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Review article

Utilization of immunohistochemistry in gynecologic tumors: An expert review

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ABSTRACT

The use of immunohistochemistry (IHC) and molecular pathology has been widely adopted over the past 3 decades and has aided in the precision of diagnosing gynecologic tumors. While many tumors can be diagnosed by histologic appearance on routine hematoxylin and eosin stained slides, the use of IHC has dramatically changed practice, leading to a better understanding and subtyping of gynecologic tumors. This detailed classification of tumors has aided in the implementation and development of targeted therapies. Available IHC stains and their applications continue to rapidly evolve. Our review aims to provide updated information on the use of IHC in gynecologic tumors. We will also address the rationale for preferred therapeutic regimens that are personalized based on IHC.

1. Ovarian carcinoma

1.1. Identifying the primary tumor

Identification of the primary origin of a tumor is a challenge in the practice of gynecologic oncology but is crucial in providing the appropriate treatment (Kalampokas et al., 2018). The ovaries are commonly involved by metastatic tumors, most frequently originating from the gastrointestinal tract, breast, uterus, fallopian tube, and peritoneum (primary peritoneal carcinoma). Histologic features alone may not allow for identifying the origin of the primary neoplasm. (See Table 1.).

PAX8 expression in ovarian carcinoma (OC) has been seen in more than 70 % of cases. PAX8 has utility in identifying OC as it is expressed in serous ovarian carcinoma, endometrioid ovarian carcinoma and clear cell ovarian carcinomas with a sensitivity of over 90 %. This increases the utility of PAX8, especially compared to WT1 which has limited expression in non-serous histology OC. PAX8 is not expressed in carcinomas of the colon, bile duct, stomach, hepatocellular, pancreas, or esophagus. PAX8 is also negative in mammary carcinomas. PAX8 is emerging as the leading IHC for distinguishing primary OC (Kuhn and Ayhan, 2018 Feb).

CK7 is a useful marker for making the distinction between OC and metastatic colorectal carcinoma. CK7 is predominantly negative in metastatic colorectal cancer to the ovary, whereas it is positive in cases of primary OC. CK20 positive staining is almost universal in colorectal carcinomas. In contrast, CK20 is typically negative in primary serous OC but positive in colorectal neoplasms metastatic to the ovary (Chu et al., 2000 Sep). Rarely, CK20 positivity is found in mucinous ovarian carcinomas (Kuhn and Ayhan, 2018 Feb). In these cases, morphology, distribution of disease, and status should be reviewed together to make the correct diagnosis. The addition of SATB2 is useful in differentiating between OC and metastatic colorectal carcinoma as it is usually positive in metastatic colorectal carcinoma and negative in OC (Moh et al., 2016).

Conversely, CK7 positivity can be observed in breast carcinoma metastatic to the ovary (McCluggage and Wilkinson, 2005). In the event of a clinical history of breast carcinoma, GATA-3 IHC can aid in identification as it will be positive in breast carcinoma and negative in OC (Espinosa et al., 2015 May). This differentiation cannot be made in the rare circumstance of an ovarian mesonephric carcinoma as these tumors

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Table 1
Summary of recommended IHC panels for diagnosis and expected staining patterns.

