ESR1 hotspot mutations in endometrial stromal sarcoma with high-grade transformation and endocrine treatment

Kimberly Dessources, Kathryn M Miller, Elizabeth Kertowidjojo, Arnaud Da Cruz Paula, Youran Zou, Pier Selenica, Edaise M da Silva, Ryma Benayed, Charles W Ashley, Nadeem R Abu-Rustum, Snjezana Dogan, Robert A Soslow, Martee L Hensley, Britta Weigelt, Sarah Chiang

1Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY
2Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY
3Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY

Abstract

High-grade endometrial stromal sarcomas (HGESSs) are more aggressive and have higher rates of resistance to endocrine therapy than low-grade endometrial stromal sarcomas (LGESSs). The pathogenesis of hormonal resistance in these lesions has yet to be defined. Here we sought to histologically and genetically characterize 3 LGESSs and their recurrences that underwent histologic high-grade transformation following endocrine therapy. For this, DNA from primary tumors and select subsequent recurrences were subject to massively parallel sequencing targeting 468 cancer-related genes. Somatic mutation analyses were performed using validated bioinformatics methods. In addition, RNA from each case was evaluated for the presence of gene fusions using targeted RNA-sequencing. All patients initially presented with LGESS, developed HGESS recurrences, and received at least 2 lines of hormonal suppressive therapy. Gene fusions classically described as associated with LGESS were identified in all 3 cases, including JAZF1-PHF1, EPC1-PHF1 and JAZF1-SUZ12 fusions for Cases 1, 2 and 3, respectively. Targeted sequencing analysis revealed that none of the primary LGESS, however the HGESS recurrences of Cases 1 and 3, and the LGESS and HGESS recurrences of Case 2 post endocrine treatment harbored ESR1 p.Y537S hotspot mutations. These ESR1 ligand-binding domain mutations have...
been found as a mechanism of acquired endocrine resistance in breast cancer. Also, a reduction in estrogen receptor (ER) expression was observed in recurrences. Our findings suggest that the ESR1 p.Y537S hotspot mutation in LGESS with histologic high-grade transformation may be associated with endocrine resistance in these lesions. Furthermore, our data suggest that genetic analyses may be performed in recurrent LGESS following hormonal therapy, development of high-grade morphology, and/or altered/diminished ER expression.

Keywords
Endometrial stromal sarcoma; high grade transformation; hormonal resistance; sequencing; gene fusion

INTRODUCTION
Endometrial stromal sarcomas (ESSs) are rare, accounting for ~15% of uterine sarcomas, and are classified as low- (LGESS) or high-grade (HGESS) based on histologic, immunophenotypic, and molecular genetic features [1]. LGESS are characterized by spindle cells with mild nuclear atypia, low mitotic activity, CD10, estrogen receptor (ER), progesterone receptor (PR) expression, and recurrent chromosomal rearrangements often involving JAZF1 [2–4]. HGESS consist of round and/or spindle cells demonstrating moderate nuclear atypia, brisk mitotic activity, and loss of CD10 and/or ER and PR expression with cyclin D1 and BCOR overexpression [1]. Most HGESS harbor YWHAE and BCOR fusions or BCOR internal tandem duplications [3,4]. Rare tumors harbor LGESS-associated gene fusions and demonstrate increased nuclear atypia and mitotic index warranting classification as HGESS [5].

Prognostication of ESS, particularly high-grade variants, is difficult due to the evolving uterine sarcoma classification with increased utility of massively parallel sequencing. LGESS are indolent tumors treated with surgery, hormonal blockade, or a combination thereof [6,7]. While LGESS has a 5-year disease-free survival rate of >90%, up to 60% of patients develop late recurrences [8–10]. Some LGESS patients develop resistance to hormonal treatment and may benefit from cytotoxic chemotherapy [6,11,12]. HGESS, including histologically transformed ESS with LGESS-associated fusions, appear to have a more aggressive behavior compared to LGESS and is treated by surgery and chemotherapy with improved response using anthracycline-based regimens in tumors harboring YWHAE fusions [13]. Mechanisms of hormonal resistance in LGESS have not been previously explored, and the pathogenesis of histologically transformed LGESS is unknown. Here we sought to histologically and genetically characterize 3 LGESSs and their recurrences that underwent histologic high-grade transformation following endocrine therapy.

