original reports

CDKN2C-Null Leiomyosarcoma: A Novel, Genomically Distinct Class of *TP53*/*RB1*–Wild-Type Tumor With Frequent *CIC* Genomic Alterations and 1p/19q-Codeletion

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abstract

PURPOSE Leiomyosarcoma (LMS) harbors frequent mutations in *TP53* and *RB1* but few actionable genomic alterations. Here, we searched for recurrent actionable genomic alterations in LMS that occur in the absence of common untreatable oncogenic drivers.

METHODS Tissues from 276,645 unique advanced cancers, including 2,570 uterine and soft tissue LMS, were sequenced by hybrid-capture–based next-generation DNA and RNA sequencing/comprehensive genomic profiling of up to 406 genes. We characterized clinicopathologic features of relevant patient cases.

RESULTS Overall, 77 LMS exhibited homozygous copy loss of *CDKN2C* at chromosome 1p32.3 (3.0% of LMS). Genomic alterations (GAs) in *TP53*, *RB1*, and *ATRX* were rare compared with the remainder of the LMS cohort (11.7% *v* 73.4%, 0% *v* 54.5%, 2.6% *v* 24.5%, respectively; all $P < .0001$). *CDKN2C*-null LMS patient cases were significantly enriched for GAs in *CIC* (40.3% *v* 1.4%) at 19q13.2, *CDKN2A* (46.8% *v* 7.0%), and *RAD51B* (16.9% *v* 1.7%; all $P < .0001$). Chromosome arm-level aneuploidy analysis of available LMS patient cases (n = 1,284) found that 81% (58 of 72) of *CDKN2C*-null LMS exhibited 1p/19q-codeletion, a significant enrichment compared with 5.1% in the remainder of the LMS cohort ($P < .0001$). In total, 99% of *CDKN2C*-null LMS were in women; the median age was 61 years at surgery (range, 36-81 years). Fifty-five patient cases were uterine primary, four were nonuterine, and the remaining 18 were of uncertain primary site. Sixty percent of cases showed at least focal epithelioid variant histology. Most patients had advanced-stage disease, with 62% of confirmed uterine primary LMS at International Federation of Gynecology and Obstetrics stage IVB. We further validated our findings in two publicly available datasets: The Cancer Genome Atlas and the Project GENIE initiative.

CONCLUSION *CDKN2C*-null LMS defines a genomically distinct tumor that may have prognostic and/or therapeutic clinical implications, including possible use of specific cyclin-dependent kinase inhibitors.

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ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

Leiomyosarcoma (LMS), a neoplasm defined by smooth muscle differentiation, is the most common form of uterine sarcoma.[1] LMS is aggressive and resists standard therapy, with high rates of recurrence and progression. Multiple studies have shown an overall 5-year survival of 25%-76%, with survival for patients with metastatic disease at presentation approaching 10%-15%.[2] Stage of disease, as defined by the International Federation of Gynecology and Obstetrics (FIGO)[3] or the American Joint Committee on Cancer (AJCC),[4] at the time of diagnosis, is the most important prognostic factor for uterine LMS.[1] Surgery is the standard of care for localized tumors, with hormonal and cytotoxic chemotherapy reserved for advanced stages.[5]

Genomic studies of LMS have demonstrated notable mutational heterogeneity, frequent inactivation of *TP53* and *RB1* through varied mechanisms, and widespread copy number alterations.[6] LMS is often associated with complex karyotypes with numerous chromosomal gains and losses.[7] LMS has demonstrated occasional potentially targetable genomic alterations (GAs), but novel targeted therapeutic agents have not been widely used.[8-10] Herein, we describe a novel recurrent genomic signature of cyclin-dependent kinase inhibitor-2C gene (*CDKN2C*) homozygous loss in LMS primarily

CONTEXT

Key Objective

Leiomyosarcoma (LMS), an aggressive tumor with limited curative options, shows frequent mutations in *TP53* and *RB1* but few actionable genomic alterations. Here, we searched for recurrent actionable genetic alterations in LMS.

Knowledge Generated

A novel, genomically distinct class of LMS (3.0%; 77 of 2,570 cases) harbor homozygous loss of *CDKN2C*, which encodes the cyclin-dependent kinase inhibitor-2C. *CDKN2C*-null LMS lack typical *TP53* and *RB1* mutations; show concurrent homozygous deletion of *CIC*, *CDKN2A*, and *RAD51B*; and show frequent 1p/19q-codeletion.

Relevance

The finding of recurrent *CDKN2C*-null LMS provides insight into tumor biology and raises the possibility for use of specific cyclin-dependent kinase inhibitors in this aggressive disease.

from the uterus, with significantly low frequency of *TP53* and *RB1* GAs.

METHODS

Cohort and Genomic Analyses

Comprehensive genomic profiling was performed in a Clinical Laboratory Improvement Amendments–certified, College of American Pathologists–accredited laboratory (Foundation Medicine, Cambridge, MA). Approval for this study, including a waiver of informed consent and a HIPAA waiver of authorization, was obtained from the Western Institutional Review Board (Protocol No. 20152817). The pathologic diagnosis of each patient case was confirmed on routine hematoxylin and eosin (H&E)–stained slides. Sections were macrodissected to achieve > 20% estimated percent tumor nuclei in each case, for which the percent tumor nuclei equals 100 times the number of tumor cells divided by total number of nucleated cells. In brief, ≥ 60 ng of DNA was extracted from 40-μm sections of tumor samples in formalin-fixed, paraffin-embedded tissue blocks. The samples were assayed by adaptor ligation hybrid capture, performed for all coding exons of 236 (v1), 315 (v2), or 405 (v3) cancer-related genes plus select introns from 19 (v1), 28 (v2), or 31 (v3) genes frequently rearranged in cancer (Appendix Table A1).[11,12] For samples with available RNA, targeted RNA sequencing was performed for rearrangement analysis in 265 genes.[12] Sequencing of captured libraries was performed using the Illumina HiSeq 4000 System (Illumina, San Diego, CA) to a mean exon coverage depth of targeted regions of > 500×, and sequences were analyzed for GAs, including short variant alterations (base substitutions, insertions, and deletions), copy number alterations (focal amplifications and homozygous deletions), and select gene fusions or rearrangements.[11,13,14] To maximize mutation detection accuracy (sensitivity and specificity) in impure clinical specimens, the test was previously optimized and validated to detect base substitutions at a ≥ 5% mutant allele frequency, indels with a ≥ 10% mutant allele frequency with ≥ 99% accuracy, and fusions occurring within baited introns/exons with > 99% sensitivity.[11] Germline and somatic status of pathogenic alterations was not delineated. Tumor mutational burden (TMB; mutations/Mb) was determined on 0.8-1.1 Mb of sequenced DNA.[14] Microsatellite instability was determined on up to 114 loci.[15]

Copy number analysis. Copy number analysis to detect gene-level amplifications at > 6-8 copies depending on tumor ploidy and homozygous deletions was performed as previously described.[11] In brief, the aligned DNA sequences of each tumor specimen were normalized against a process-matched normal, producing log-ratio and minor allele frequency data. Next, whole-genome segmentation was performed using a circular binary segmentation algorithm on the log-ratio data. A Gibbs sampler fitted copy number model and a grid-based model were fitted to the segmented log-ratio and minor allele frequency data, producing genome-wide copy number estimates. Finally, the degrees-of-fit of candidate models returned by Gibbs sampling and grid sampling were compared, and the optimal model was selected by an automated heuristic.

Signal-to-noise ratios for each genomic segment were used to determine gain or loss per chromosome arm on the basis of tumor purity and ploidy; the sum of segment sizes determined the fraction of each arm gained or lost. Chromosomes were assessed for arm-level aneuploidy, defined as positive if > 50% of the arm was altered. This threshold was previously validated on 109 *IDH1/2*-mutant glioma samples with 1p/19q-codeletion fluorescence in situ hybridization (FISH) results available. Patient cases were blinded to FISH results, and 1p/19q-codeletion status was determined via arm-level aneuploidy analysis. Concordance was 95%, sensitivity was 91%, and positive predictive value was 100%. A query for chromosome 1p and 19q arm-level aneuploidy was performed on LMS patient cases with available aneuploidy data (n = 1,284), with positive patient cases defined as 1p/19q-codeleted.