Ovarian Tumors	
Epithelial Ovarian Cancers	***General Panel: WT1, ER, PR, p16, p53, Napsin A***
High Grade Serous Carcinoma	Diffuse WT1+, p53 aberrant (overexpressed, null phenotype, and cytoplasmic expression), ER/PR +/-, p16+, Napsin A-
Clear Cell Carcinoma	WT1-, p53 wild type or aberrant, ER/PR-, p16 +/-, Napsin A+, HNF1B
Endometrioid Carcinoma	WT1-, p53 wild type or aberrant, ER/PR+, p16+/-, Napsin A-, PAX 8+/-, CK7+, CK20 and CDX-2focal positivity, SATB2-
Mucinous Carcinoma	WT1-, p53 wild type or aberrant, ER/PR-, p16-, Napsin A-
Low Grade Serous Carcinoma	WT1+, p53 wild type, ER/PR+, p16-, Napsin A-
Sex Cord Stromal Tumors	***General Panel: CD56, SF-1, WT1, calretinin, a-inhibin MART-1, CD99***
Sertoli Cell Tumor	CD56+, SF-1+, WT1+, Calretinin+/-, a-inhibin+, MART-1-, CD99+
Granulosa Cell Tumor	CD56+, SF-1+, WT1+/-, Calretinin+, a-inhibin+, MART-1-, CD99+
Fibroma	CD56+, SF-1+, WT1+, Calretinin+/-, a-inhibin+/-, MART-1-, CD99-
Leydig Cell Tumor	CD56+, SF-1+, WT1+, Calretinin+, a-inhibin+, MART-1+, CD99
Sertoli-Leydig Cell Tumor	CD56+, SF-1+, WT1+, Calretinin+/- a-inhibin+, MART-1-, CD99+
Germ Cell Tumors	***General Panel: PLAP, OCT4, SALL4, AFP, Glypican 3, c-kit***
Immature Teratoma	PLAP+, OCT4+/-, SALL4+/-, Glypican 3+/-, c-kit+
Dysgerminoma	PLAP+, OCT4+, SALL4+, c-kit+
Yolk Sac Tumor	SALL4+, AFP+, Glypican 3+
Gonadoblastoma	PLAP+, SALL4+
Embryonal Carcinoma	PLAP+, OCT4+, SALL4+, patchy Glypican 3+, c-kit-
Uterine Tumors	
Uterine Serous Carcinoma	Patchy WT1+, p53 overexpression, ER/PR-, p16+, B-catenin −, E-cadherin+
Carcinosarcoma	WT1 focal/weak+, p16+, p53 aberrant
Clear Cell Carcinoma	WT1-, p53 wild type, or aberrant ER/PR-, patchy p16+, Napsin A+
Uterine Sarcomas	**General Panel: CD10, SMA, Vimentin, desmin, h-caldesmon, ER/PR, c-kit, cyclin D1**
Leiomyosarcoma	Vimentin+, CD10+/-, SMA+, desmin+, h-caldesmon+, ER/PR +/-, c-kit +/-, cyclin D1-
Low-Grade Endometrial Stromal Sarcoma	Vimentin +, CD10+, SMA + . desmin +/-, h-caldesmon-, ER/PR+, patchy cyclin D1+
High-Grade Endometrial Stromal Sarcoma	Vimentin+, CD10-, h-caldesmon-, desmin-, ER/PR-, c-kit+, diffuse cyclin D1+
Undifferentiated Sarcoma	Vimentin+, CD10+, desmin-, ER/PR-, cyclin D1+
Gestational Trophoblastic Neoplasia	***General Panel: HSD3B, p63, hPL, B-hCG, Cyclin E, Ki-67, p57***
Choriocarcinoma	HSD3B+, B-hCG+, diffuse Ki-67+
Placental Site Trophoblastic Tumor (PSTT)	HSD3B+, p63-, HPL+, B-hCG-, mild Ki-67+, Cyclin E+
Epithelioid Trophoblastic Tumor (ETT)	HSD3B+, p63+, HPL-, B-hCG-, mild Ki-67+, Cyclin E+
Cervical Tumors	
Cervical Adenocarcinoma	CEA+, Vimentin-, ER/PR-, p16+, p63-
Cervical Squamous Cell Carcinoma	CEA+, Vimentin-, ER/PR-, p16+, p63+
Vulvar Tumors	
Vulvar Carcinoma	PDL1, p16 and p53
Vulvar Paget's Disease	CK7+, CK-20-, Her2/neu +, HMB45, Melan A, CAM5.2
Vulvar melanoma	S100, SOX10, NGFR, MART1, HMB45, vimentin

can show GATA-3 positivity. To differentiate between this histologic subtype of OC and metastatic breast carcinoma, alternative IHC such as TTF-1 (to diagnose a mesonephric carcinoma) or ER can be used (if the breast carcinoma was ER positive) (Koh et al., 2022 Jan 27).

1.2. Epithelial ovarian cancers

The FIGO staging system merges ovarian, fallopian tube, and primary peritoneal carcinomas into the broader description of epithelial ovarian cancer (OC), however when possible the primary site should be identified. Originally thought to be indistinguishable, now certain characteristic features can help to differentiate OC from fallopian tube carcinoma (FTC). FTC can be characterized by a dominant fallopian tube mass, the presence of tubal carcinoma in situ, or minimal ovarian involvement. Additionally, FTCs are usually accompanied by an in situ lesion which not seen with OC or primary peritoneal carcinoma. Primary peritoneal carcinoma (PPC) can be differentiated when the ovaries are minimally involved by carcinoma (Chivukula, 2011).

The next challenge in diagnosing primary OC is determining the histologic subtype. Histologic typing has been shown to be more challenging than establishing grade, especially in the case of poor to moderately differentiated tumors. OC can be classified into 5 main histologic types which are high grade serous carcinoma (HGSC), clear cell carcinoma, endometrioid carcinoma, mucinous carcinoma, and low grade serous carcinoma (Köbel et al., 2016 Sep). To streamline the use of IHC and for practical application, various investigators have proposed algorithms composed of 4–8 IHC markers used to type OC. One study found that using an 8-marker panel yielded a 93 % accuracy in predicting OC. This panel consists of WT1, p53, p16, Napsin A, PR, TFF3, ARID1A, and vimentin. An alternative algorithm includes WT1, p53, Napsin A, PR, HNF1B, AMACr, and ER to approach diagnosing the same 5 subtypes of OC (Kuhn and Ayhan, 2018 Feb).

