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Clinicopathological and genomic characterization of *BCORL1*-driven high-grade endometrial stromal sarcomas

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BCORL1 is a transcriptional corepressor homologous to *BCOR*. We describe 12 *BCORL1*-altered uterine sarcomas with striking resemblance to *BCOR*-altered endometrial stromal sarcoma (*BCOR*-ESS), including 5 with *BCORL1* rearrangements (*JAZF1-BCORL1*, *EP300-BCORL1*, or internal *BCORL1* rearrangement), 5 with inactivating *BCORL1* mutations (T513fs*22, P600fs*1, R945*, R1196*, or R1265fs*4) and 2 with homozygous *BCORL1* deletion. The median patient age was 57.5 years (range 33–79). An association with aggressive clinical behavior was identified. Diagnoses assigned prior to genomic testing varied: 7 tumors were previously diagnosed as ESS, 2 as high-grade uterine sarcomas, 2 as myxoid uterine leiomyosarcomas, and 1 as a uterine spindle cell neoplasm consistent with leiomyosarcoma. Tumors harbored frequent gelatinous, mucomyxoid-like appearance by gross examination and unique histology with morphological overlap with *BCOR*-ESS. Key microscopic features included (1) a spindle cell appearance, most often with at least focal myxoid stroma, (2) variable amounts of hypocellular fibromyxoid spindle areas with lower grade atypia and/ or (3) variable amounts of epithelioid areas with higher grade atypia. Specifically, spindle and epithelioid components were present in 100 and 75% of sarcomas, respectively; myxoid stroma was identified in 83%, collagen plaques or fibrosis in 50%, and high-grade nuclear atypia was present in 42%. Like *BCOR*-ESS, 50% of *BCORL1*-altered sarcomas exhibited *CDK4* amplification or *CDKN2A* loss. In contrast, 33% harbored *NF1* alterations, while 25% had other alterations in the *NF2-mTOR* pathway, expanding potential therapeutic targets. In conclusion, inactivating *BCORL1* genomic alterations may define a distinct subset of high-grade endometrial stromal sarcomas with biological overlap with *BCOR*-ESS, both of which may mimic myxoid leiomyosarcomas.

INTRODUCTION

Rare cases of endometrial stromal sarcomas with *BCORL1* alterations have been reported [1, 2]; however, the clinicopathological features and mutational landscape of *BCORL1*-altered uterine sarcomas have not been systematically investigated. BCORL1 is a transcriptional corepressor homologous to BCOR; both have related biological functions during transcriptional regulation as they may interchangeably form polycomb repression complex 1 (PRC1) variants [3]. Recently, uterine sarcomas with genomic alterations in *BCOR* via rearrangements or internal tandem duplication (ITD) have become a newly recognized, distinct subtype of high-grade endometrial stromal sarcomas with aggressive behavior and unique morphology [4–6]. Because of its homology and related biological function to *BCOR*, we hypothesized that molecular alterations of *BCORL1* may also similarly define a distinct subset of uterine sarcomas.

Low-grade endometrial stromal sarcomas are generally considered to be indolent tumors with late recurrences, while high-grade endometrial sarcomas exhibit more aggressive behavior with poorer outcomes [7]. Low-grade endometrial stromal sarcomas are often associated with *JAFZ1* or *MEAF6*, *EPC1*, *EPC2* rearrangements partnering with either *SUZ12* or *PHF1*, both of which are subunits of polycomb repressive complex 2 (PRC2), a

histone methyltransferase [8]. These rearrangements give rise to fusion products such as *JAZF1-SUZ12*, *JAZF1-PHF1*, *MEAF6-PHF1*, *EPC1-PHF1*, or *EPC2-PHF1* [9–11]. Morphologically, low-grade endometrial sarcoma is classically characterized by islands of cells resembling endometrial stroma with oval to fusiform nuclei, scant cytoplasm, low grade nuclear atypia, variable mitotic activity, with infiltration into the myometrium in a tongue-like fashion. In contrast, high-grade endometrial stromal sarcomas exhibit poor prognosis and may harbor *YWHAE* or *BCOR* rearrangements, or alternatively, *BCOR* ITD, as well as high-grade features. *YWHAE-NUTM2A/B* fusion-positive uterine sarcomas demonstrate round cells with eosinophilic cytoplasm and high-grade nuclear atypia in the form of irregular nuclear contours, vesicular chromatin, and variably distinct nucleoli.

BCOR-altered endometrial stromal sarcomas exhibit unique morphology, which may be dependent on the type of *BCOR* molecular alteration. *ZC3H7B-BCOR* fusion is the most common *BCOR* genomic alteration in uterine sarcomas, and most tumors with *ZC3H7B-BCOR* fusions feature spindle cells in a fascicular growth pattern with uniform nuclei, mild-to-moderate atypia, and frequent myxoid and collagenous stromal changes [4, 12]. In addition, tumors with rare *BCOR* rearrangements involving non-*ZC3H7B* partners or with *BCOR* ITD demonstrate a mix of spindle,

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epithelioid or small round cell components and varying degrees of fascicular growth and myxoid or collagenous stroma [6]. Because of prominent myxoid features, a subset of *BCOR*-altered endometrial stromal sarcoma has previously masqueraded as myxoid uterine leiomyosarcomas [4, 12]. Molecularly, *BCOR*-rearranged endometrial stromal sarcomas harbor frequent *MDM2* amplification and activation of the cyclin D1-CDK4 pathway via *CDK4* amplification, *CDKN2A* deletion or cyclin D1 protein overexpression [6].

Similar to *BCOR*, disruption of PRC1 via genomic alterations of the homologous *BCORL1* has been reported in several cancer types, such as ossifying fibromyxoid tumor with *CREBBP-BCORL1* fusion [13], myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) with inactivating *BCORL1* truncating mutations [14], and hepatocellular carcinoma with *BCORL1-ELF4* fusion [15]. In addition, *JAZF1-BCORL1* fusions have been previously reported in a case of uterine adenosarcoma [16] as well as in rare cases of endometrial stromal sarcoma [1, 17]. However, the morphological spectrum and biological behavior of *BCORL1*-altered uterine sarcomas are not well defined.

While inactivating truncating *BCORL1* short variant mutations, such as nonsense and frameshift mutations, are present in MDS and AML, they have not been previously reported in uterine sarcoma. Due to the identification of inactivating mutations in leukemia, *BCORL1* has been postulated to be a tumor suppressor gene, whose loss of function leads to disruption of the PRC1 complex and dysregulation of proper transcription control [18]. Considering the homology and related biological functions of *BCOR* and *BCORL1*, we aimed to (1) investigate the mutational landscape of *BCORL1* in uterine sarcomas, (2) determine whether molecular alterations of *BCORL1* drive the oncogenesis of a distinct subset of endometrial stromal sarcomas, and (3) compare the clinicopathological and molecular features of *BCOR*- versus *BCORL1*-altered uterine sarcomas.

