Ancillary studies (Non-core)

Ancillary testing (chiefly immunohistochemistry and/or molecular testing) may be of value in the diagnosis of uterine malignant and potentially malignant mesenchymal tumours. The results of ancillary tests should be interpreted in the overall context of the clinical setting, macroscopic pathology and microscopic pathology. The most recent World Health Organization (WHO) Classification[1] defines two potential roles for ancillary tests for certain tumours: 1) to serve as essential diagnostic criteria, required for establishing the diagnosis; or 2) to serve as supportive criteria that are desirable but not essential to establish the diagnosis. To harmonise with the latest WHO Classification,[1] the International Collaboration on Cancer Reporting Uterine Sarcoma Dataset Authoring Committee recommends adopting a similar strategy, acknowledging that some of these ancillary tests may not be available in all practice settings. Discussion of the detailed immunophenotype of the various uterine sarcomas or use of ancillary testing to resolve specific differential diagnoses is beyond the scope of these recommendations. Among the tumours with a defining molecular alteration, gene fusion is the main pathologic mechanism; thus, fluorescent in situ hybridisation or RNA sequencing are the primary types of molecular diagnostic tools, with a few rare exceptions of tumours characterised by inactivating mutations.

Leiomyosarcoma and smooth muscle tumour of uncertain malignant potential (STUMP) are expected to exhibit a smooth muscle immunophenotype (positive for desmin, h-caldesmon, smooth muscle myosin and smooth muscle actin), although it is not uncommon for only some of the smooth muscle stains to be positive or for staining to be patchy, particularly in myxoid and epithelioid variants. While mutation of *TP53*, *MED12*, and/or *ATRX* occur in a minority of leiomyosarcomas, these alterations are not specific to leiomyosarcoma. A recent study has shown that p53 immunohistochemistry may be useful in distinguishing translocated associated sarcomas from other non-translocated associated sarcomas with the former more often showing wild-type staining.[2] A minority of myxoid leiomyosarcoma may exhibit PLAG1 immunoreactivity and *PLAG1* fusion. Low grade endometrial stromal sarcoma is expected to exhibit diffuse strong CD10 and estrogen receptor (ER) immunoreactivity The diagnosis can be supported by demonstrating a gene fusion involving *JAZF1* and/or *PHF1* but since only about two-thirds of these tumours harbor such a gene fusion, molecular testing is not essential for the diagnosis nor does a negative result exclude the diagnosis. High grade endometrial stromal sarcoma encompasses a range of tumours that are subclassified by one of a variety of distinct gene fusions, thus requiring molecular testing for their diagnosis. The high grade component of *YWHAE-NUTM2A/B* high grade endometrial stromal sarcoma typically exhibits absent CD10 and ER immunoreactivity, positive cyclin D1, CD117, CD56, CD99 and BCOR immunoreactivity,[3] and the *YWHAE-NUTM2A/B* gene fusion. *ZC3H7B-BCOR* high grade endometrial stromal sarcoma retains CD10 immunoreactivity, exhibits variable ER immunoexpression, positive cyclin D1 immunoreactivity, variable BCOR immunoreactivity, and *ZC3H7B-BCOR* gene fusion. High grade endometrial stromal sarcoma with *BCOR* internal tandem duplication (ITD) exhibit variable CD10 immunoexpression, loss of ER immunoreactivity, positive cyclin D1 and BCOR immunoreactivity, and *BCOR* ITD by molecular sequencing techniques.

SMARCA4-deficient uterine sarcoma is defined by loss of SMARCA4 (BRG1) immunoreactivity or, rarely, loss of SMARCB1 (INI1) immunoreactivity. The diagnosis can be supported by demonstrating inactivating mutation or deletion of *SMARCA4.* IMT is defined by positive ALK immunoreactivity; demonstration of *ALK* fusion by molecular testing can support the diagnosis but is not essential if the ALK immunostain is positive. Perivascular epithelioid cell tumour (PEComa) is defined by dual melanocytic (HMB45, cathepsin K, melan A, MITF, and/or PNL2) and myoid (smooth muscle actin, desmin, h-caldesmon) immunoreactivity. It is recommended that at least two melanocytic markers be positive given the lack of specificity of any one marker for PEComa. The subset of PEComas that harbour a *TFE3* fusion exhibit TFE3 immunoreactivity along with melanocytic marker

immunoreactivity, although smooth muscle marker immunoreactivity may be limited or absent. The diagnosis of PEComa can be supported by demonstration of an inactivating mutation of *TSC1* or *TSC2* or by demonstrating *TFE3* or *RAD51B* fusion.[4] Uterine tumour resembling ovarian sex cord tumour (UTROSCT) is characterised by polyphenotypic immunoreactivity of epithelial markers (keratin, epithelial membrane antigen (EMA)), sex cord markers (FOXL2, SF1, calretinin, inhibin, WT1, and/or melan A), myoid markers (smooth muscle actin, desmin and h-caldesmon) and hormone receptors (ER and progesterone receptor (PR)). The diagnosis can be supported by demonstrating *ESR1* or *GREB1* fusion; however such alterations are not present in all cases, so a negative result does not exclude the diagnosis. *NTRK* uterine sarcoma is defined by a gene fusion involving *NTRK1, NTRK2,* or *NTKR3.* S100 and CD34 are usually positive and immunoreactivity of these markers can be used as a screening tool to identify tumours that merit *NTRK* molecular testing; smooth muscle markers, CD10 and hormone receptors are usually negative. Pan-TRK immunoreactivity can also be used to triage testing for a *NTRK* fusion. However, high grade endometrial stromal sarcoma may show NTRK immunoreactivity in the absence of an *NTRK* fusion.[5] Uterine adenosarcomas do not have a unique immunophenotype and so the diagnosis is mainly based on morphologic criteria. The tumour cells usually exhibit CD10 and ER immunoreactivity but these markers may be absent in areas of high grade stroma/sarcomatous overgrowth. Rhabdomyosarcoma is expected to exhibit immunoreactivity of desmin, myogenin, and/or myoD1. Both rhabdomyosarcoma and adenosarcoma with rhabdomyosarcomatous differentiation may harbor *DICER1* mutations.

References

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