METHODS

Cases
The patients provided informed consent for a Memorial Sloan Kettering Cancer Center (MSK) Institutional Review Board-approved tumor genomic profiling study. Between 2014
and 2018, 18 diagnostically confirmed LGESS and HGESS were subjected to clinical sequencing at our institution. Three cases with ESR1 mutations and available tissues were identified. Formalin-fixed paraffin-embedded tissues of primary tumors and matched recurrences were obtained. All available hematoxylin and eosin and ER, PR, BCOR, and cyclin D1 immunohistochemical stained slides were reviewed by expert pathologists (S.C., R.A.S.). Cases were histologically subtyped using the World Health Organization classification system [14]. ER and PR expression were interpreted as positive if ≥1% of tumor cells showed nuclear staining, and intensity of staining was recorded [2]. ER and PR Allred scores were calculated as the sum of proportion and intensity scores. The proportion score was assigned as 0 (0%), 1 (<1%), 2 (1–10%), 3 (11–33%), 4 (34–66%), and 5 (67–100%). The intensity score was assigned as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong) [15]. BCOR and cyclin D1 were interpreted as positive if ≥50% of tumor cells showed nuclear staining [3,4]. Clinical data, including demographics, treatment, and follow-up, were extracted from the electronic medical record.

**Targeted massively parallel sequencing and validation**

Representative 8 μm-thick sections from representative LGESS and HGESS formalin-fixed paraffin-embedded (FFPE) tumor blocks were subjected to microdissection using a sterile needle under a stereomicroscope when appropriate to ensure >80% tumor cell content, as previously described [16,17]. Genomic DNA was extracted from the tumor samples and matched normal blood or normal tissue devoid of neoplastic cells using the DNeasy Blood and Tissue Kit (Qiagen) according to manufacturers’ instructions [18]. Tumor and matched normal DNA samples were subjected to massively parallel sequencing targeting 468 genes using MSK-Integrated Mutational Profiling of Actionable Cancer Targets (MSK-IMPACT) at a median depth of 595x (range, 584–625x) [16,19], and sequencing data were analyzed using validated bioinformatics tools as previously described [16,17]. In brief, somatic single nucleotide variants (SNVs) were identified using MuTect (v1.17) [20], and small insertions and deletions (indels) using Strelka (v1.0.15), VarScan 2 (v2.3.7), Lancet (v1.0.0) and Scalpel (v0.5.3) [21–24]. Mutations identified in the primary LGESS or recurrence from a given patient were interrogated in the respective LGESS and/or recurrence(s) by manual inspection of BAM files using mpileup files (SAMtools version 1.2 htslib 1.2.1) [25]. Copy number alterations (CNAs) and loss of heterozygosity (LOH) were defined using FACETS [26], as previously described [16,17]. Cancer cell fractions (CCFs) of somatic mutations identified were defined using ABSOLUTE (v1.0.6) [27], and a mutation was classified as clonal if its probability of being clonal was >50% or if the lower bound of the 95% confidence interval of its CCF was >90%, as previously described [16,17]. Mutational hotspot annotation was performed according to Chang et al [28].

ESR1 p.Y537S mutations identified by MSK-IMPACT sequencing were subjected to orthogonal validation using Sanger sequencing, as previously described [29].

In brief, PCR amplification was performed using the AmpliTaq Gold 360 Master Mix kit (Life Technologies, ThermoFisher Scientific; primers: forward 5’-CCTTTCTGTGTCTTCCCACCT, reverse 3’-AGTGGCTTTGGGTCCGTC), and PCR fragments were cleaned using ExoSAP IT (ThermoFisher Scientific).
**Targeted fusion gene detection**

Tumor samples were subjected to the MSK-Fusion Solid Panel assay (Archer FusionPlex) utilizing Archer Anchored Multiplex PCR technology to detect 85 validated gene fusions in solid tumors, as previously described [5]. For this, tumor RNA was extracted from 5 μm-thick tumor sections, followed by cDNA synthesis and library preparation. Final targeted amplicons were sequenced, and data analyzed using the Archer analysis software V5.0.

**RESULTS**

**Case 1**

Case 1 was a 47-year-old woman who, after resection, was found to have FIGO stage IIIB LGESS with residual miliary disease (Figure 1A). The primary uterine (Case1-Prim) and extraterine tumor demonstrated predominately conventional LGESS morphology characterized by small monomorphic spindle cells with round to oval nuclei, inconspicuous nucleoli, scant cytoplasm, and a mitotic index of <1 per 10 high power fields (HPF; Figure 1B); focal variant decidual change was present. Strong ER and PR expression was observed in >90% of tumor cells (ER and PR Allred scores 8 and 8, respectively; Figure 1B). She received post-resection megestrol acetate and remained radiographically disease-free for 6.5 years. A large recurrent jejunal mass was partially resected in the setting of a small bowel obstruction (Case1-Recur1), and she received post-resection anastrozole for 3.5 years, at which time attempted resection of persistent disease was not successful (Case1-Recur2). Treatment was changed to letrozole with disease control for 2 years, after which the patient had successful complete resection of the intra-colonic mass (Case1-Recur3) and peritoneal disease.