Serous carcinomas of the ovary, fallopian tube, and uterus are challenging to identify the primary organ based on histology alone. Clinically, serous carcinomas have a propensity for invasion, peritoneal spread, and a poor prognosis regardless of the origin, which further complicates identifying the origin of disease. Although WT1 is expressed in all serous carcinomas of gynecologic origin, WT1 expression is usually more diffuse and strong staining in ovarian, tubal, and primary peritoneal tumors, and is weak and focal in endometrial serous carcinomas. WT1 positivity is only noted in one fifth of endometrial serous carcinomas (Acs G, 2024). WT1 is usually negative in breast, GI, and pancreatico-biliary tumors (Mittal et al., 2008).

Additional IHC can be used to delineate high grade from low grade serous OC. In addition to WT1 expression, high grade serous carcinomas will show aberrant p53 expression seen as overexpression, null expression, or cytoplasmic expression (Kuhn and Ayhan, 2018 Feb). Most high grade serous carcinomas demonstrate p16 positivity with higher expression than low grade or endometrioid OC (O'Neill et al., 2007 May). p16 can also be used to differentiate HPV associated endocervical carcinomas metastatic to the ovary from primary mucinous ovarian carcinoma as p16 will be positive in the case of endocervical carcinoma along with the clinical history and testing for high risk HPV RNA in situ hybridization (Vang et al., 2007). The use of p16 IHC in HPV associated cancers is discussed further in the discussion of cervical cancer below.

Steroid hormones play a role in the carcinogenesis of tumors. Therefore, estrogen receptor (ER) and progesterone receptor (PR) status are evaluated in OC. Low grade serous OC express significantly higher levels of ER and PR than high-grade serous ovarian tumors (Wong et al., 2007 Oct). Hormone receptor positive tumors generally have a better overall prognosis. Women with hormone receptor positive endometrioid OC have a better overall survival than those with hormone receptor negative tumors. High grade serous OC that are strongly positive for PR have an improved overall survival compared to weakly or negative PR (Sieh et al., 2013). A large percentage of low grade serous OC are positive for both ER and PR, indicating a possibility of hormonal manipulation in their treatment (Wong et al., 2007 Oct). Because serous carcinomas may express ER and PR, these markers are not useful in distinguishing serous OC from endometrioid OC however ER negativity is helpful in diagnosing clear cell carcinomas (Rabban JT, 2010).

Napsin A can be used to differentiate histologic subtypes of ovarian cancer, showing a predominance in ovarian clear cell carcinoma with intense staining. Positivity is also seen in ovarian endometroid carcinoma while it is not expressed in high grade serous OC (Yamashita et al., 2015 Jan). Although an ovarian clear cell carcinoma diagnosis can typically be made through H&E staining alone, high grade serous OC can show characteristics of clear cell carcinoma including papillary architecture, clear cytoplasm, and hobnail cells, creating a diagnostic challenge. Having a definitive diagnosis is critical, as clear cell OC has a different clinical response to chemotherapy, prognosis and recurrence pattern. HNF1B gene is over-expressed in clear cell OC exhibiting a gain in function mutation that is silenced in serous OC with loss of function (Shen et al., 2013). This subsequently correlates with an oncogenic role in clear cell disease causing proliferation, migration, and invasiveness. Patients with clear cell OC have a poor prognosis, in part due to low response to platinum based chemotherapy. Clear cell OC is also characterized by a high frequency of mutations resulting in the loss of function of the ARID1A gene, represented in IHC by loss of ARID1A staining (Wiegand et al., 2010). This mutation in ARID1A is a current area of investigation in clinical trials as a potential target for treatment for clear cell OC (Kuroda et al., 2019 Dec).

The reliability of staining patterns for mucinous OC is less consistent as there are different types of primary ovarian mucinous carcinomas. However, the majority of mucinous OC are intestinal or enteric type (McCluggage, 2012 Jul). Mucinous OC with intestinal-type neoplasia are typically ER/PR, WT1, and CA 125 negative (Tabrizi et al., 2010 Mar). The heterogenous nature of primary mucinous OC creates a void in composing an expected IHC staining pattern. However, the diagnosis is usually possible on morphology and distinguishing primary mucinous OC vs a metastatic lesion to the ovary can usually be accomplished through careful pathologic evaluation and the distribution of disease (McCluggage, 2012 Jul). In these cases, gastrointestinal cancer should also be ruled out clinically with the addition of colonoscopy and esophagogastroduodenoscopy (EGD). Although it is less readily available and utilized, TFF3 can be used to discern primary mucinous OC in most unclear cases (Köbel et al., 2016 Sep).

