRCA1*, *BRCA2*, *PALB2*, *PTEN*, *STK11*, *TP53, CHEK2* (truncating variants), and *ATM* (truncating variants plus ATM c.7271T>G, p.(Val2424Gly) [9].
7. **What are the testing methods for *BRCA* mutations?**
   - The predominant genetic test for *BRCA1* and *BRCA2* mutations was BRACA*nalysis* (Myriad Genetic Laboratories, Utah, USA):
     - The testing is performed via forward and reverse sequencing of amplified DNA aliquots obtained from patient's buccal mucosa or peripheral blood sample.
     - Variants of the BRACA*nalysis* test include (1) BRACA*nalysis* rearrangement test, which is indicated for patients who are suspected of having a *BRCA* mutation; (2) single-site BRACA*nalysis*, which is indicated for patients with a known familial mutation; and (3) multisite 3 BRACA*nalysis*, which is indicated for patients with Ashkenazi Jewish heritage.
   - Since 2013, genetic options to include gene mutation panels have been expanded by university-based and private laboratories.
   - The standard method for the laboratory assessment of BRCA genes includes comprehensive sequencing and testing of broad genomic rearrangements including next-generation multi-gene sequencing.
   - If the patient has a relative with a particular mutation, a single-site targeted mutation analysis can also be performed.
   - The results are broadly described in three ways: (1) positive for a deleterious mutation, (2) genetic variant, and (3) no deleterious mutations.
8. **What are the limitations of the genetic testing for hereditary breast cancer [8, 9]?**
   - It is only one of several tools for assessing breast cancer risk and the result is not always straightforward with clear guideline. The negative result does not necessarily mean that they are not at increased risk for developing breast cancer, and other contributing factors such as age, medical history, family history, life style, and exposure should also be considered.
   - Testing a larger number of genes will result in finding more variants of uncertain significance, which causes difficulty in interpreting and explaining the results, and can leave families with more questions than answers.
   - Finding a pathogenic variant in a moderate-risk gene in the context of a high-risk family history does not

always aid clinical management since the variant cannot be assumed to account for all of the genetic risks in the family.

9. **What is the molecular subclassification of breast cancer? What are the clinicopathologic features of breast cancers by molecular subclassification?**
   - Breast cancers have traditionally been classified based on clinicopathological features, mainly histologic type, histologic grade, and tumor stage. With the advancement in detecting biomarker expression profile, breast cancer can be classified into ER positive, ER negative, HER2 positive, HER2 negative, and triple-negative categories. This simplified molecular classification system remains the most important and informative molecular breast cancer taxonomy to date for clinical management in routine practice [10].
   - In 2000, Perou and colleagues [11] studied 8,102 human genes on 65 breast specimens by using complementary DNA (cDNA) microarray and unsupervised cluster analysis. Their results indicated each tumor was unique and had distinct gene expression signatures, which led to a selection of 496 "intrinsic gene subset" and subsequently revealed five distinct classes of breast carcinomas: luminal A, luminal B, ERBB2 (HER2)-enriched, basal-like, and normal breast-like in an extended analysis [12, 13].
     - The luminal tumors were named as such because of the high expression of genes normally expressed by luminal epithelium of the breast. These luminal tumors also express ER and ER-related genes.
     - The ERBB2 (HER2)-enriched tumors were characterized by high expression of several genes in the *ERBB2* amplicon at 17q22.24 including ERBB2, GRB7, and TRAP100.
     - The basal-like tumors were named due to the high expression of KRT5, KRT17, annexin 8, CX3CL1, and TRIM29 and were completely negative for the luminal/ER cluster of genes.
     - The normal breast-like group has been shown to have the highest expression of many genes known to be expressed by adipose tissue and other non-epithelial cell types, and it is unclear whether these tumors represent poorly sampled tumor tissue or a distinct, clinically important group [14].
   - In addition, other molecular subtypes including claudin-low and molecular apocrine types have later been identified, and both groups are considered defined molecular subgroups of triple negative breast cancer [15–17].
   - Each of these molecular subtypes is characterized by different clinical features such as significant difference in overall survival, relapse-free survival, and pattern of recurrence, independent of traditional pathologic features. Table 5.1 lists the clinicopathologic characteristics and key molecular features of most common molecular subtypes of breast cancer.
10. **What is the relationship between triple-negative and basal-like subtype breast cancers?**
    - The triple-negative breast cancers (TNBC) are characterized by the absence of ER and PR expression and lack of overexpression/amplification of HER2.
    - Basal-like subtype of breast cancer in the above-mentioned molecular subclassification refers to a distinct gene expression signature characterized by high expression of basal epithelial markers such as cytokeratin 5, 6, and 17. Both TNBCs and triple-like breast cancers are associated with poor prognosis and show disproportionally higher prevalence in African women.
    - The majority (~70%) of TNBCs are found to be basal-like by gene expression, and recent studies using hierarchical clustering have identified four stable TNBC subtypes including two basal-like, mesenchymal, and luminal androgen receptor subtypes [23, 24].
    - Most basal-like cancers (50–77%) are triple negative in nature [25, 26].
11. **When should the biomarkers in breast cancers be assessed?**
    - According to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines [27, 28]:
      - The evaluation of biomarkers including ER, PR, and HER2 should be performed in all patients with newly diagnosed invasive breast cancer and in recurrent and metastatic breast cancers when the tissue sample is available.
      - The evaluation of ER in cases of newly diagnosed ductal carcinoma in situ (DCIS, without association invasion) is recommended and PR testing is considered optional in DCIS.
12. **Why does the ASCO/CAP recommend testing PR status in breast cancers although there is no targeted therapy?**
    - Upon binding by progesterone, the progesterone receptors dimerize, bind progesterone responsive elements (PRE) in the promoters of a number of genes, and thus induce transcription of these genes including those regulating proliferation.
    - PR status serves as an indicator of intact ER function since PR expression can be regulated by ER. The possible mechanisms of PR loss in the ER positive breast cancers include the aberrant

Table 5.1 The clinicopathologic characteristics and key molecular features of most common molecular subtypes of breast cancer

Molecular subtypes	Luminal A	Luminal B	HER2 enriched	Basal-like
% of breast cancer	~30–40%	~20–30%	10–15%	15–20%
Age	Older age	Younger	Younger	Younger
Histologic grade	1–2	2–3	2–3	Typically 3
Common histologic types	IC-NST(well-differentiated), classic lobular, tubular, cribriform, mucinous, neuroendocrine	IC-NST, micropapillary	IC-NST, apocrine, pleomorphic lobular	IC-NST, medullary features, metaplastic, adenoid cystic, secretory
ER status by IHC	Positive (high)	Positive (maybe low)	Negative	Mostly negative
PR status by IHC	Usually positive	Negative or low positive	Negative	Mostly negative
HER2 by IHC or FISH	Negative	Positive in ~40% (luminal HER2)	Positive (Classic HER2)	Negative
Ki-67	Low (<10%)	Typically high (>14%)	Typically high (>20%)	Typically very high (>50%)
CK5/6 or EGFR	Negative	Negative	Occasionally positive	50–85% positive
Key molecular features [1, 18]	*PIK3CA* mutations, *MAP3K1* mutations, *ESR1* high expression, *XBP1* high expression, *GATA* mutations, *FOXA1* mutations; quiet genomes, gain of 1q, 8q, loss of 8p, 16q	*TP53* mutations, *PIK3CA* mutations, *Cyclin* D1 amplifications, *MDM2* amplifications, *ATM* loss, enhanced genomic instability, focal amplifications	*ERBB2* amplifications, *TP53* mutations, *PIK3CA* mutations, *FGFR4* high expression, *EGFR* high expression, *APOBEC* mutations, *Cyclin D1* amplifications, high genomic instability	*TP53* mutations, *RB1* loss, *BRCA1* loss, high expression of DNA repair proteins, *FOXM1* activation, high genomic instability, focal amplifications (e.g., 8q24)
Natural history	Indolent, possible late recurrence	More aggressive than luminal A	Worse natural history; sensitive to HER2-targeted therapy	Worse natural history except for some special types, early recurrence more likely
Local recurrence [19]	0.8–8%	1.5–8.7%	4–15% (without HER2 targeted therapy)	3–17%
Neoadjuvant response (pCR rate) [20, 21]	~2%	~6%	≥ 57%	~34%
Type of therapy [22]	Endocrine therapy alone	Endocrine ± systemic chemotherapy Luminal HER2: Add HER2-targeted therapy	Systemic chemotherapy+ targeted therapy	Systemic chemotherapy

EGFR epidermal growth factor receptor, *FISH* fluorescent in situ hybridization, *ER* estrogen receptor, *HER2* human epidermal growth factor receptor 2, *IC-NST* invasive carcinoma of no special type, *IHC* immunohistochemistry, *pCR* pathologic complete response, *PR* progesterone receptor

ER-alpha signaling pathway, loss of PR gene, or downregulation by HER2 [29, 30].

- PR status has predominant prognostic values in the ER-positive breast cancers. When ER-positive invasive breast carcinoma has low or negative PR expression, it is usually high-grade tumor with higher mitotic activity (luminal type B), and the prognosis is worse than the ER+/PR+ breast cancers and is less responsive to hormonal therapy.
- Therefore, although only ER should be used as a predictor of benefit from adjuvant endocrine therapy, the ASCO/CAP updated ER/PR testing guidelines continue to recommend routine PR testing in invasive breast cancers [27].

13. **When should we consider repeating biomarker testing in breast cancers on the resection specimen?**
    - Initial core biopsy shows borderline, insufficient, equivocal, unusual, or discordant with clinicopathologic findings.
    - The invasive tumor shows high grade morphology with negative HER2 result on the initial core.
    - Tumor shows morphologic heterogeneity or high grade on the resection specimen.
    - Limited tumor cells on core biopsy.
    - Any suspicions on the tissue handling or testing errors on core biopsy.

14. **Should the biomarker status be repeated in the breast specimen status/post neoadjuvant therapy?**
    - Neoadjuvant chemotherapy (NAC) has been increasingly used for breast cancer. Studies have reported that the discordance rates between pre- and post-neoadjuvant therapy were up to 46% for ER and up to 43% for HER2 [see Reviews 31, 32]. The possible explanations for these discrepancies include intra-tumoral heterogeneity, treatment effect of targeted therapy, different antibody clones or methods, specimen handling/processing, and result interpretation variability.
    - Currently there are no guidelines regarding whether the biomarker studies should be repeated in the residual tumor after NAC. Whether to retest the biomarkers after NAC is an institutional-dependent decision or on a case by case basis.
    - Retesting of these biomarkers must be performed if previously unknown or if required by clinical trial. One may consider retesting if the pre-therapy results were negative, the pre-therapy tumor sample was insufficient, residual tumor shows heterogenous morphology, there are multiple tumors with different morphologic appearance, or if requested by clinicians [33].
    - It needs to be noted that a few studies have suggested that fluorescence in situ hybridization (FISH) analysis is preferred to immunohistochemistry (IHC) when retesting for HER2 because FISH analysis is more stable than IHC [34, 35].
15. **What are the key points of ASCO/CAP recommendations for pre-analytic variables for biomarker assessment in breast cancers? What are the common pre-analytic, analytic, and post-analytic factors that could affect the biomarker testing in breast cancer?**
    - According to the most updated ASCO/CAP recommendations, the key points for pre-analytic variables for biomarker (ER, PR and HER2) assessment in breast cancers include [27, 28]:
      - Minimize cold ischemic time (time intervals between tissue removal from patient to exposure to formalin fixation) to 1 h or less.
      - Use 10% neutral buffered formalin as the standard fixative.
      - Tissue fixation time is at least 6 h, but no more than 72 h. This applies to both core biopsy and resection specimens. For specimens fixed longer than 72 h in which negative test results are obtained, the report should state that prolonged fixation could be a possible cause for the negative result, and alternative testing methods should be considered (e.g., FISH for HER2; gene expression assay for ER). For HER2 testing, labs should also consider confirming by FISH on any specimen fixed longer than 72 h, especially when it is not HER2 positive by IHC (score of 3+).
      - Unstained slides cut more than 6 weeks should not be used for analysis.
      - The common pre-analytic, analytic, and post-analytic factors that could affect the biomarker testing in breast cancer include prolonged cold ischemic times (>1 h), strong decalcification process or inadequate fixation, test methodology differences and standards or test interpretation criteria and methods, and under/over interpretation.
16. **How to handle a bone specimen if it is suspicious for metastatic breast cancer?**
    - Bone is the most common site of breast cancer involvement and the bone specimen always requires decalcification.
    - The current ASCO/CAP guidelines do not recommend a specific decalcification process for biomarker testing. The guidelines state that the sample with decalcification artifacts should be rejected, and the sample that was decalcified in a strong acid solution may be rejected [28].
    - It has been demonstrated that EDTA decalcification is the preferred method since it has been shown to be molecular friendly and that it minimally affects the biomarker expression results [36].
17. **Do the ASCO/CAP guidelines for biomarker tests in breast cancer exclude testing of cytology specimens (fluids and aspirates) that have been fixed in 95% ethanol rather than formalin?**
    - According to CAP [37], fixatives other than formalin are not precluded by the guidelines.
    - For tissue specimens, laboratories that choose to use a fixative other than neutral buffered formalin must validate that fixative's performance against the results of testing of the same samples fixed in neutral buffered formalin and tested with the identical assay.
    - Since cytology specimens are not ordinarily fixed in formalin such concordance studies are not practical, but labs performing testing on such specimens must document that they validated their methods and achieved acceptable concordance, perhaps by comparing staining of alcohol-fixed cytology specimens with subsequently excised routinely processed, formalin-fixed, surgical pathology specimens.