All recurrences (Case1-Recur1/Recur2/Recur3) demonstrated LGESS morphology with foci of high-grade histologic transformation characterized by nuclear enlargement, prominent nucleoli, and increased mitotic activity of 9 to 11 mitoses per 10 HPF (Figure 1B). The first and second recurrences (Case1-Recur1/Recur2) demonstrated ER and PR expression with variable intensity in >90% of tumor cells, including histologically high-grade foci (ER and PR Allred scores 7 and 7 in Case1-Recur1, respectively; ER and PR Allred scores 8 and 7 in Case1-Recur2, respectively). The third recurrence (Case1-Recur3) demonstrated strong PR but weak ER expression in 90% of tumor cells (ER and PR Allred scores 6 and 8, respectively). Cyclin D1 and BCOR were negative in all recurrences (Case1-Recur1/Recur2/Recur3).

A **JAZF1-PHF1** fusion was detected. Targeted massively parallel sequencing revealed the presence of a somatic **KDM5C** frameshift mutation in the primary and second and third recurrent tumors (Case1-Prim/Recur2/Recur3; Figure 1C); the first recurrence (Case1-Recur1) had insufficient tissue for sequencing. Notably, the third recurrence (Case1-Recur3) acquired a **STK40** missense p.R128W mutation and an **ESR1** p.Y537S hotspot mutation (variant allele fraction (VAF), 40%) not present in the primary tumor or second recurrence (Case1-Prim/Recur2; Figure 1C). We next defined the cancer cell fraction (CCF; i.e. the bioinformatically inferred percentage of tumor cells harboring the mutation in the sample) of the **ESR1** mutation, taking into account the tumor purity, ploidy and local copy number.
[27], which revealed that the ESR1 p.Y537S hotspot mutation was clonal (i.e. present in 100% of the cancer cells; Figure 1C). With the finding of an ESR1 mutation in the third recurrence (Case1-Recur3), the patient was treated with fulvestrant and continues to have no measurable disease for 48 months, 15 years from initial diagnosis (Figure 1A).

**Case 2**

A 56-year-old woman with presumed fibroids underwent subtotal abdominal hysterectomy with morcellation and was found to have FIGO stage IB LGESS (Figure 2A). Completion staging was subsequently performed with residual mass resected from the vaginal cuff (Case2-Prim), and the patient was treated with post-resection letrozole and remained disease-free for 23 months, at which time imaging showed recurrent pelvic disease. Megestrol acetate was added to the letrozole, but there was rapid progression of disease. She was subsequently treated with gemcitabine-docetaxel, but the disease continued to progress. She underwent resection of multi-site peritoneal and retroperitoneal disease (Case2-Recur1) followed by observation for 11 months. For subsequent progression, she underwent another resection (Case2-Recur2) then progressed 4 months later. She was treated with doxorubicin but had progression of disease.

The primary tumor and first recurrence (Case2-Prim/Recur1) showed conventional and variant sex cord-like LGESS morphology and a mitotic index of 2/10 HPF (Figure 2B). PR expression was strong in >90% of tumor cells in the primary tumor and first recurrence (Case2-Prim/Recur1; PR Allred scores of 8 and 8, respectively); however, ER expression was strong in >90% of tumor cells in the primary tumor (ER Allred score 8; Case2-Prim) and weak in 10% of tumor cells of the first recurrence (ER Allred score 3; Case2-Recur1; Figure 2B). The second recurrence (Case2-Recur2) involved multiple peritoneal and retroperitoneal sites mostly demonstrating conventional and variant sex cord-like LGESS morphology with moderate ER expression in 60% of tumor cells and strong PR expression in >90% of tumor cells (ER and PR Allred scores of 6 and 8, respectively). The para-aortic recurrence (Case2-Recur2), however, demonstrated round cells with enlarged nuclei, abnormal chromatin, and 12 mitoses per 10 HPF consistent with histologic high-grade transformation (Figure 2B). These foci demonstrated strong ER/PR expression in >90% of tumor cells (ER and PR Allred scores of 8 and 8, respectively; Figure 2B) and were positive for BCOR, while negative for cyclin D1.