1.3. Sex cord stromal tumors

The morphology of sex-cord stromal tissues usually distinguishes them from other ovarian tumors. While the frequency and expression can vary amongst the different types of tumors, the most sensitive markers for sex-cord stromal tumors are inhibin, calretinin, SF-1, and WT1. Alpha inhibin is diagnostically the most sensitive IHC marker for ovarian sex-cord stromal tumors, with positivity above 90 % in most tumor subtypes (Zhao et al., 2009 Mar). Calretinin is at least as sensitive as inhibin for marking sex cord stromal tumors, but it is a more useful marker for fibromas and fibro-thecomas, however both can be present in other tumors requiring their use within a larger panel (Deavers et al., 2003 Jun). FOXL2 somatic gain of function mutations are common in sex cord stromal tumors. FOXL2 IHC staining has been adapted as a more practical diagnostic tool to detect these mutations with sensitivity and specificity approaching 80 % and 99 %, respectively (Al-Agha et al., 2011 Apr). CD56 is a useful marker for identifying sex-cord stromal tumors as it is ubiquitously expressed across all morphologies, demonstrating strong and diffuse staining. While CD56 is a sensitive marker for sex-cord stromal tumors, its use is limited as it is also positive amongst neuroendocrine tumors, which are frequently on the differential diagnosis when evaluating these neoplasms (McCluggage et al., 2007 Jul). CD99 is commonly expressed in Sertoli-Leydig and Granulosa cell tumors, but negative in fibromas, thecomas, and Leydig cell tumors. However, CD99 also has positivity in other ovarian tumors, making it a less sensitive and specific marker that cannot be used independently for establishing the diagnosis of a sex cord-stromal tumor (Baker and Oliva, 2005 Jan).

1.4. Germ cell tumors

A wide panel for the identification of germ cell tumors has been used including cytokeratin, PLAP, a-fetoprotein, B-hCG, chromogranin, thyroglobulin, OCT-4, CD30, c-kit, and HepPAR-1 (Baker and Oliva, 2005 Jan). When considering the diagnosis of an ovarian germ cell tumor, SALL4 and PLAP has been considered a first line choice for identification (Rabban and Zaloudek, 2013 Jan).

AFP positivity can be used to confirm the diagnosis of a yolk sac tumor, although it is not specific. Complementary to AFP is the use of Glypican 3, which is also secreted by the early yolk sac and may be positive amongst AFP-negative tumors. More commonly, the pluripotent antibody SALL4 has strong expression in the nuclei of yolk sac tumor (Nogales et al., 2014 Mar). Additionally, yolk sac tumors will have positive staining for placental alkaline phosphatase (PLAP), Cam 5.2, and AE1/AE3 (Rabban and Zaloudek, 2013 Jan).

Ovarian dysgerminomas have excellent survival rates, therefore use of IHC is paramount to assist in ruling out another germ cell tumor with a worse prognosis. A panel of SALL4, PLAP, c-kit, and OCT4 is most useful for diagnosing ovarian dysgerminomas (McCluggage and Young, 2005 Feb). Both dysgerminoma and embryonal carcinoma are positive for PLAP, OCT4, and SALL4. C-kit can be used for differentiation as it is positive in dysgerminoma and negative in embryonal carcinoma. The additional use of IHC to identify CD30 is most reliable to identify embryonal carcinoma (Nogales et al., 2014 Mar).

2. Uterine carcinoma

2.1. Endometrial cancer

2.1.1. Endometrioid endometrial carcinoma

The most prevalent form of endometrial cancer (EC) is low grade, hormone-receptor positive, endometrioid endometrial carcinoma that is associated with a good prognosis (Morice et al., 2016 Mar 12). Endometrioid EC are usually easily identified on pathology based on the glandular architecture with crowded, stratified, columnar cells with atypia, and eosinophilic cytoplasm (Goebel et al., 2018 Jun). Most low

grade endometrioid tumors have a characteristic appearance and do not require additional IHC staining for characterization. When further investigation is warranted, diagnosing endometrioid EC utilizes a IHC panel of estrogen receptor (ER), vimentin, CEA, and p16. Endometrial involvement by metastatic tumors is an uncommon occurrence, but in the case of breast metastasis, PAX8 and GATA3 can be used to diagnose a metastatic lesion from the breast. In the case of identifying colon metastasis PAX8, CK7, CK20 are utilized to differentiate metastases from a primary EC (Djordjevic et al., 2012 Dec 1).

Although ER/PR status is not currently utilized to personalize treatment, staining information on these biomarkers can identify high-risk patients. For example, loss of ER/PR expression in complement with high risk histology can identify patients with poor prognosis. (Trovik et al., 2013).