18. **How should we interpret the ER/PR staining results in breast cancers?**
    - According to the ASCO/CAP guidelines, ER/PR in breast invasive cancers and carcinoma in situ should be interpreted [27]:
      - ER- or PR-positive cancer is one in which ≥1% of invasive carcinoma/carcinoma in situ cell nuclei show immunoreactivity. If 1–10% of invasive tumor cell nuclei are immunoreactive for ER, the sample should be reported as ER low positive with a recommended comment (This does not apply to PR).
      - ER- or PR-negative cancer is one in which <1% or 0% of invasive carcinoma/carcinoma in situ cell nuclei show immunoreactivity, regardless of staining intensity.
      - ER and/or PR status is not interpretable if the sample is inadequate (insufficient cancer or severe artifacts present), if the external and internal controls do not show appropriate and acceptable staining, or if pre-analytic variables have interfered with the assay's accuracy.
      - Interpretation of any ER result should include evaluation of the concordance with the histologic findings of each case.
      - For cases without internal controls present and with positive external controls, an additional report comment is recommended.
19. **What are the semiquantitative scoring methods for immunohistochemical assessment of ER and PR in breast cancers?**
    - The H score is also called "histo" score and is a method of assessing the extent of nuclear immunoreactivity. The score is calculated by the formula: 3 × percentage of strongly staining nuclei +2 × percentage of moderately staining nuclei +1 × percentage of weakly staining nuclei +0 × percentage of no staining nuclei, giving a range of 0–300.
    - The Allred score is calculated by adding the proportion score (PS, score 0–5 depends on the proportion of tumor cells which are stained) and the intensity score (IS, score 0–3 depends on the intensity of staining), giving a final score of 0–8.
    - The Quickscore is similar to the Allred score system, but the final score is calculated by multiplying the percentage score (score 0–6 depends on the proportion of tumor cells which are stained) and intensity score (score 0–3 depends on the intensity of staining), together giving a final score of 0–18.
20. **What is the significance of the ER low positivity in breast cancer?**
    - The newly released ASCO/CAP updated ER/PR testing guidelines specifically addressed the low ER-expressing breast cancers.
    - Low ER positive breast cancer refers to the tumor cells showing 1–10% ER expression by immunohistochemistry, which accounts for ~2–3% of breast cancers [38].
    - Recent studies showed that the breast cancers with low ER positivity is a heterogenous group, more often to have basal-like intrinsic subtype than luminal subtype including high-grade morphology, sheet-like growth pattern, and the presence of tumor necrosis. These low-ER positivity breast cancers usually fail to show survival benefit from hormonal therapy although the overall prognosis is slightly better than the ER negative breast cancers [27, 39, 40].
    - When one encounters such a case, other clinicopathologic variables such as age, histologic grade, tumor size, PR status, and molecular assays such as PAM50 or BluePrint may be helpful in making clinical decisions.
21. **Are other ER expression assays acceptable for identifying patients likely to benefit from endocrine therapy?**
    - According to the ASCO/CAP updated ER/PR testing guidelines [27], validated IHC is the recommended gold standard test for predicting benefit from endocrine therapy in patients with breast cancer, and no other assay types are recommended as the primary screening test.
    - Data on the ability of new methods of ER testing such as mRNA testing in the panel-based gene expression assays to predict endocrine therapy benefit for breast cancer as an initial screening test are limited.
22. **What are the possible causes for the discordant ER status by IHC and RT-PCR (Oncotype Dx result)?**
    - Earlier studies have demonstrated good agreement (93–98.9%) for ER status between RT-PCR-based methods and IHC, with IHC being slightly more sensitive [41, 42]. However, the tumor rarely can be ER positive by IHC and negative by RT-PCR quantitative result on an Oncotype DX assay.
    - The possible causes for this discordance include low ER positive/borderline result, false positive IHC result due to mis-interpretation, or low cancer cellularity (such as what might be seen with invasive lobular carcinoma) that causes false negative RT-PCR results.
    - It is always important to correlate the ER-testing results with other histopathologic features.
23. **Which method is better for HER2 testing in breast cancers, immunohistochemistry (IHC), or in situ hybridization (ISH)?**
    - Currently, there are several FDA-approved methods to evaluate HER2 status in breast cancer including

IHC assessment of HER2 protein expression and in situ hybridization (ISH) for gene amplification, most commonly FISH.

- Both IHC and ISH are clinically validated to help predict the response of the tumor to the HER2-targeted therapy, and each method has its own advantages and disadvantages.
- The ASCO/CAP guidelines do not recommend one test over the other, and both assays could be used to assess the HER2 status of breast cancers if the test had been appropriately validated and the laboratory follows the recommendations of the guidelines.
- Worldwide, most laboratories use IHC as the primary test with reflex FISH analysis in the equivocal cases or discordant cases. It is also acceptable to do ISH testing as first-line testing method (with appropriate reflex IHC testing) or to do IHC and ISH co-testing in all cases.
- The main advantages of IHC are that it is easy to perform, is relatively inexpensive, and has permanent storage. The major disadvantages of IHC include considerable interobserver variability, less reliability since IHC can be significantly affected by specimen handling, antigen retrieval methods, and antibody specificity and sensitivity. These disadvantages have largely decreased since the introduction of the highly standardized Hercep test, the use of fully automated staining system, and the published ASCO/CAP testing guidelines [43].
- The main advantages of FISH include more objective and quantitative results, more accurate and less impacted by pre-analytical factors. The major disadvantages of FISH include being labor intensive and costly, more time-consuming, requirement of costly equipment, faded signals, and possible missed tumor heterogeneity [43].

24. **When should the breast cancer case be considered for HER2 ISH testing, if using IHC as the primary test?**
    - HER2 is scored 2+ by IHC.
    - HER2 IHC shows granular, cytoplasmic staining that is difficult to interpret the extent of membranous staining.
    - Cases with significant crush artifact that disrupts the membranous staining.
    - HER2 IHC shows moderate-strong but incomplete staining or basolateral pattern.
    - HER2 IHC shows heterogeneity.
25. **What are single-probe and dual-probe assays in HER2 in situ hybridization assay? Which assay is better?**
    - In breast cancer, the ISH assay is used to quantify the HER2 gene copy number within tumor cell nuclei. ISH can be performed either as a single-probe assay (HER2 probe only) or dual-probe assay (using differentially labeled HER2 and chromosome 17 centromere probes simultaneously).
    - Single-probe assays: If single-probe assays are used, only the average HER2 signals per cell are counted. If the average HER2 signals per cell≥ 6, the tumor is considered as HER2 positive; if the average HER2 signals per cell<4, the tumor is considered as HER2 negative. For cases with ≥4 but <6 average HER2 signals per cell, the concurrent HER2 IHC on the same block need to be reviewed. If the concurrent IHC shows positive (3+), then it is considered as ISH positive; if the concurrent IHC shows negative (0 or 1+), it is ISH negative. If the concurrent IHC shows equivocal (2+), then a dual-probe ISH needs to be performed for a final result.
    - Dual-probe assays: When using the dual-probe assays, one probe for the HER2 gene and one probe for the control gene in chromosome 17 will be counted. The interpretations for dual-probe ISH are based on the HER2/CEP17 ratio and the average HER2 signals/cell, and the result will fall into one of five result groups (see detailed below in Question 26).
    - The current ASCO/CAP testing guidelines also recommend the use of dual-probe instead of single-probe methods, although it recognizes that several single-probe ISH assays have regulatory approval in many parts of the world.
26. **What are the HER2 ISH groups in breast cancers on dual-probe assays? What are the unusual HER2 groups?**
    - There are five HER2 ISH groups in breast cancers when using dual-probe assays:
      - Group 1 (positive): HER2/CEP17 ratio≥2.0 and average HER2 signals per cell ≥4.0
      - Group 2: HER2/CEP17 ratio ≥ 2.0 and average HER2 copy number < 4.0 signals per cell
      - Group 3: HER2/CEP17 ratio <2.0 and average HER2 copy number ≥ 6.0 signals per cell
      - Group 4: HER2/CEP17 ratio < 2.0 and average HER2 copy number ≥ 4.0 and < 6.0 signals per cell
      - Group 5 (negative): HER2/CEP 17 ratio <2.0 and average HER2 signals per cell <4.0
    - The unusual HER2 ISH groups refer to groups 2, 3, and 4 on dual-probe assays (see Table 5.2).
    - ISH workup of an IHC HER2 equivocal (2+) breast carcinoma case using a dual-probe assay. Between 2013 and 2018, cases with equivocal HER2 ISH results were often sent for additional testing using alternative probes. However, this strategy was challenged based on the evidence that the indiscriminate use of alternative control probes to calculate

Table 5.2 HER2 ISH unusual group by dual-probe assay

HER2 ISH result	Group 2 (Monosomy)	Group 3 (Co-amplified/polysomy)	Group 4
Incidence (ISH testing)	0.4–3.7%	0.4–3.0%	1.9–14.2%
Reasons	Amplification of the *HER2* gene and an associated increased HER2 copy number, with a loss of chromosome 17 copy number	Either polysomy of chromosome 17 or more commonly co-amplification of both the *HER2* and *CEP17* genes	Mainly heterozygous deletions
Histologic features	Usually ER+ (~80%) Majority histologic grade 2 and 3	Typically ER+ (75%) Majority histologic grade 2 and 3	Usually ER+ (~82%) Majority histologic grade 2 and 3
HER2 IHC	Negative to equivocal:~88% IHC3+: ~12.4%	Negative to equivocal:~48% IHC3+:~31.7%	Negative to equivocal:~92.7% IHC3+:~7.3%

ER estrogen receptor, *IHC* immunohistochemistry, *ISH* in situ hybridization

HER2 ISH ratios in HER2-equivocal breast cancers may lead to false-positive interpretations of HER2 status, resulting from unrecognized heterozygous deletions of these alternative control genomic sites and incorrect HER2 ratio determinations [44, 45]. The 2018 ASCO/CAP guidelines discontinued the recommendation for using an alternative control probe to resolve ISH equivocal cases. Figure 5.2 illustrates the ISH workup of an IHC HER2 equivocal (2+) breast carcinoma case using a dual-probe assay, according to the most recent ASCO/CAP guidelines.

27. **What are the possible causes for the discordant HER2 results between IHC and ISH analyses?**
    - False-negative IHC results due to tissue handling.
    - False-positive IHC results due to analytic and post-analytic errors.
    - Unusual ISH positive group (such as HER2/CEP17 ratio>2 and the average HER2 copy number between 4–6, group 1b) or tumor heterogeneity.
    - It has been recommended to use HER2 IHC slides as the guide map for ISH analysis and to coordinate between IHC and ISH results for HER2 interpretation.
28. **What are the common discordances of biomarker status with histology or other clinicopathologic findings? What are the possible causes for these discordances? How to solve these discordances?**
    - ER negative or HER2 positive (score 3+, by IHC) results in a grade 1 invasive ductal or lobular cancers, pure tubular, cribriform, or mucinous cancers.
    - The possible causes include, but are not limited to, incorrect initial histologic classification, false negative/positive result, or specimen mix-up.
    - When this discordance occurs, reviewing the original H&E slide for confirmation of histologic discordance, repeating testing on the same or different blocks, sending the specimen for HER2 ISH analysis, or exploring the possible pre-analytic, analytic, and post-analytic causes can be helpful.
    - It needs to be noted that rare low-grade breast cancers can be HER2 positive, such as ~5–6% of classical or nonpleomorphic invasive lobular carcinomas are HER2 positive [46, 47]. Fig. 5.3 demonstrates a classical invasive lobular carcinoma with positive HER2 expression.
29. **What are the possible causes of discordant biomarker status between primary and metastatic breast tumors?**
    - It is widely accepted that receptor conversion occurs during metastatic progression of breast cancer and the reported incidence is variable. For example, Schrijver et al. reported in their meta-analysis that the pooled percentage of positive to negative conversion during metastasis in ER, PR, and HER2 was 22.5%, 49.4%, and 21.3%, respectively; conversely, the percentage of negative to positive conversion was 21.5%, 15.9%, and 9.5% [48]. In the study of Woo et al., positive to negative conversion was found in 5.3%, 24.3%, and 5.9% while negative to positive conversion was found in 0.7%, 2.0%, and 2.0% for ER, PR, and HER2, respectively [49].
    - The possible causes of discordance of biomarker status between primary and metastatic breast tumors include heterogeneity for the biomarker expression in tumor cells, loss of ER expression due to clonal selection and disease progression, treatment effect of targeted therapy, unusual or borderline/equivocal results for HER2, false-negative result for ER in the metastatic tumor due to tissue handling, false-negative initial HER2 result, or different testing methods for HER2.

