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Cyclin D1 as a diagnostic immunomarker for endometrial stromal sarcoma with *YWHAE-FAM22* rearrangement

Cheng-Han Lee[1,↑], **Rola H Ali**[1,↑], **Marjan Rouzbahman**[2], **Adrian Marino-Enriquez**[3], **Meijun Zhu**[3], **Xiangqian Guo**[4], **Alayne L Brunner**[4], **Sarah Chiang**[5], **Samuel Leung**[1], **Nataliya Nelnyk**[6], **David G. Huntsman**[6], **C Blake Gilks**[1], **Torsten O. Nielsen**[1], **Paola Dal Cin**[3], **Matt van de Rijn**[4], **Esther Oliva**[5], **Jonathan A Fletcher**[3], and **Marisa R Nucci**[3]

[1]Department of Pathology and Laboratory Medicine, and Genetic Pathology Evaluation Center, Vancouver General Hospital and University of British Columbia, Vancouver, Canada

[2]Department of Pathology, Brigham and Women's Hospital, Boston, United States

[3]Department of Pathology, Toronto General Hospital, Toronto, Canada

[4]Department of Pathology, Stanford University Medical Center, Stanford, United States

[5]Department of Pathology, Massachusetts General Hospital, Boston, United States

[6]Centre for Translational and Applied Genomics (CTAG), British Columbia Cancer Agency, Vancouver, Canada

Abstract

Endometrial stromal sarcoma (ESS) characterized by *YWHAE-FAM22* genetic fusion is histologically higher-grade and clinically more aggressive than ESS with *JAZF1-SUZ12* or equivalent genetic rearrangements, hence it is clinically important to recognize this subset of ESS. To identify diagnostic immunomarkers for this biologically-defined ESS subset, we compared gene expression profiles from *YWHAE-FAM22* ESS, *JAZF1*-rearranged ESS and uterine leiomyosarcomas. These studies showed consistent upregulation of cyclin D1 in *YWHAE-FAM22* ESS compared to *JAZF1-SUZ12* ESS. Immunohistochemically, the high-grade round cell component of all 12 *YWHAE-FAM22* ESS demonstrated diffuse (≥70%) moderate-to-strong nuclear cyclin D1 staining and this diffuse positivity was not seen in 34 ESS with *JAZF1* and equivalent genetic rearrangements or in 21 low-grade ESS with no demonstrable genetic rearrangements. In a series of 243 non-ESS pure uterine mesenchymal and mixed epithelial-mesenchymal tumors, only 2 of 8 undifferentiated endometrial sarcomas with nuclear uniformity and 1 of 80 uterine leiomyosarcomas demonstrate diffuse cyclin D1 immunoreactivity. Both cyclin D1-positive undifferentiated endometrial sarcomas showed diffuse strong CD10 staining, which is consistently absent in the high-grade round cell component of *YWHAE-FAM22* ESS. The low-grade spindle cell component of *YWHAE-FAM22* ESS showed a spatially heterogeneous cyclin D1 staining pattern that was weaker and less diffuse overall. Our findings indicate that

Corresponding authors: Marisa R. Nucci, M.D., Associate Professor, Department of Pathology, Harvard Medical School, Associate Pathologist, Brigham and Women's Hospital, 75 Francis Street, Amory 3, Boston, MA, 02115; Cheng-Han Lee, M.D., Ph.D., Clinical Assistant Professor, Department of Pathology and Laboratory Medicine, University of British Columbia Consultant pathologist, Anatomical Pathology, Vancouver General Hospital JP1400, 910 West 10th Ave, Vancouver, BC, Canada. V5Z 4E3.
[↑]Both authors contributed equally to the study

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cyclin D1 is a sensitive and specific diagnostic immunomarker for *YWHAE-FAM22* ESS. When evaluating high-grade uterine sarcomas, cyclin D1 can be included in the immunohistochemical panel as an indicator of *YWHAE-FAM22* ESS.