Approximately 30–40 % of endometrioid EC will have a loss of DNA mismatch repair proteins either due to MLH1 promotor hypermethylation or due to Lynch Syndrome (Morice et al., 2016 Mar 12). IHC staining of the markers MLH1, MSH2, PMS2, and MSH6 is used to diagnose microsatellite instability phenotypes. In tumors where staining shows a loss of MLH1 expression, MLH1 promoter hypermethylation testing should performed. In the setting of loss of MSH2, PMS2, and MSH6 and loss of MLH1 with negative hypermethylation testing, patients should be referred for genetic testing to rule out a germline mutation. IHC staining is an overall low cost, simple method for identifying the loss of MMR protein expression and plays an important role in the screening of Lynch Syndrome. MMR status plays an important role in clinical decision making regarding treatment with immunotherapy. Several recent studies have been published showing survival benefit with use of immunotherapy in advanced and recurrent endometrial cancer with a more pronounced benefit in mismatch repair deficient (dMMR) when compared to mismatch repair proficient (pMMR) tumors (Di Dio et al., 2023 Feb).

2.2. Uterine serous carcinoma

Differentiating disseminated serous carcinoma originating from the uterus (USC) versus the ovary can be difficult. While WT1 is specific to serous tumors, its staining pattern differs from the diffuse marking in high grade serous OC to patchy to weak involvement in (USC). Like in serous OC, p53 overexpression is seen in the majority of USC (Kuhn and Ayhan, 2018 Feb). In the case where p53 is not expressed, strong and diffuse staining of MIB-1 is suggestive but does not definitively diagnose USC (Mittal et al., 2008). USC has demonstrated strong E-cadherin expression where EC has weak E-cadherin expression with strong nuclear B-catenin (Schlosshauer et al., 2002 Oct). USC also demonstrates uniformly diffuse and moderate staining of p16 not seen amongst endometrioid EC, although the underlying molecular mechanism causing expression in unknown (Yemelyanova et al., 2009 Oct). If p53 shows wild type expression but the specimen is morphologically consistent with USC and p16 shows diffuse strong positivity then molecular testing can be performed to confirm the diagnosis of USC.

Although USC represents approximately 10 % of all uterine carcinomas, it is one of the most aggressive cancers, with tendencies for deep myometrial invasion, lympho-vascular space invasion, distant metastases and recurrence. USC is responsible for 40 % of EC related deaths. Following the identification of USC, all tumors should undergo testing for Her2/neu as data has proven over-expression is associated with advanced surgical stage, poor survival, and extra-uterine spread and is a target for therapy (Sarmadi et al., 2019). Her2neu testing should also be performed in the setting of endometrial carcinosarcoma as well as grade 3 endometrioid endometrial adenocarcinomas as a portion of these tumors will also overexpress the gene product. Her2neu expression is measured by an IHC scoring system where 3+ (greater than 30 % immunostaining) is considered positive. In instances of 2+ (greater than or equal to 10 % immunostaining) staining, this is considered an equivocal result and should trigger a reflex to perform fluorescence in situ hybridization (FISH) to assess gene status as a potential therapeutic target. Multiple studies have investigated Her2neu as a therapeutic target in advanced and recurrent endometrial cancer. Enhertu recently received FDA approval for Her2neu positive tumors and Trastuzumab in combination with Carboplatin/Paclitaxel is currently being investigated as another therapeutic option (Plotkin et al., 2024).

2.3. Carcinosarcoma

Uterine carcinosarcoma (CS) is an aggressive subtype of EC with an overall poor prognosis. It comprises approximately 4 % of all uterine cancers. CS is most frequently diagnosed by morphologic findings of a biphasic tumor composed of both epithelial and mesenchymal elements (Chen et al., 2017 Sep). P16 has been demonstrated to be a useful IHC marker for differentiating between subtypes of low grade and high grade EC, with p16 over-expression found in both the carcinomatous and sarcomatous components of the CS (Buza and Tavassoli, 2009 Nov). Similarly, p53 has demonstrated concordant staining amongst the different tumor components, both supporting monoclonal tumorigenesis and contributing to the staining profile for diagnosing CS as most carcinosarcomas are p53 aberrant. Rounding out the panel of IHC staining for CS is PAX8, with positive staining in both the epithelial and stromal components (Chen et al., 2017 Sep).

2.4. Clear cell endometrial carcinoma

Clear cell endometrial carcinoma is a rare and aggressive subtype of EC. The morphologic appearance of clear cell carcinoma is characterized by clear, eosinophilic cells with hobnailing appearance. Their precursor lesion is not yet defined but their etiology is thought to be different than endometrioid type endometrial carcinomas (Olawaiye and Boruta, 2009 May). Napsin A is frequently expressed in clear cell EC and either infrequently expressed or absent in USC and EC. Although the presence of Napsin A cannot definitively confirm a diagnosis of clear cell carcinoma, its sensitivity and specificity make it a useful tool to aid in the diagnosis of a challenging histology (Fadare et al., 2014 Feb). Clear cell EC typically are high Ki-67, ER/PR negative, p16 positive (strong, diffuse or patchy), with a wild type (most commonly) or aberrant p53 expression (Olawaiye and Boruta, 2009 May). AMACR and HNF1B may also help as expression of hepatocyte nuclear factor-1beta (HNF-1beta) in clear cell tumors and endometriosis of the ovary. (Fadare et al., 2014 Feb).