An EPC1-PHF1 fusion was detected. Massively parallel sequencing revealed the presence of an ESR1 p.Y537S hotspot mutation in the recurrences (Case2-Recur1, VAF 43%; Case2-Recur2, VAF 29%) but not in the primary tumor (Case2-Prim; Figure 2C). Analysis to define the CCFs showed that the ESR1 hotspot mutation was clonal and present in 100% of the cancer cells of both recurrences. Noting the ESR1 mutation on the most recent pathology (Case2-Recur2), the patient was treated with fulvestrant with radiographic response sustained for 1 year. At further progression, she was changed to anastrozole, then deceased 6 months later, 6 years from initial diagnosis.
Case 3

A 56-year-old underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and debulking for abdominal masses and was found to have LGESS (Case3-Prim; Figure 3A). She was subsequently treated with post-resection megestrol acetate for 11 years. Over 8 years since her initial surgery, she underwent resection of an intrabdominal mass and hernia repair, followed by splenectomy and then a small bowel resection for small bowel obstruction. While no recurrent disease was found in the splenectomy specimen, the pathology from the other 2 surgeries were not reviewed. She presented to our institution 11 years after initial diagnosis with 2 large recurrent abdominal masses which were debulked (Case3-Recur1) and remained disease-free while on post-resection letrozole for 6 years. She underwent surgery, including removal of an infected mesh and small bowel partial resection, with no evidence of recurrent disease intraoperatively. Letrozole was resumed, but she was found to have peritoneal nodules in the left upper quadrant on imaging a year later. She underwent resection of the left upper quadrant abdominal masses and partial omentectomy for recurrent disease (Case3-Recur2).

The primary tumor demonstrated LGESS morphology and a mitotic index of <1/10 HPF; strong ER and PR staining was seen in >90% of tumor cells (ER and PR Allred scores of 8 and 8, respectively; Figure 3B). The first recurrence (Case3-Recur1) demonstrated epithelioid and spindled cells with enlarged nuclei with moderate to severe atypia, abundant eosinophilic cytoplasm, prominent myxoid change, and <1 mitotic figure per 10 HPF, associated with a prominent lymphocytic infiltrate; PR expression was strong in >90% of tumor cells, while ER was weak in <5% of cells in the first recurrence (PR and ER Allred scores of 8 and 3, respectively; Case3-Recur1; Figure 3B). The second recurrence (Case3-Recur2) demonstrated spindle cells with uniform moderate cytologic atypia, including nuclear enlargement and prominent nucleoli, and a mitotic index of 10/10 HPF, consistent with high-grade transformation; no epithelioid or myxoid change was present (Figure 3B). ER and PR expression was strong in 80% and >90% of cells, respectively (ER and PR Allred scores of 8 and 8, respectively).

A JAZF1-SUZ12 fusion was detected. Massively parallel sequencing revealed the presence of a frameshift mutation in SMARCB1 in the first recurrence (Case3-Recur1) and a clonal ESR1 p.Y537S hotspot mutation in the second recurrence (Case3-Recur2, VAF 43%; Figure 3C). Akin to Case 1 and Case 2, the ESR1 hotspot mutation was clonal in the second recurrence (Case3-Recur2), with all cancer cells harboring this mutation (CCF 100%; Figure 3C). Noting the ESR1 mutation on the most recent pathology (Case3-Recur2), the patient completed 3 cycles of fulvestrant and is alive with disease, 19 years from initial diagnosis.

DISCUSSION

Here we report on likely de novo ESR1 p.Y537S hotspot mutations in the recurrences of 3 patients who were initially diagnosed with LGESS and subsequently developed recurrent disease demonstrating histologic high-grade transformation after hormonal treatment.

The ESR1 gene codes for the estrogen receptor 1 (ERalpha), a ligand-activated transcription factor implicated in the tumorigenesis of many ER-positive cancers including ESS [30,31].
ESR1 p.Y537S hotspot mutations are activating and enable ER coactivator binding in the absence of estrogen [32,33]. Mutations in the ESR1 ligand-binding domain have been identified as an important mechanism of acquired endocrine resistance and occur under the selective pressure from hormonal therapies [34,35]. Particularly in breast cancer, ESR1 mutations have been found in ~1% of primary ER-positive breast cancers and 10–50% of metastatic breast cancers previously treated with hormonal blockade [34,36,37]. Also, a de novo ESR1 hotspot mutation in a patient with endometrial carcinoma treated with an aromatase inhibitor has been reported [38].

Other mechanisms of endocrine resistance include ESR1 amplification/fusion and alterations in receptor tyrosine kinases, PI3K and MAPK pathway components, regulators of gene expression, and DNA repair genes. We explored these alterations in the remaining 15 ESS of the 18 diagnostically confirmed ESS subjected to clinical sequencing at our institution (see Methods). Somatic genetic alterations associated with endocrine resistance were detected in only 2 cases: a recurrent HGESS harboring a YWHAE rearrangement showed TSC2 and MTOR deep deletions as well as AKT2 amplification. AKT1 amplification and PMS2 deletion were detected in a primary LGESS with a JAZF1-SUZ12 fusion. Among the 15 ESS with sequencing data, 3 were histologically confirmed LGESS lacking ESR1 mutations, and none of these 3 patients had evidence of hormonal resistance.