Clinicopathologic analysis of LMS cohort harboring homozygous* CDKN2C *deletion. The cohort of *CDKN2C*-null LMS comprised 77 cases, each from a different patient, that were submitted to Foundation Medicine for comprehensive genomic profiling during routine clinical care. Human investigations were performed after approval by a local human investigations committee and in accordance with an assurance filed with and approved by the Department of Health and Human Services, when appropriate. Clinicopathologic data, including patient age, sex, tumor site, and FIGO stage or AJCC (8th edition) stage, were extracted from the accompanying pathology report.[4,16] Primary site data were not available for a subset of patient cases ("indeterminant primary"). The histopathology was assessed on routine H&E-stained slides of tissue sections submitted for genomic profiling by two board-certified pathologists (E.A.W., D.I.L.).

Quantitative data were analyzed using the Fisher exact test because of the categoric quality of the data and the size of the cohort. For the age and TMB comparisons between two groups, the nonparametric Mann-Whitney *U* test was used. A two-tailed *P* value of $< .05$ was considered statistically significant; the Bonferroni correction was applied for multiple simultaneous comparisons.

Review of publicly available datasets. The Cancer Genome Atlas (TCGA) Network's sarcoma genomic dataset[17] and the American Association for Cancer Research (AACR) Project GENIE Consortium dataset (v7.0-public)[18] were interrogated for LMS with homozygous loss of *CDKN2C*. Histopathology of TCGA patient cases was reviewed by two board-certified pathologists (E.A.W., D.I.L.).

TABLE 1. Clinical Characteristics of Patients With *CDKN2C*-Null Leiomyosarcoma

Characteristic	No. (%)
No. of patients	77
Median (range) age at diagnosis, years	61 (36-81)
Sex	
Female	76 (99)
Male	1 (1)
Primary site	
Uterine	55 (71)
Soft tissue	4 (5)
Indeterminant	18 (23)
FIGO staging (uterine primary)	
IB	6 (11)
IIA	2 (4)
IIB	4 (7)
IIIA	2 (4)
IIIB	1 (2)
IIIC	4 (7)
IVB	34 (62)
Unknown	3 (6)
AJCC staging (soft tissue or indeterminant primary)	
IA	1 (5)
IV	19 (86)
Unknown	2 (9)

Abbreviations: AJCC, American Joint Committee on Cancer; FIGO, International Federation of Gynecology and Obstetrics.

RESULTS

A Novel Class of *CDKN2C*-Null LMS: Clinicopathologic Features

From an internal series of 276,645 unique advanced cancers, including 2,570 LMS, of which 939 were of confirmed uterine origin, we identified 77 LMS with homozygous copy loss of *CDKN2C* at chromosome 1p32.3 (3.0% of all LMS [77 of 2,570], 5.9% of uterine LMS [55 of 939]). Clinical characteristics of the 77 patients with this novel class of CDKN2C-null LMS are summarized in Table 1. These patients were significantly older than the remainder of the LMS cohort (median age, 61 *v* 57 years; $P = .0009$, Mann-Whitney *U* test). Patients were enriched for female sex compared with the remainder of the LMS cohort (99% [76 of 77] *v* 79% [1,968 of 2,493]; $P < .0001$, Fisher's exact test). Six female patients had a prior history of leiomyomatosis (n = 2) or uterine smooth muscle tumor of uncertain malignant potential (STUMP; n = 4). The majority of *CDKN2C*-null LMS patients showed clinically advanced/metastatic disease, with 62% of confirmed uterine primary occurrences documented at FIGO stage IV (n = 34 of 55) and 86% of indeterminant or soft tissue primary cases documented at AJCC stage IV (n = 19/22), as summarized in Table 1. Locations of the sequenced tumor specimens are summarized in Appendix Table A2.

Comprehensive Genomic Profiling of *CDKN2C*-Null LMS

The distribution of GAs in the 77 *CDKN2C*-null LMS is displayed in Figure 1. *TP53*, *RB1*, and *ATRX* GAs were rare in comparison with the remainder of the LMS cohort (Table 2; Appendix Table A3). *CDKN2C*-null LMS comprised 14% (68 of 486) of *TP53*/*RB1*–wild-type LMS. The most frequent GAs were identified in *CIC* at 19q13.2, *CDKN2A*, and *RAD51B* (Table 2), and unsupervised analysis showed significant enrichment of these alterations in the *CDKN2C*-null cohort (Appendix Fig A1). Eighty-five percent (60 of 71) of patient cases evaluated for *FAF1* showed homozygous deletion of *FAF1* at 1p32.3, a gene adjacent to *CDKN2C* (9.7 kb apart). No *CDKN2C*-null LMS in our cohort had inactivating GAs in *FUBP1* or pathogenic alterations in *IDH1/2* or *TERTp*.

The median TMB was 2.4 mutations/Mb (range, < 0.8-9.6; Q1-Q3, 1.6-3.2), similar to the remainder of the LMS cohort (median, 2.4 mutations/Mb; range, < 0.8-203; Q1-Q3, 1.6-4.0) but slightly lower overall ($P = .0425$, Mann-Whitney

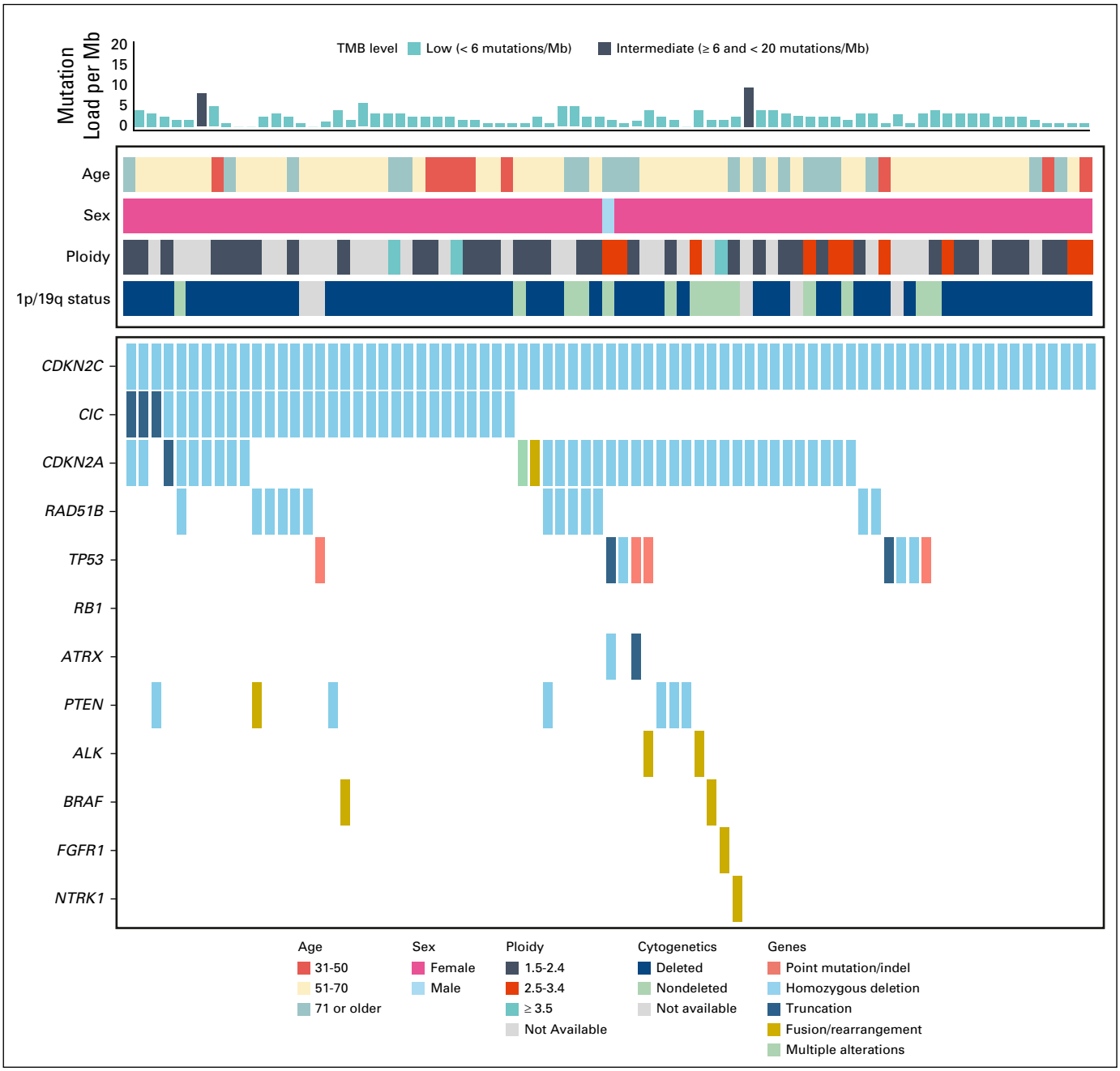

FIG 1. Mutational landscape of *CDKN2C*-null leiomyosarcoma. Summary tile plot of pathogenic variants identified in 77 cases of *CDKN2C*-null leiomyosarcoma. Each column represents data for a single patient. Age, sex, tumor ploidy, and 1p/19q-codeletion status are also provided for each case. The histogram on top shows tumor mutational burden (TMB; mutations/megabase).