Keywords

Endometrial stromal sarcoma; round cell; YWHAE-FAM22; cyclin D1; JAZF1-SUZ12

Introduction

Endometrial stromal sarcoma (ESS) is a histologically and genetically heterogeneous group of uterine sarcoma. The majority, particularly those with a classic low-grade histologic appearance, contain a genetic fusion involving *JAZF1* and members of polycomb complex gene (*SUZ12, PHF1, EPC1*) most frequently resulting from a chromosomal translocation, t(7;17).[6, 13, 16, 26, 28, 29, 31] We recently described and characterized a series of ESS with *YWHAE-FAM22A/B* (*YWHAE-FAM22*) genetic fusion resulting from t(10;17)(q22;p13).[22] Clinically, the majority of patients with *YWHAE-FAM22* ESS present with evidence of extrauterine spread, in contrast to patients with *JAZF1* ESS (encompassing *JAZF1/SUZ12/PHF1/EPC1*-rearranged tumors), in which the disease is usually confined to the uterus at initial presentation. Patients with *YWHAE-FAM22* ESS also experience more frequent recurrences compared to those with *JAZF1* ESS. In contrast to *JAZF1* ESS, *YWHAE-FAM22* ESS nearly always display a cellular high-grade round cell morphologic appearance with larger nuclei, more irregular nuclear contour, high mitotic activity and frequent necrosis. This high-grade round cell component is accompanied in about half of the cases by a low-grade bland spindle cell component (with low mitotic activity) with a fibrous to fibromyxoid stromal background.[21] The high-grade round cell area of *YWHAE-FAM22* ESS exhibits an undifferentiated appearance immunohistochemically, being negative for estrogen receptor (ER), progesterone receptor (PR) and CD10, in contrast to the frequent diffuse ER, PR and CD10 immunoreactivity seen in *JAZF1* ESS while the low-grade spindle cell component is consistently immunoreactive for ER, PR and CD10, an immunohistochemical profile that overlaps with that seen in *JAZF1* ESS. The consistent lack of ER and PR expression in the high-grade round cell component implies that hormonal therapy, which has been used as a systemic therapy for ESS, will likely be ineffective against *YWHAE-FAM22* ESS. [34]

Because of the difference in clinical behavior and its potential therapeutic implications, the distinction of *YWHAE-FAM22* ESS from *JAZF1* ESS is important. However, while *YWHAE-FAM22 ESS* exhibits higher grade histologic features compared to *JAZF1* ESS, it can in some instances be challenging to distinguish these different ESS subsets on histologic grounds alone, particularly given their rarity. Furthermore, *YWHAE-FAM22* ESS also needs to be distinguished from other high-grade typically pleomorphic uterine sarcomas including undifferentiated endometrial sarcoma (UES) and leiomyosarcoma, which in contrast to *YWHAE-FAM22* ESS, are highly aggressive with a five year survival of less than 50%.[1, 18] While molecular confirmation (RT-PCR or FISH analysis) currently represents the standard to establish a definitive diagnosis of *YWHAE-FAM22* ESS, these tests are presently offered only at a few centers. Thus, there is a need to identify diagnostic immunomarker(s) that would be more accessible to practicing pathologists in general. In order to potentially identify such a marker, we analyzed published gene expression data of *YWHAE-FAM22* ESS and *JAZF1* ESS. Following identification of a potential marker, we then rigorously evaluated its diagnostic utility by examining its sensitivity and specificity for *YWHAE-FAM22* ESS in the context of uterine mesenchymal and mixed epithelial-mesenchymal tumors.

Methods

Tumor samples

Formalin-fixed paraffin-embedded tumor tissues were obtained from ESS in which FISH studies had demonstrated either *YWHAE-FAM22* rearrangement (n=12), *JAZF1/SUZ12/PHF1/EPC1* rearrangement (n=34) or no demonstrable genetic rearrangement (n=21); these were from the pathology archives at Brigham and Women's Hospital, Massachusetts General Hospital, Vancouver General Hospital and Toronto General Hospital. The histologic features of all tumors were reviewed by two authors (C.H. Lee and M. R. Nucci) and previously described.[21] A comparison group consisted of tissue microarrays of 8 undifferentiated endometrial sarcomas with nuclear uniformity (UES-U),[18] 13 undifferentiated endometrial sarcoma with nuclear pleomorphism (UES-P), 119 uterine leiomyosarcomas (LMS, 80 primary and 39 metastatic from uterine primary), 26 uterine adenosarcomas (8 with sarcomatous overgrowth), 13 uterine carcinosarcomas, 4 uterine PEComas, 49 uterine leiomyomas, 7 polypoid endometriosis, 3 endometrial stromal nodules and 2 uterine tumors resembling ovarian sex cord tumor (UTROSCT).[6, 20, 23] All the tumors in the comparison group lacked *YWHAE* rearrangement by FISH study. The study was approved by the Institutional Review Boards at the respective institutions.

Analysis of gene expression data

3′ end sequencing-based gene expression profile data on a series of 3 *YWHAE-FAM22* ESS and 4 *JAZF1* ESS was previously published by our group.[22] Significance of microarray analysis was used to identify differentially expressed genes between different tumor groups (37) and a false-discovery rate (FDR) of <1% was considered to be significant.