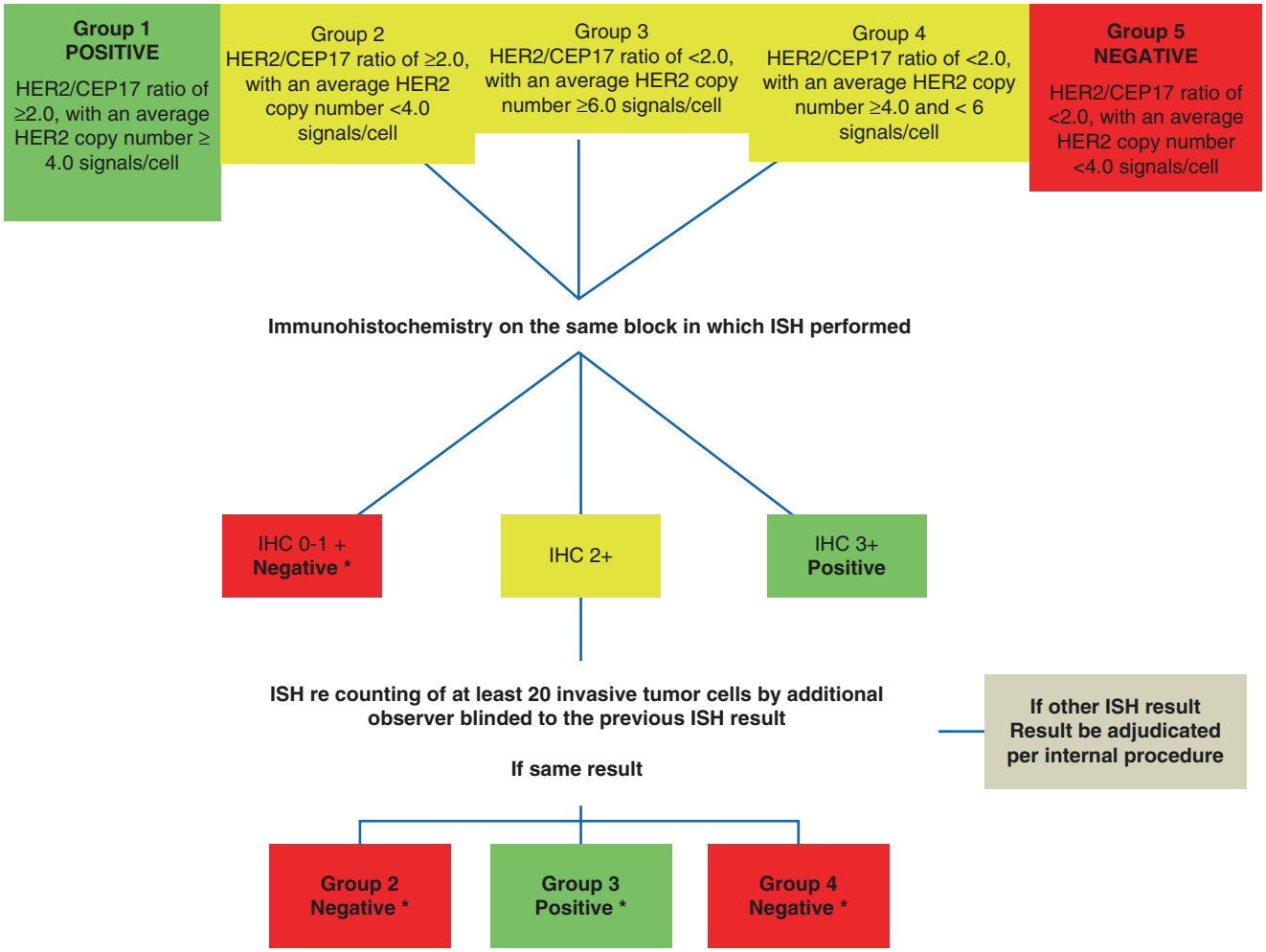

Fig. 5.2 The ISH workup of an IHC HER2 equivocal (2+) breast carcinoma case using a dual-probe assay. Used with permission: Zhang H, Moisini I, Ajabnoor RM, Turner BM, Hicks DG. Applying the New Guidelines of HER2 Testing in Breast Cancer. Curr Oncol Rep. 2020;22:51

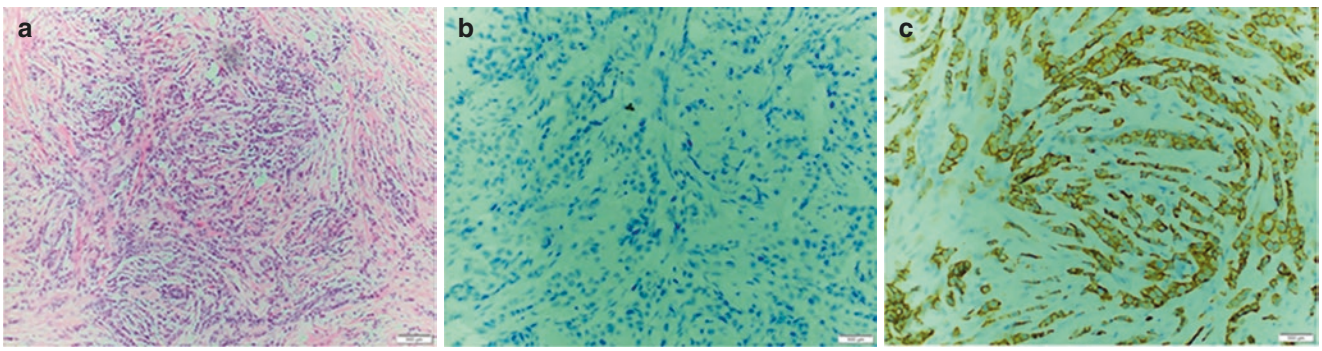

Fig. 5.3 Classical invasive lobular carcinoma with positive HER2 expression. (**a**) H&E: ×200; (**b**) Tumor cells are negative for E-cadherin (×200); (**c**) Tumor cells are positive for HER2 by immunohistochemistry (score 3+, ×200)

30. **What are gene signature and molecular profiling of cancers? What are the roles of molecular profiling tests in early-stage ER+ breast cancers? What are the commercially available molecular profiling tests for prognostication in early-stage ER+ breast cancers?**
    - A gene signature refers to a group of genes in a cell whose combined expression pattern is uniquely characteristic of a biological phenotype or medical condition. Molecular profiling refers to the assessment of DNA, RNA, and/or protein within an individual patient's tumor using cells obtained from a tumor biopsy or through the capture of tumor cells circulating in the bloodstream. The results of molecular profiling test can (1) reveal the genetic characteristics and any unique biomarkers; (2) give the risk score and the recurrence free survival rate for each patient; and (3) identify and create targeted therapies that are designed to work better for a specific cancer tumor profile.
    - In clinical practice, ER positive/HER2 negative breast cancer is the most commonly encountered but also the most challenging group for treatment due to significant clinical heterogeneity. It is crucial to identify those tumors with more aggressive biology that may benefit from additional chemotherapy from those tumors that can be treated by endocrine therapy alone. Molecular profiling tests can help determine the prognosis for an individual cancer patient with ER positive cancer, which is either node negative (largest group) or node positive, with the goal being to identify the low-risk group, in whom risks of adjuvant systemic chemotherapy outweigh the predicted benefit. Molecular profiling also identifies the patients with high risk, who are likely to respond to systemic chemotherapy. The result of molecular profiling tests plays an important role in clinical decision making in the era of precision medicine.
    - The commercially available molecular profiling tests for ER positive breast cancer include Oncotype Dx test, MammaPrint test, Prosigna test, EndoPredict test, Breast Cancer Index test, Mammostrate, and IHC4 (see Table 5.3).
    - When choosing the block for molecular profiling tests, the most ideal sample should have the largest area of invasive carcinoma available (usually resection specimen is preferred), avoiding the areas of biopsy site changes, inflammation, carcinoma in situ, or normal tissue as much as possible. Cases with only microinvasion or blocks from metastatic carcinoma in a lymph node are generally not appropriate for molecular profiling test due to the interference of non-cancer tissue and the potential to skew the assay results.
31. **When should we raise the suspicion for a major discordance between pathologic findings and Oncotype recurrence risk result? What shall we do if there is major discordance between the pathology result and Oncotype DX recurrence score (RS) result?**
    - The major discordance between pathologic findings and oncotype recurrence risk result should be concerned:
      - When the recurrence score of a tumor is higher than the expected such as a high RS score in a low-grade tumor with low proliferation index
      - When the quantitative ER result on Oncotype DX is negative or much lower compared to IHC result
    - If such major discordance is present, the pathologist can:
      - Review the original H&E section for the tested block to see whether there is large areas of biopsy site changes or inflammation
      - Retesting on additional material, either from different block without biopsy site changes or inflammation, or the biopsy material
32. **What is the role of testing *PIK3CA* mutation in breast cancer?**
    - *PIK3CA* is the most frequently mutated gene in ER+/HER2– breast cancer and up to 40% of these cancers carry a *PIK3CA* mutation [50].
    - In results from the phase III SOLAR-1 study, patients with *PIK3CA* mutations and prior endocrine therapy had significantly improved progression-free survival when treated with PI3K alpha-specific inhibitors alpelisib and fulvestrant compared to fulvestrant alone (11.0 vs. 5.7 months) [51].
    - *PIK3CA* mutation CDx testing is an FDA-approved qualitative companion diagnostic assay performed on DNA extracted from FFPE breast tissue to detect 11 mutations in exons 8, 10, and 21 of the *PIK3CA* gene (NM_006218.4; transcript ID: ENST00000263967.4), and this test is intended to identify *PIK3CA* mutations in patients with advanced HR+/HER2– breast cancer who may be candidates for therapy with alpelisib.
33. **How can molecular testing help in the diagnosis of breast tumors?**
    - In addition to providing prognostication and treatment response predication, molecular markers are also used to help the diagnosis of breast lesions.
      - Immunohistochemical stain for ER has been utilized frequently in differentiating between usual

Table 5.3 Comparison of selected commercially available molecular profiling tests for prognostication in ER-positive breast cancer

Test	Material	Method	Centralized	Component genes	Indication	Molecular subtyping	Result	AJCC staging	ASCO/NCCN recommendation	Involved in score assessment
Oncotype DX (Genomic Health, Redwood City, CA, USA)	FFPE	RT-PCR	Yes	21 genes	ER+, HER2- and 0-3 positive nodes	No	Low risk (RS < 18), intermediate risk (RS 18-31), high risk (RS > 31)	Yes	Yes (strong)	No
MammaPrint (Agendia, Amsterdam, The Netherlands)	Fresh or FFPE	Microarray	Yes	70 genes	ER+/−, HER2-, Stage 1-2, 0-3 positive nodes	Yes (BluePrint, 80 genes)	Low risk and high risk	Yes	Yes (strong)	Yes (Adjuvant! online)
Prosigna (NanoString Technologies Inc. Seattle, WA, USA)	FFPE	RT-PCR	No	50 genes	ER+, HER2-, Stage 1-2, 0-3 positive node	Yes	Node -: Low risk (0–40); intermediate risk (41–60); high risk (61–100) Node +: Low risk (0–40); High risk (41–100)	Yes	Yes (moderate)	Yes (Proliferation score, tumor size)
EndoPredict (Sividon Diagnostics GmbH, Koln, Germany)	FFPE	RT-PCR	No	12 genes	ER+, HER2-, 0-3 positive nodes	No	Low risk: EPclin Risk score <3.3287; High risk: EPclin Risk score >3.3287	Yes	Yes (moderate)	Yes (tumor size and nodal status EPclin Risk score)
Breast Cancer Index (Biotheranostic, San Diego, CA, USA)	FFPE	RT-PCR	Yes	Combine 2 independent biomarkers (HOXB13:IL17BR [H/I] and the 5 gene molecular grade index (MGI)	ER/PR+, HER2-, node 0-3 positive	No	BCI prognostic score: Low or high risk; BCI predictive score: High or low likelihood of benefit from extended endocrine therapy	Yes	Yes (moderate)	No

AJCC American Joint Committee on Cancer, *ASCO* American Society of Clinical Oncology, *ER* estrogen receptor, *FFPE* formalin fixed paraffin embedded, *HER2* human epidermal growth factor receptor 2, *NCCN* National Comprehensive Cancer Network, *RS* recurrence score, *RT-PCR* reverse transcription polymerase chain reaction

ductal hyperplasia and neoplastic clonal epithelial proliferation (atypical ductal hyperplasia/low grade ductal carcinoma in situ), between microglandular adenosis and well-differentiated carcinoma and in determination of tumor origin.
  - As the product of the *CDH1* gene, immunohistochemical stain for E-cadherin helps distinguish the ductal and lobular phenotype of breast lesions due to the *CDH1* gene aberrations in lobular lesions, as well as serves as a prognostic marker in breast cancers.
  - Some uncommon, special type mammary carcinomas show specific translocations which characterize these tumors and can be used as diagnostic adjunct. Secretory carcinoma of the breast is characterized by a balanced translocation of genetic material between chromosomes 12 and 15 [t(12:15)] which produce *ETV6-NTRK3* fusion gene [52]. Adenoid cystic carcinomas show a specific translocation [t(6:9)(q22-23;p23-24)] and create *MYB-NFIB* transfusion gene [53].