U test). No microsatellite-unstable patient cases were present in the *CDKN2C*-null cohort.

Within the cohort of *CDKN2C*-null LMS, comparison of patients < 61 years of age with patients ≥ 61 years revealed significant differences in frequency of *CIC* alterations (54% [20 of 37] *v* 28% [11 of 40]; $P = .022$) and *RAD51B* alterations (5% [2 of 37] *v* 28% [11 of 40]; $P = .0136$). No other significant differences based on age were identified. Comparison of patient cases on the basis of clinical stage, history of lower grade smooth muscle neoplasm, or primary site did not reveal any significant differences in GAs.

Three patients had two separate tissue specimens analyzed (Appendix Table A4). For all three patients, each initial sequencing result, including *CDKN2C* loss, was identified in the subsequent paired-specimen result. One patient had sequencing of both the primary uterine LMS and a subsequent lung metastasis. The lung mass showed additional homozygous loss of *CIC*.

TABLE 2. Comparative Demographics and Percent Frequency of Genomic Alterations Stratified by *CDKN2C* Status

Variable	CDKN2C-Null LMS	Remaining LMS Cohort	*P*
Female sex, % (n/total N)	99 (76/77)	79 (1,968/2,493)	**< .0001**
Median (range) age, years	61 (36-81)	57 (< 1 to > 89)	**.0009**
TMB (Q1-Q3), mut/Mb, % (n/total N)	2.4 (1.6-3.2)	2.4 (1.6-4.0)	.0425
MSI high, % (n/total N)	0 (0/63)	0.2 (5/2,093)	1.0000
Genomic alteration, % (n/total N)			
1p/19q-codeletion	85 (33/39)	5 (62/1,212)	**< .0001**
CIC	40 (31/77)	1 (35/2,473)	**< .0001**
CDKN2A	47 (36/77)	7 (175/2,493)	**< .0001**
RAD51B	17 (13/77)	2 (43/2,473)	**< .0001**
TP53	12 (9/77)	73 (1,830/2,493)	**< .0001**
RB1	0 (0/77)	55 (1,359/2,493)	**< .0001**
ATRX	3 (2/77)	25 (606/2,473)	**< .0001**
PTEN	9 (7/77)	16 (399/2,493)	.113
ALK fusion	3 (2/77)	2 (41/2,493)	.371
BRAF fusion	3 (2/77)	0.2 (4/2,493)	.0123
FGFR1 fusion	1 (1/77)	0.1 (2/2,493)	.0873
NTRK1 fusion	1 (1/77)	0.1 (3/2,493)	.115

NOTE. For percent values, number of positive cases over the total number of evaluated cases is included in parentheses. The Bonferroni correction for 16 simultaneous comparisons was applied; significant *P* values (< .003) are in bold.

Abbreviations: LMS, leiomyosarcoma; MSI, microsatellite instability; TMB, tumor mutational burden.

A query and review for chromosome 1p and 19q arm-level aneuploidy in available LMS patient cases (n = 1,284) revealed that 99% (71 of 72) of *CDKN2C*-null LMS patient cases had whole-arm aneuploidy of the short arm of chromosome 1, and 81% (58 of 72) had aneuploidy of the long arm of chromosome 19 and 1p/19q-codeletion. Significant enrichment for 1p/19q-codeletion was identified in comparison with the remainder of the evaluated LMS cohort (81% [58 of 72] *v* 5% [62 of 1,212]; $P < .0001$). Copy number plots of two exemplary cases of *CDKN2C*-null LMS exhibiting 1p/19q-codeletion are shown in Figures 2A and 2B. Additional recurrent chromosomal arm-level changes were identified in the 72 patient cases available, including most frequently aneuploidy of chromosomes 6p (n = 35), 9p (n = 19), 10q (n = 28), 11p (n = 39), 13q (n = 46), 14q (n = 46), and 16q (n = 52).

A review of 1p/19q-codeletion status in available LMS patient cases without homozygous deletion of *CDKN2C* (n = 1,212) revealed 62 1p/19q-codeleted LMS (5%; Fig 2C). These 62 *CDKN2C*-retained LMS showed GAs in *TP53* (52%; n = 33), *RB1* (45%; n = 28), *ATRX* (16%; n = 10), and *PTEN* (13%; n = 8). GAs were also identified in *CDKN2A* (23%; n = 14) and *ALK* (10%; n = 6; all activating rearrangement events). A minority showed GAs in *RAD51B* (8%; n = 5), *CIC* (7%; n = 4; all homozygous loss), and *FAF1* (5%; n = 3). Three of the four patient cases with homozygous deletion of *CIC* also showed homozygous deletion of both *RAD51B* and *FAF1*. All four occurred in uterine LMS (one of which with a history of STUMP).

All non-LMS sarcoma patient cases in the Foundation Medicine dataset (n = 12,097) were evaluated for *CDKN2C* status. Twenty-two of 1,297 gastrointestinal stromal tumors (GISTs) were *CDKN2C*-null (1.7% of GISTs). Twenty-one had a *KIT* mutation, and the single remaining GIST had a *PDGFRA* mutation. None of the 14 *CDKN2C*-null GIST cases with 1p/19q data had 1p/19q codeletion. Nineteen additional sarcoma occurrences with homozygous deletion of *CDKN2C* were identified (0.18% of non-LMS non-GIST sarcomas). These included diverse sarcoma diagnoses, including six high-grade sarcomas not otherwise specified, two osteosarcomas, two malignant peripheral nerve sheath tumors, and two inflammatory myofibroblastic tumors. Genomics were also varied, with alterations identified in *CDKN2A* (68%; n = 13), *TP53* (42%; n = 8), *NF1* (26%; n = 5), *NF2* (26%; n = 5), and *ALK* (16%; n = 3; all activating rearrangement events). No GAs in *CIC* or *RAD51B* were identified. Eleven of the 19 patient cases had 1p/19q-codeletion data available; two (18%) of the 11 had 1p/19q-codeletion. Both were *ALK* rearrangement–positive tumors in women (ages, 63 and 72 years).

We also searched our entire LMS cohort (N = 2,570) for cases with pathogenic alterations in *CDKN2C* other than homozygous deletion. Only one case was identified, in a 52-year-old woman with an estrogen receptor–positive, progesterone receptor–positive (per report, by immunohistochemistry) uterine LMS with a truncating mutation in *CDKN2C* (p.R68*). Concurrent homozygous deletions of *CIC*