Immunohistochemistry

Immunohistochemical analysis was performed on whole sections of 12 *YWHAE-FAM22* ESS and on whole sections or tissue microarray sections of 298 other gynecologic tumors. Cyclin D1 (Thermoscientific, clone SP4, rabbit monoclonal, 1:50) immunostaining was performed following heat-induced antigen retrieval using CC1 antigen retrieval buffer (Ventana Medical Systems, AZ, USA). After incubation with the primary antibodies, sections were stained on the Ventana automated slide stainer (NEXES, Tucson, AZ) using the Ventana diamino-benzidine (DAB) detection kit (Ventana Medical Systems, Tucson, AZ). Only nuclear staining was considered positive. Strong cyclin D1 staining was defined as having the same intensity as cyclin D1-positive mantle cell lymphoma control, moderate as definite staining that is visible at low power (4x objective) but weaker than cyclin D1-positive mantle cell lymphoma control, and weak as nuclear staining that require high power (20-40x) examination to recognize (Figure 2C). Percentage of nuclei showing moderate to strong nuclear cyclin D1 staining was assessed by evaluating representative high-power fields (for whole tissue sections) or the entire tissue microarray cores. Tumors showing ≥ 70% moderate to strong nuclear staining for cyclin D1 were considered to be positive.

Results

Analysis of the 3′end sequencing gene expression profiles

The result of filtered 3′end sequencing performed on a series of 3 *YWHAE-FAM22* ESS and 4 *JAZF1* ESS was previously described.[22] We performed SAM analyses comparing *YWHAE-FAM22* ESS to *JAZF1* ESS and identified a number of differentially expressed genes with a false discovery rate of < 0.01% (Table 1). *CCND1* (which encodes Cyclin D1) was among the top five most upregulated genes in *YWHAE-FAM22* ESS compared to *JAZF1* ESS. Given the general availability of cyclin D1 in pathology laboratories, this

potential diagnostic biomarker was selected for immunohistochemistry validation studies in *YWHAE-FAM22* ESS.

Cyclin D1 immunostaining in YWHAE-FAM22 ESS and JAZF1 ESS

Cyclin D1 immunohistochemistry was evaluated in 12 *YWHAE-FAM22* ESS, 4 of which also contained a low-grade fibrous/fibromyxoid spindle cell component in the material available for the study, while the other 8 contained pure high-grade round cell morphology. All tumors showed ≥70% moderate to strong cyclin D1 nuclear staining of the tumor cells (average 91%) with an homogenous staining in the high-grade round cell components (Figure 1). In the 4 cases where a low-grade spindle cell component was present, cyclin D1 immunostaining demonstrated significant intra-tumoral variability in all cases (Figure 2), which ranged from less than 1% to more than 50% nuclear staining in different areas of the same tumor. The staining intensity in the low-grade spindle cell component was weaker (weak to moderate intensity) in contrast to the strong staining intensity seen in the high-grade round cell component (Figure 2).

A series of 34 *JAZF1* ESS were also examined and 31 displayed absent to very focal cyclin D1 nuclear staining (≤5%), typically in the form of scattered positive single cells (Figure 3 and 4). In histologically classic low-grade ESS with no detectable genetic rearrangements, the pattern of cyclin D1 staining was similar to that seen in *JAZF1* ESS, with 19 of 21 showing ≤5% nuclear staining (Figure 4).

Cyclin D1 immunostaining in other uterine mesenchymal and mixed epithelial/mesenchymal tumors

The cyclin D1 immunostaining results of other gynecologic mesenchymal and mixed epithelial/mesenchymal tumors are summarized in Table 2 and illustrated in Figure 5. A total of 8 UES with nuclear uniformity (UES-U) lacking *YWHAE* or *JAZF/PHF1* rearrangements were studied and 2 showed diffuse cyclin D1 staining (Figure 5C). Both cyclin D1 positive UES-U displayed strong diffuse CD10 immunoreactivity and were negative for ER and PR. All 13 UES with nuclear pleomorphism (UES-P) showed focal (<20%) cyclin D1 staining (Figure 5D). Other than UES-U, diffuse strong cyclin D1 was rarely seen in other uterine mesenchymal or mixed epithelial-mesenchymal tumors; only 1 of 80 primary uterine leiomyosarcomas (and 0 of 39 metastatic leiomyosarcomas) examined showed ≥70% cyclin D1 nuclear staining (Figure 5E-F); this tumor had a prominent histiocytic and lymphocytic infiltrate (Figure 5F). Thus, in this series, cyclin D1 immunostaining showed a sensitivity of 100% and specificity of 99% as a diagnostic marker for *YWHAE-FAM22* ESS, with positive and negative predictive values of 80% and 100%, respectively.

Discussion

We recently described the histologic features of *YWHAE-FAM22* ESS. The tumors were characterized by the presence of a high-grade round cell component where the tumor cell nuclei are larger in size, more irregular in contour and demonstrate greater mitotic activity and necrosis compared to the tumor cells in *JAZF1* ESS. Even though RT-PCR and/or FISH analysis of *YWHAE-FAM22* genetic rearrangement should be considered the gold standard for diagnosis, our studies show that cyclin D1 immunohistochemistry is an informative diagnostic adjunct, which can be used to support a provisional diagnosis of *YWHAE-FAM22* ESS and screen-in cases for confirmatory molecular *YWHAE-FAM22* analyses.

