

Genome profiling is an efficient tool to avoid the STUMP classification of uterine smooth muscle lesions: a comprehensive array-genomic hybridization analysis of 77 tumors

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The diagnosis of a uterine smooth muscle lesion is, in the majority of cases, straightforward. However, in a small number of cases, the morphological criteria used in such lesions cannot differentiate with certainty a benign from a malignant lesion and a diagnosis of smooth muscle tumor with uncertain malignant potential (STUMP) is made. Uterine leiomyosarcomas are often easy to diagnose but it is difficult or even impossible to identify a prognostic factor at the moment of the diagnosis with the exception of the stage. We hypothesize, for uterine smooth muscle lesions, that there is a gradient of genomic complexity that correlates to outcome. We first tested this hypothesis on STUMP lesions in a previous study and demonstrated that this 'gray category' could be split according to genomic index into two groups. A benign group, with a low to moderate alteration rate without recurrence and a malignant group, with a highly rearranged profile akin to uterine leiomyosarcomas. Here, we analyzed a large series of 77 uterine smooth muscle lesions (from 76 patients) morphologically classified as 19 leiomyomas, 14 STUMP and 44 leiomyosarcomas with clinicopathological and genomic correlations. We

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confirmed that genomic index with a cut-off = 10 is a predictor of recurrence ($P < 0.0001$) and with a cut-off = 35 is a marker for poor overall survival ($P = 0.035$). For the tumors confined to the uterus, stage as a prognostic factor was not useful in survival prediction. At stage I, among the tumors reclassified as molecular leiomyosarcomas (ie, genomic index ≥ 10), the poor prognostic markers were: 5p gain (overall survival $P = 0.0008$), genomic index at cut-off = 35 (overall survival $P = 0.0193$), 13p loss including *RB1* (overall survival $P = 0.0096$) and 17p gain including *MYOCD* gain (overall survival $P = 0.0425$). Based on these findings (and the feasibility of genomic profiling by array-comparative genomic hybridization), genomic index, 5p and 17p gains prognostic value could be evaluated in future prospective chemotherapy trials.

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Uterine sarcomas are rare neoplasms accounting for 4% of all uterine tumors with an estimated incidence of 0.86 per 100 000 women.¹ Uterine smooth muscle lesions include very common benign tumors such as leiomyomas and malignant as leiomyosarcomas.² The overall incidence of leiomyomas is 70–80% by 50 years of age.³ Leiomyomas have a benign behavior despite the benign metastasizing leiomyoma and leiomyomatoses, whereas leiomyosarcomas eventually lead to death through recurrences in up to 75% of cases.^{4,5}

The diagnosis of uterine smooth muscle lesions, based on the Stanford criteria (three morphological features: presence of cytologic atypia, mitotic count and tumor cell necrosis),⁶ is straightforward in the majority of the cases. However, sometimes morphology is confusing and introduces a degree of subjectivity in the interpretation of these criteria. External factors such as prior treatments (hormonal therapies) or a non-optimal fixation of the sample may pose diagnostic challenges. Hence, such lesions are usually classified as smooth muscle tumors of uncertain malignant potential (STUMP).² This classification could result in a risk of under or over diagnosis with clinical consequences for the treatment that could impact the patient.

The FIGO stage is still the most important uterine leiomyosarcomas prognostic factor and forms the basis of the therapeutic strategy.^{4,7–9} However, FIGO staging fails to identify patients with high risk of death who could potentially be eligible for chemotherapy.^{10–13} Even if this disease is clinically very aggressive,⁵ it is difficult to predict the outcome especially when the diagnosis is made at stage I (confined to the uterus). Recently, a specific uterine leiomyosarcomas nomogram for predicting post-resection 5-year overall survival using seven clinicopathological items (age, tumor size, tumor grade, cervical involvement, loco-regional metastases, distant metastases and mitotic index) was published¹⁴ and was subsequently validated on an independent series.¹⁵ In this nomogram, the mitotic index and the tumor grade were taken into account as biological parameters. Nevertheless, the tumor grading in uterine leiomyosarcomas is controversial because by definition leiomyosarcomas diagnosed on the basis of Stanford criteria⁶ are of high grade. Contrary to other soft tissue sarcomas, the tumor grade does

not show a prognostic value in uterine leiomyosarcomas.¹⁶ Among the seven parameters, three (presence of regional metastasis, distant metastasis and size) are strictly linked to the FIGO stage.¹⁴