METHODS
BCORL1-altered uterine sarcoma cohorts
Approval for this study was obtained from the Western Institutional Review Board (Protocol No. 20152817). A retrospective database search of a CLIA-certified and CAP-accredited reference molecular laboratory was performed for uterine sarcomas with genomic profiles containing all classes of pathogenic *BCORL1* genomic alterations. The samples were previously sent to Foundation Medicine from medical care facilities across North America for detection of targetable genetic alterations during routine clinical and oncologic care at various institutions. At the time of the study, the Foundation Medicine research database originally contained 1,445 uterine sarcomas (278 endometrial stromal sarcomas, 963 uterine leiomyosarcoma, and 204 uterine sarcomas NOS). The tumors were previously assayed with comprehensive genomic profiling (CGP) via both DNA- and RNA-based targeted next-generation sequencing (NGS). Clinicopathological data including age, sites of metastases, and immunohistochemistry were extracted from pathology reports and available clinical documents. The pathologic diagnosis of uterine sarcoma, endometrial stromal sarcoma and associated morphological features were centrally re-evaluated on routine H&E slides of tissue sections submitted for genomic profiling by Dr. Douglas Lin.

Genomic profiling and biomarker analysis
NGS-based CGP was performed on hybridization-captured, adaptor ligation–based libraries using DNA and RNA extracted from formalin-fixed paraffin-embedded tumor tissue. All samples forwarded for DNA and RNA extraction contained a minimum of 20% tumor cells. The samples were assayed for all coding exons from up to 406 cancer-related genes, plus select introns from up to 31 genes. Patient samples were sequenced and evaluated for all classes of genomic alterations including base substitutions, insertions, deletions, copy number alterations (amplifications and homozygous deletions), and for fusions/rearrangements, as previously described [19–21]. Oncoprints of genomic data were generated with the cBio portal [22, 23]. Tumor mutational burden (TMB) was determined on 0.79–1.14 Mb of sequenced DNA using a mutation burden estimation algorithm [24]. In this study, low TMB was defined as <10 mut/Mb, since a score of at least 10 mut/Mb is currently FDA-approved companion diagnostic biomarker for immunotherapy [25]. Assessment of microsatellite instability was performed from DNA next-generation sequencing across 114 loci [24].

Statistical analysis
Differences among categorical variables were assessed using Fisher's exact test. Statistical tests were 2-sided and used a significance threshold of $p < 0.05$. Reported p values were not adjusted for multiple testing.

RESULTS
BCORL1-altered endometrial stromal sarcomas
We retrospectively identified 12 uterine sarcomas with *BCORL1* genomic alterations from the archives of Foundation Medicine. The 12 *BCORL1*-mutated uterine sarcomas comprised 1% of all uterine sarcomas in our research database, which originally contained 1,445 uterine sarcomas (278 endometrial stromal sarcomas, 963 uterine leiomyosarcomas and 204 uterine sarcomas NOS) with comprehensive genomic profiling data between 2010 and 2020. The 12 uterine sarcomas harbored different classes of *BCORL1* genomic alterations, which included 5 tumors with *BCORL1* rearrangements or fusions (*JAZF1-BCORL1, EP300-BCORL1*, or internal *BCORL1* rearrangement), 5 with truncating *BCORL1* short variant, nonsense or frameshift mutations (T513fs*22, P600fs*1, R945*, R1196*, or R1265fs*4) and 2 tumors with homozygous *BCORL1* gene deletion (Fig. 1 and Table 1). The sarcoma with internal *BCORL1* alteration exhibited a chromosome X deletion, resulting in loss of exon 8 and intron 9 of *BCORL1*. Out of the uterine sarcomas with *BCORL1* fusions, three harbored recurrent *JAZF1-BCORL1* or reciprocal *BCORL1-JAZF1*-fusions and one harbored a novel *EP300-BCORL1* fusion (Fig. 1 and Table 1).

In our cohort of 12 *BCORL1*-altered sarcomas, the median patient age was 57.5 years (range 33–79) (Table 1). There were 3 primary uterine resections, 2 initial non-uterine diagnostic biopsies (1 vagina, 1 pelvis) and 7 recurrences. Most tumors were aggressive with extra-uterine spread and/or recurrences after total hysterectomy (Table 1). Available tumor staging is provided on Table 1. Sites of metastasis or recurrences included peri-aortic and obturator lymph nodes, pelvic sidewall, omentum, pancreas, small and large intestine, bladder, rectum and vagina. Of patients with available clinical follow-up, 4 died of disease at 11, 11, 64 and 73 months after total abdominal hysterectomy, respectively (Table 1, case #1, #5, #7 and #12).

Primary tumor size was available for 3 of 12 tumors (3.0, 7.4 and 14.5 cm). Size of recurrences was available for 6 cases and ranged from 4.5 to 25 cm. Gross descriptions were available for 9 tumors (3 primary samples and 6 metastasis) and not available for 3 samples since they were core biopsies or fragmented specimens. Two of three primary tumors exhibited gross necrosis and one of three had gelatinous, mucomyxoid-like areas. Of the 6 metastasis, 4 cases exhibited gross hemorrhage and necrosis, and 2 cases had mucomyxoid-like gelatinous areas. Lymph node metastasis was identified in one sample; however, not all patients underwent a lymph node dissection.

Microscopically, of the 3 primary tumors, 2 had evaluable borders. 1 case exhibited tongue-like infiltration, dissecting into the myometrium (Fig. 2A), while the second tumor had a broad front, pushing border (Fig. 2B). One of three sarcomas extended into the cervical stroma. When it could be evaluated, tongue-like pattern of infiltration into adjacent pelvic soft tissue structures was also identified in one recurrence.