Among uterine tumors, the differential diagnosis for the high-grade round cell area of *YWHAE-FAM22* ESS includes other genotypes of ESS with increased mitotic activity and/

or necrosis (that correspond to either *JAZF1* ESS or ESS without demonstrable genetic rearrangements), undifferentiated endometrial sarcomas (particularly UES-U), leiomyosarcoma (particularly epithelioid variant), PEComa and sarcoma-predominant carcinosarcoma. As shown here, diffuse strong cyclin D1 immunoreactivity is consistently observed in the high-grade round cell component of *YWHAE-FAM22* ESS. Although diffuse cyclin D1 immunostaining was seen in a subset of UES-U and rare case of uterine leiomyosarcoma in this study, we have not seen this in *JAZF1* ESS, ESS without demonstrable rearrangements, undifferentiated endometrial sarcomas with nuclear pleomorphism (UES-P), PEComa, leiomyosarcoma, leiomyoma, adenosarcoma (including cases with sarcomatous overgrowth) and carcinosarcoma. Two of 8 UES-U in our current series showed diffuse cyclin D1 immunoreactivity comparable to that in *YWHAE-FAM22* ESS, but lacked *YWHAE (or JAZF1/PHF1)* rearrangements by FISH analysis. Importantly, both cyclin D1 positive UES-U showed diffuse strong CD10 staining, in contrast to the high-grade round cell component of *YWHAE-FAM22* ESS, which consistently lacked substantial CD10 staining. Therefore, when a combined panel of cyclin D1 and CD10 is applied to a histologically high-grade but non-pleomorphic uterine sarcoma, the finding of diffuse strong cyclin D1 in the absence of CD10 staining appears to be highly sensitive and specific for *YWHAE-FAM22* ESS.

Cyclin D1 immunoreactivity has been previously evaluated by Kurihara *et al* in both endometrial stromal tumors and UES, and they observed similar staining patterns to our present study.[19] None of the 8 endometrial stromal nodules (4 positive for *JAZF1-SUZ12* genetic fusion by RT-PCR assay), 16 low-grade ESS (5 positive for *JAZF1-SUZ12* genetic fusion) and 6 UES-P showed ≥70% nuclear cyclin D1 staining. Among 7 UES-U, 3 showed ≥70% nuclear cyclin D1 staining. However, it is unknown whether any of the UES-U harboured *YWHAE* rearrangement or exhibited CD10 immunoreactivity. Cyclin D1 immunoreactivity has also been previously examined in uterine carcinosarcoma.[7, 14] De Jong *et al* found 7 of 31 tumors to show cyclin D1 positivity in the mesenchymal component,[7] however, these authors used a much lower nuclear cut-off staining (10%). In our series, 4 of 13 carcinosarcomas would have been classified as positive for cyclin D1 if the same lower cut-off would have been used. Kanthan *et al* reported no significant cyclin D1 expression of the mesenchymal component of 23 uterine carcinosarcomas,[14] which is in concordance with the present findings.

While the low-grade spindle cell component of *YWHAE-FAM22* ESS is seen next to the high-grade round cell component in about half of the tumors, it is possible that a biopsy may sample only the low-grade spindle cell area. The histologic differential for the low-grade spindle cell component of *YWHAE-FAM22* ESS includes fibrous/fibromyxoid variant of ESS, well-differentiated leiomyosarcoma and the sarcomatous overgrowth of an adenosarcoma. The low-grade spindle cell component of *YWHAE-FAM22* ESS showed overall weak and focal cyclin D1 nuclear staining with significant intra-tumoral heterogeneity, ranging from absent to diffuse weak/moderate staining. Cyclin D1 immunostaining is therefore of limited diagnostic value for identifying *YWHAE-FAM22* ESS when the tissue sample shows a low-grade spindle cell proliferation in a fibrous/fibromyxoid background. Further studies are necessary to determine whether there is significant difference in cyclin D1 immunostaining pattern between the low-grade spindle cell component of *YWHAE-FAM22* ESS and fibrous/fibromyxoid variant of ESS lacking known genetic rearrangement.[9, 13, 30, 40] With these considerations in mind, we propose diagnostic algorithm incorporating morphologic and immunophenotypic features to identify uterine *YWHAE-FAM22* ESS.