2.5. IHC surrogates for TCGA subgroups

Historically, endometrial cancer (EC) has been classified using different stratification schemas. The goal of classification has been to group tumors based on prognosis. The integration of the molecular classification of EC aids in identifying the high-risk subgroups and tailoring therapy. Therefore, there is a strong prognostic value in including the molecular classification of all cases of EC. Most recently, EC has been divided based on their molecular classification groups from The Cancer Genome Atlas (TCGA) Project. The TCGA study performed genomic characterization of EC and found that these molecular subgroups strongly correlate with the histologic subtypes of EC. The groups include POLE ultramutated (POLE), microsatellite instability-high/hypermutated (MSI-H), copy-number high (CNH) and copy-number low (CNL) (Kandoth et al., 2013 May 2). Although panels for molecular testing are becoming more widely available, there are IHC surrogates that can help to classify these tumors into their respective groups.

POLE mutated tumors overall have a good prognosis compared to other subtypes with the group mostly made up of endometrioid endometrial cancers. MSI-H tumors most frequently include endometrioid EC or undifferentiated carcinomas and have an intermediate prognosis. CNH have nearly universal p53 mutations (95 %) and generally correspond with endometrial serous carcinomas with a poor prognosis. Up to

25 % of these tumors show high grade endometrioid or carcinosarcoma histology. CNL includes mostly endometrioid endometrial and clear cell carcinomas. A readily applicable IHC pathway for defining these groups includes MMR and p53 IHC with a molecular test for pathogenic POLE mutations. (Berek et al., 2023) Aberrant p53 staining can vary from strong nuclear expression (>80 %), to the absence of expression or unequivocal cytoplasmic expression. (WHO, 2020) Although molecular subgroups can help to classify tumors and provide information about prognosis, histology along with IHC remains an important tool in correctly identifying endometrial carcinomas.

2.6. Uterine sarcomas

Uterine sarcomas account for approximately 3–7 % of all uterine cancers. Leiomyosarcomas are the most common subtype of uterine sarcoma. They are aggressive, high-grade tumors with a poor prognosis regardless of if apparently confined to the uterus. IHC using p16, p53, and Ki-67 is useful for making the distinction between leiomyosarcoma and benign smooth muscle tumors like leiomyomas (Chen and Yang, 2008 Jul). Additionally, leiomyosarcomas have variable expression of estrogen and progesterone receptors. High-grade endometrial stromal sarcomas are often diagnosed in advanced stage, with extensive invasion and poor prognosis. Conversely, low-grade endometrial stromal sarcomas are less aggressive and associated with better long-term survival. Their growth pattern is more indolent when compared to high-grade endometrial stromal sarcomas (Zhang et al., 2019 Jan). The distinction between low and high-grade endometrial stromal sarcoma is clinically relevant as high-grade tumors lack hormone receptors and therefore hormonal therapy cannot be considered (Nucci, 2016 Jan).

Regardless of the subtype, all sarcomas are positive for vimentin and are commonly immuno-positive for SMA. A wide panel of IHC have been explored for uterine sarcomas, including vimentin, AE1/AE3, smooth muscle actin (SMA), desmin, h-caldesmon, actin, Myf4, CD10, CD31, CD68, CD177, factor VIII, HMB-45, and S-100 protein (Abeler and Nenodovic, 2011 May). CD10 is a reliable marker of the endometrial stroma, therefore it is frequently found to be positive amongst sarcoma specimens (McCluggage et al., 2001 Sep).

For leiomyosarcoma, the initial panel of p53, Rb, PTEN, and ATRX is applied, followed by a panel of DAXX, MTAP, and MDM2 in cases without abnormalities. The combined staining of SMA positive with desmin or h-caldesmon, yields a positive diagnosis for leiomyosarcoma of 96 % and 92 %, respectively (Abeler and Nenodovic, 2011 May). High-grade endometrial stromal sarcomas are CD10 negative, ER/PR negative, with strong cyclin D1 immuno-reactivity, and commonly c-kit positive (Nucci, 2016 Jan). C-kit is expressed in a subset of leiomyosarcomas and may identify those tumors that will respond to tyrosine kinase inhibitors (Wang et al., 2003 Aug).

Low grade endometrial stromal sarcomas are anticipated to have IHC with strongly immuno-reactive CD10, SMA, patchy ER/PR, occasionally desmin or cyclin D1 positive, and h-caldesmon negative. Additionally, undifferentiated uterine sarcoma will be CD10, p53 and cyclin D1 positive, but negative ER/PR markers (Nucci, 2016 Jan).