II **Gynecologic tumors**

1. **What are the roles of molecular testing in gynecologic pathology?**
   - Same as in breast pathology, molecular testing has become increasingly important in the diagnosis and management of gynecologic tumors in the era of precision medicine. The applications of molecular testing in gynecologic pathology include:
     - Helping in the understanding of the tumor pathogenesis especially in ovarian, endometrial, and cervical carcinomas.
     - Testing for hereditary DNA mutation in patients with endometrial and ovarian/fallopian tube/peritoneal cancers to define hereditary cancer syndrome and identify patients for selected therapy.
     - Subclassifying molecular tumor types in endometrial cancers.
     - Identifying biomarkers that can predict or monitor the response to treatment.
     - Testing for specific markers and translocations to facilitate the diagnosis of certain gynecologic tumors.
2. **Why testing *BRCA1* and *BRCA2* genomic status is important in patients with ovarian carcinomas?**
   - *BRCA1* and *BRCA2* encode essential proteins for DNA homologous recombination repair (HRR) (see question 3 below).
   - Germline mutations in *BRCA1* and *BRCA2* have been identified in approximately 17% of high-grade ovarian serous carcinomas and somatic mutations in additional 3% [54].
   - Women with non-serous ovarian carcinomas including endometrioid, clear cell, low-grade serous, or carcinosarcoma subtypes also have appreciable rates of carrying *BRCA* mutations [55].
   - In additional to identifying patients with hereditary *BRCA* mutations for high-risk surveillance and management for patients and their affected family members, *BRCA*-mutated ovarian cancer displays enhanced sensitivity to DNA-damaging agents (platinum-based chemotherapy) or to novel agents that block parallel DNA repair pathways, including PARP inhibitors [55–58]. PARP inhibition blocks the repair of DNA single-strand breaks and results in stalling of replication fork progression by trapping PARP on the DNA break [59].
3. **What is homologous recombination deficiency (HRD)?**
   - DNA double-strand break (DSB) is one of the most cytotoxic DNA lesions and causes chromosomal aberration and ultimately cell death if not adequately repaired. The ability to restore DSBs depends on the activity of HRR apparatus, which copies the respective undamaged, homologous DNA of the sister chromatid to reconstruct the corrupted double strand during S and G2 phases. If HRR fails, the process is ended by the so-called non-homologous end joining, an error-prone process of random end-to-end fusion of damaged strands, and leads to accumulation of additional mutations and chromosomal stability, as well as increasing risk of malignant transformation.
   - The function of this HRR apparatus relies on the interaction of a complex set proteins such as the BRCA1, BRCA2, RAD51C, RAD51D, BRIP1, PALB2, and the MMR proteins.
   - Any dysfunctional protein involved may induce phenotypical homologous recombination deficiency (HRD).
   - The Cancer Genome Atlas (TCGA) data showed that approximately half of the high-grade ovarian serous cancers have aberrations in HRR and a majority of them harbor *BRCA1* or *BRCA2* germline or somatic mutations [54]. However, approximately 30% of high-grade serous carcinomas show *BRCA* wild-type status but are associated with alteration of HRR apparatus and cause the phenotypical-deficient cell behavior [54, 60, 61].

4. **How to diagnose HRD?**
   - Testing for HRD can define a subset of high-grade serous carcinoma patients who are most likely to benefit from PARP inhibitor therapy in the first-line and recurrent settings. Currently, there are several different strategies to test for HRD. Since all proposed methods lack broad prospective validation, currently no specific assay may be generally recommended.
     – Germline mutation testing of genes related to HRR.
     – Somatic mutation screening of genes related to HRR.
     – Genomic scarring assays: These assays aim to quantify large genomic aberrations, which represent the genomic instability secondary to HRD, by next-generation whole genome sequencing. The "CDx BRCA LOH" (Foundation Medicine, Cambridge, MA, USA) detects the percentage of loss of heterozygosity throughout the genome and mutations in *BRCA1* or *BRCA2*. The "myChoice" HR deficiency test (Myriad Genetics Inc., Salt Lake City, UT, USA) calculates a score based on the presence of heterozygosity, large scale transitions, and telomeric allelic imbalance. All the clinical trials relied on these genomic scarring assays.
     – HRDetect test: A whole-genome sequencing-based classifier designed to predict *BRCA1* and *BRCA2* deficiency based on six HRD-associated mutational signatures, this test identifies *BRCA1/BRCA2*-deficient tumors with 98.7% sensitivity [62]. Limitations include the need for whole-genome sequencing and, therefore, increased expense, possibly longer turn-around time, and the requirement of a tumor cell percentage greater than 50%.
     – RAD51 foci assays: A functional assay for detecting HRD in tumor samples by immunohistochemistry or immunofluorescence. *RAD51* encodes a recombinase with an essential role in HRR. RAD51 forms distinct subnuclear foci after DNA damage, and the inability to form RAD51 foci is a common feature of HRD. HRD scores based on RAD51 foci formation assays have been shown to correlate to chemosensitivity, PARP inhibitor sensitivity, and overall survival [63, 64].
5. **In which individual should risk evaluation, counseling, and genomic testing for germline and somatic tumor alterations in ovarian cancer be performed?**
   - According to the recent ASCO guidelines [65], the following patients are recommended to have genomic testing for germline and somatic alterations:
     – All women diagnosed with epithelial ovarian cancer should be offered germline genetic testing for *BRCA1*, *BRCA2*, and other ovarian cancer susceptibility genes, irrespective of their clinical features or family cancer history.
     – Somatic tumor testing for *BRCA1* and *BRCA2* pathogenic or likely pathogenic variants should be performed in women who do not carry a germline pathogenic or likely pathogenic *BRCA1/2* variant.
     – Women diagnosed with clear cell, endometrioid, or mucinous ovarian cancer should be offered somatic testing for mismatch repair deficiency (dMMR).
     – Testing for dMMR may be offered to women diagnosed with other histologic types of epithelial ovarian cancer.
     – First- or second-degree blood relatives of a patient with ovarian cancer with a known germline pathogenic cancer susceptibility gene mutation or variant should be offered individualized genetic risk evaluation, counseling, and genetic testing.
6. **What is the best approach for testing *BRCA* mutations in patients with ovarian/tubal/primary peritoneal carcinoma?**
   - Women with ovarian/fallopian tube/primary peritoneal carcinomas should be offered testing at the time of diagnosis. If the patients have not had testing at the time of diagnosis, they should be offered germline genetic testing if possible.
   - The most sensitive approach for *BRCA* mutation in patients with ovarian/tubal/primary peritoneal carcinoma is the sequencing of germline DNA.
   - If germline mutation DNA is negative for *BRCA* mutation, the DNA from tumor tissue should be sequenced since additional 5–6% of patients have somatic *BRCA* mutations [54, 65–67].
   - Due to the less sensitivity of somatic testing, the decision to sequence germline DNA should not depend on finding a mutation in tumor tissue. Up to 5% of germline mutations will be missed if using tumor somatic mutation results to determine whether to sequence germline DNA [65].
   - The expert panel from the ASCO guidelines recommends that germline sequencing of *BRCA1* and *BRCA2* can be performed in the context of a multigene panel that includes, at minimum, *BRCA1, BRCA2, RAD51C, RAD51D, BRIP1, MLH1, MSH2, MSH6, PMS2*, and *PALB2* [65].

7. **What is the significance of detecting deficient mismatch repair (dMMR) in patients with ovarian carcinomas?**
   - Overall dMMR has been identified in approximately 10–12% of unselected epithelial ovarian cancers. It is more common in endometrioid ovarian cancer (~13–20%) but can also be found in clear cell carcinomas (~2.4%) [68–70].
   - The identification of dMMR status can provide additional treatment options such as immunotherapy for patients with recurrent ovarian, fallopian tube, or primary peritoneal cancers [65].
8. **What are the pathologic features of Lynch syndrome-associated endometrial cancer?**
   - The experience with the pathologic features of Lynch syndrome-associated endometrial cancer is less compared to those of colorectal cancer. It has been found that endometrial cancer associated with Lynch syndrome tends to show the following pathologic features:
     - Histologically diverse and include a much greater proportion of mixed and nonendometrioid morphologies, and frequent dedifferentiated/biphasic morphology in Lynch syndrome-related endometrial cancer [71–73].
     - Present in a relatively young age with a mean age of 46.4 years at the diagnosis.
     - Cancer arising from the lower uterine segment (LUS). A large series of endometrial cancers demonstrated that the prevalence of Lynch syndrome in patients with LUS endometrial carcinoma (29%) is much greater than that of the general endometrial cancer patient population (1.8%) or in endometrial cancer patients younger than age 50 years (8–9%) [74].
     - Presence of prominent tumor-infiltrating lymphocytes and peritumoral lymphocytes.
9. **Why testing for mismatch repair protein is important in patients diagnosed with endometrial carcinomas?**
   - Appropriately 2–5% of endometrial cancers are due to Lynch syndrome, which results from germline mutation in one of mismatch repair protein genes: *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*.
   - Patients with Lynch syndrome have 40–60% lifetime risk for endometrial and colon cancers and are at risk for other cancers in the ovary, stomach, small bowel, and liver.
   - Among women with Lynch syndrome who have both colon and endometrial cancers, about half present first with endometrial cancer, which makes endometrial cancer the most common sentinel cancer in Lynch syndrome [75, 76].
   - Identification of patients with Lynch syndrome may allow for screening and prevention strategies for patients themselves and their affected family members.
   - US FDA-approved PD-L1 inhibitor pembrolizumab for the treatment of unresectable or metastatic dMMR cancers including endometrial cancer.
10. **Who should be screened for Lynch syndrome on tissue when diagnosed with endometrial cancer?**
    - Currently, the practice of tissue testing for screening of Lynch syndrome in endometrial cancer is highly variable across institutions and countries. There are three approaches for assessing the possibility of Lynch syndrome in a woman with a diagnosis of endometrial cancer [77]:
      - Perform tissue testing on endometrial cancer from a woman identified to be at risk through a systemic screen that includes a focused personal and family history.
      - Perform tumor testing on all endometrial cancers irrespective of age of diagnosis.
      - Perform tumor testing on all endometrial cancers diagnosed before age 60 years.
      - A universal Lynch-syndrome-screening algorithm has been proposed by Mills and Longacre [78].
11. **What are the common testing methods for screening Lynch syndrome?**
    - Immunohistochemistry method to assess the expression status of key MMR proteins including MLH1, MSH2, MSH6, and PMS2. It is simple, cost-effective, and is the most-commonly used first-line method. The major disadvantage of the IHC method is to miss dMMR tumors due to mutations that lead to loss of MMR function but still maintain antigenicity [79].
    - Polymerase chain reaction (PCR)-based microsatellite instability testing. Microsatellite instability testing has been shown to be less sensitive than IHC, which is due to the failure to detect many *MSH6* germline mutation carriers and fails to identify the putative causative gene/protein deficiency.
    - Both IHC and MSI tests have high sensitivity. The false-negative rates in both tests are 5–10%.
    - *MLH1* promoter methylation analysis: *MLH1* promoter methylation accounts for up to 96.9% of endometrial cancer occurrences that have an absence of MLH1 and PMS2 on IHC [75]. If the tumor demonstrates an absent expression of MLH1 and PMS2 on IHC, an *MLH1* promoter methylation needs to be done before germline genetic testing.
    - Germline mutation testing is the most conclusive method. Next-generation sequencing has also been

used to predict microsatellite status by focusing on targeted sequencing of known microsatellite loci or analysis of microsatellite regions using novel informatics algorithms [80, 81].

12. **How to interpret the immunohistochemical stains for MMR proteins? What are the pitfalls in the interpretation of MMR on IHC? How to resolve these pitfalls?**
    - Immunohistochemical stains for MMR proteins are performed on a tumor sample.
    - The result should be reported as either positive or negative.
    - Unequivocal nuclear staining of MMR protein in viable tumor cells in the presence of appropriate internal positive controls is considered as intact protein expression. Strong nuclear staining in the surrounding endometrial stroma, myometrium, lymphocytes, or normal endometrium should serve as an internal positive control. The absence of nuclear staining of MMR protein in viable tumor cells in the presence of appropriate internal positive controls is considered as negative for the MMR protein.
    - The presence of dot-like nuclear, cytoplasmic, and other potentially "artifactual" staining patterns should be disregarded in the interpretation of status.
    - Figure 5.4 shows a representative case of MMR intact endometrial cancer by immunohistochemistry. Figure 5.5 shows heterogenous expression of MMR by immunohistochemistry on a dedifferentiated endometrial cancer with loss of nuclear expression of MLH1 and PMS2 in the undifferentiated carcinoma component, while intact expression of MLH1 and PMS2 in the well-differentiated endometrioid component.
    - When interpreting the MMR status using IHC method, there are some pitfalls:
    - False-negative nuclear staining in the tumor cells occurs in the setting of inadequate internal positive controls. This may be resolved by repeating the IHC with consideration of increasing antigen exposure time or using different tissue blocks from the same specimen.
    - Presence of endometrial stromal cells or lymphocytes on the IHC sections may cause difficulties in MMR interpretation. Correlation with the corresponding H&E sections should resolve this problem.
    - Heterogenous staining of MSH6 expression was reported in 0.17% cases of colorectal, endometrial, and sebaceous tumors [82]. This is not a typical feature of Lynch syndrome; however, a possibility of germline mutation in the other MMR genes cannot be excluded.
    - Subclonal loss of MMR protein: Predominantly MLH1 is identified by recognizing an area with retained expression of MMR in tumor cell nuclei