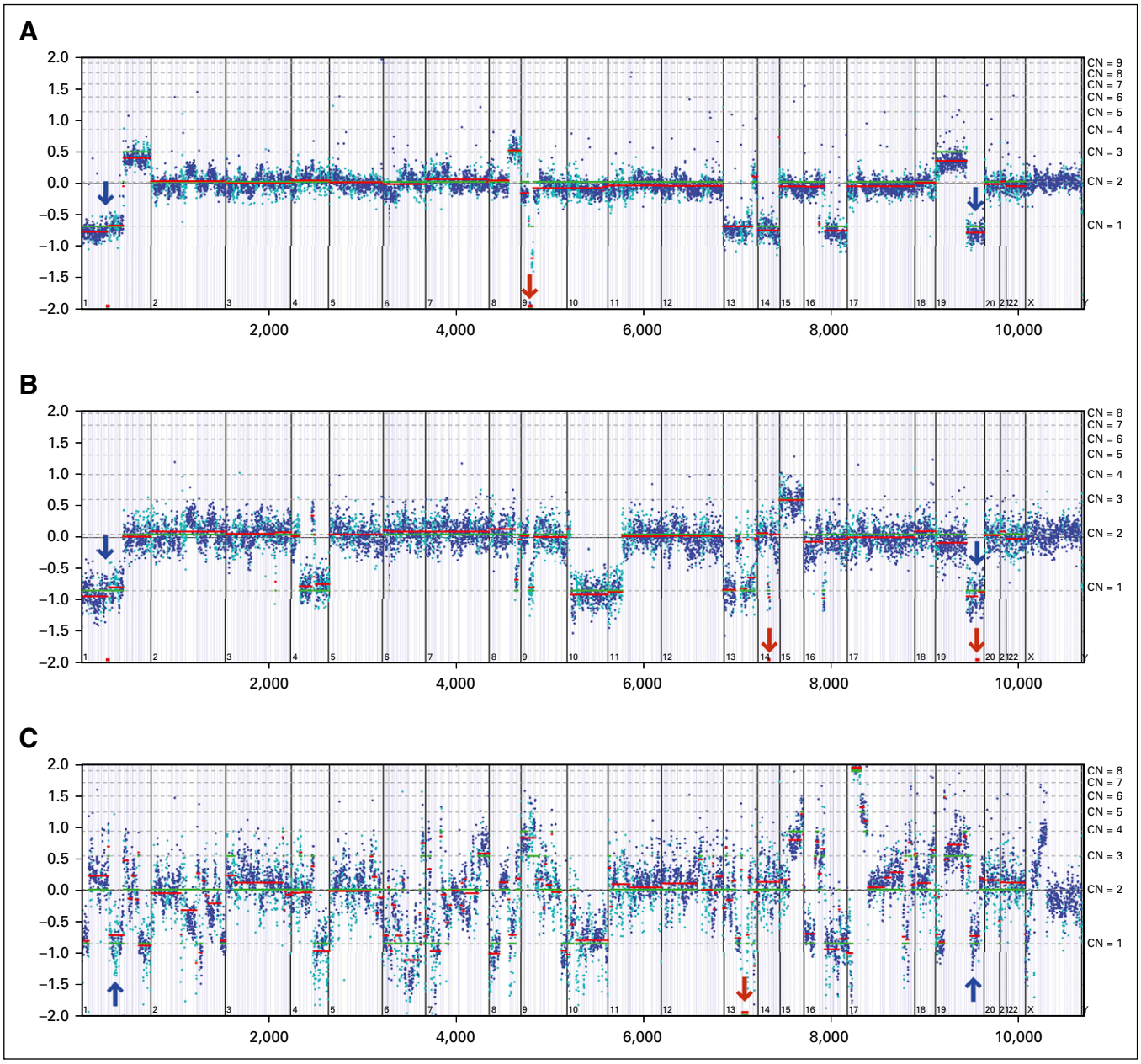

FIG 2. Copy number (CN) plots of three leiomyosarcomas (LMS) with known *CDKN2C* status and 1p/19q-codeletion (blue arrows). The *y*-axes display log-ratio measurements of coverage from each case as compared with a normal reference sample, with assessed CNs denoted by dashed horizontal lines. Each dot represents a genomic region evaluated by the assay (cyan, single-nucleotide polymorphism; blue, exon), which are organized by genomic position. Red lines designate average log-ratio in a segment, and green lines represent model prediction. (A) *CDKN2C*-null LMS with a truncating variant in *CIC* (p.Q907*) and homozygous deletion of *CDKN2A* at chromosome 9p21.3 (red arrow). (B) *CDKN2C*-null LMS with homozygous deletion of *CIC* at chromosome 19q13.2 (red arrow) and *RAD51B* at chromosome 14q24.1 (red arrow). (C) *CDKN2C*-retained LMS with deep deletion of *RB1* at chromosome 13q14.2 (red arrow) and a CN plot of high complexity.

and *RAD51B* were identified; 1p/19q status was not available.

Histopathology

Histopathologic evaluation was performed on all available high-resolution digital pathology H&E slides of our cohort of *CDKN2C*-null LMS (n = 70). Histology was heterogeneous, as shown in Figure 3. Twenty-seven cases (39%) were epithelioid LMS. Twenty-three cases (33%) were spindle cell LMS. Nineteen cases (27%) showed mixed histology, including 11 mixed spindle and epithelioid LMS; four mixed spindle and myxoid LMS; two mixed epithelioid and myxoid LMS; and two mixed spindle, epithelioid, and myxoid LMS. A single case showed small round cell morphology.

Per immunohistochemistry reports, *CDKN2C*-null LMS showed diffuse positivity for estrogen receptor (29 of 29 LMS) and

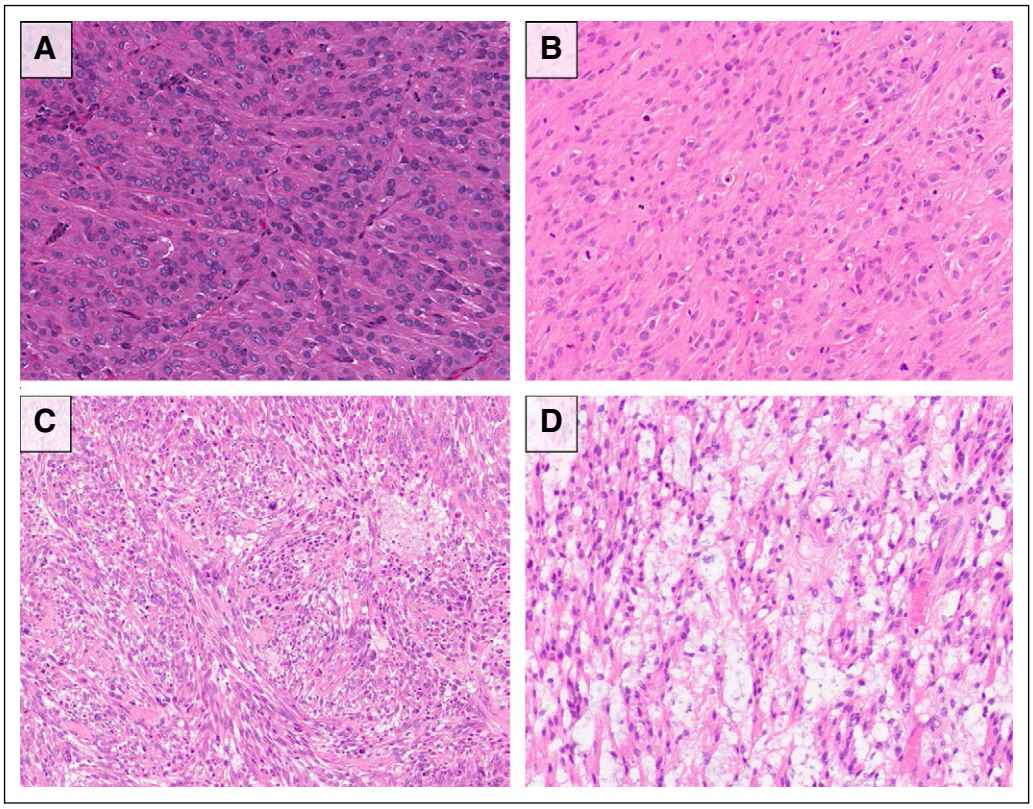

FIG 3. Histopathology of *CDKN2C*-null leiomyosarcoma ranged from (A, B) epithelioid to (C) spindled (hematoxylin & eosin [H&E] stains, 200×). (D) Occasional cases showed focal myxoid histology (H&E stain, 200×).

progesterone receptor (24 of 26; remaining two with focal positivity). *CDKN2C*-null LMS were also positive for desmin (30 of 33; remaining three with focal positivity), smooth muscle actin (25 of 25), muscle-specific actin (11 of 11), and caldesmon (6 of 6). HMB-45 was focally positive in two of nine cases, and CD10 was positive in one of 14 cases. Tumors were reportedly negative for S100 (n = 15), various keratin markers (n = 19), CD34 (n = 11), and CD117 (n = 11).

Publicly Available Datasets

The frequency of *CDKN2C*-null cases in LMS in our dataset prompted us to interrogate the sarcoma genomic dataset of TCGA Network[17] and the AACR Project GENIE Consortium dataset (v7.0-public).[18] A total of 12 *CDKN2C*-null LMS patient cases were identified (TCGA: n = 3 [4%] of 80; GENIE: n = 9 [2%] of 449; Table 3). The *CDKN2C*-null LMS patient cases were enriched for female sex (n = 12 of 12), uterine origin, and epithelioid histology. Patient cases showed frequent homozygous loss of *CIC* (n = 5 [42%] of 12), *CDKN2A* (n = 4 [33%] of 12), and *RAD51B* (n = 3 [25%] of 12), and all were wild type for *TP53*, *RB1*, and *ATRX* (n = 12 of 12).