Histologically, most tumors were characterized by spindle cells and variable amounts of epithelioid cells. No small cell morphology was identified in any tumor. (Table 2). A spindle cell component was present in 100% (12 of 12) of tumors and was
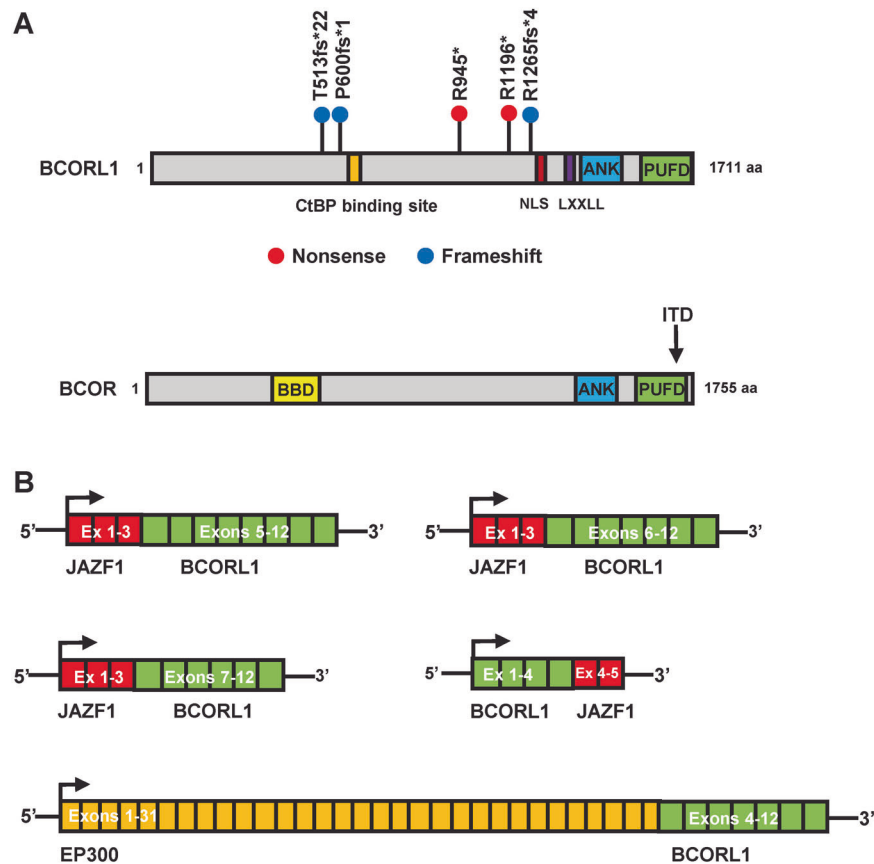
Fig. 1 **Mutational landscape of *BCORL1* across our endometrial stromal sarcoma cohort. A** Top: schematic representation of BCORL1 and positions and types of mutations in *BCORL1* (NM_021946) identified in our uterine sarcomas. Protein domains: CTBP1 binding site, NLS (nuclear localization signal), LXXL (Leu-Xaa-Xaa-Leu-Leu motif), ANK (ankyrin repeats) and PUFD domains. Bottom: Homologous BCOR protein domains for comparison and location of previously described *BCOR* internal tandem duplications (ITD, denoted by arrow, BBD: BCL6 binding domain). **B** Diagrams demonstrating *BCORL1* fusions identified in our cohort. Reference sequences: *JAZF1* (NM_175061), *BCORL1* (NM_021946), *EP300* (NM_001429), *BCOR* (NM_017745). Two additional uterine sarcomas also harbored homozygous *BCORL1* gene deletion and one additional tumor had an internal *BCORL1* rearrangement, which are not represented in this figure.

characterized by either cellular areas some of which were interspersed with collagen plaques, fibrosis or by hypocellular spindle areas with myxoid stroma (Figs. 3, 4 and 5). Spindle cells had scant cytoplasm, predominantly uniform nuclei, even chromatin pattern and inconspicuous nucleoli (Figs. 3, 4 and 5). Occasionally, focal areas with increased atypia and pleomorphism were noted in the spindle and myxoid components (Fig. 3F). In contrast, an epithelioid component was identified in 75% (9 of 12) tumors and featured cells with abundant clear to pale cytoplasm, round nuclei, often with increased nuclear atypia, coarser chromatin and conspicuous nucleoli (Figs. 3, 4 and 5). Rhabdoid morphology was also identified within the epithelioid component in 42% (5 of 12) of sarcomas (Fig. 5E) with the amount of rhabdoid component ranging from 5 to 80% of the tumors. No sex cord stromal elements were identified in any tumor.

Overall, 58% of tumors had uniform nuclei and mild to moderate atypia, while 42% harbored severe nuclear atypia, characterized by nuclear enlargement, condensed chromatin and prominent nucleoli, more often associated with the epithelioid component (Figs. 3, 4 and 5). Myxoid stroma was present in 83% of tumors, while stromal fibrosis or collagen plaques were present in 50%. Tumor necrosis and spiral arterioles were present in 42 and 25% of sarcomas, respectively (Table 2). One sarcoma exhibited perivascular tumor cell whorling; and none demonstrated hemangiopericytoma-like vessels or larger thick-walled vessels. Ossification, characterized by benign bone formation, was present in one tumor. Mitotic count across the twelve uterine

sarcomas with *BCORL1* alterations varied and ranged from 2 to 25 mitoses per 10 high power fields (mean = 9.5, median = 8.5 mitoses, range 2–25 mitoses per 10 HPF). 42% (5 out of 12) of tumors had mitotic indices of at least 10 mitotic figures/10 HPF (Table 2). One uterine sarcoma (8%, 1 of 12) with a *BCORL1* R1196* mutation (case #6) exhibited classic, conventional low-grade endometrial stromal sarcoma morphology with low-grade spindle cells in a tongue-like pattern of infiltration and devoid of myxoid, collagen plaques or epithelioid components. Interestingly, this case also harbored an *EPC1-PHF1* fusion, which is characteristic of low-grade endometrial stromal sarcoma.

By stratifying according to the type of *BCORL1* genomic alteration (i.e. fusion/rearrangement, short variant alteration or homozygous gene deletion), 100% of uterine sarcomas (7 of 7) harboring *BCORL1* fusions or homozygous gene deletion exhibited an epithelioid component with at least moderate nuclear atypia (Fig. 3, Fig. 4 and Table 2). In contrast, tumors featuring *BCORL1* short variant alterations (i.e. frameshift or nonsense mutations) exhibited predominant spindle cell morphology with uniform nuclei, and an epithelioid component was present in 40% (2 of 5) (Fig. 5 and Table 2). In addition, 40% (2 of 5) of tumors with *BCORL1* short variant alterations also harbored fusions classically associated with low-grade endometrial stromal sarcoma, such as *JAFZ1-SUZ12* or *EPC1-PHF1*, in contrast to 0% of tumors containing *BCORL1* fusion or homozygous gene deletion. The case with a *JAFZ1-SUZ12* fusion had a co-occurring *BCORL1* R1265fs*4 frameshift mutation and demonstrated an associated epithelioid

Table 1. Clinicopathological features of *BCORL1*-altered uterine sarcomas.