The association between ER expression and ESR1 mutation in our cohort is unclear. In Cases 1 and 2, ER expression was significantly attenuated in the recurrences that harbored ESR1 mutations. In Case 3, however, ER expression was diminished in the first recurrence with wild-type ESR1. Interestingly, in Cases 2 and 3, the recurrences that initially showed reduced ER expression were followed by recurrences with strong levels of ER expression.

In all cases studied here, histologically high-grade transformation of LGESS was observed during disease progression as evidenced by increased mitotic rates and nuclear atypia in the recurrences, which paralleled the detection of ESR1 p.Y537S hotspot mutations. While Case 2 displayed endocrine resistance at first recurrence, in Cases 1 and 3 prolonged disease control was observed on a selective ER degrader (SERD; fulvestrant) following resistance to a selective ER modulator (SERM)/aromatase inhibitor (AI). In fact, work in breast cancer has shown that the mechanism of resistance to SERMs/AIs is distinct from that of SERDs [39,40]. All 3 patients had some degree of clinical benefit from fulvestrant, which was administered after detection of ESR1 hotspot mutations in the recurrences. Further studies are warranted to assess whether patients with ESR1-mutant ESS may benefit from SERDs over other therapies.

High-grade transformation of LGESS is rare, and data on the molecular underpinnings of these lesions are limited. The majority of LGESS are driven by PHF1 and JAZF1 translocations [5,41], as the 3 cases described in this study, whereas YWHAE and BCOR genetic abnormalities are associated with HGESS [42]. In Case 1, this occurred prior to the identification of the ESR1 mutation and in Cases 2 and 3, the high-grade transformation was identified after the emergence of an ESR1 mutation. Notably in Case 3, however, there was increased nuclear atypia despite low mitotic activity in the first recurrence (Case3-Recur1),
suggesting early transformation at that time. The interplay between ER-alpha signaling and histologic high-grade transformation of LGESS remains unknown and warrants further investigation.

Ligand independent activation of ER receptors results in increased translational activity outside of the downstream affects typically attributed to estrogen binding [43,44]. For example, *ESR1* mutations were found to result in increased expression of non-ER regulation genes associated with breast cancer cell migration such as *WNT11* [45] and *RET* [46], suggesting that these mutations may contribute to increased metastatic potential of these tumors [33]. This theory is supported by identification of these mutations preferentially in distant metastatic sites in comparison to locoregional recurrences [37,47]. Thus, the presence of an *ESR1* mutation may have prognostic implications separate from the limitation on effective therapies.

This study has several limitations. Given the rarity of LGESS in general, and of those undergoing high-grade transformation in particular, the number of cases studied is small, however the identification of potential mechanisms of endocrine resistance contributes to the body of knowledge in this disease. In addition, ER and PR immunohistochemical analyses were performed at the referring institution (Case 1) or at our clinical and research laboratories (Cases 1–3), which may contribute to differences in staining intensities.

In summary, de novo *ESR1* hotspot mutations may occur in LGESS following histologic high-grade transformation and resistance to endocrine treatment. Larger series are required to further investigate the frequency of *ESR1* mutations and their role in endocrine treatment resistance. Our findings suggest that genetic analyses may be performed in recurrent LGESS following hormonal therapy, development of high-grade morphology, and/or altered/diminished ER expression.

**ACKNOWLEDGEMENTS**

This work was funded in part the NIH/NCI Cancer Center Support Grant P30-CA008748. B. Weigelt was funded in part by Cycle for Survival and Breast Cancer Research Foundation grants.

**CONFLICTS OF INTEREST**

N.R. Abu-Rustum reports institutional grants from Stryker/Novadaq and GRAIL, outside the submitted work. M.L. Hensley reports spouse employment by Sanofi, advisory board consulting for Tesaro, Glaxo Smith Kline. B. Weigelt reports ad hoc membership of the scientific advisory board of Repare Therapeutics, outside the current study. S. Chiang reports consulting for AstraZeneca. The remaining authors have no conflicts of interest to declare.

**REFERENCES**

1. WHO classification of tumours editorial board. Female genital tumours: WHO classification of tumours, 5th ed, Vol 4 (International Agency for Research on Cancer: Lyon, 2020).
2. Toki T, Shimizu M, Takagi Y, Ashida T, Konishi I. CD10 is a marker for normal and neoplastic endometrial stromal cells. Int J Gynecol Pathol 21, 41–47 (2002) [PubMed: 11781522]
3. Chiang S, Oliva E. Cytogenetic and molecular aberrations in endometrial stromal tumors. Hum Pathol 42, 609–617 (2011) [PubMed: 21420714]
4. Momeni-Boroujeni A, Chiang S. Uterine mesenchymal tumours: recent advances. Histopathology 76, 64–75 (2020) [PubMed: 31846533]
5. Zou Y, Turashvili G, Soslow RA, Park KJ, Croce S, McCluggage WG, et al. High-grade transformation of low-grade endometrial stromal sarcomas lacking YWHAE and BCOR genetic abnormalities. Mod Pathol 33, 1861–1870 (2020) [PubMed: 32317704]