2.7. Gestational trophoblastic disease

Gestational trophoblastic disease (GTD) encompasses several different disease entities with multiple classifications. GTD can typically be identified based on distinctive histologic features alone, but in challenging cases IHC can be used for clarity. Multiple trophoblast associated IHC markers have been identified to distinguish each diagnosis from another. HSD3B is used as a preliminary stain to distinguish GTD from non-GTD disease. HSD3B1 demonstrates immunoreactivity in all trophoblastic tumors and lesions, including placental site trophoblastic tumor (PSTT), epithelioid trophoblastic tumor (ETT), placental site nodule, exaggerated placental site, choriocarcinomas, and complete moles making it a highly specific marker for distinguishing gestational trophoblastic disease. HLA-G is exclusively expressed in the intermediate trophoblast cells of normal and molar placentas making it an immunoreactive target in most gestational trophoblastic lesions, with no expression in non-trophoblastic uterine tumors. Additionally, all subtypes of trophoblastic cells stain positive for GATA3 and as such may help in distinguishing trophoblastic tumors from other Mullerian epithelial malignancies (IeM, 2007 Jun).

Choriocarcinoma can arise from the trophoblasts of any gestational event and results in a highly malignant, although treatable, tumor. Choriocarcinoma is composed of mononucleate trophoblastic and sheets of syncytiotrophoblast cells, therefore trophoblastic markers serve as targets for IHC. Although identification of choriocarcinoma is usually accomplished by histology alone, when the diagnosis is unclear, hPL, b-hCG, p63, p40 and HSD3B1 can be useful for clarification (IeM, 2007 Jun). SALL4 is particularly useful for differentiating choriocarcinoma from PSTT and ETT, as these tumors do not express the marker (Stichelbout et al., 2016 Aug).

PSTT and ETT are recognized separate entities of GTN, with distinct IHC patterns. PSTTs are generally strongly Mel-CAM and HPL positive with focally positive PLAP. Intermediate trophoblastic cells of choriocarcinoma are also recognizable with Mel-Cam IHC. P63 and hPL are used to distinguish ETT and PSTT. Additionally, combined use of p63 and HPL can differentiate implantation site trophoblastic disease from chorionic disease and choriocarcinoma as implantation site tumors will be noted to have negative staining for p63 and chorionic disease will be strongly positive. ETT is distinguished by positivity for pan-cytokeratin, epithelial membrane antigen, E-cadherin, PLAP, and p63, Cyclin E and focal positivity for HPL, Mel-CAM, and beta-hCG (Horowitz et al., 2017). Beta-hCG staining is depicted as single to small clusters of cells, which differs from its presentation in choriocarcinoma with positive staining in the trophoblastic cells and negative amongst the cytotrophoblasts (Shih and Kurman, 2001 Jan).

3. Cervical carcinoma

The tumor suppressor protein p16 is commonly mutated in cancers leading to dysregulation of cell cycle progression. In cervical cancer there is a well-established relationship between cervical dysplasia and p16 staining intensity. More than 90 % of squamous cell carcinomas contain HPV DNA (Waggoner, 2003 Jun 28). In HPV infected cells, oncoprotein E7 causes inactivation of retinoblastoma protein leading to multiple downstream effects that promote cell cycle progression including the release of the p16 gene from transcriptional inhibition leading to an increase in p16 protein that can be detected by IHC. Diffuse and strong cytoplasmic and nuclear staining of p16 is reflective of high risk HPV infections, and sensitive and specific to differentiate high and intermediate risk HPV strains from low risk HPV. Ki-67 has many roles in affecting cell cycle progression, and therefore predicts the malignant potential of tumors (Yu et al., 2019 Jun 2). The co-expression of p16 and Ki-67 can be used as a surrogate marker for cell-cycle deregulation caused by HPV infection. Due to the highest sensitivity and specificity of p16/Ki-67 testing, it has been proposed for use when making colposcopy referrals. Women who are p16 and Ki-67 negative can defer invasive testing and opt for retesting (Petry et al., 2011 Jun 1).

Most forms of cervical cancer are associated with HPV infection; however, 3 % to 8 % of cases are HPV-negative. Although SCC of the cervix is usually HPV-positive, 15 % to 38 % of all cervical adenocarcinomas are HPV-negative. HPV-negative cervical cancer is a poorly understood disease entity with poorer patient outcomes compared with HPV-positive cervical cancer. Cervical cancer biologic characteristics, genomic alterations, and immunogenic responses may vary by HPV status. P16 immuno-staining is an easy and effective technique to identify HPV related tumors (Santos et al., 2004 Jul). No FDA approved targeted therapies are currently available for HPV-positive or HPV-negative cervical cancer. However, HPV-positive cervical cancers are considered immunologically hot, as HPV infection can increase immune

targets, such as PD-1, PD-L1 or CTLA4. HPV-negative cervical cancers are considered immunologically cold and unresponsive to immunotherapy. Thus, HPV-negative cervical cancer must be further investigated to identify molecular etiologies that could serve as future therapeutic targets.