| n	*BCORL1* genomic alteration	Age	Submitting diagnosis	Biopsy proven sites of metastasis or extension	Follow-up
1	*JAZF1-BCORL1* fusion	59	Recurrent endometrial stromal sarcoma	Recurrence in pelvic peritoneum, omentum and pancreas	Died of disease at 64 months after hysterectomy
2	*EP300-BCORL1* fusion	56	Metastatic endometrial stromal sarcoma	Recurrence in peri-aortic lymph node	Not available
3	Internal *BCORL1* rearrangement	72	Metastatic endometrial stromal sarcoma	Recurrence in small intestine	Not available
4	*JAZF1-BCORL1* fusion	46	Low to high grade endometrial stromal sarcoma	Recurrence in small and large intestine, right obdurator node	Not available
5	*BCORL1* R1265fs*4	66	Recurrent low grade endometrial stromal sarcoma	Recurrence in bladder and rectum	Died of disease at 73 months after hysterectomy
6	*BCORL1* R1196*	66	Low grade endometrial stromal sarcoma	Not applicable, primary resection, stage I	Alive at 28 months after hysterectomy.
7	*BCORL1* homozygous deletion	38	High grade endometrial stromal sarcoma	Pelvis initial diagnostic biopsy; stage IV per test requisition sheet	Died of disease at 11 months after diagnosis
8	*JAZF1-BCORL1* fusion	79	High grade uterine sarcoma	Vagina initial diagnostic biopsy; stage IV per test requisition sheet	Not available
9	*BCORL1* homozygous deletion	48	High grade uterine sarcoma	Recurrence in pelvis, omentum, retroperitoneum. Initial stage II per clinical records	Alive at 22 months after hysterectomy with recurrence refractory to gemcitabine and docetaxel followed by adriamycin
10	*BCORL1* T513fs*22	33	Myxoid uterine leiomyosarcoma	Cervix, right uterine sidewall. Primary resection, stage II	Not available
11	*BCORL1* R945*	56	Myxoid uterine leiomyosarcoma	Recurrence in right adnexa, left pelvic sidewall. Stage IV per requisition sheet	Alive at 12 months after hysterectomy
12	*BCORL1* P600fs*1	74	Spindle cell neoplasm most consistent with leiomyosarcoma	Primary resection stage I. Stage IV per test requisition sheet	Died of disease at 11 months after hysterectomy
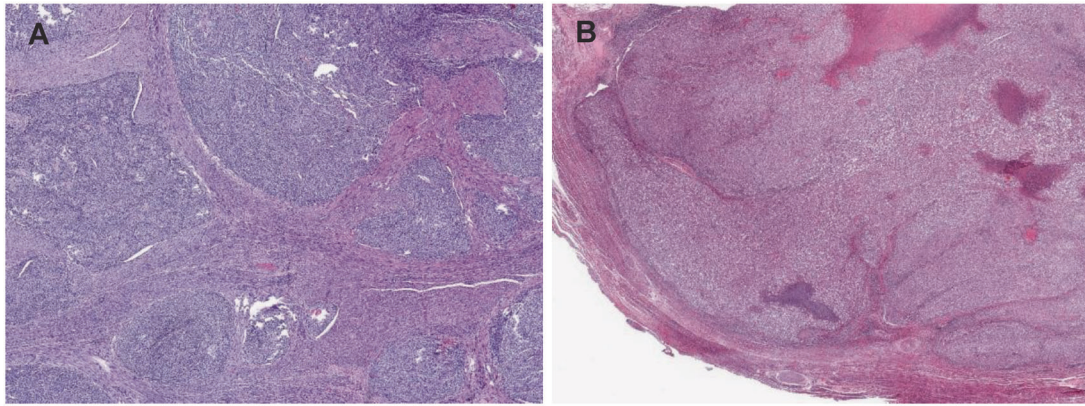
Fig. 2 Myometrial infiltration patterns of *BCORL1*-altered uterine sarcomas. A. Tongue-like. **B.** Broad front, pushing border.

component with higher grade nuclear atypia as well as myxoid and stromal fibrosis components (Figs. 5A, 5B and 5C).

The submitted diagnoses from referring outside institutions included 7 endometrial stromal sarcomas, 2 high-grade uterine sarcomas, 2 myxoid uterine leiomyosarcomas and 1 uterine spindle cell neoplasm consistent with leiomyosarcoma (Table 1). Based on genomics and morphological similarity to *BCOR*-altered endometrial stromal sarcomas, the 3 cases with prior diagnoses of leiomyosarcoma were reclassified as *BCORL1*-altered endometrial stromal sarcoma, as previously described for *BCOR*-altered ESS that mimicked myxoid leiomyosarcomas [4, 6], based on morphology and lack of known driver mutations commonly found in either conventional uterine leiomyosarcoma (i.e., lack of *TP53*, *ATRX*, *PTEN* or *MED12* alterations [26–28]) or in myxoid uterine leiomyosarcoma (i.e., lack of *TP53* and *BRCA2* mutations or *PLAG1* rearrangements [29–31]). For instance, case #10 was previously diagnosed as myxoid uterine leiomyosarcoma; however, the frameshift *BCORL1* T513fs*22 short variant mutation was the only genomic alteration identified with no other co-occurring molecular alterations.

Comprehensive genomic profiling revealed that *BCORL1*-altered uterine sarcomas were microsatellite stable and had a low tumor mutation burden. In addition to *BCORL1* alterations, high frequency of homozygous deletion of *CDKN2A* and *CDKN2B* was present in 33% of *BCORL1*-altered uterine sarcomas, while *CDK4* and *MDM2* gene amplification was present in 17% of cases (Fig. 6). Therefore, overall genomic alterations leading to the activation of the CDK4 kinase pathway, via *CDK4* amplification or *CDKN2A* loss, occurred in 6 of 12 (50%) *BCORL1*-altered uterine sarcoma cases, at a comparable rate as seen with previously described *BCOR*-rearranged endometrial stromal sarcomas by our group using the same methodology [6] (50% vs. 65%, p = 0.5).

Other targetable alterations in *BCORL1*-altered uterine sarcomas included homozygous deletion or inactivating truncating mutations of *NF1* (W267* and E291*) in 33% of tumors (Fig. 6). The frequency of *NF1* alterations in *BCORL1*-mutated uterine sarcoma was higher than that seen in *BCOR*-rearranged endometrial stromal sarcoma (33% vs. 5%, p = 0.02). Another notable difference was a higher frequency of alterations in the NF2-mTOR pathway in 25% of *BCORL1*-altered uterine sarcoma compared with 2.5% in *BCOR*-rearranged endometrial stromal sarcoma (p = 0.03) (Fig. 6). Specific alterations in the NF2-mTOR pathway included *NF2* K550fs*2 and *PIK3R1* D560Y in one case as well as *mTOR* C1483Y or *AKT1* E17K in two additional cases.

BCORL1 alterations in other uterine mesenchymal tumors

In contrast to *BCORL1* fusions or rearrangements which were specific to uterine sarcomas in the Foundation Medicine genomic database, two short variant *BCORL1* mutations were also identified in one conventional uterine leiomyosarcoma and one uterine inflammatory myofibroblastic tumor. The conventional uterine leiomyosarcoma had a *BCORL1* V1096I missense mutation, as well as *TP53*, *RB1*, and *MED12* co-alterations and classic leiomyosarcoma morphology and without myxoid features. The uterine inflammatory myofibroblastic tumor harbored a *BCORL1* R1196* nonsense mutation, a co-occurring *RANBP2-ALK1* fusion and myxoid features. In these two cases, the morphology and genomic profiles were compatible with conventional leiomyosarcoma and inflammatory myofibroblastic tumors with co-occurring *BCORL1* short variant mutations. Notably, the type of *BCORL1* mutation identified in the conventional uterine leiomyosarcoma was a point mutation, *BCORL1* V1096I, in contrast to the nonsense and frameshift mutations that were identified in the other *BCORL1*-mutated uterine sarcomas with myxoid features.