6. Chu MC, Gor G, Lim C, Zheng W, Parkash V, Schwartz PE. Low-grade endometrial stromal sarcoma: hormonal aspects. Gynecol Oncol 90, 170–176 (2003) [PubMed: 12813589]

7. Yamaguchi M, Erdenebaatar C, Saito F, Matohara T, Miyahara Y, Tashiro H, et al. Long-Term Outcome of Aromatase Inhibitor Therapy With Letrozole in Patients With Advanced Low-Grade Endometrial Stromal Sarcoma. Int J Gynecol Cancer 25, 1645–1651 (2015) [PubMed: 26495759]

8. Amant F, Woestenborghs H, Vandenbroucke V, Berteloot P, Neven P, Moerman P, et al. Transition of endometrial stromal sarcoma into high-grade sarcoma. Gynecol Oncol 103, 1137–1140 (2006) [PubMed: 16919712]

9. Kurihara S, Oda Y, Ohiishi Y, Iwasa A, Takahira T, Kaneki E, et al. Endometrial stromal sarcomas and related high-grade sarcomas: immunohistochemical and molecular genetic study of 31 cases. Am J Surg Pathol 32, 1228–1238 (2008) [PubMed: 18580489]

10. Lee CH, Marino-Enriquez A, Ou W, Zhu M, Ali RH, Chiang S, et al. The clinicopathologic features of YWHAE-FAM22 endometrial stromal sarcomas: a histologically high-grade and clinically aggressive tumor. Am J Surg Pathol 36, 641–653 (2012) [PubMed: 22456610]

11. Pink D, Lindner T, Mrozek A, Kretzschmar A, Thuss-Patience PC, Dorken B, et al. Harm or benefit of hormonal treatment in metastatic low-grade endometrial stromal sarcoma: single center experience with 10 cases and review of the literature. Gynecol Oncol 101, 464–469 (2006) [PubMed: 16368128]

12. Hemming ML, Wagner AJ, Nucci MR, Chiang S, Wang L, Hensley ML, et al. YWHAE-rearranged high-grade endometrial stromal sarcoma: Two-center case series and response to chemotherapy. Gynecol Oncol 145, 531–535 (2017) [PubMed: 28390819]

13. Cree IA, White VA, Indave BI, Lokuhetty D. Revising the WHO classification: female genital tract tumours. Histopathology 76, 151–156 (2020) [PubMed: 31846528]

14. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 11, 155–168 (1998) [PubMed: 9504686]

15. da Silva EM, Fix DJ, Sebastiao APM, Selenica P, Ferrando L, Kim SH, et al. Mesonephric and mesonephric-like carcinomas of the female genital tract: molecular characterization including cases with mixed histology and matched metastases. Mod Pathol 34, 1570–1587 (2021) [PubMed: 33772212]

16. Martelotto LG, De Filippo MR, Ng CK, Natrajan R, Fuhrmann L, Cyrtu J, et al. Genomic landscape of adenoid cystic carcinoma of the breast. J Pathol 237, 179–189 (2015) [PubMed: 26095796]

17. Cheng DT, Mitchell TN, Zehir A, Shah RH, Benayed R, Syed A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A Hybridization Capture-Based Next-Generation Sequencing Clinical Assay for Solid Tumor Molecular Oncology. J Mol Diagn 17, 251–264 (2015)

18. Cibulskis K, Lawrence MS, Carter SL, Sivachenko A, Jaffe D, Sougnez C, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nat Biotechnol 31, 213–219 (2013) [PubMed: 23396013]

19. Saunders CT, Wong WS, Swamy S, Becq J, Murray LJ, Cheetham RK, Strelau: accurate somatic small-variant calling from sequenced tumor-normal sample pairs. Bioinformatics 28, 1811–1817 (2012) [PubMed: 22581179]

20. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L, et al. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res 22, 568–576 (2012) [PubMed: 22300766]
23. Narzisi G, Corvelo A, Arora K, Bergmann EA, Shah M, Musunuri R, et al. Genome-wide somatic variant calling using localized colored de Bruijn graphs. Commun Biol 1, 20 (2018) [PubMed: 30271907]

24. Narzisi G, O’Rawe JA, lossifov I, Fang H, Lee YH, Wang Z, et al. Accurate de novo and transmitted indel detection in exome-capture data using microassembly. Nat Methods 11, 1033–1036 (2014) [PubMed: 25128977]

25. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25, 2078–2079 (2009) [PubMed: 19505943]

26. Shen R, Seshan VE. FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. Nucleic Acids Res 44, e131 (2016) [PubMed: 27270079]

27. Carter SL, Cibulskis K, Helman E, McKenna A, Shen H, Zack T, et al. Absolute quantification of somatic DNA alterations in human cancer. Nat Biotechnol 30, 413–421 (2012) [PubMed: 22544022]

28. Chang MT, Bhattarai TS, Schram AM, Bielski CM, Donoghue MTA, Jonsson P, et al. Accelerating Discovery of Functional Mutant Alleles in Cancer. Cancer Discov 8, 174–183 (2018) [PubMed: 29247016]

29. Weinreb I, Fiscuoglio S, Martelotto LG, Waggott D, Ng CK, Perez-Ordonez B, et al. Hotspot activating PRKD1 somatic mutations in polymorphous low-grade adenocarcinomas of the salivary glands. Nat Genet 46, 1166–1169 (2014) [PubMed: 25240283]

30. Blanchard Z, Vahrenkamp JM, Berrett KC, Arnesen S, Gertz J. Estrogen-independent molecular actions of mutant estrogen receptor 1 in endometrial cancer. Genome Res 29, 1429–1441 (2019) [PubMed: 31362937]

31. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2, 401–404 (2012) [PubMed: 22588877]

32. Li S, Shen D, Shao J, Crowder R, Liu W, Prat A, et al. Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. Cell Rep 4, 1116–1130 (2013) [PubMed: 24055055]

33. Fanning SW, Mayne CG, Dharmarajan V, Carlson KE, Martin TA, Novick SJ, et al. Estrogen receptor alpha somatic mutations Y537S and D538G confer breast cancer endocrine resistance by stabilizing the activating function-2 binding conformation. Elife 5, (2016)

34. Toy W, Shen Y, Won H, Green B, Sakr RA, Will M, et al. ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. Nat Genet 45, 1439–1445 (2013) [PubMed: 24185512]

35. Chandarlapaty S, Chen D, He W, Sung P, Samoila A, You D, et al. Prevalence of ESR1 Mutations in Cell-Free DNA and Outcomes in Metastatic Breast Cancer: A Secondary Analysis of the BOLERO-2 Clinical Trial. JAMA Oncol 2, 1310–1315 (2016) [PubMed: 27532364]

36. Spoerke JM, Gendreau S, Walter K, Qiu J, Wilson TR, Savage H, et al. Heterogeneity and clinical significance of ESR1 mutations in ER-positive metastatic breast cancer patients receiving fulvestrant. Nat Commun 7, 11579 (2016) [PubMed: 27174596]

37. Jeselsohn R, Buchwalter G, De Angelis C, Brown M, Schiff R. ESR1 mutations-a mechanism for acquired endocrine resistance in breast cancer. Nat Rev Clin Oncol 12, 573–583 (2015) [PubMed: 26122181]

38. Morel A, Masliah-Planchnon J, Bataillon G, Becette V, Morel C, Antonio S, et al. De Novo ESR1 Hotspot Mutation in a Patient With Endometrial Cancer Treated With an Aromatase Inhibitor. JCO Precision Oncology 3, 1–3 (2019)

39. Ingle JN, Suman VJ, Rowland KM, Mirchandani D, Bernath AM, Camoriano JK, et al. Fulvestrant in women with advanced breast cancer after progression on prior aromatase inhibitor therapy: North Central Cancer Treatment Group Trial N0032. J Clin Oncol 24, 1052–1056 (2006) [PubMed: 16505423]

40. Toy W, Weir H, Razavi P, Lawson M, Goeppert AU, Mazzola AM, et al. Activating ESR1 Mutations Differentially Affect the Efficacy of ER Antagonists. Cancer Discov 7, 277–287 (2017) [PubMed: 27986707]

41. Ali RH, Rouzbahman M. Endometrial stromal tumours revisited: an update based on the 2014 WHO classification. J Clin Pathol 68, 325–332 (2015) [PubMed: 25595274]
42. Sciallis AP, Bedroske PP, Schoolmeester JK, Sukov WR, Keeney GL, Hodge JC, et al. High-grade endometrial stromal sarcomas: a clinicopathologic study of a group of tumors with heterogenous morphologic and genetic features. Am J Surg Pathol 38, 1161–1172 (2014) [PubMed: 25133706]

43. Rothenberger NJ, Somasundaram A, Stabile LP. The Role of the Estrogen Pathway in the Tumor Microenvironment. Int J Mol Sci 19, (2018)