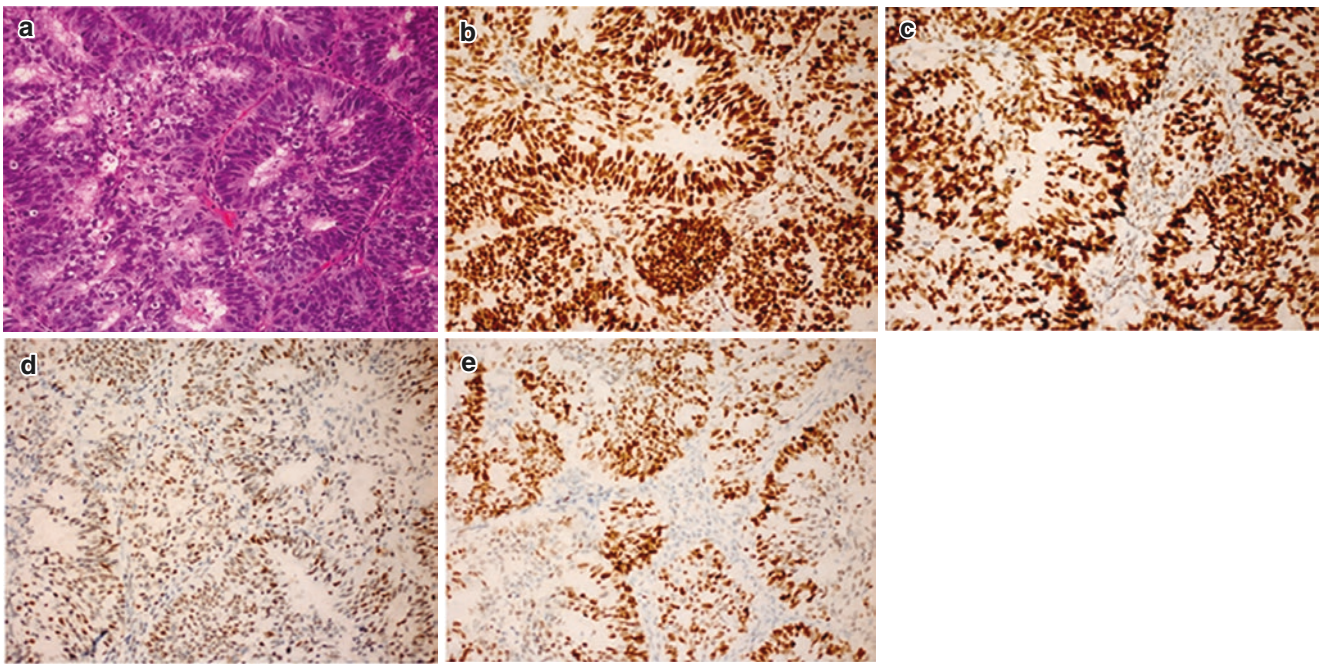

Fig. 5.4 A 68-year-old woman with endometrial adenocarcinoma. The tumor shows endometrioid histotype (**a**, H&E, ×100) and intact MLH1(**b**, ×100), MSH2 (**c**, ×100), PMS2 (**d**, ×100), and MSH6 (**e**, ×100)

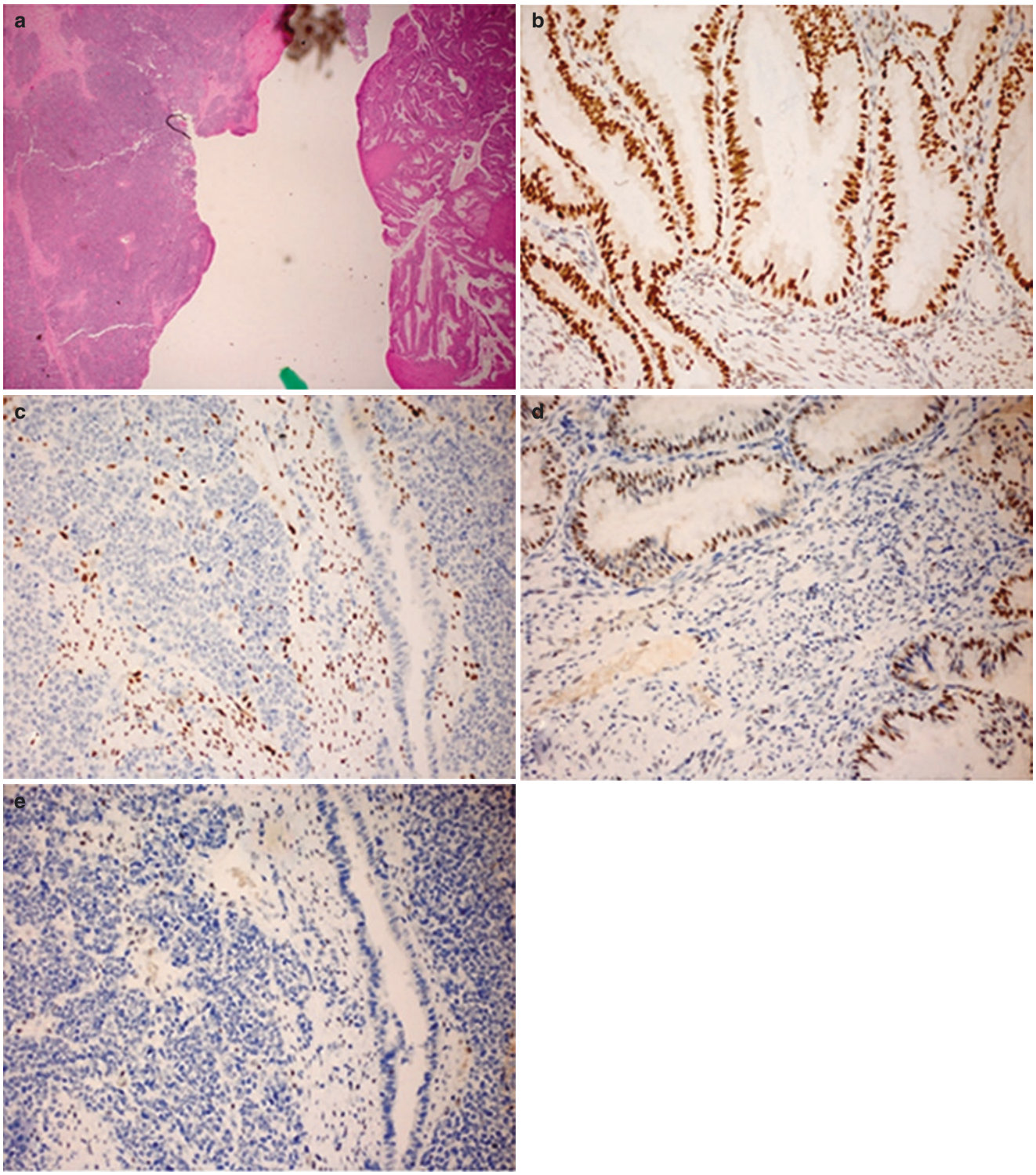

Fig. 5.5 A 56-year-old woman with endometrial adenocarcinoma. The tumor shows dedifferentiated histotype with well-differentiated endometrioid adenocarcinoma and abrupt transition into solid growth of medium-sized, discohesive tumor cell population (**a**, H&E, ×20). MMR immunohistochemistry shows heterogenous expression with loss of nuclear expression of MLH1 (**c**, ×200) and PMS2 (**e**, ×200) in the undifferentiated carcinoma component, while intact expression of MLH1 (**b**, ×200) and PMS2 (**d**, ×200) in the well-differentiated endometrioid component. *MLH1* promoter methylation by PCR shows positive *MLH1* promoter methylation, indicating sporadic endometrial cancer

and an abrupt transition to a clearly delineated regional area with complete loss of expression. The subclonal loss of MMR protein can be seen in appropriately 7% of endometrial carcinomas and it is associated with epigenetic silencing of the *MLH1* promoter by methylation rather than a germline *MLH1* mutation [83]. Subclonal loss can be distinguished from the inadequate fixation or an error in the staining process by identifying staining in positive internal control cells. In exceptional cases, heterogeneous MLH1 loss can be due to mutations in DNA polymerase epsilon (*POLE*) gene [84].

13. **Should the mismatch repair protein testing be repeated in cases of tumor recurrence?**
    - The role of MMR testing has evolved from identifying Lynch syndrome patients to predicting response to the immune checkpoint inhibitors. This may lead to request from clinical providers to retest recurrences of MMR-proficient primary tumors in the hope that the recurrence may show a different MMR status and may qualify the patient for additional treatment.
    - Aird et al. recently evaluated recurrent tumors from 137 patients with MMR-proficient primary tumors of the gynecological and gastrointestinal tracts, and they did not identify any cases with a genuine discordance between the primary and recurrent cases. Therefore, the authors do not advocate repeating MMR IHC on recurrences when the primary tumor shows intact MMR staining [85].
    - Ta et al. found that advanced endometrial cancer may rarely (~7%) exhibit discordant somatic MMR loss compared to primary tumor and the discordant metastatic endometrial carcinoma may be derived from an MMR-deficient subclone. Their results indicated MMR testing of recurrent tumor or metastasis should be considered for guiding immunotherapy if primary uterine tumor exhibits abnormal subclonal MMR loss [86].
14. **What is microsatellite instability (MSI)? What are the MSI testing methods? What are MSI-high, MSI-low, and MSI stable?**
    - Microsatellites are short-repeated sequences of DNA that are composed of repeating sequence of nucleotides of one to six base pairs in length (e.g., AAAAA or CGCGCGCG).
    - Microsatellite instability (MSI) is a form of genomic instability resulting in the accumulation of insertions or deletions (indels) in microsatellites during replication due to an impaired MMR protein function.
    - MSI can be tested by PCR-based technology and next-generation sequencing.
    - The PCR-based analysis is the commonly used method for screening MSI in patients with endometrial cancer. It compares the sizes of microsatellite marker sets in tumor DNA with corresponding DNA isolated from a normal tissue sample from the same patients via electrophoresis. A range of markers may be used but the core panel recommended five microsatellite markers consisting of three dinucleotide repeats (D2S123, D5S346, D17S250) and two mononucleotide repeats (BAT26, BAT25) [87].
    - Next-generation sequencing-based analyses have comprehensively characterized MSI-positive cancers, and different panels demonstrated the clinical validity of specific methodology and the NGS approach for detection of MSI for Lynch syndrome [88–91].
    - Definition of MSI-high varies by the panel used and the reference standard depends upon the purpose of the test. By using PCR-based MSI testing method, tumor with ≥2 of core panel, or >30% of markers for other panels showing instability is considered as MSI-high. Tumor with 1 of core markers, or <30% of markers for other panels showing instability is considered as MSI-low. MSI-stable refers to the tumor with 0 marker showing instability.
15. **What is *MLH1* promoter methylation? How to test *MLH1* promoter methylation?**
    - Gene inactivation through an epigenetic process marked by promoter region hypermethylation associated with transcriptional loss is an alternative mode for cancer development.
    - In endometrial cancers with loss of MLH1 protein expression, approximately 65–96.9% of these tumors are due to MLH1 promoter methylation [92–94].
    - MLH1 promoter methylation analysis is used to distinguish sporadic endometrial cancers from Lynch syndrome in tumors that are MLH1-deficient by IHC staining and/or high level of MSI-H.
    - MLH1 promotor methylation is detected by methylation specific real-time PCR. In this assay, extracted tumor tissue DNA (typically from the same block in the IHC/MSI assay) is exposed to a bisulfate compound, which converts unmethylated cytosines to uracil, whereas methylated cytosines are resistant to this conversion, allowing for creation of different PCR reaction primers that can differentiate these two types of sequences. The absence of *MLH1* promotor methylation in tumors demonstrating loss of MLH1 protein expression and/or MSI-H may suggest a *MLH1* mutation associated with Lynch syndrome and genetic testing for

germline *MLH1* mutation is recommended. The presence of *MLH1* promotor hypermethylation in tumor tissue is suggestive of sporadic MSI and not associated with Lynch syndrome.

- Rare cases of co-*MLH1* promotor methylation and *MLH1* germline mutations have been reported [95, 96]. Constitutional epimutations that result in heritable *MLH1* germline mutation has also been reported, which cause Lynch syndrome phenotype in the absence of primary sequence alterations in the *MLH1* gene [97]. If there is a clinical suspicion of germline *MLH1* promotor methylation, a germline *MLH1* promotor methylation testing on peripheral blood is recommended.