Reported immunohistochemistry

We extracted immunohistochemistry (IHC) results that were performed at outside institutions, if they were available, from the submitting pathology reports. All tested tumors were reportedly negative for cytokeratins, while all tumors (100%, 6 of 6) were positive for CD10. The majority was positive for ER (71%, 5 of 7) and PR (83%, 5 of 6). BCOR IHC was not available for any tumor. One of three tumors (33%) was positive for cyclin D1, and there was variable staining for muscle markers, including for desmin (75%, 3 of 4), smooth muscle actin (75%, 3 of 4). Tumors were also reportedly negative for caldesmon (n = 2), CD117 (n = 2), DOG1 (n = 2), S100 (n = 2) and CD34 (n = 1). However, these results need to be confirmed in a larger series and in a centralized laboratory.

DISCUSSION

BCORL1 is a transcriptional corepressor that is homologous to *BCOR*. We assessed whether molecular alterations in *BCORL1* defined a distinct subset of uterine sarcomas similar to *BCOR*. To test this hypothesis, we examined the molecular landscape of *BCORL1* genomic alterations across various uterine mesenchymal malignancies and characterized the clinicopathological and genomic features of uterine sarcomas that are driven by *BCORL1* molecular alterations. The identified cohort of *BCORL1*-altered uterine sarcomas had several similarities to the findings previously described for *BCOR*-rearranged high grade endometrial stromal sarcomas [4, 6] including: (1) similar median age of mid-50 years and age range of 20–30 s to 70 s in both types, (2) aggressive behavior with refractory recurrences, (3) striking morphological resemblance with hypocellular fibromyxoid areas, collagen plaques, stromal fibrosis, and high-grade epithelioid components,

Table 2. Morphological features of *BCORL1*-altered uterine sarcomas.

| n	*BCORL1* alteration	Atypia	Nuclei	Spindle cells	Epithelioid cells	Small cells	Myxoid stroma	Collagen plaques	Hypocellular fibromyxoid	Spiral arterioles	Necrosis	Mitotic count
1	*JAZF1-BCORL1* fusion	Moderate to severe	Mild pleomorphism	Present	Present	Absent	Present	Absent	Present	Absent	Absent	25 per 10 HPF
2	*EP300-BCORL1* fusion	Moderate	Mild pleomorphism	Present	Present	Absent	Present	Absent	Absent	Absent	Present	2 per 10 HPF
3	Internal *BCORL1* rearrangement	Mild to moderate	Uniform	Present	Present	Absent	Absent	Present	Present	Absent	Absent	8 per 10 HPF
4	*JAZF1-BCORL1* fusion	Moderate	Mild pleomorphism	Present	Present	Absent	Focal	Absent	Absent	Absent	Present	17 per 10 HPF
5	*BCORL1* R1265fs*4	Moderate to severe	Uniform	Present	Present	Absent	Present	Present	Present	Absent	Absent	5 per 10 HPF
6	*BCORL1* R1196*	Mild to moderate	Uniform	Present	Absent	Absent	Absent	Absent	Absent	Present	Absent	2 per 10 HPF
7	*BCORL1* homozygous deletion	Mild to moderate	Uniform	Present	Present	Absent	Present	Focal	Present	Present	Present	15 per 10 HPF
8	*JAZF1-BCORL1* fusion	Moderate to severe	Focal pleomorphism	Present	Present	Absent	Present	Focal	Present	Absent	Present	10 per 10 HPF
9	*BCORL1* homozygous deletion	Moderate to focal severe	Focal pleomorphism	Present	Focal	Absent	Present	Present	Present	Present	Absent	9 per 10 HPF
10	*BCORL1* T513fs*22	Moderate	Uniform	Present	Absent	Absent	Present	Present	Present	Absent	Absent	2 per 10 HPF
11	*BCORL1* R945*	Mild to moderate	Uniform	Present	Absent	Absent	Present	Absent	Present	Absent	Absent	4 per 10 HPF
12	*BCORL1* P600fs*1	Moderate to severe	Mild pleomorphism	Present	Focal	Absent	Focal	Absent	Absent	Absent	Present	15 per 10 HPF
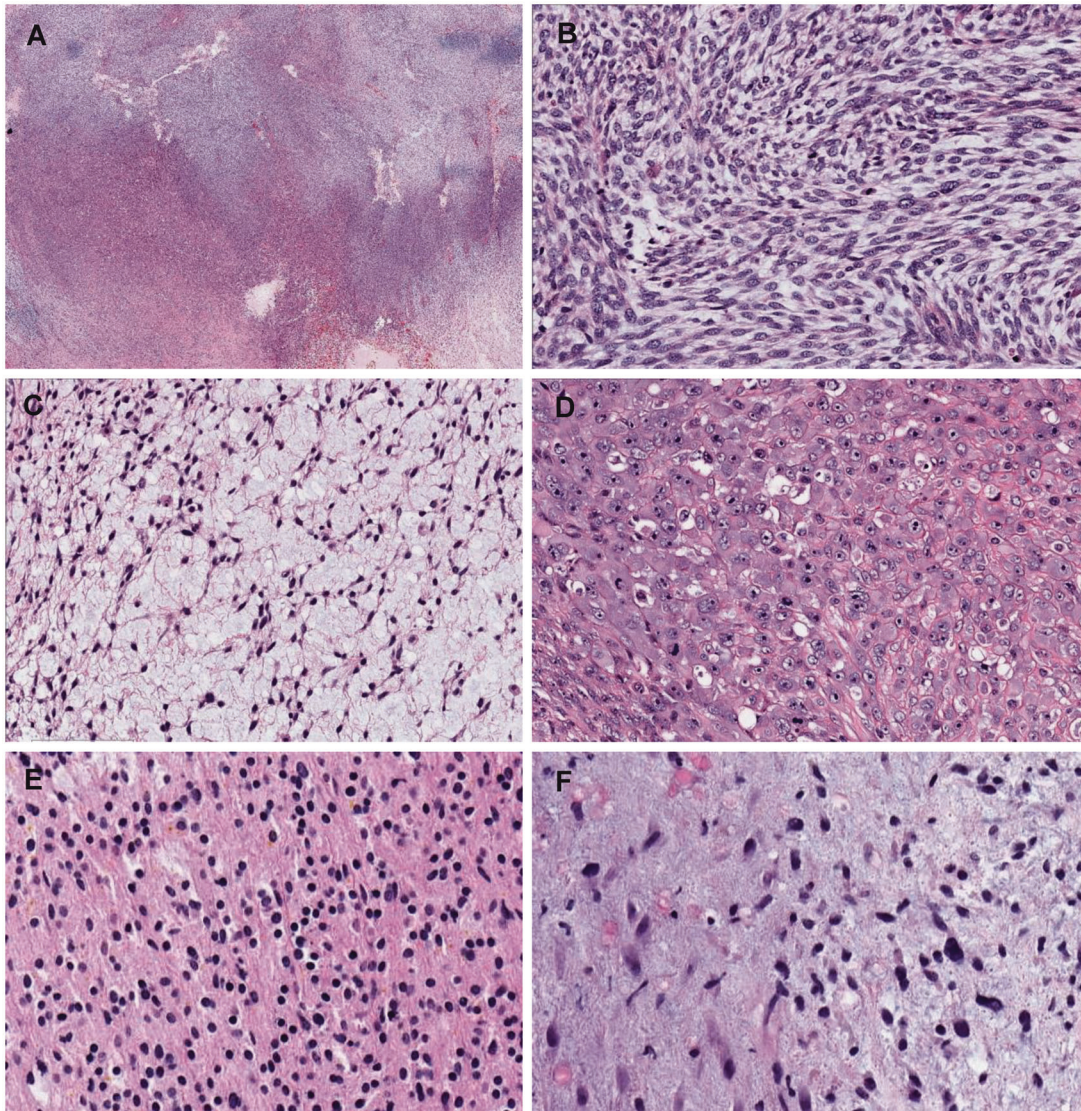
Fig. 3 Morphological spectrum of endometrial stromal sarcomas with *BCORL1* fusions. A-D: Case #1 with *JAZF1-BCORL1* demonstrating (**A**) alternating hypercellular and hypocellular areas and myxoid stroma on low power view, (**B**) hypercellular area with spindle cells and mild to moderate atypia, (**C**) hypocellular and spindle cell area with low-grade atypia and myxoid stromal change, and (**D**) hypercellular, epithelioid area with high-grade nuclear atypia and prominent nucleoli. **E**-**F**: Case #2 with *EP300-BCORL1* rearrangement characterized by (**E**) epithelioid morphology with pink cytoplasm and (**F**) hypocellular myxoid and spindle cell areas.