Among non-gynecologic type sarcomas that may present as a pelvic mass, Ewing sarcoma/peripheral neuroectodermal tumor, gastrointestinal stromal tumors (GIST),

rhabdomyosarcoma (both alveolar and embryonal type), malignant solitary fibrous tumors, and less frequently epithelioid malignant peripheral nerve sheath tumors (MPNST) and round cell liposarcomas can possess histologically high-grade round cell areas that could potentially resemble *YWHAE-FAM22* ESS. Nuclear cyclin D1 immunostaining has been found to be focal (<50% nuclear staining) in malignant solitary fibrous tumors,[3] round cell liposarcomas,[8] MPNST,[17] alveolar and embryonal rhabdomyosarcomas.[36] Nakamura *et al* observed an average of 12% nuclear immunostaining (standard deviation of 21%) in a series of 88 GIST, but rare cases (number of cases not specified) showed ≥70% nuclear staining.[27] Strong membranous/cytoplasmic KIT and DOG1 immunoreactivity would suggest a diagnosis of GIST.[24] In Ewing sarcomas, a significant subset (13 of 31) can show diffuse strong nuclear cyclin D1 immunostaining.[10] Furthermore, *YWHAE-FAM22* ESS can also show strong diffuse CD99 immunostaining.[2] This overlap in immunophenotype does present a diagnostic challenge when one encounters a malignant round cell tumor in the biopsy of a pelvic mass. While the presence of a concurrent low-grade fibromyxoid spindle cell component and/or the presence of moderate eosinophilic cytoplasm in the round cell component would favor *YWHAE-FAM22* ESS, these features may not be present in the tumor or be represented in the sampled tissue. Moreover, rosette formation may occur in Ewing's sarcoma (although not described to date in those of the uterus) and *YWHAE-FAMM 22* ESS, therefore, this histologic finding is of little utility in this distinction.[2] In this selected scenario, molecular analysis (FISH or RT-PCR) is necessary to confirm the diagnosis of *YWHAE-FAM22* ESS versus Ewing sarcoma. In contrast to *YWHAE-FAM22* ESS with its t(10;17), the vast majority of Ewing's sarcoma show a t(11;22)(q24;q12), which results in the fusion of *EWS* with *FLI1*; in a minority, *EWS* is involved with variant partners: t(21;22)(q12;q12) and t(7;22)(p22;q12), leading to EWS-ERG and EWS-ETV1 fusions, respectively. In the scenario of uterine tumor consisting purely of monomorphic high-grade round cells with a cyclin D1-positive and CD10/ER/PR-negative immunoprofile, the presence of a myopermeative growth pattern would indicate a *YWHAE-FAM22* ESS, as all molecularly confirmed uterine Ewing sarcomas reported to date formed an expansile uterine mass and lacked the extensive and characteristic finger-like growth pattern that is consistently observed in uterine *YWHAE-FAM22* ESS.[4, 33, 35]

Cyclin D1 (also known as BCL1) is a diagnostic immunomarker best known for its utility in the diagnosis of mantle cell lymphoma.[5, 39] In the case of mantle cell lymphoma, genetic rearrangement between *IGH@* and *CCND1* results in upregulated cyclin D1 expression at mRNA and protein levels.[32, 38] Cyclin D1 regulates cell cycle progression in G1/S transition and elevated cyclin D1 is observed in various types of human malignancy including sarcomas.[11, 12, 15, 25] The mechanisms underlying cyclin D1 upregulation in tumors include chromosomal translocation, amplification and increased protein stability.[15] Notably, although cyclin D1 immunostaining extent and intensity in *YWHAE-FAM22* ESS is comparable to that in mantle cell lymphoma, the mechanism of cyclin D1 overexpression in *YWHAE-FAM22* ESS has not been determined. Further studies are ongoing to address this issue.

In conclusion, we have identified and demonstrated in this study the utility of cyclin D1 as a sensitive and specific diagnostic immunomarker for *YWHAE-FAM22* ESS. Given the general availability of cyclin D1, this approach is useful in supporting a provisional diagnosis of *YWHAE-FAM22* ESS when encountering a uterine tumor in which the differential of a histologically high-grade ESS is considered.

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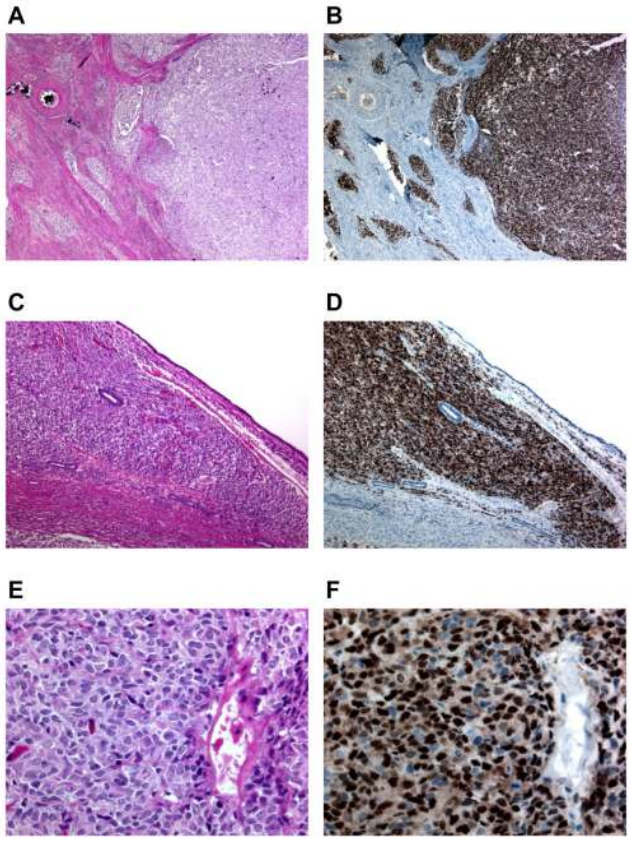