DISCUSSION

In 2,570 patient cases of LMS, *CDKN2C*-null LMS (n = 77; 3.0%) comprised a genomically distinct molecular subgroup. *CDKN2C*-null LMS typically lacked mutations in *TP53, RB1*, and *ATRX* but showed frequent 1p/19q-codeletion (81%), and nearly half (40.3%) showed homozygous deletion or inactivating truncations of *CIC*. Clinical features were significantly different from other LMS: patients were slightly but statistically significantly older, and the vast majority (76 of 77 patients) were women. Most were of uterine primary site of origin. A high percentage demonstrated epithelioid variant features on histology, and limited clinical data suggest a possible association with and progression from lower-grade uterine smooth tumors, such as leiomyomatosis and STUMP.

CDKN2C at 1p32.3 encodes the homologous p18INK4C cell cycle regulatory protein that blocks cell cycle progression by inhibiting the cyclin D–dependent kinases CDK4 and CDK6.[19-21] Loss of *CDKN2C* results in loss of potent inhibition of CDK4/6 in the cyclin D-CDK4/6-INK4-Rb pathway. CDKN2C is also a key factor for ATM/ATR-mediated activation of the tumor suppressor p53, and *CDKN2C* loss has been shown to block p53 induction in response to DNA damage.[22,23] *CDKN2C* loss has been documented in a subset of diverse tumor types, including multiple myeloma, pituitary adenoma, and thyroid carcinoma.[24-26] *CDKN2C* loss has also been documented in a small percentage of oligodendroglioma.[27,28] The adjacent *FAF1* gene at 1p32.3 encodes FAS-associated factor 1,

TABLE 3. *CDKN2C*-Null Leiomyosarcoma From Two Independent Cohorts

Sample	Age	Sex	Uterine	Additional Genomic Alterations	Histology	Disease Status	Survival Status
TCGA-K1-A42X	62	F	Yes	Homozygous loss of *CIC* and *RAD51B*; amplification of *CCND1* and *FGF19*	Epithelioid and spindled	Local recurrence at 67 months	Living at 123 months
TCGA-FX-A3RE	65	F	Yes	Homozygous loss of *CIC*, *CDKN2A*, and *RAD51B*; amplification of *MET, BRAF, EZH2,* and *RHEB*	Epithelioid and spindled	Disease free at 21 months	Living at 21 months
TCGA-IW-A3M6	59	F	Yes	Homozygous loss of *CDKN2A* and *KMT2C*; amplification of *MDM4, NTRK1, ALK, IKBKE, AKT3, MCL1,* and *DDR2*	Epithelioid and spindled	Disease free at 22 months	Living at 22 months
GENIE-DFCI-024530	73	F	Yes	None	Epithelioid, per report	Not available	Not available
GENIE-DFCI-090524	79	F	Unknown	Homozygous loss of *CIC*; *ARID1A* p.Q1095Afs*10; amplification of *ERBB2* and *PPM1D*	Not available	Not available	Not available
GENIE-DFCI-108895	66	F	Yes	Homozygous loss of *CDKN2A* and *CDKN1A*	Not available	Not available	Not available
GENIE-DFCI-118367	72	F	Unknown	Homozygous loss of *CHEK2*	Not available	Not available	Not available
GENIE-DFCI-118421	54	F	Yes	Homozygous loss of *CIC*, *PTEN*, and *FAS*; *RNF43* p.L17Afs*24	Epithelioid, per report	Not available	Not available
GENIE-MSK-P-0028037	36	F	Yes	Homozygous loss of *BBC3* on 19q13.32	Not available	Not available	Not available
GENIE-MSK-P-0030528	60	F	Yes	*CIC-ERF* fusion	Not available	Not available	Not available
GENIE-MSK-P-0034923	44	F	Yes	Homozygous loss of *CIC*, *ERF*, *ERCC2*, and *BBC3* at 19q13; homozygous loss of *RAD51B* and *PTEN; CIC* p.H505Pfs*9, *HOXB13* X201_splice	Not available	Not available	Not available
GENIE-VICC-439861	71	F	Unknown	Homozygous loss of *CDKN2A*; amplification of *CCND1* and *FGF19*; *CHEK2* p.K287Rfs*17	Not available	Not available	Not available

NOTE. TCGA rows were identified from The Cancer Genome Atlas,[17] from a total of 80 leiomyosarcoma. GENIE rows were identified from project GENIE dataset,[18] from a total of 449 leiomyosarcoma with copy number alteration data.

which enhances FAS-mediated apoptosis, and its loss may contribute to tumor pathogenesis.[29]

The *CIC* gene on chromosome 19q13.2 represses genes induced downstream to RTK pathway activation.[30] In the absence of RTK signaling, CIC blocks transcription of genes that have diverse effects on cellular proliferation, metabolism, and migration.[31] Along with single copy loss of *CIC* on 19q, concurrent inactivating mutations in *CIC* are identified in a high percentage of oligodendrogliomas.[32]

Whole-arm 1p/19q-codeletion, with concurrent mutation in *IDH1* or *IDH2*, is entity-defining for oligodendrogliomas.[33-35] Oligodendrogliomas are associated with relatively long overall survival, and treatment strategies are often stratified on the basis of 1p/19q status.[36-38] The codeletion is a result of unbalanced translocation between two chromosomes, with subsequent loss of der(1;19)(p10;q10), likely because chromosomes 1 and 19 are near each other in the nonrandom organization of the nucleus.[39-41] A large percentage of oligodendrogliomas also show *CIC* and *FUBP1* mutations.[31] Our cohort of *CDKN2C*-null LMS shows notable similarities and differences to oligodendroglioma; 40% of our cohort showed an inactivating alteration in *CIC*, most commonly homozygous deletion. Although *FUBP1* at 1p31.1 is somatically mutated in a subset of oligodendroglioma, no *CDKN2C*-null LMS patient cases in our cohort had inactivating GAs in *FUBP1* or pathogenic alterations in *IDH1*/2 or *TERTp*. The recurrent chromosomal arm-level losses in our cohort may indicate that additional tumor suppressor genes are located on these arms. Rare sarcoma-like tumors originating from oligodendrogliomas have been reported, with documented *IDH1* mutation and 1p/19q-codeletion, and have been termed "oligosarcoma."[42-44] Rodriguez et al[43] identified 6 of 7 patient cases of oligosarcoma with at least focal smooth muscle actin positivity of the sarcomatous component by immunohistochemistry and one patient case with smooth muscle differentiation by electron microscopy. These results indicate a similarity in differentiation to our cohort of LMS.

Among all sarcomas with *CDKN2C* loss, 1p/19q-codeletion appears to be nearly exclusive to LMS. In our overall LMS cohort, however, occasional *CDKN2C*-retained LMS showed *TP53* and *RB1* alterations and were positive by the 1p/19q-codeletion detection algorithm. We speculate that, given the complexity of these genomically unstable occurrences, occasional *CDKN2C*-retained LMS satisfy these criteria (Fig 2C). As such, identification of homozygous deletion of *CDKN2C* may be the most specific distinguishing feature.

Cytogenetic findings in LMS and leiomyoma have been previously reported, although without characterization of *CDKN2C* status. A greater frequency of 1p loss has been documented in metastasized LMS.[45] From a cytogenetics study of 800 uterine leiomyomata, nine diploid occurrences with 1p loss were identified, with other associated alterations, particularly chromosome 19 and/or chromosome 22 loss.[46] Transcriptional profiling of two of the 1p-deleted leiomyomas in that study showed alignment with malignant LMS in a hierarchical clustering analysis.[46] In another study, 1p loss was identified in approximately one quarter of uterine cellular leiomyomata.[47] Three reports on a total of eight pulmonary-based "benign metastasizing leiomyoma" reported 19q and 22q terminal deletion in each case.[48-50] Rare uterine leiomyomas with GAs in *RAD51B* have also been identified.[51] The overlap in GAs between our cohort of *CDKN2C*-null LMS and a subset of leiomyoma of uncertain *CDKN2C* status in the literature suggests a possible connection between these entities.