16. **What are the possible causes for normal tissue testing result in patients with known MMR gene mutations? What are the possible causes for abnormal MSI/IHC results with non-detectable MMR gene mutations?**
    - In some endometrial cancer patients with known MMR gene mutations, the MMR IHC or MSI testing shows normal results. The possible causes include:
      - Patients with full-length but non-functional MMR protein resulting from missense mutations in MMR genes [98].
      - In endometrial cancer, the proportion of unstable microsatellite marker is lower (0.27 for endometrium vs 0.45 for colon per average tumor), the allelic shifts in BAT loci is shorter, and a greater proportion of tumor shows MSS [98, 99].
      - Patients with *MSH6* germline mutation tend to have tumors that are disproportionally MSI-low or MSS [100–102].
    - In some patients with abnormal MSI/IHC results indicative of Lynch syndrome, while the genetic analysis fails to reveal a pathologic mutation in the MMR gene, the possible causes include the following:
      - The presence of MMR gene variants of undetermined significance
      - The presence of MSH2 inversion (exons 1–7) [103]
      - The presence of EPCAM germline mutation, resulting in hypermethylation of the MSH2 promoter and subsequent transcriptional silence of an otherwise normal MSH2 gene [104]
      - The presence of bi-allelic somatic DNA mutations in MMR genes [105, 106]

17. **What are the advantages and disadvantages of IHC method, PCR-based MSI testing, and NGS-based MSI testing for Lynch syndrome screening?**
    - The advantages of IHC method for Lynch syndrome screening include:
      - High sensitivity and nearly perfect specificity
      - Cheap and available in most laboratories
      - Only requires tumor sample (not matched tumor/normal samples as required by PCR for MSI)
      - Can identify the candidate protein/gene most likely to be affected

      The disadvantages of IHC method for Lynch syndrome screening include:
      - Presence of false-negative results where protein function is impaired but still present such as MLH1 promoter hypermethylation cases which may show loss of nuclear staining for MLH1 and PMS2
      - Variation in tissue fixation and other pre-analytic and analytic issues
      - Presence of tumor heterogeneity in endometrial cancer
      - Less reliable on small tissue
    - The advantages of PCR-based MSI testing for Lynch syndrome testing include:
      - Complementary with IHC
      - Identifies MSI status regardless of protein function
      - Can be performed on small samples
      - High reproducibility

      The disadvantages of PCR-based MSI testing for Lynch syndrome testing include:
      - Time consuming due to microdissection and subsequent molecular analysis
      - Require both tumor tissue and normal tissue
      - Additional testing needed to identify the candidate gene
      - dMMR tumor detection depends on the proposed cut-off and not all tumors with dMMR are necessarily MSI-H, especially in *MSH6* and *PMS2* mutated tumors
    - The advantages of NGS-based MSI testing for Lynch syndrome testing include the following:
      - Does not require tumor microdissection
      - Can be performed on small samples
      - Potentially faster result than PCR-based methods
      - Potentially more accurate result than MSI PCR-based method for detection of MSI-H status in some cancers

- Can perform large-scale testing, especially when looking for dMMR in the low-incidence cancers
- Can integrate results such as MSI status, tumor mutation burden within the same test

The disadvantages of NGS-based MSI testing for Lynch syndrome testing include:

- Clinical utility as a pan-tumor assay is not widely-established
- Currently, no evidence available directly in support of predicative purpose for immunotherapy

18. **What is the TCGA molecular classification of endometrial carcinomas?**
    - Endometrial carcinoma is a clinically heterogenous disease with diverse underlying molecular alterations.
    - The Cancer Genome Atlas (TCGA) Research Network performed an integrated genomic, transcriptomic, and proteomic characterization of endometrial carcinomas, using array- and sequencing-based assays. The study included 307 endometrioid, 53 serous, and 13 mixed histology cases and classified these endometrial carcinomas into four distinct molecular subtypes based on the somatic copy number alterations and tumor mutational burden: polymerase epslon (*POLE*) ultramutated, microsatellite instability hypermutated, copy-number low, and copy-number high [107].
    - This molecular classification provides clinically relevant and prognostic information, with the potential to influence the clinical management [107–109]. Table 5.4 shows the clinicopathological features, outcomes, and clinical management of high-risk endometrial cancers by the molecular subtypes.

Table 5.4 The clinicopathological features, outcomes, and clinical management of high-risk endometrial cancers by the molecular subtypes

Molecular subgroups	*POLE* ultramutated	Microsatellite instability hypermutated	Copy number low (NSMP)	Copy number high (p53 aberrant)
Incidence	12.4%	33.4%	31.5%	22.7%
Molecular alterations	*POLE , DMD, CSMD1, FAT4, PTEN*	*PTEN,PIK3CA,PIK3R1, RPL22,ARID1A*	*PTEN, PIK3CA,CTNNB1, ARID1A*	Frequently *TP53;* Focal amplifications of *MYC*, *ERBB2*,and *CCNE1*
Somatic copy number alterations	Very low	Low	Low	High
Mutation frequency	High	Moderate to High	Low	Low
Diagnostic test	NGS; Sanger sequencing	See question 11		P53 IHC; NGS
Histology	Typically, high grade endometrioid type or morphologically ambiguous, superficially broad front invasion, scattered tumor giant cells, prominent TILS	Mostly endometrioid type, abundant TILs, "MELF" pattern of invasion may present	Usually endometrioid type with squamous differentiation and ER/PR positivity	Most serous and mixed histology, a minority of endometrioid type
Surrogate IHC markers		MMR protein		P53
Prognosis	Excellent	Intermediate	Mixed	Poor
Potential utility in surgical planning	May skip the lymphadenectomy			More aggressive surgery with lymph node assessment and omentectomy
Adjuvant treatment	No benefit of CTRT over RT	No significant benefit of CTRT vs CT	No significant benefit of CTRT vs CT, although a trend toward benefit from CTRT	Significant benefit from CTRT
Suggested Treatment in recurrent/ metastatic disease	Checkpoint inhibitors	Checkpoint inhibitors	Hormonal therapy/mTOR inhibitors	HER2-targeted therapy in serous carcinoma

CTRT combined adjuvant chemotherapy and radiotherapy, *ER* estrogen receptor, *MELF* microcystic elongated and fragmented, *NGS* next-generation sequencing, *NSMP* non-specific molecular profile, *POLE* polymerase epslon; PR: progesterone receptor, *RT* radiotherapy, *TILs* tumor-infiltrating lymphocytes

19. **What are the roles of molecular testing in endometrial stromal tumors?**
    - The understanding of endometrial stromal sarcomas has evolved dramatically since the discovery of several recurrent cytogenetic aberrations occurring in low- and high-grade endometrial stromal sarcomas.
    - Low-grade endometrial stromal sarcomas bear close histopathological resemblance to proliferative-type endometrial stroma and approximately 50% of cases harbor gene rearrangement of t(7:17)(p15;q21), which causes *JAZF1-SUZ12* fusion [110, 111]. Less common rearrangements involving PHD finger protein-1 (PHF1) and multiple fusion partners, including JAZF1, EPC1, EPC2, MRAF6, and MBTD1, have also been reported [112].
    - The term "high-grade endometrial stromal sarcoma" was recently re-introduced in the classification of endometrial stromal tumors after the discovery of t(10;17)(q22;p13) resulting in *YWHAE-NUTM2A/B* fusion and is associated with distinct morphological characteristics [113–115].
20. **What are the biomarker tests for endometrial carcinoma? How should these biomarkers be reported?**
    - The biomarker tests for endometrial carcinomas includes ER, PR, HER2, MMR proteins/MSI, and p53.
    - CAP offers the templates for reporting results of biomarker testing in specimens from patients with endometrial carcinoma.
21. **What is the role of HER2 testing in uterine serous carcinoma?**
    - The HER2 overexpression in the uterine serous carcinoma is variable, between 14% and 80%, and HER2 overexpression/amplification has been linked to poor prognosis in endometrial cancer [116].
    - The gynecologic oncologists request HER2 testing in uterine serous carcinomas given the proven benefit of adding Trastuzumab to the traditional regimen of carboplatin-paclitaxel increased the progression-free survivals in patients with advanced or recurrent uterine serous carcinoma [117].
    - Currently, there are no HER2 testing guidelines for endometrial cancer, and CAP offers a template for prognosis marker reporting results for cases with uterine carcinomas, by using the breast guideline.
22. **What is molecular human papillomavirus (HPV) testing in the cervical cytology specimen?**
    - Approximately 95% cervical cancers are caused by 12–15 high-risk human papillomavirus (hr-HPV) infections.
    - Molecular HPV testing on liquid-based cervical cytology specimens has been approved by the US FDA since 2001 from initially being as a reflex testing, to a routine co-tests in women aged 30 years and above, and to a primary screening test.
    - The molecular HPV testing is performed using assays that detect viral DNA or RNA within the cells.
    - There are at least 254 distinct commercial HPV tests and at least 425 testing variants on the global market in 2020. These tests include hr-HPV DNA screening tests, hr-HPV DNA screening tests with concurrent partial genotyping tests (HPV16/18/45), HPV DNA full genotyping tests, HPV DNA type- or group-specific genotyping tests, hr-HPV E6/E7 mRNA tests, in situ hybridization DNA in mRNA-based HPV tests, and HPV DNA tests targeting miscellaneous HPV types [118].
    - The US FDA has approved five testing modalities for the detection of HPV in cervical cytology specimens [119]: Hybrid Capture 2 HPV DNA test by Qiagen (Hilden, Germany, 2001), Cervista HPV DNA test by Hologic (Marlborough, Massachusetts, 2009), Cobas 4800 HPV DNA test by Roche (Basel, Switzerland, 2011), Aptima HPV RNA assay by Gen Probe (San Diego, California, 2011, purchased by Hologic in 2012), and BD Onclarity HPV DNA assay by Becton Dickinson (Franklin Lakes, New Jersey, 2018). Table 5.5 lists the comparisons among FDA-approved hrHPV testing platforms.
23. **When should p16 immunostaining be performed in lower anogenital squamous lesions?**
    - The Lower Anogenital Squamous Terminology (LAST) Standardization Project only recommended immunostaining of p16, a biomarker that is recognized in the context of HPV biology to reflect the activation of E6/E7-driven cell proliferation can be used as an adjunctive diagnostic tool in the lower anogenital squamous lesions [120]:
      – When the H&E morphologic differential diagnosis is between precancer (high-grade squamous intraepithelial lesion, -IN2 or –IN3) and its mimics such as immature squamous metaplasia, atrophy, reparative epithelial changes, tangential cutting.
      – To clarify the situation if the pathologist is entertaining an H&E morphologic interpretation of -IN2, the equivocal lesions falling between low grade lesions and precancer lesions.
      – When there is a professional disagreement in histologic specimen interpretation, with the caveat that the differential diagnosis includes a precancerous lesion (-IN2 or-IN3).

Table 5.5 Comparisons among FDA-approved hrHPV testing platforms

HPV testing	HC2	Cervista	Cobas 4800	Aptima	Onclarity
Manufacturer	Qiagen	Hologic	Roche	Hologic	Becton Dickinson
Approval in ASC-US triage	2001	2009	2011 (TP) 2016 (SP)	2011	2018
Primary screening, co-testing	Yes	Yes	Yes	Yes	Yes
Primary screening, HPV alone	N/A	N/A	Yes	N/A	Yes
Preparation	TP	TP	TP and SP	TP	SP
Method	DNA (non-PCR based); signal amplification: full-genome probe	DNA (non-PCR based) signal amplification: L1, E6, and E7 gene targets	DNA (PCR based); target amplification: L1 gene target	mRNA (PCR based); target amplification: E6/E7 gene target	DNA (PCR based); target amplification: E6/E7 gene target
Genotype detected	13 genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68)	14 genotypes (13 genotypes as HC2, adding 66)	14 genotypes (same as Cervista with genotyping of 16 and 18)	14 genotypes (same as Cervista; genotyping [16,18/45] as separate test	14 genotypes (same as Cervista; simultaneous identification of 16, 18, and 45)
Clinical validation	Extensive	Limited	Limited	Limited	Limited
Sensitivity for CIN2/3	63.6–100%	92.8–100%	71.1–99%	55.3–100%	85.7–100%
Specificity for CIN2/3	6.2–98.4%		24–86.2%	28.8–99.2%	17–98.8%
Built-in internal control	No	Yes (HIST2H2BE)	Yes (ß-globin)	Yes	Yes (ß-globin)
Main limitations	Cross-reactivity with low-risk HPV types and bacterial plasmid pBR322 False-negative due to low levels of HPV infection, insufficient cellular material, high concentrations of anti-fungal cream, contraceptive jelly, or douche	Cross-reactivity with HPV 67/70 False-negative due to presence of high levels of contraceptive jelly and/or anti-fungal creams False-positive due to high levels of human DNA	False negative due to high concentration of blood contamination False positive due to cross-contamination Non-epithelial cell-specific internal control	Interfered by the presence of lubricants containing polyquaternium 15 or anti-fungal medications containing tioconazole	False negative due to high concentration of mucin acyclovir cream, or clindamycin vaginal cream

CIN cervical intraepithelial neoplasia, *HC2* hybrid capture 2, *Hr-HPV* high-risk human papillomavirus, *N/A* not applicable, *PCR* polymerase chain reaction, *SP* SurePath (BD, Franklin Lakes, NJ, USA), *TP* ThinPrep (Hologic, Marlborough, MA, USA)

- When biopsy specimens interpreted as ≤-IN1 that are at high risk for missed high-grade disease, which is defined as a prior cytologic interpretation of HSIL, ASC-H, ASC-US/HPV16+ or AGC (NOS).
- In these scenarios, the strong and diffuse block-positive p16 results support a categorization of precancerous disease and negative or non-block-positive staining favors an interpretation as low-grade disease (low-grade squamous intraepithelial lesion, -IN1) or a non-HPV-associated pathology.
- Positive p16 IHC (strong and diffuse block) is defined as "continuous strong nuclear or nuclear plus cytoplasmic staining of the basal cell layer with extension upward involving at least one third of the epithelial thickness."
- Negative p16 IHC is defined as "focal or patchy nuclear staining, and all other staining patterns, including cytoplasmic only, wispy, blob-like, puddled, scattered, single cells."