Figure 1.
Cyclin D1 immunostaining in high-grade round cell component of *YWHAE-FAM22* ESS. The representative H&E and immunostaining images illustrate the diffuse strong nuclear cyclin D1 staining at low (A-D) and high (E-F) magnifications in the high-grade round cell component of an *YWHAE-FAM22* ESS.

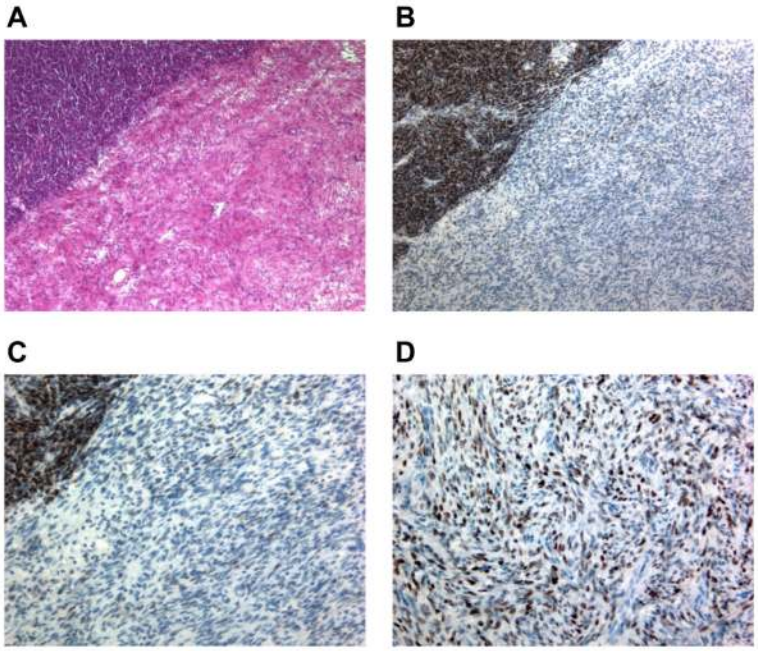

Figure 2.
Cyclin D1 immunostaining in the low-grade spindle cell component of *YWHAE-FAM22* ESS. The low-grade spindle cell component of *YWHAE-FAM22* ESS (bottom right) displays weaker cyclin D1 immunostaining compared to the high-grade round cell component (A-C). The staining in the low-grade spindle cell component is variable, ranging from very focal and weak (C) to diffuse weak to moderate staining intensity (D) in the same tumor.

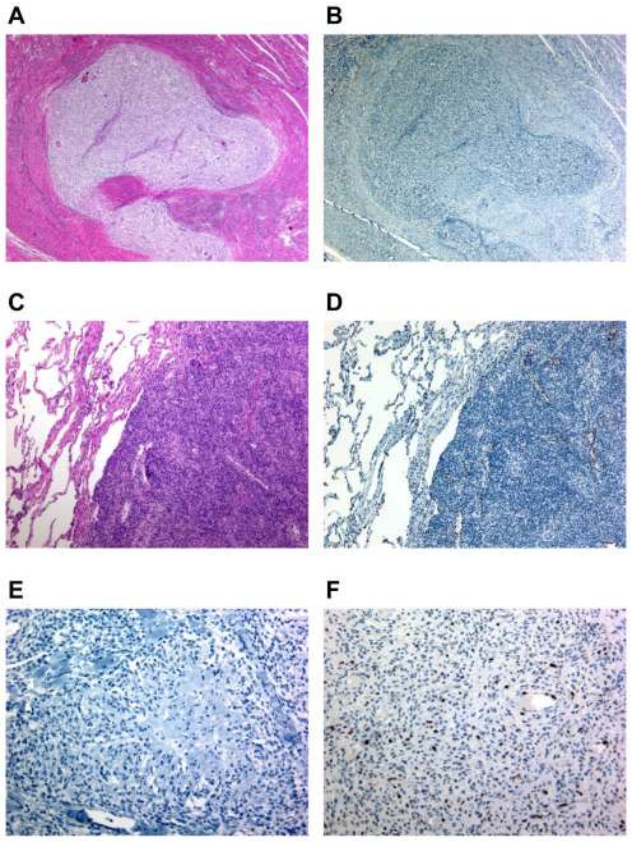

Figure 3.
Cyclin D1 immunostaining in *JAZF1* ESS. Panels A and B depict the H&E and cyclin D1 immunostaining of a primary uterine *JAZF1* ESS. Panel C and D depict the H&E and cyclin D1 immunostaining of a pulmonary metastasis of a *JAZF1* ESS. Panels E and E depict cyclin D1 immunostaining in two different uterine *JAZF1* ESS.