Evaluations of GAs in epithelioid or myxoid uterine smooth muscle neoplasms are limited in the literature.[52-56] Although a high percentage of *CDKN2C*-null LMS in our study demonstrated epithelioid features on histology, histology was also varied. Immunohistochemistry results extracted from pathology reports were typical for uterine LMS, with expression of characteristic smooth muscle markers.[57]

Given the overall low response rate of LMS to standard therapies, the identification of this targetable alteration in *CDKN2C* may be useful for treatment decisions. CDK4/6 inhibitors have previously shown effectiveness in a LMS with a *CDKN2A* alteration.[10] Nearly half of the *CDKN2C*-null LMS harbored loss of *CDKN2A*; CDK4/6 inhibitors may be effective in replacing the loss of inhibition of CDK4/6 that results from *CDKN2C* and *CDKN2A* loss in these patient cases that recur after standard chemotherapy regimens. A minor subset of *CDKN2C*-null and/or 1p/19q-codeleted LMS harbored activating fusions in *ALK*, *BRAF*, *FGFR1*, and *NTRK1*, for which targeted inhibitors may be of utility.[9]

Limitations of this study include its retrospective nature and the enrichment for aggressive tumors, mostly metastatic to distant sites. The latter may be due to collection bias from submission of specimens later in the disease course.

Additional studies will be needed to correlate the finding of *CDKN2C* loss in LMS with prognostic data and treatment outcomes. If clinically indicated, future studies are needed to evaluate other diagnostic modalities, such as *CDKN2C* testing through immunohistochemical surrogates[58,59] or 1p/19q FISH testing. Future studies are also needed to identify the gene expression profile of this novel genomic subtype.[60] Comprehensive genomic profiling of LMS may provide insights into LMS biology and potentially inform therapeutic options, including specific cyclin-dependent kinase inhibitors.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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APPENDIX

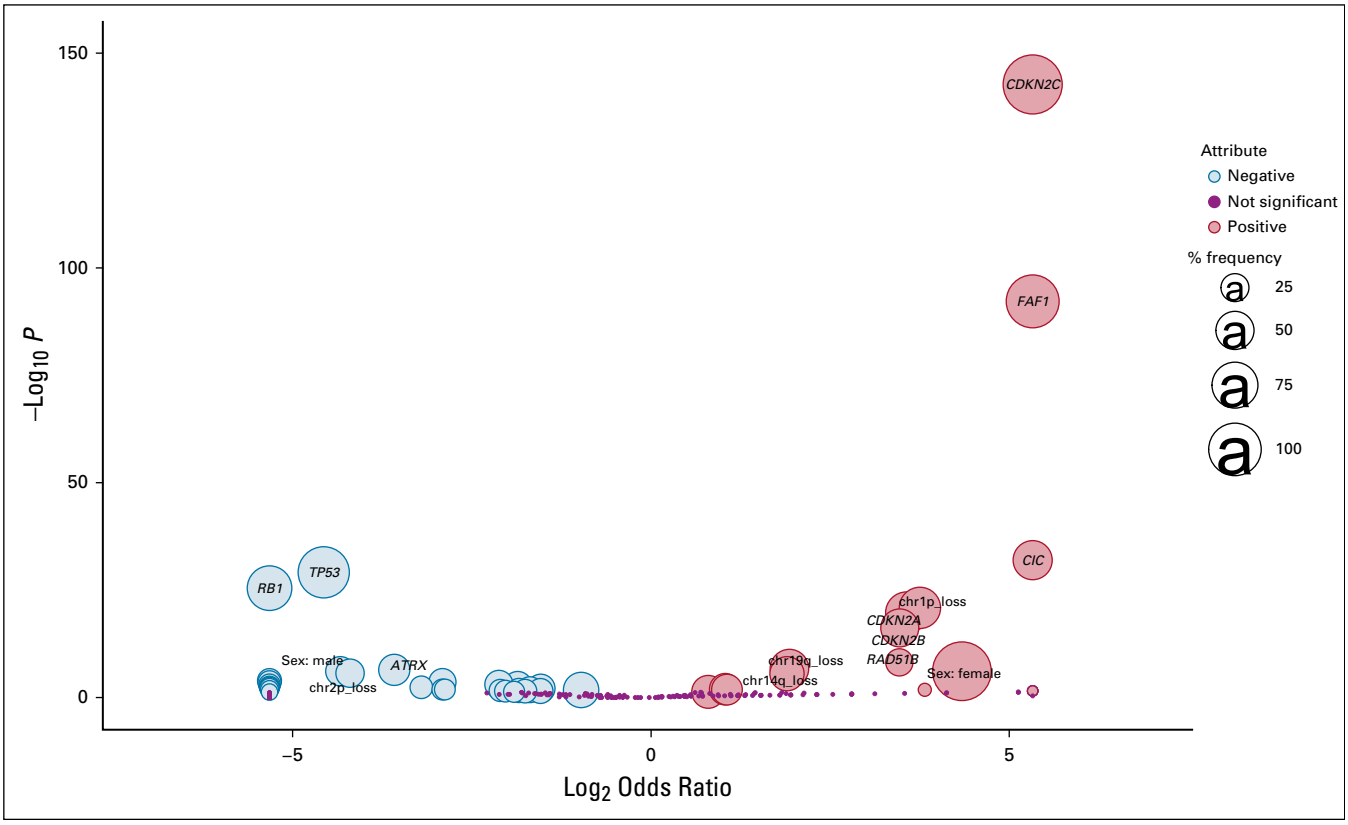

FIG A1. Volcano plot for *CDKN2C*-null leiomyosarcoma. Attributes with *P* value < .0001 are labeled. Red and blue indicate positive and negative correlation, respectively, in *CDKN2C*-null leiomyosarcoma (n = 77) compared with the remainder of the leiomyosarcoma cohort (n = 2,493). Chromosomal arm-level aneuploidy analysis was available in a subset of *CDKN2C*-null leiomyosarcoma (n = 72) and *CDKN2C*-retained leiomyosarcoma (n = 1,212).