24. **What are the roles of molecular testing in sex cord-stromal tumors of the ovary?**
    - Molecular testing has potential roles in the pathological diagnosis of some ovarian sex-cord stromal tumors and helps to recognize patients with inherited cancer susceptibility syndromes (see review article [121]).

- *FOXL2* mutation testing: *FOXL2* (chromosome 3q23) encodes a transcription factor that is a member of the forehead box (FOX) family of proteins. A somatic missense mutation (402 C→G) (C134W) of *FOXL2* was reported in the majority (61–97%) of adult granulosa cell tumors (AGCT), 5–10% of thecomas, and less than 10% of juvenile GCT cases, but not in other ovarian tumors. The methods used to test for the FOXL2 C134W missense mutation include Sanger sequencing, targeted next-generation sequencing, and allele-specific quantitative amplification assays such as Taqman.
- *DICER1* mutation testing: *DICER1* (chromosome 14q32.13) encodes an RNA endoribonuclease that cleaves (i.e., dices) microRNA precursors to create mature miRNAs, which subsequently regulate the translation of a broad array of endogenous and exogenous RNAs. Germline mutation of *DICER1* gene is associated with DICER1 syndrome. This condition causes benign and malignant tumors in the lungs, kidneys, ovaries, and thyroid. In the gynecologic tumors, somatic mutation of *DICER1* is commonly found in ovarian moderately or poorly differentiated Sertoli-Leydig cell tumors (SLCTs) and cervical embryonal rhabdomyosarcomas as well as in a minority of juvenile granulosa cell tumor (JGCTs), gynandroblastomas, and germ cell tumors. Determining *DICER1* mutation status in SLCT cases can assist with the diagnosis and risk assessment for DICER1 syndrome. Sanger sequencing or targeted NGS of the relevant exons is the mainstay of DICER1 mutation testing.
- *CTNNB1* mutation testing: *CTNNB1* (chromosome 3p22.1) encodes β-catenin, which is an adherens junction protein that is critical for the establishment and maintenance of epithelial layers. Mutations in *CTNNB1* are seen in a wide range of cancers, including hepatocellular carcinoma, colorectal carcinoma, breast cancer, and glioblastoma. In the gynecologic tumors, a somatic mutation of *CTNNB1* exon 3 has been reported in 73% of microcystic stromal tumors (MCSTs). Interestingly, immunohistochemical expression of β-catenin did not perfectly correlate with mutation status. The presence of nuclear staining in MCSTs is associated with *CTNNB1* mutation in approximately three-quarters of cases, and nearly all MCSTs with *CTNNB1* mutations show β-catenin nuclear staining. Sanger sequencing or targeted NGS of exon 3 is the mainstay of *CTNNB1* mutation testing.

25. **What are the roles of molecular testing in the diagnosis of gestational trophoblast disease?**

- The diagnostic accuracy of various gestational trophoblast disease (GTDs) has been significantly improved by the application of molecular testing. An algorithmic approach combining histology and the ancillary tests has been proposed by Buza and Hui to provide the best practice in the diagnosis of hydatidiform moles [122].
- Ploidy analysis: By determining the number of complete haploid sets of chromosomes, ploidy analysis can separate diploid gestations from triploid, tetraploid, or other aneuploid ones. It can be performed by conventional karyotyping, flow cytometry, or polymorphic deletion probe (PDP) fluorescent in situ hybridization. The results cannot separate triploid partial moles from non-molar digynic triploidy, and diploid complete moles from diploid non-molar hydropic abortions.
- Short tandem repeat (STR) genotyping: By comparing the alleles of maternal and villous tissue at each STR locus, the presence and relative proportion (copy number) of maternal and paternal alleles in the villous tissue can be determined. The genotypic profile of a complete hydatidiform mole contains exclusively paternal alleles of either homozygous or heterozygous pattern in at least two informative STR loci. Monospermic (homozygous) partial moles show one maternal allele and a duplicate quantity of one paternal allele at every STR locus, while two unique paternal alleles in addition to one maternal allele in at least two loci is diagnostic of dispermic (heterozygous) partial hydatidiform mole. A balanced biallelic profile of both maternal and paternal genetic contributions is seen in non-molar hydropic abortions.
- P57 immunohistochemistry: p57 is paternally imprinted and expressed from the maternal allele. In a complete hydatidiform mole, the cytotrophoblasts and villous trophoblasts lack p57 immunoreactivity while its expression is retained in intervillous intermediate trophoblasts, villous endothelial cells, and maternal decidua. P57 IHC can separate a complete hydatidiform mole from its mimics including partial moles, hydropic non-molar abortions, and trisomies.

Case Presentations

Case 1

Case History

A 70-year-old woman with vague hypoechoic mass on screening mammogram

Histologic Finding

H&E sections show small uniform, round glands haphazardly distributed in the fibrofatty stroma with luminal eosinophilic secretions. The glands are lined by a monolayer of flat to cuboidal epithelial cells that lack a myoepithelial layer (see Fig. 5.6a–b).

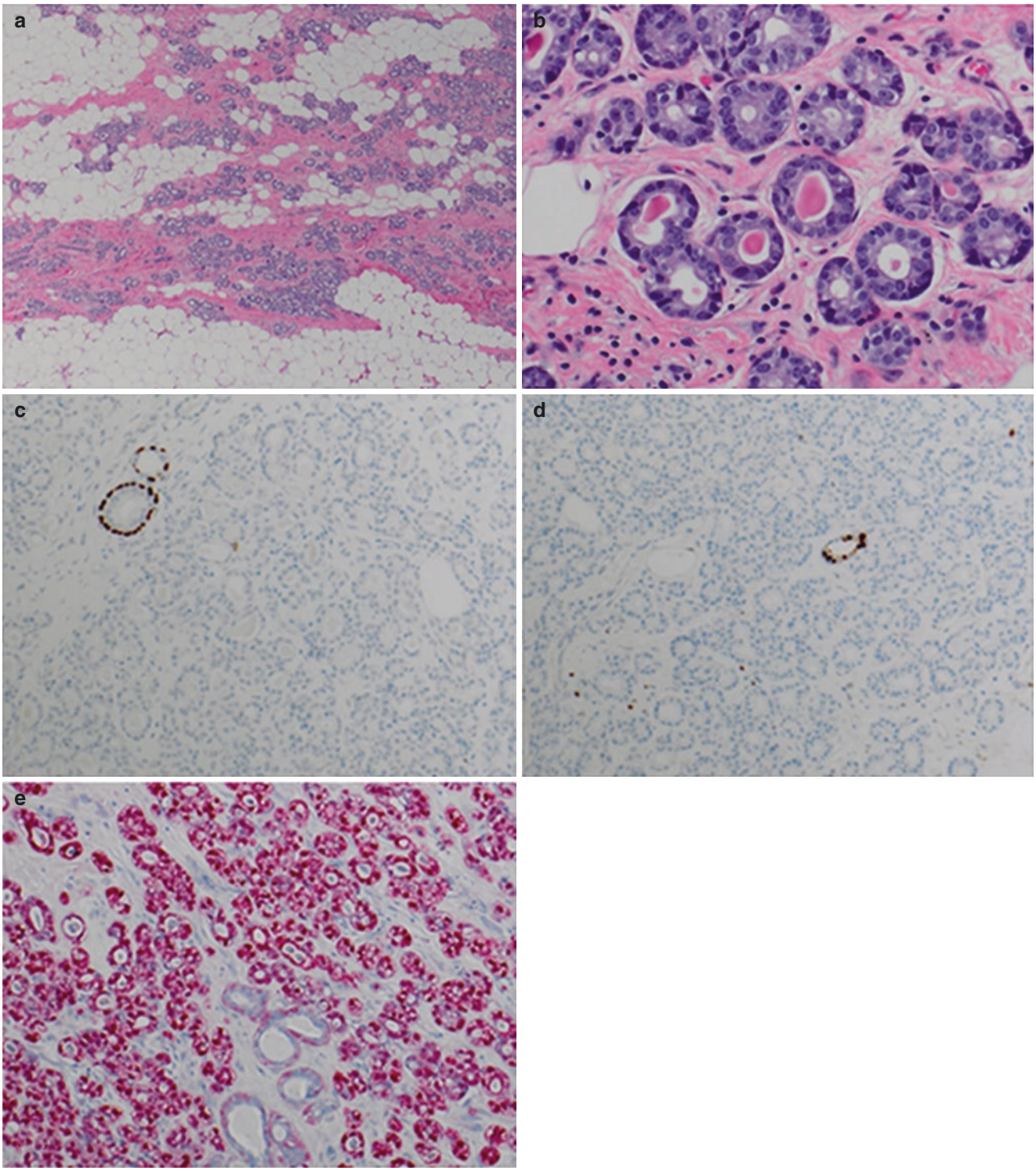

Fig. 5.6 A case of microglandular adenosis demonstrating the diagnostic value of ER/PR. A and B: Morphology of the lesion (**a** H&E, ×40 and **b**, H&E, ×400). The areas of interest are negative for p63 (**c**, ×200), ER (**d**, ×200), and PR and are positive for S100 (**e**, ×200)

Differential Diagnosis

Well-differentiated carcinoma (tubular carcinoma), microglandular adenosis

Ancillary Studies

The areas of interest are negative for p63, ER, and PR and positive for S100 (see Fig. 5.6c–e).

Final Diagnosis

Microglandular adenosis

Discussion

This case demonstrates that microglandular adenosis can closely mimic well-differentiated breast cancer both clinically and pathologically. Immunohistochemistry for ER/PR may be very helpful in this scenario since negative ER and PR expressions are very unusual in the well-differentiated breast carcinoma. Expression of S100 protein is additional information supporting a diagnosis of microglandular adenosis. An absence of the myoepithelial cell layer needs to be interpreted with caution since both well differentiated carcinomas and microglandular adenosis will show a lack of staining for myoepithelial cells.

Case 2

History

A 59-year-old woman was found to have a mass on a screening mammogram. The core biopsy shows invasive ductal carcinoma, histologic grade 3 (see Fig. 5.7a).

HER2 Testing

- **Immunohistochemistry:** Initial HER2 IHC shows ~40% of tumor cells with complete, strong intensity and ~40% with complete moderate to strong intensity (score 3+); however, there is weak to moderate staining in the benign breast glands. A repeated HER2 IHC shows absent staining in the benign breast glands and negative HER2 staining in the tumor cells (score 1+). A reflex HER2 FISH analysis was performed (see Fig. 5.7b–c).

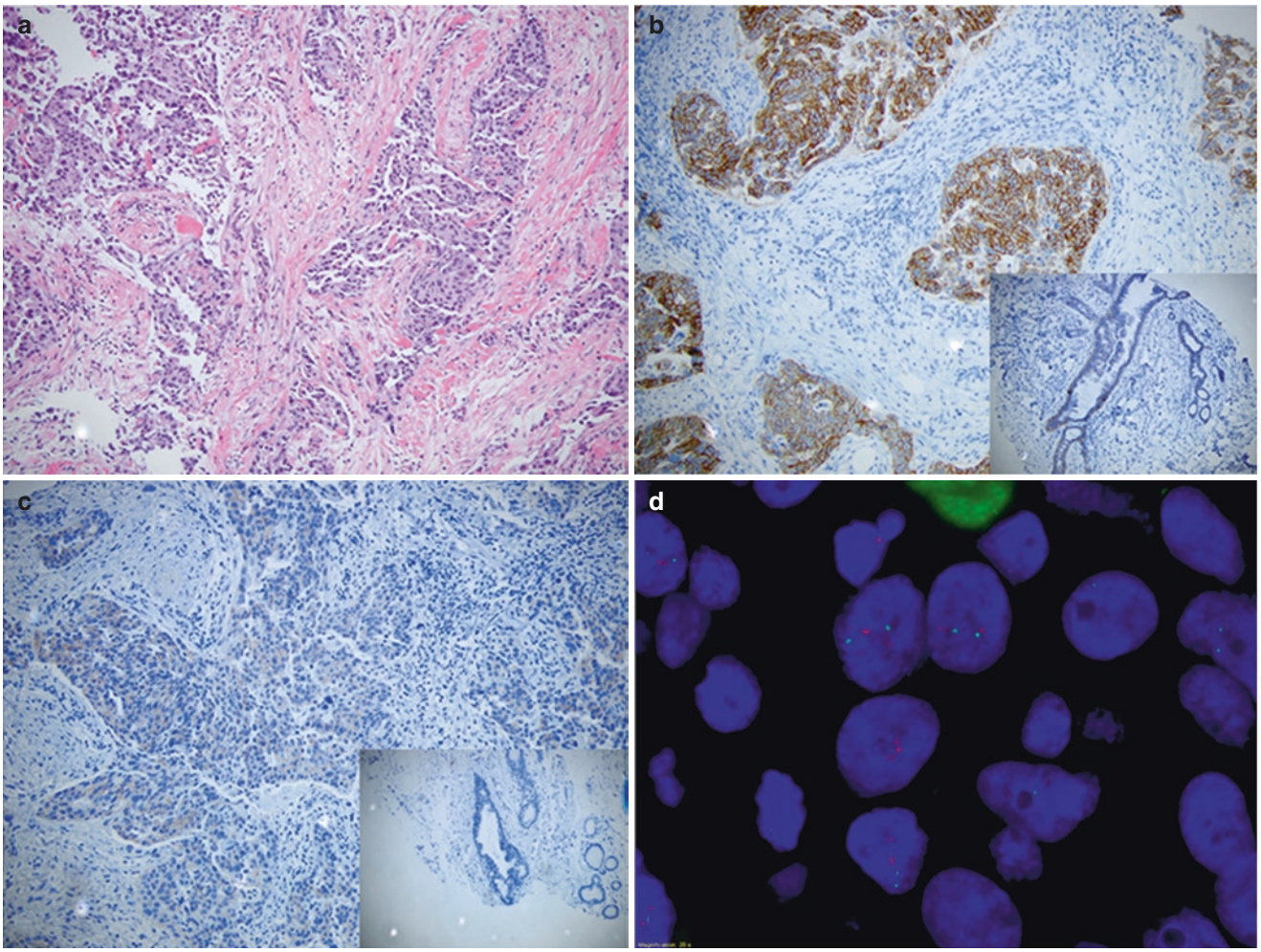

Fig. 5.7 Interpretation of HER2 immunohistochemistry. **a**: H&E (×100); **b**: Initial HER2 IHC (inlet: benign breast ducts) (×200); **c**: Repeated HER2 IHC (inlet: benign breast ducts) (×200), and D: FISH: non-amplified (**d**, ×1,000)

- **FISH:** Non-amplified (HER2 copy number: 3.7/cell and HER2/CEP17 ratio: 1.5) (see Fig. 5.7d).