Ascertaining whether an adenocarcinoma is primary cervical or endometrial can be challenging, especially in the case of a tumor that invades both the uterus and cervix. In this case a panel of ER, vimentin, monoclonal CEA, and p16 can be utilized to identify the primary tumor. Endocervical adenocarcinoma can be distinguished from EC with diffusely positive CEA and p16 (McCluggage, 2007 Feb).

Investigation into the use of p63 staining is limited, however, it may have a useful role in distinguishing squamous cell carcinoma although p40 is more commonly used. Amongst small cell neuroendocrine, large cell endocrine and adenocarcinoma, the staining pattern is typically negative or focally positive, differing from squamous cell which demonstrates diffuse expression (Houghton and McCluggage, 2009 Sep).

Tumor cells have adapted overexpression of PD-L1 to avoid immunologic surveillance and facilitate further tumor growth. PD-L1 protein expression can be detected on standard IHC and is quantified using a combined positive score (CPS) which measures the number of PD-L1 staining cells in a sample. PD-L1 expression has been utilized as a target for novel immune checkpoint inhibitors, with clinical responses even being seen amongst PD-L1 negative tumors. Pembrolizumab, an anti PD-1 antibody, has been approved in the first line and recurrent settings of cervical cancer in patients with CPS scores greater than or equal to 1. (Monk et al., 2022). Differences in PD-L1 expression have been observed amongst cervical cancer subtypes, with more frequent expression in squamous cell carcinoma than adenocarcinoma. Further, diffuse PD-L1 expression in squamous cell carcinoma and the presence of PD-L1 positive tumor-associated macrophages in adenocarcinoma correlate with poor disease prognosis (Heeren AM, 2016).

4. Vulvar cancers

As discussed previously, p16 immuno-staining is an easy and effective technique identify HPV related vulvar tumors, as HPV related neoplasms reliably show strong p16 expression (Santos et al., 2004 Jul). HPV independent, differentiated vulvar intraepithelial neoplasia (dVIN) associated vulvar cancers can be identified using an IHC panel including p53, Ki67, and p16 (Wang et al., 2023). P53 staining can have variable expression in dVIN with four mutation patterns: basal overexpression, diffuse overexpression, absent expression and cytoplasmic expression. P53 overexpression is not seen, however, in HPV independent vulvar squamous cell carcinoma (Tessier-Cloutier et al., 2020 Aug). CK17 is used together with p53 and Ki-67 to aid in diagnosis of dVIN to identify vulvar dysplasia and therefore serves as an adjunct for the diagnosis of dVIN (Wang et al., 2023). The use of immune checkpoint inhibitors in metastatic or recurrent vulvar cancer has been extrapolated from treatment of cervical cancer. Due to the role of immune checkpoint inhibitors, PDL1 testing may be performed routinely in primary vulva cancers to help guide incorporation of treatment with immunotherapy (Garganese et al., 2021 Dec 19).

IHC is also utilized in differentiating primary extra-mammary Paget disease from extra-mammary Paget disease secondary to malignancy, as the primary disease has a significantly better prognosis (Perrotto et al., 2010 Apr). Findings usually include positivity for CK7 and CAM 5.2 as well as occasional positivity for CK20 and negative for Melan A. HMB-45 is used to rule out melanoma and CK5/6 to rule out squamous cell carcinoma. CK7 is positive amongst both primary and secondary Paget disease (Ohnishi and Watanabe, 2000 Feb). CK20 usually demonstrates a negative or focally positive staining pattern in primary vulvar Paget disease, and a strongly diffuse staining is suggestive of an underlying carcinoma.

Vulvar melanoma accounts for less than 5 % of all vulvar malignancies and 1–2 % of all melanomas. Invasive melanoma can be identified through IHC with positive staining for S100, SOX10 and nerve growth factor receptor (NGFR). Melanotic lesions have a characteristic appearance but melanocytic markers such as MART1 (MelnA) can help to identify invasion. Amelanotic lesions may require additional staining for identification, especially if they show pleomorphism within the tumor. In this case, a panel of HMB45, S100, MART1, and vimentin can be used to identify these lesions (Falcicchio et al., 2022 Oct 25).

CRediT authorship contribution statement

Arielle H. Katcher: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Michelle P. Greenman:** Writing – original draft, Investigation. **Sudarshana Roychoudhury:** Writing – review & editing, Supervision. **Gary L. Goldberg:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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