44. Bahreini A, Li Z, Wang P, Levine KM, Tasdemir N, Cao L, et al. Mutation site and context dependent effects of ESR1 mutation in genome-edited breast cancer cell models. Breast Cancer Res 19, 60 (2017) [PubMed: 28535794]

45. Dwyer MA, Joseph JD, Wade HE, Eaton ML, Kunder RS, Kazmin D, et al. WNT11 expression is induced by estrogen-related receptor alpha and beta-catenin and acts in an autocrine manner to increase cancer cell migration. Cancer Res 70, 9298–9308 (2010) [PubMed: 20870744]

46. Gattelli A, Nalvarte I, Boulay A, Roloff TC, Schreiber M, Carragher N, et al. Ret inhibition decreases growth and metastatic potential of estrogen receptor positive breast cancer cells. EMBO Mol Med 5, 1335–1350 (2013) [PubMed: 23868506]

47. Niu J, Andres G, Kramer K, Kundra MN, Alvarez RH, Klimant E, et al. Incidence and clinical significance of ESR1 mutations in heavily pretreated metastatic breast cancer patients. Onco Targets Ther 8, 3323–3328 (2015) [PubMed: 26648736]
Figure 1: Histologic and genomic analysis of Case 1.

(A) Clinical, surgical and treatment history including histology and estrogen receptor (ER)/progesterone receptor (PR) status of the primary low-grade endometrial stromal sarcoma (LGESS) (Case1-Prim) and recurrences (Case1-Recur1/Recur2/Recur3). (B) Micrographs of representative hematoxylin and eosin (H&E)-stained sections and ER/PR expression by immunohistochemistry of the primary LGESS (Case1-Prim) and each recurrence (Case1-Recur1/Recur2/Recur3). Magnification 20X. (C) Fusion gene and non-synonymous somatic mutations identified in the primary LGESS (Case1-Prim) and the second (Case1-Recur2) and third (Case1-Recur3) recurrences. Mutation types (middle), including the variant allele fraction for the \( ESR1 \) mutation, and cancer cell fraction of mutations identified (bottom) are color-coded according to the legend. Note the clonal \( ESR1 \) hotspot mutation was
only detected in the last recurrence (Case1-Recur3). The Allred scores for ER and PR are provided in the phenobar. LGESS, low-grade endometrial stromal sarcoma; HGESS, high-grade endometrial stromal sarcoma.
Figure 2: Histologic and genomic analysis of Case 2.

(A) Clinical, surgical and treatment history including histology and estrogen receptor (ER)/ progesterone receptor (PR) status of the primary low-grade endometrial stromal sarcoma (LGESS) (Case2-Prim) and recurrences (Case2-Recur1/Recur2). (B) Micrographs of representative hematoxylin and eosin (H&E)-stained sections and ER/PR expression by immunohistochemistry of the primary LGESS (Case2-Prim) and each recurrence (Case2-Recur1/Recur2). For Case2-Recur2, the low-grade component of the HGESS was subjected to ER and PR immunohistochemical analysis separately. Magnification 20X. (C) Fusion gene and non-synonymous somatic mutations identified in the primary LGESS (Case2-Prim) and first (Case2-Recur1) and second (Case2-Recur2) recurrences. Mutation types (middle),
including the variant allele fraction for the *ESR1* mutation, and cancer cell fraction of mutations identified (bottom) are color-coded according to the legend. Note the clonal *ESR1* hotspot mutation was identified in both recurrences (Case2-Recur1/Recur2). The Allred scores for ER and PR are provided in the phenobar. LGESS, low-grade endometrial stromal sarcoma; HGESS, high-grade endometrial stromal sarcoma; POD, progression of disease; PR, partial response.
Figure 3: Histologic and genomic analysis of Case 3.

(A) Clinical, surgical and treatment history including histology and estrogen receptor (ER)/ progesterone receptor (PR) status of the primary low-grade endometrial stromal sarcoma (LGESS) (Case3-Prim) and recurrences (Case3-Recur1/Recur2). (B) Micrographs of representative hematoxylin and eosin (H&E)-stained sections and ER/PR expression by immunohistochemistry of the primary LGESS (Case3-Prim) and each recurrence (Case3-Recur1/Recur2). Magnification 20X. (C) Fusion gene and non-synonymous somatic mutations identified in the primary LGESS (Case3-Prim) and first (Case3-Recur1) and second (Case3-Recur2) recurrences. Mutation types (middle), including the variant allele fraction for the ESR1 mutation, and cancer cell fraction of mutations identified (bottom) are color-coded according to the legend. Note the clonal ESR1 hotspot mutation was identified only the second recurrence (Case3-Recur2). The Allred scores for ER and PR are provided in the phenobar. LGESS, low-grade endometrial stromal sarcoma; HGESS, high-grade endometrial stromal sarcoma.