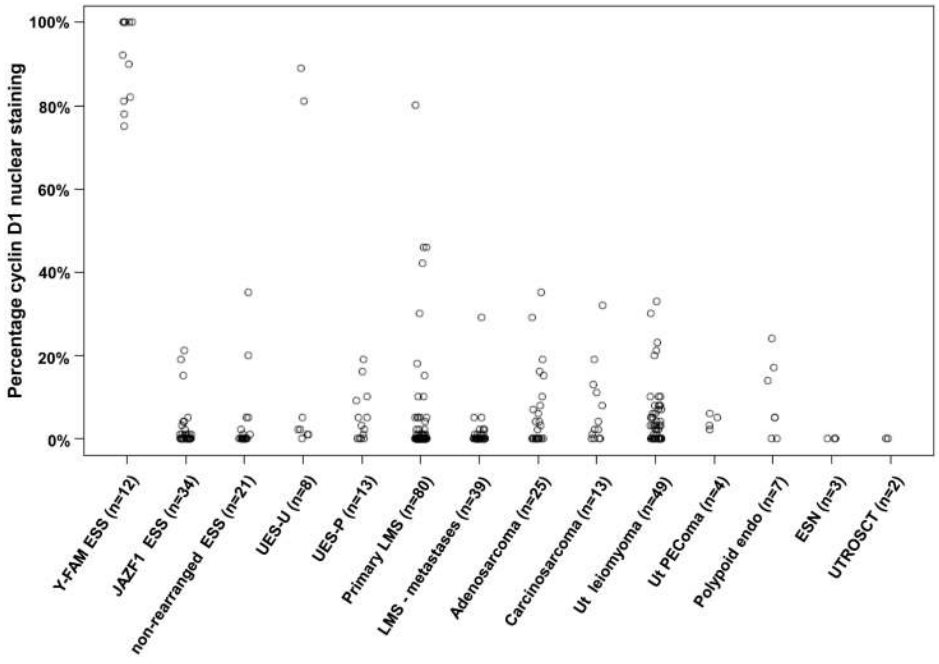

Figure 4.

Scatter plot of the extent of cyclin D1 immunostaining (% moderate to strong nuclear staining) in a series of gynecologic mesenchymal and mixed epithelial and mesenchymal tumors (n = 310). ESS: endometrial stromal sarcoma; UES-U: undifferentiated endometrial sarcoma with nuclear uniformity; UES-P: undifferentiated endometrial sarcoma with nuclear pleomorphism; LMS: leiomyosarcoma; Ut: uterine; endo: endometriosis; ESN: endometrial stromal nodule; UTROSCT: uterine tumor resembling ovarian sex-cord tumor.

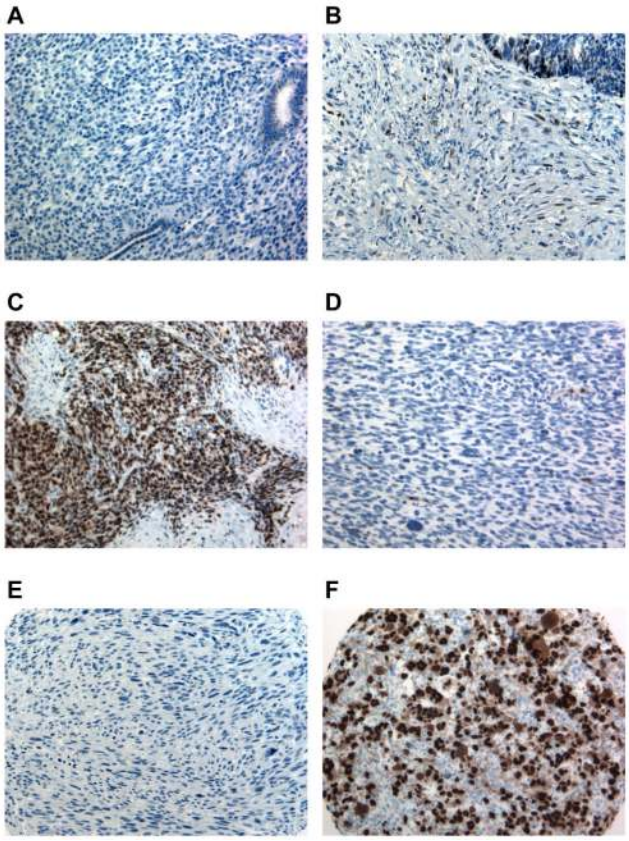

Figure 5.