(4) frequent myxoid stroma, some of which mimicked and were previously diagnosed as myxoid uterine leiomyosarcoma, and (5) frequent co-occurring genomic alterations leading to the activation of the CDK4 kinase pathway, via *CDK4* amplification or *CDKN2A* loss.

Overall, 11 of 12 cases demonstrated striking resemblance to previously described *BCOR*-altered endometrial stromal sarcomas in the form of myxoid stromal change, collagen plaques or stromal fibrosis and/or epithelioid components [4, 6]. One exception resembled low-grade ESS and harbored a *BCORL1* frameshift mutation and an *EPC1-PH1* fusion. It remains to be determined whether low-grade tumors with *BCORL1* short variant mutations and EPC1-PH1 fusions have a worse prognosis or increased propensity to high grade transformation than typical low-grade ESS. A limitation to our study is lack of long-term follow-up for the entire cohort to assess prognosis and survival. Nevertheless, four patients succumbed to disease within six years following hysterectomy, two of which died <1 year following hysterectomy. Our results suggest that similarly to *BCOR*, genomic alterations in

BCORL1 drive the pathogenesis of a distinct subset of high-grade endometrial stromal sarcomas. The high-grade nature of this new uterine sarcoma subtype is supported by both morphological features and short patient survival following hysterectomy when follow-up was available. The endometrial stromal origin is supported by positivity for CD10, which is traditionally viewed as a marker of endometrial stromal differentiation.

Identification of *BCORL1*-altered high-grade endometrial stromal sarcomas may be difficult without performing molecular analysis, as highlighted by the fact that 25%, 3 of 12 tumors were previously diagnosed as leiomyosarcomas, all of which exhibited myxoid features on re-review. Key clues that may raise an index of suspicion for a *BCORL1*-endometrial stromal sarcoma are: 1) frequent gelatinous, mucomyxoid-like appearance by gross examination, 2) microscopic spindle cell appearance, most often with at least focal myxoid stromal change, 3) variable amounts of hypocellular fibromyxoid spindle areas with lower grade atypia and/or 4) variable amounts of epithelioid areas with higher grade nuclear atypia. Notably, epithelioid or rhabdoid components were
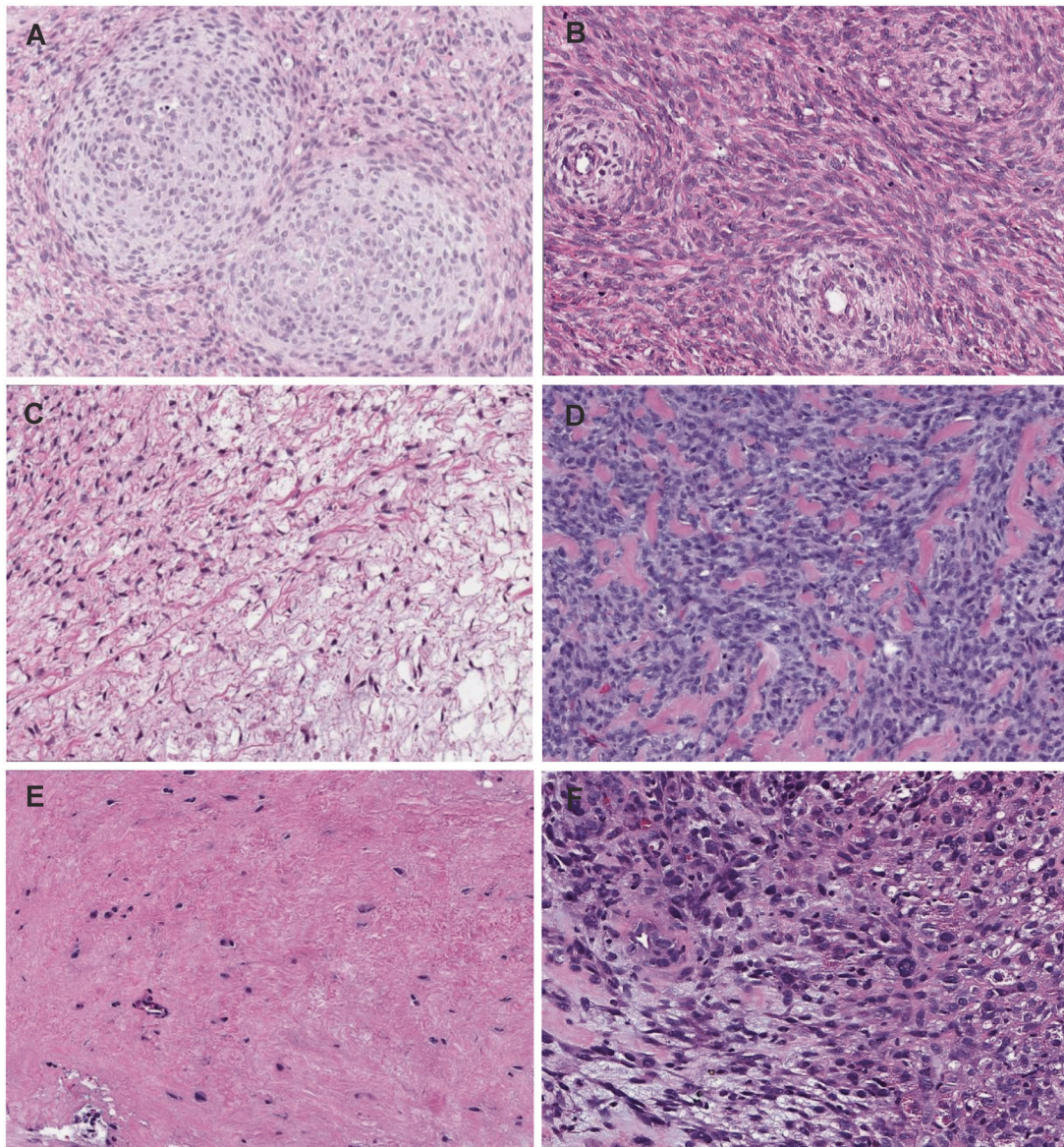
Fig. 4 Morphological features of endometrial stromal sarcomas with homozygous *BCORL1* gene deletion. A-B-C) Case #7 with epithelioid and spindle areas, spiral arterioles, myxoid stroma and mild to moderate atypia. **D-E-F**) Case #9 with hypercellular spindle cell area with collagen plaques, hypocellular fibromyxoid areas, and focal epithelioid areas with high-grade atypia adjacent to spindle cell and myxoid areas.

never identified in isolation and were always associated with a spindle cell component.