Final HER2 Interpretation
Negative

Discussion
Accurate assessment of HER2 status in breast cancer is critically important and clinically relevant. This case demonstrates the importance of knowing IHC rejection criteria when interpreting HER2 IHC, which include (1) controls are not as expected, (2) artifact involves most of the sample, and (3) sample has strong membrane staining of normal breast ducts (internal controls). If the sample meets the IHC rejection criteria, repeating IHC or sending for FISH analysis can be performed to assure the accurate interpretation of HER2 status.

Case 3

History
A 55-year-old female presented with bloating and pelvic pressure. Pelvic ultrasound revealed a 3 cm cystic mass within the uterus that had been persistent over at least 4 months, suggestive of a possible hemangioma or arteriovenous malformation. A definitive surgical management with a hysterectomy and bilateral salpingo-oophorectomy was performed.

Gross Findings
There is a 4 × 4 cm purple-gray, irregularly shaped, ill-defined, cystic membranous lesion in both the anterior and posterior aspects of the uterus serosa and the left paraovarian tissue, extending 1.2 cm into the myometrium from the lateral aspect and comes within 0.1 cm from the endometrial lining. The endometrial cavity is not grossly involved.

Histologic Findings
H&E sections show small, monotonous ovoid cells inflitrate between bundles of myometrium, in the background of prominent arterioles (see Fig. 5.8a–b).

Differential Diagnosis
Vascular lesion, smooth muscle tumor (vascular leiomyoma), endometrial stromal tumor

Ancillary Studies
- Immunohistochemistry: The cells of interest are positive for CD10 and ER and are negative for CAM5.2, AE1/AE3, PAX8, CD31, CD34, desmin, h-caldesmon, SMA, and beta-catenin (see Fig. 5.8c–e).
- FISH analysis: The result indicates an unbalanced rearrangement involving the *PHF1* gene region with loss of the 5'PHF1 probe. Rearrangement of the *PHF1* gene at 6p21 has been observed in endometrial stromal sarcoma and ossifying fibromyxoid tumor. No rearrangement involving the *JAZF1* and *YWHAE* gene regions was identified.

Final Diagnosis
Low-grade endometrial stromal sarcoma

Discussion
Molecular testing can be very helpful in some challenging cases in gynecologic pathology. In this case, due to the unusual gross and morphologic findings, it would be difficult to render the diagnosis of endometrial stromal sarcoma without the FISH findings.

Case 4

History
A 54-year-old female with history of ductal carcinoma in situ of breast, s/p partial mastectomy, and hormonal therapy presented with postmenopausal bleeding. Pelvic ultrasound revealed a mildly thickened endometrium. Endometrial biopsy showed atypical hyperplasia. A hysterectomy and bilateral salpingo-oophorectomy was performed.

Histologic Findings
Endometrioid adenocarcinoma, FIGO grade 1 (see Fig. 5.9a)

MMR Studies
The tumor demonstrates loss of expression of PMS2. There is retained nuclear expression of MLH1, MSH2, and MSH6 (see Fig. 5.9b–e).

Genetic Testing
Sequence analysis identified one copy (heterozygous) of *PMS2* mutation S46I(137G > T) (genetic variant, suspected deleterious).

Follow-up
- Patient developed urothelial carcinoma 3 years later.
- Genetic follow-up detected a variant of unknown significance in the *ATM* gene, and negative *BRCA1/2*, *CDH1*, *CHECK2*, *PALB2*, *PTEN*, and *TP53*.

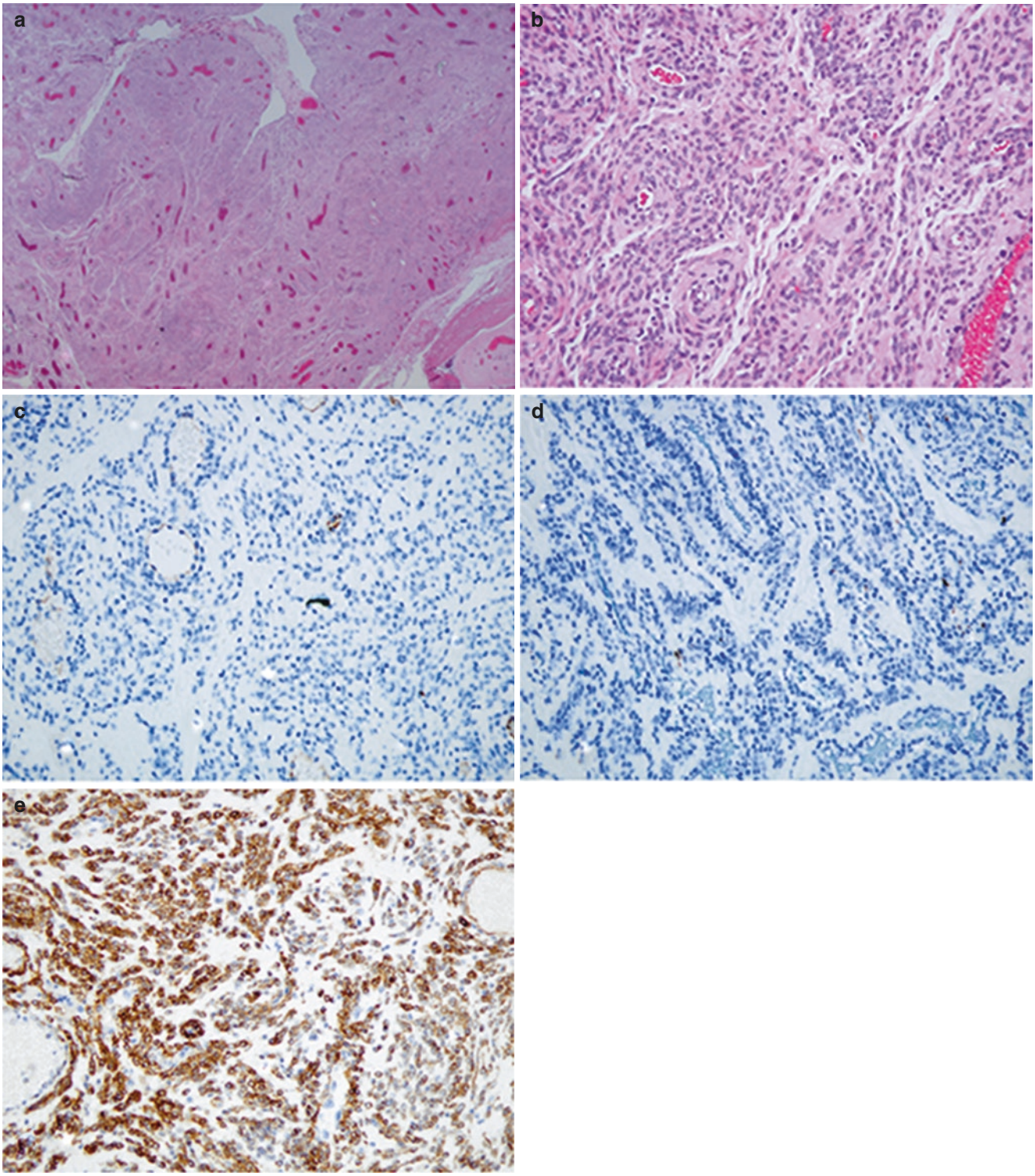

Fig. 5.8 A case of low-grade endometrial stromal sarcoma, demonstrating the diagnostic value of molecular testing. **a** and **b**: Morphology of the lesion (**a**, H&E, ×20; **b**, H&E, ×200). The cells of interest are positive for CD10 (**e**, ×200) and are negative for CD34 (**c**, ×200) and caldesmon (**d**, ×200)

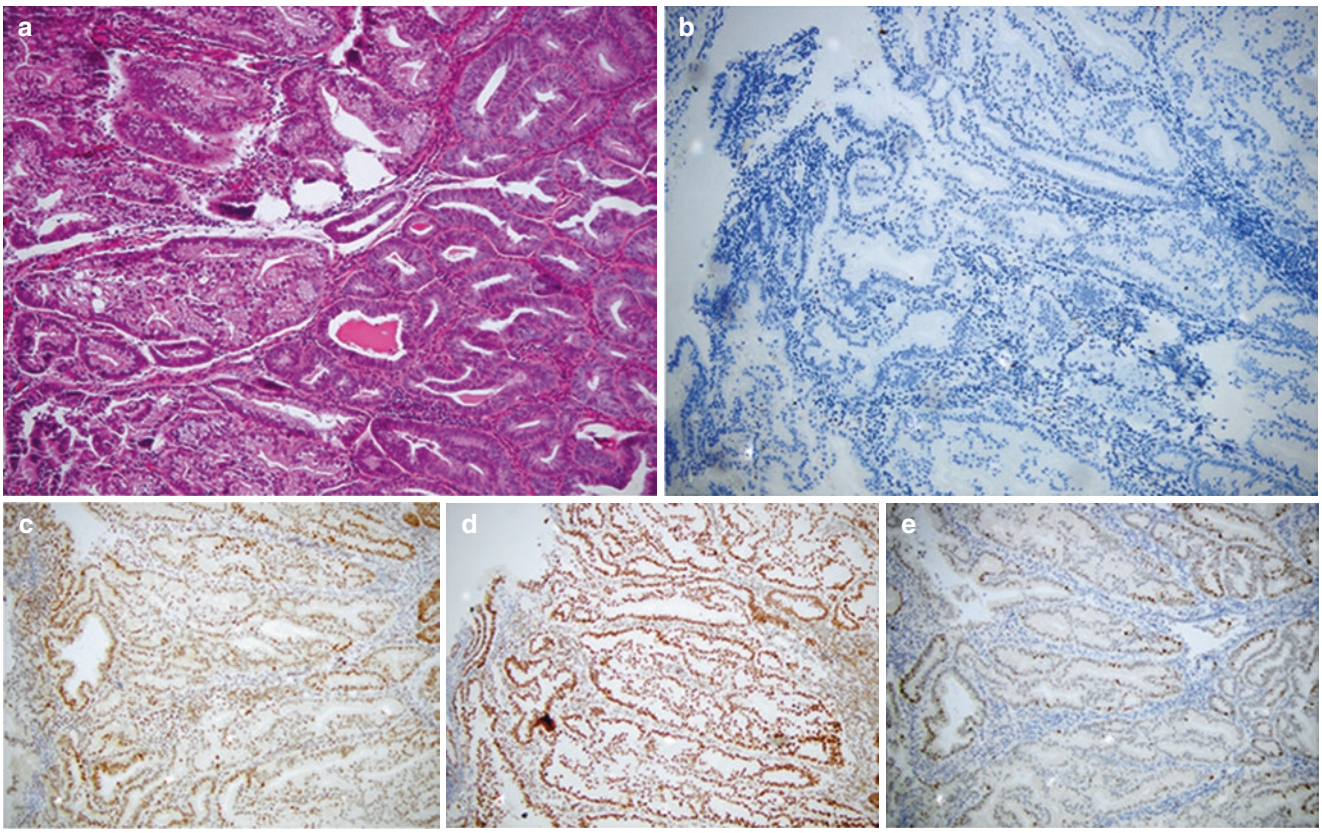

Fig. 5.9 A case of endometrioid adenocarcinoma, demonstrating the significance of routine MMR testing in women with endometrial cancer. **a**: Morphology of tumor (H&E, ×100); the tumor cells show loss of expression of PMS2 (**b**, ×200) and retained nuclear expression of MLH1 (**c**, ×200), MSH2 (**d**, × 200), and MSH6 (**e**, ×200)

- Normal surveillance colonoscopy, gastric and pancreatic cancer screenings.
- The patient's daughter also showed *PMS2* mutation S46I(137G > T) and undergoes surveillance.

Discussion

Identification of patients with Lynch syndrome may allow for screening and prevention strategies for patients themselves and their affected family members. This case illustrates the importance of routine MMR testing in women with endometrial cancer. As mentioned in Part 1, immunohistochemistry of MMR proteins is a simple and cost-effective method for Lynch syndrome screening.

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