Representative images of cyclin D1 immunostaining in other uterine tumor. Absent or very focal cyclin D1 immunostaining is seen in the mesenchymal component of an adenosarcoma (A) and a carcinosarcoma (B). Panel C depicts a UES-U with diffuse cyclin D1 immunostaining (one of the two cyclin D1 positive UES-U). Panel D and E show the typically weak and focal staining in a UES-P and a uterine leiomyosarcoma respectively. Panel F depicts the single case of uterine leiomyosarcoma with diffuse cyclin D1 staining; this case contains substantial histiocytic and lymphocytic infiltrates.

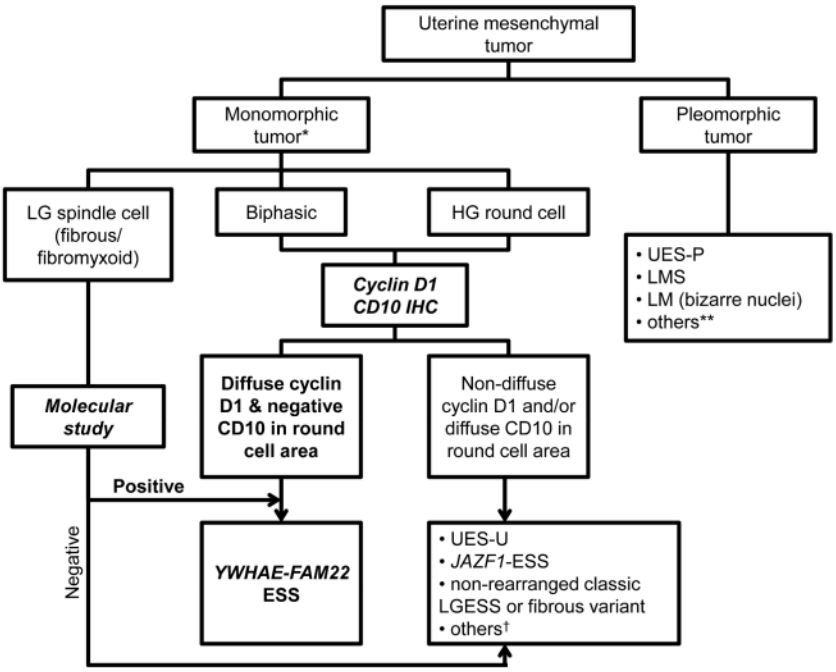

Figure 6.

Diagnostic algorithm for work-up of suspected *YWHAE-FAM22* ESS among uterine mesenchymal tumors. * with myopermeative growth pattern; ** the other differential includes sarcoma-predominant carcinosarcoma, adenosarcoma with HG sarcomatous overgrowth, rhabdomyosarcoma; † the other differential includes that were discussed in the text of the discussion.

Table 1

Significance analysis of microarrays (SAM) comparison between *YWHAE-FAM22* ESS and *JAZF1* ESS.

	SAM comparison (FDR < 1%)
Genes upregulated in *YWHAE-FAM22* ESS compared to *JAZF1* ESS	*TMEM132E*, ***CCND1***, *CEBPA, TPI1, LSMD1, MMP15, FAM20C, ANKRD19, QARS*
Genes downregulated in *YWHAE-FAM22* ESS compared to *JAZF1* ESS	*C15orf43, SPINT1, DPP7, PPAPDC2, GCGR, AMN*

Table 2

Summary of cyclin D1 immunostaining results (cyclin D1 positivity defined as ≥70% moderate to strong nuclear staining)

Tumor type	Number of cases studied	Cyclin D1 positive cases
ESS with *YWHAE-FAM22* rearrangement	12	12 (100%)
ESS with *JAZF1/SUZ12/PHF1/EPC1* rearrangement	34	0 (0%)
ESS with no demonstrable rearrangement	21	0 (0%)
UES - with nuclear pleomorphism (UES-P)	13	0 (0%)
UES - with nuclear uniformity (UES-U)	8	2 (25%)
Uterine leiomyosarcoma *	119	1 (1%)
Adenosarcoma †	25	0 (0%)
Carcinosarcoma	13	0 (0%)
PEComa	4	0 (0%)
Leiomyoma	49	0 (0%)
Polypoid endometriosis	7	0 (0%)
Endometrial stromal nodule	3	0 (0%)
Uterine tumors resembling ovarian sex cord tumor	2	0 (0%)

* 80 primary and 39 metastatic tumors

† 8 of 25 adenosarcomas show sarcomatous overgrowth.