Most of the short variant *BCORL1* alterations in the uterine sarcomas were nonsense mutations or out-of-frame insertions that are predicted to encode truncated proteins lacking the last C-terminal LXXLL nuclear receptor recruitment motif and ANK and PUFD domains, which are involved in protein-protein interactions and transcriptional regulation [32] (Fig. 1). In addition, we identified 2 uterine sarcomas with homozygous *BCORL1* deep gene deletion. These results suggest that a subset of uterine sarcomas is driven by *BCORL1* loss of function within the PRC1 transcriptional complex, leading to transcriptional de-regulation. Supporting this notion, a *BCORL1*-mutated tumor in our study also exhibited co-occurring *BCOR* alteration (Fig. 6), likely further contributing to PRC1 transcriptional dysfunction. In addition, none of the *BCORL1* short variant alterations were subclonal (defined as *BCORL1* next-generation sequencing variant allele frequency less than 10% of tumor purity) and therefore were consistent with driver alterations. While loss of heterozygosity of second the *BCORL1* could not be

determined, it is worth noting that *BCORL1* is located on the X chromosome, and the second *BCORL1* allele may be subject to complete chromosome X inactivation in females [33].

While *JAFZ1-BCORL1* or *EP300-BCORL1* fusions and homozygous *BCORL1* deletion appeared specific to uterine sarcomas, a *BCORL1* point mutation and a *BCORL1* nonsense mutation were also identified in one conventional uterine leiomyosarcoma and one myxoid inflammatory myofibroblastic tumor. However, the morphology and genomic profiles of these 2 tumors were different than *BCORL1*-altered uterine sarcomas and compatible with the submitting diagnoses. In addition, short variant *BCORL1* frameshift or nonsense mutations were also identified in endometrial carcinomas and carcinosarcomas, especially in a microsatellite instability setting (not shown). Defects in the mismatch repair system often results in frameshift mutations, and therefore most of the *BCORL1* short variant mutations in endometrial carcinoma or carcinosarcoma were likely secondary mutations due to microsatellite instability rather than primary driver mutations as seen in microsatellite stable uterine sarcomas.
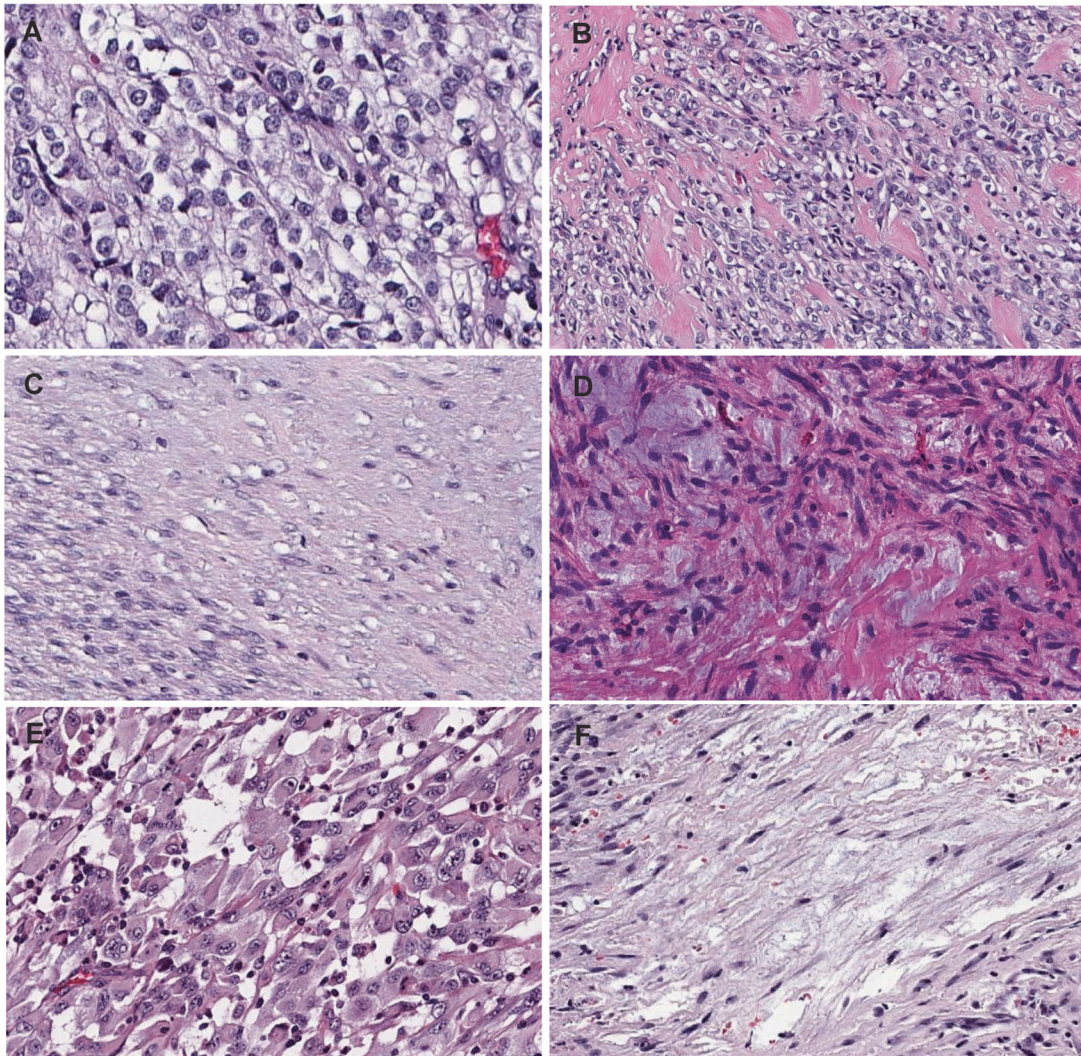
Fig. 5 Morphology of uterine sarcomas with short variant *BCORL1* mutations. A-C: Case #5 with *BCORL1* R1265fs*4 frameshift mutation, previously diagnosed as low grade endometrial stromal sarcoma, characterized by (**A**) epithelioid morphology with clear to pale cytoplasm and moderate to high grade atypia, (**B**) spindle to epithelioid areas with collagen plaques, and (**C**) spindle cell areas with myxoid stroma and lower grade atypia. **D.** Case #10, previously diagnosed as myxoid leiomyosarcoma, harboring *BCORL1* T513fs*22 frameshift mutation as the only oncogenic genomic alteration, exhibiting spindle cell morphology with myxoid stroma and stromal fibrosis. **E-F:** Case #12, previously diagnosed as spindle cell neoplasm consistent with leiomyosarcoma with *BCORL1* P600fs*1 frameshift mutation, featuring epithelioid and rhabdoid areas with high grade atypia, and (**E**) spindle cell area with lower grade atypia and myxoid change.