TABLE A1. List of Sequenced Genes in the FoundationOne CDx and F1H Platforms

Gene Description	Gene Symbol																		
In FoundationOne CDx panel																			
With full coding exonic regions for detection of substitutions, indels, and copy number alterations	*ABL1*	*ACVR1B*	*AKT1*	*AKT2*	*AKT3*	*ALK*	*AMER1*	*APC*	*AR*	*ARAF*	*ARFRP1*	*ARID1A*	*ASXL1*	*ATM*	*ATR*	*ATRX*	*AURKA*	*AURKB*	*AXL*	*BAP1*
	BARD1	*BCL2*	*BCL2L1*	*BCL2L2*	*BCL6*	*BCOR*	*BCORL1*	*BRAF*	*BRCA1*	*BRCA2*	*BRD4*	*BRIP1*	*BTG1*	*BTG2*	*BTK*	*C11orf30*	*CALR*	*CARD11*	*CBFB*	*CBL*
	CCND1	*CCND2*	*CCND3*	*CCNE1*	*CD22*	*CD274*	*CD70*	*CD79A*	*CD79B*	*CDC73*	*CDH1*	*CDK12*	*CDK4*	*CDK6*	*CDK8*	*CDKN1A*	*CDKN1B*	*CDKN2A*	*CDKN2B*	*CDKN2C*
	CEBPA	*CHEK1*	*CHEK2*	*CIC*	*CREBBP*	*CRKL*	*CSF1R*	*CSF3R*	*CTCF*	*CTNNA1*	*CTNNB1*	*CUL3*	*CXCR4*	*DAXX*	*DDR2*	*DNMT3A*	*DOT1L*	*EED*	*EGFR*	*EP300*
	EPHA3	*EPHB1*	*ERBB2*	*ERBB3*	*ERBB4*	*ERG*	*ERRFI1*	*ESR1*	*EZH2*	*FAM123B*	*FAM46C*	*FANCA*	*FANCC*	*FANCG*	*FANCL*	*FAS*	*FBXW7*	*FGF10*	*FGF14*	*FGF19*
	FGF23	*FGF3*	*FGF4*	*FGF6*	*FGFR1*	*FGFR2*	*FGFR3*	*FGFR4*	*FH*	*FLCN*	*FLT1*	*FLT3*	*FOXL2*	*FUBP1*	*GABRA6*	*GATA3*	*GATA4*	*GATA6*	*GID4*	*GNA11*
	GNA13	*GNAQ*	*GNAS*	*GRM3*	*GSK3B*	*H3F3A*	*HDAC1*	*HGF*	*HNF1A*	*HRAS*	*HSD3B1*	*ID3*	*IDH1*	*IDH2*	*IGF1R*	*IKBKE*	*IKZF1*	*INPP4B*	*IRF2*	*IRF4*
	IRS2	*JAK1*	*JAK2*	*JAK3*	*JUN*	*KDM5A*	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KEL*	*KIT*	*KLHL6*	*KMT2A*	*KMT2D*	*KRAS*	*LYN*	*MAF*	*MAP2K1*	*MAP2K2*
	MAP2K4	*MAP3K1*	*MAPK1*	*MCL1*	*MDM2*	*MDM4*	*MED12*	*MEF2B*	*MEN1*	*MET*	*MITF*	*MLH1*	*MMSET*	*MPL*	*MRE11*	*MRE11A*	*MSH2*	*MSH3*	*MSH6*	*MTAP*
	MTOR	*MUTYH*	*MYC*	*MYCL*	*MYCN*	*MYD88*	*NF1*	*NF2*	*NFE2L2*	*NFKBIA*	*NKX2-1*	*NOTCH1*	*NOTCH2*	*NOTCH3*	*NPM1*	*NRAS*	*NSD2*	*NSD3*	*NT5C2*	*NTRK1*
	NTRK2	*NTRK3*	*P2RY8*	*PALB2*	*PARK2*	*PAX5*	*PBRM1*	*PDCD1*	*PDCD1LG2*	*PDGFRA*	*PDGFRB*	*PDK1*	*PIK3C2B*	*PIK3CA*	*PIK3CB*	*PIK3R1*	*PIM1*	*PMS2*	*POLD1*	*POLE*
	PPP2R1A	*PRDM1*	*PRKAR1A*	*PRKCI*	*PRKN*	*PTCH1*	*PTEN*	*PTPN11*	*PTPRO*	*QKI*	*RAC1*	*RAD21*	*RAD51*	*RAF1*	*RARA*	*RB1*	*RBM10*	*RET*	*RICTOR*	*RNF43*
	ROS1	*RPTOR*	*SDHA*	*SDHB*	*SDHC*	*SDHD*	*SETD2*	*SF3B1*	*SGK1*	*SMAD2*	*SMAD4*	*SMARCA4*	*SMARCB1*	*SMO*	*SNCAIP*	*SOCS1*	*SOX2*	*SOX9*	*SPEN*	*SPOP*
	SRC	*STAG2*	*STAT3*	*STK11*	*SUFU*	*SYK*	*TBX3*	*TERC*	*TET2*	*TGFBR2*	*TNFAIP3*	*TNFRSF14*	*TP53*	*TSC1*	*TSC2*	*U2AF1*	*VEGFA*	*VHL*	*WHSC1*	*WHSC1L1*
	WT1	*WTX*	*XPO1*	*ZNF217*	*ZNF703*															
With select intronic regions	*ALK*	*BCL2*	*BCR*	*BRAF*	*BRCA1*	*BRCA2*	*EGFR*	*ETV4*	*ETV5*	*ETV6*	*EWSR1*	*FGFR1*	*FGFR2*	*FGFR3*	*KIT*	*KMT2A*	*MSH2*	*MYB*	*MYC*	*NOTCH2*
	NTRK1	*NTRK2*	*NUTM1*	*PDGFRA*	*RAF1*	*RARA*	*RET*	*ROS1*	*SLC34A2*	*TERT*	*TMPRSS2*									
In F1H DNA panel																			
With full coding exonic regions for detection of substitutions, indels, and copy number alterations	*ABI1*	*ACTB*	*ADGRA2*	*AKT1*	*AKT2*	*AKT3*	*ALK*	*AMER1*	*APC*	*APH1A*	*AR*	*ARAF*	*ARFRP1*	*ARHGAP26*	*ARID1A*	*ARID2*	*ASMTL*	*ASXL1*	*ATM*	*ATR*
	ATRX	*AURKA*	*AURKB*	*AXIN1*	*AXL*	*B2M*	*BAP1*	*BARD1*	*BCL10*	*BCL11B*	*BCL2*	*BCL2L2*	*BCL6*	*BCOR*	*BCORL1*	*BIRC3*	*BLM*	*BRAF*	*BRCA1*	*BRCA2*
	BRD4	*BRIP1*	*BRSK1*	*BTG1*	*BTG2*	*BTK*	*BTLA*	*C11orf30*	*C17orf39*	*CAD*	*CARD11*	*CBFB*	*CBL*	*CCND1*	*CCND2*	*CCND3*	*CCNE1*	*CCT6B*	*CD22*	*CD274*
	CD36	*CD58*	*CD70*	*CD79A*	*CD79B*	*CDC73*	*CDH1*	*CDK12*	*CDK4*	*CDK6*	*CDK8*	*CDKN1B*	*CDKN2A*	*CDKN2B*	*CDKN2C*	*CEBPA*	*CHEK1*	*CHEK2*	*CIC*	*CIITA*
	CKS1B	*CPS1*	*CREBBP*	*CRKL*	*CRLF2*	*CSF1R*	*CSF3R*	*CTCF*	*CTNNA1*	*CTNNB1*	*CUX1*	*CXCR4*	*DAXX*	*DDR2*	*DDX3X*	*DNM2*	*DNMT3A*	*DOT1L*	*DTX1*	*DUSP2*
	DUSP9	*E2A*	*EBF1*	*ECT2L*	*EED*	*EGFR*	*ELP2*	*EMSY*	*EP300*	*EPHA3*	*EPHA5*	*EPHA7*	*EPHB1*	*ERBB2*	*ERBB3*	*ERBB4*	*ERG*	*ESR1*	*ETO*	*ETS1*
	ETV6	*EXOSC6*	*EZH2*	*FAF1*	*FAM123B*	*FAM46C*	*FANCA*	*FANCC*	*FANCD2*	*FANCE*	*FANCF*	*FANCG*	*FANCL*	*FAS*	*FBXO11*	*FBXO31*	*FBXW7*	*FGF10*	*FGF14*	*FGF19*
	FGF23	*FGF3*	*FGF4*	*FGF6*	*FGFR1*	*FGFR2*	*FGFR3*	*FGFR4*	*FHIT*	*FLCN*	*FLT1*	*FLT3*	*FLT4*	*FLYWCH1*	*FOXL2*	*FOXO1*	*FOXO3*	*FOXP1*	*FRS2*	*GADD45B*
	GATA1	*GATA2*	*GATA3*	*GID4*	*GNA11*	*GNA12*	*GNA13*	*GNAQ*	*GNAS*	*GPR124*	*GRAF*	*GRIN2A*	*GSK3B*	*GTSE1*	*HDAC1*	*HDAC4*	*HDAC7*	*HGF*	*HIST1H1C*	*HIST1H1D*
	HIST1H1E	*HIST1H2AC*	*HIST1H2AG*	*HIST1H2AL*	*HIST1H2AM*	*HIST1H2BC*	*HIST1H2BJ*	*HIST1H2BK*	*HIST1H2BO*	*HIST1H3B*	*HNF1A*	*HRAS*	*HSP90AA1*	*ICK*	*ID3*	*IDH1*	*IDH2*	*IKBKE*	*IKZF1*	*IKZF2*
	IKZF3	*IL7R*	*INHBA*	*INPP4B*	*INPP5D*	*IRF1*	*IRF4*	*IRF8*	*IRS2*	*JAK1*	*JAK2*	*JAK3*	*JARID2*	*JUN*	*KAT6A*	*KDM2B*	*KDM4C*	*KDM5A*	*KDM5C*	*KDM6A*
	KDR	*KEAP1*	*KIT*	*KLHL6*	*KMT2A*	*KMT2C*	*KRAS*	*LEF1*	*LMO1*	*LRP1B*	*LRRK2*	*MAF*	*MAFB*	*MAGED1*	*MALT1*	*MAP2K1*	*MAP2K2*	*MAP2K4*	*MAP3K1*	*MAP3K14*
	MAP3K6	*MAP3K7*	*MAPK1*	*MCL1*	*MDM2*	*MDM4*	*MED12*	*MEF2B*	*MEF2C*	*MEN1*	*MET*	*MIB1*	*MITF*	*MKI67*	*MLH1*	*MLL*	*MLL3*	*MMSET*	*MPL*	*MRE11*
	MRE11A	*MSH2*	*MSH3*	*MSH6*	*MTOR*	*MUTYH*	*MYC*	*MYCL*	*MYCL1*	*MYCN*	*MYD88*	*MYO18A*	*MYST3*	*NCOR2*	*NCSTN*	*NF1*	*NF2*	*NFE2L2*	*NFKBIA*	*NKX2-1*
	NOD1	*NOTCH1*	*NOTCH2*	*NPM1*	*NRAS*	*NSD1*	*NSD2*	*NT5C2*	*NTRK1*	*NTRK2*	*NTRK3*	*NUP93*	*NUP98*	*P2RY8*	*PAG1*	*PAK3*	*PALB2*	*PASK*	*PAX5*	*PBRM1*
	PC	*PCBP1*	*PCLO*	*PDCD1*	*PDCD11*	*PDCD1LG2*	*PDGFRA*	*PDGFRB*	*PDK1*	*PDL1*	*PDL2*	*PHF6*	*PIK3CA*	*PIK3CG*	*PIK3R1*	*PIK3R2*	*PIM1*	*POT1*	*POU2AF1*	*PRDM1*
	PRKAR1A	*PRKDC*	*PRSS8*	*PTCH1*	*PTEN*	*PTPN11*	*PTPN2*	*PTPN6*	*PTPRO*	*RAD21*	*RAD50*	*RAD51*	*RAF1*	*RARA*	*RASGEF1A*	*RB1*	*RELN*	*RET*	*RHOA*	*RICTOR*
	RNF43	*ROS1*	*RPTOR*	*RUNX1*	*RUNX1T1*	*S1PR2*	*SDHA*	*SDHB*	*SDHC*	*SDHD*	*SERP2*	*SETBP1*	*SETD2*	*SF3B1*	*SGK1*	*SHIP*	*SHP-1*	*SMAD2*	*SMAD4*	*SMARCA1*
	SMARCA4	*SMARCB1*	*SMC1A*	*SMC3*	*SMO*	*SOCS1*	*SOCS2*	*SOCS3*	*SOX10*	*SOX2*	*SPEN*	*SPOP*	*SRC*	*SRSF2*	*STAG2*	*STAT3*	*STAT4*	*STAT5A*	*STAT5B*	*STAT6*
	STK11	*SUFU*	*SUZ12*	*SYK*	*TAF1*	*TBL1XR1*	*TCF3*	*TCL1*	*TCL1A*	*TET2*	*TGFBR2*	*TLL2*	*TMEM30A*	*TMSB4XP8*	*TMSL3*	*TNFAIP3*	*TNFRSF11A*	*TNFRSF14*	*TNFRSF17*	*TNFRSF6*
	TOP1	*TP53*	*TP63*	*TRAF2*	*TRAF3*	*TRAF5*	*TSC1*	*TSC2*	*TUSC3*	*TYK2*	*U2AF1*	*U2AF2*	*VHL*	*WDR90*	*WHSC1*	*WISP3*	*WT1*	*WTX*	*XBP1*	*XPO1*
	YY1AP1	*ZMYM2*	*ZNF217*	*ZNF24*	*ZNF703*	*ZRSR2*	*ZSCAN3*													
With select intronic regions	*ALK*	*BCL2*	*BCL6*	*BCR*	*BRAF*	*CCND1*	*CRLF2*	*EGFR*	*EPOR*	*ETV1*	*ETV4*	*ETV5*	*ETV6*	*EWSR1*	*FGFR2*	*IGH*	*IGK*	*IGL*	*JAK1*	*JAK2*
	KMT2A	*MLL*	*MYC*	*NTRK1*	*PDGFRA*	*PDGFRB*	*RAF1*	*RARA*	*RET*	*ROS1*	*TMPRSS2*	*TRG*								