In this study, we report novel *BCORL1* rearrangements in uterine sarcomas such as *EP300-BCORL1* and as well as a case with internal *BCORL1* gene rearrangement without another gene partner. Interestingly, similar *EP300-BCOR* and internal *BCOR* rearrangements have also been previously identified in *BCOR*-rearranged high-grade endometrial stromal sarcomas, which also demonstrated similar spindle, epithelioid and myxoid morphology [6].

Like *BCOR*-rearranged endometrial stromal sarcomas, *BCORL1*-altered uterine sarcomas exhibited high frequencies of *CDK4* amplification and *CDKN2A* homozygous gene deletion. Genomic alterations leading to the activation of the CDK4 kinase, via *CDK4* amplification or *CDKN2A* loss, occurred in 50% *BCORL1*-altered uterine sarcomas. Future investigation would be of interest to determine whether refractory tumors with *CDK4* and *CDKN2A* alterations are responsive to CDK4 inhibitors, such as palbociclib, ribociclib, and abemaciclib, in patients that fail conventional therapy and in which current standard treatments may no longer be clinically effective.

CDK4 inhibitors are currently FDA-approved for the treatment of ER-positive and HER2-negative breast carcinomas with minimal side effects [34, 35].

In contrast to *BCOR*-rearranged uterine sarcomas in which only a small minority exhibited *NF1* (5%) or *NF2* (2.5%) alterations [6], *BCORL1*-altered uterine sarcomas harbored inactivating *NF1* mutations or alterations of the *mTOR-NF2-AKT* pathway in 33 and 25% of cases, respectively. For these reasons, based on response in other tumor types [36, 37], off-label use of MEK inhibitors or mTOR inhibitors as single agents or in combination with other therapies could also be considered in a subset of *BCORL1*-mutated uterine sarcomas that are refractory to conventional cytotoxic chemotherapy.

Our study demonstrates the value of next-generation sequencing-based comprehensive genomic profiling for identifying *BCORL1*-mutated uterine sarcomas since the *BCORL1* nonsense and frameshift mutations seen in our study would not have been detected by either FISH or sarcoma fusion panels, similarly to the
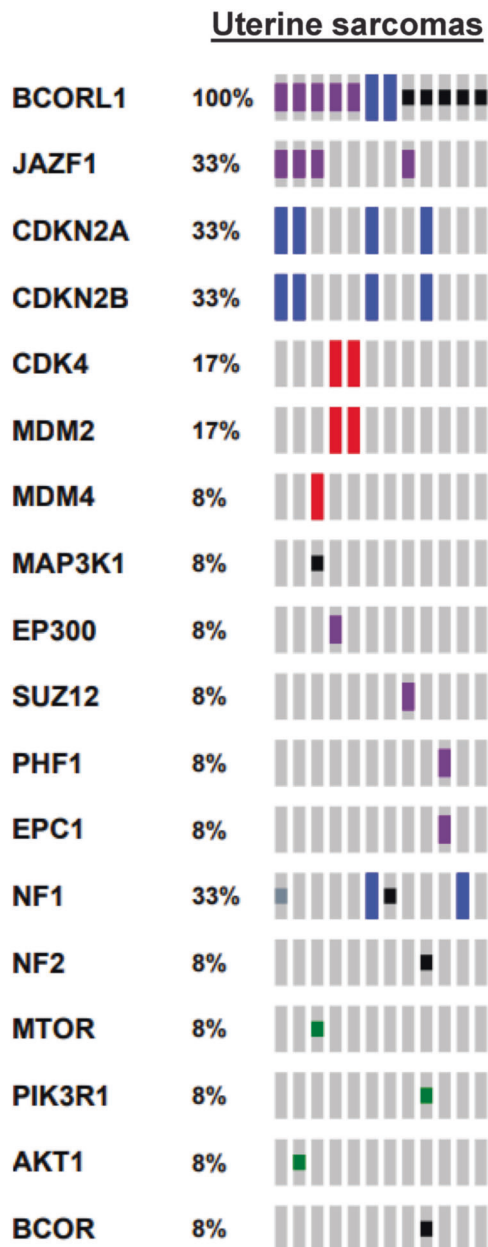
Uterine sarcomas

Fig. 6 Oncoprint of our *BCORL1*-altered uterine sarcomas demonstrating genomic profiles with frequent activation of the CDK4 kinase pathway via *CDK4* amplification and *CDKN2A* deletion. Purple rectangle: gene rearrangement. Blue rectangle: homozygous deletion; red rectangle: amplification; black square: truncating or frameshift short variant mutation; Green square: oncogenic missense mutation.

gene structures, related biological functions of *BCORL1* and *BCOR* and similar clinicopathological features, *BCORL1*-altered uterine sarcomas may represent a new subtype of high-grade endometrial stromal sarcomas. This new group of aggressive *BCORL1*-mutated uterine sarcomas may mimic myxoid leiomyosarcomas and should also be considered in the differential diagnosis of myxoid uterine mesenchymal neoplasms.

DATA AVAILABILITY
All data generated or analyzed during this study are included in this published article.

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previously described *BCOR* internal tandem duplications within exon 15 of *BCOR* in high-grade endometrial stromal sarcomas. Therefore, during the pathological work-up of an uterine sarcoma case, a negative result for either FISH or sarcoma fusion panel for *BCOR* or *BCORL1* may warrant reflex testing to a next-generation sequencing-based assay for a more comprehensive evaluation of either *BCOR* or *BCORL1*.

In conclusion, we evaluated the clinicopathological and molecular features of uterine sarcomas driven by *BCORL1* genomic alterations. *BCORL1*-altered uterine sarcomas had striking morphological resemblance to previously described *BCOR*-rearranged high-grade endometrial stromal sarcomas. Given homologous

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AUTHOR CONTRIBUTIONS
DIL performed study concept, design, analysis, data acquisition, and manuscript writing. ND performed analysis and acquired part of the data. All authors interpreted data and provided the study materials; and all authors read, revised, and approved the paper.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
Approval for this study was obtained from the Western Institutional Review Board Protocol No. 20152817.

COMPETING INTERESTS
All authors are full-time employees of Foundation Medicine, Inc., which is a whole subsidiary of Roche, and these employees have equity interest in Roche.

ADDITIONAL INFORMATION
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