TABLE A2. Locations of Sequenced Tumor Specimens

Location	No. of Cases
Primary site	29
Uterine	25
Abdominal wall	1
Hip/gluteal	1
Sacrum	1
Small bowel	1
Metastatic site	48
Lung	7
Retroperitoneum	5
Abdominal wall	4
Limb soft tissue	4
Omentum	4
Liver	3
Paraspinal	3
Peritoneum	3
Pleura	2
Colon	2
Kidney	2
Chest wall	2
Mesentery	2
Small intestine	1
Hilar lymph node	1
Vagina	1
Posterior mediastinum	1
Heart	1

TABLE A3. Comparisons of the Frequencies of Genomic Alterations in *CDKN2C*-Null LMS Versus All Non–*CDKN2C*-Null LMS Cases, as Well as Non–*CDKN2C*-Null LMS Cases With 19/19Q-Codeletion [CIC] Mutation, *CDKN2A*, and *RAD51B* Mutations, or *ALK* Fusion

		LMS Cases Without Homozygous Deletion of *CDKN2C*					
Variable	***CDKN2C*-Null LMS**	**All *CDKN2C*-Retained LMS**	**1p/19q-Codeleted LMS**	***CIC*-Mutant LMS**	***CDKN2A*-Mutant LMS**	***RAD51B*-Mutant LMS**	***ALK*-Rearranged LMS**
No. of patient cases	77	2,493	62	35	175	43	41
Female sex, % (n/total N)	99 (76/77)	79 (1,968/2,493)	87 (54/62)	86 (30/35)	83 (146/175)	98 (42/43)	98 (40/41)
Median (range) age, years	61 (36-81)	57 (< 1 to ≥ 89)	56 (33-78)	57 (34-81)	59 (< 1-86)	57 (37-80)	58 (17-75)
Median (Q1-Q3) TMB, mut/Mb	2.4 (1.6-3.2)	2.4 (1.6-4.0)	2.5 (1.6-5.0)	2.4 (1.6-4.2)	3.2 (2.4-4.0)	3.8 (2.0-5.0)	3.2 (1.6-4.0)
MSI high	0 (0/63)	0.2 (5/2,093)	2 (1/62)	0 (0/30)	1 (1/146)	0 (0/37)	0 (0/35)
Genomic alteration, % (n/total N)							
TP53	12 (9/77)	73 (1,830/2,493)	52 (33/62)	51 (18/35)	46 (80/175)	70 (30/43)	37 (15/41)
RB1	0 (0/77)	55 (1,359/2,493)	45 (28/62)	49 (17/35)	14 (25/175)	63 (27/43)	15 (6/41)
ATRX	3 (2/77)	25 (606/2,473)	16 (10/62)	17 (6/35)	12 (20/172)	30 (13/43)	5 (2/40)
PTEN	9 (7/77)	16 (399/2,493)	13 (8/62)	17 (6/35)	9 (15/175)	19 (8/43)	5 (2/41)
1p/19q-codeletion	81 (58/72)	5 (62/1,212)	100 (62/62)	22 (4/18)	17 (14/82)	19 (5/26)	32 (6/19)
CIC	40 (31/77)	1 (35/2,473)	7 (4/62)	100 (35/35)	0 (0/172)	9 (4/43)	0 (0/40)
CDKN2A	47 (36/77)	7 (175/2,493)	23 (14/62)	0 (0/35)	100 (175/175)	5 (2/43)	63 (26/41)
RAD51B	17 (13/77)	2 (43/2,473)	8 (5/62)	11 (4/35)	1 (2/172)	100 (43/43)	5 (2/40)
ALK fusion	3 (2/77)	2 (41/2,493)	10 (6/62)	0 (0/35)	15 (26/175)	5 (2/43)	100 (41/41)
BRAF fusion	3 (2/77)	0.2 (4/2,493)	0 (0/62)	0 (0/35)	0 (0/175)	2 (1/43)	0 (0/41)
FGFR1 fusion	1 (1/77)	0.1 (2/2,493)	0 (0/62)	0 (0/35)	0 (0/175)	0 (0/43)	0 (0/41)
NTRK1 fusion	1 (1/77)	0.1 (3/2,493)	0 (0/62)	0 (0/35)	1 (2/175)	0 (0/43)	0 (0/41)

NOTE. For percent values, the number of positive cases over the number of evaluated cases is included in parentheses.
Abbreviations: LMS, leiomyosarcoma; MSI, microsatellite instability; TMB, tumor mutational burden.

TABLE A4. Results With Available Paired Specimens

	Initial Results		Paired Specimen Results		
Patient	**Specimen Site**	**Sequencing Results**	**Specimen Site**	**Sequencing Results**	**Time to Paired Specimen**
1	Primary uterine	***TP53* p.R342*, homozygous loss of *CDKN2C***	Right lung mass	***TP53* p.R342*, homozygous loss of *CDKN2C***, *FAF1* exon 1-2, *CIC* exon 1, and *RB1* exon 9-17	13 months
2	Ileum (from uterine primary)	**Homozygous loss of *CDKN2C*, *FAF1*, *CDKN2A*, *CKDN2B*, and *TP53***	Peritoneal mass	**Homozygous loss of *CDKN2C*, *FAF1*, *CDKN2A*, *CKDN2B*, *TP53***, *FAS*, and *FANCA*. *FANCD2-EMC3* rearrangement exon 34	27 months
3	Retroperitoneal mass (indeterminant primary)	**Homozygous loss of *CDKN2C***	Abdominal wall	**Homozygous loss of *CDKN2C***	22 months

NOTE. Matching genomic alterations are in bold.