Hence, there is a need to clarify these prognostic strategies. A few years ago, we published a new classification method based on genomic profiling complementary to the morphology able to distinguish within the STUMP category those uterine smooth muscle lesions with a risk of recurrence and poor outcomes from benign lesions.¹⁷

In this study, we analyzed the genomic profile by array-comparative genomic hybridization in a series of 77 uterine smooth muscle lesions (44 leiomyosarcomas, 14 STUMP and 19 leiomyomas) to validate the power of genomic index as a recurrence predictor. Furthermore, we set out to improve our previous results by studying a larger series with a broader follow-up and to identify overall survival prognostic factors in uterine leiomyosarcomas.

Materials and methods

Tumor Samples

Seventy-seven formalin-fixed and paraffin-embedded uterine smooth muscle lesions from 76 patients (for one patient, both the primary tumor and the recurrence were examined) were collected in France through the French sarcoma network (RRePS and GYN RRePS) (Institut Bergonié of Bordeaux, Centre Oscar Lambret of Lille, Hôpitaux Universitaires Lyon Sud, Centre JF LeClerc, Dijon, Hôpital Universitaire of Poitiers, Centre Jean Perrin, Clermont-Ferrand, Centre Alexis Vautrin, Vandoeuvre-les Nancy, Institut Gustave Roussy, ICO, Paul Papin, Angers), from Belgium (Hôpitaux Universitaires de Bruxelles, Catholic University of Leuven), Czech Republic (University Hospital from Prague) and Switzerland (Hôpitaux Universitaires and Argot-Lab of Lausanne). Fourteen uterine tumors diagnosed as STUMP along with 19 uterine leiomyomas previously published¹⁷ were included in the series. The tumors were diagnosed between 1977 and 2013. For each patient, 1–8 slides were available (mean: 2 slides). All cases were centrally reviewed by one of the authors (SC) and classified according to Stanford

criteria⁶ and 2014 WHO guidelines for female reproductive organs.² Cytologic atypia was evaluated at medium power magnification (objective $\times 10$) according to the presence of high nuclear size or nuclear pleomorphism and hyperchromatism,¹⁸ the mitotic count was evaluated on 10 high-power fields (objective $\times 40$, field diameter 0.53 mm) and the tumor cell necrosis defined by an abrupt transition from necrotic to non-necrotic areas without interposed granulation tissue.¹⁸ The mitotic cut-off was 10 mitoses/10 power fields for spindle cell smooth muscle tumors, ≥ 4 mitoses for epithelioid and ≥ 2 mitoses for myxoid tumors.^{2,18}

Frozen material was available for five samples. The samples from the tumor archives of each participating department were centralized in the Biological Resources Center of Institut Bergonié, which the French authorities authorized for scientific research (AC-2008-812).

DNA and RNA Extraction

Genomic DNA was extracted from formalin-fixed and paraffin-embedded tissues according to the protocol for DNA isolation from formalin-fixed and paraffin-embedded tissues (http://www.chem-agilent.com/pdf/G441090020v3_1_CGH_ULS_Protocol.pdf) (Agilent Technologies, Santa Clara, CA, USA). A cut-off of 50% of cellularity in tumor samples was set for the analysis.

Array-Comparative Genomic Hybridization Analysis

DNA was hybridized onto 8×60 K whole-genome arrays (G4450A; Agilent Technologies) according to the manufacturer's protocol. Microarray slides were scanned using a DNA Microarray Scanner, images were analyzed by Feature Extraction V10.1.1.1 followed by Agilent Cytogenomic software 4.0. The ADM-2 algorithm of the Comparative Genomic Hybridization Analytics v4.0.76 software (Agilent Technologies) was used to identify the DNA copy number anomalies at the probe level. A low-level copy number gain was defined as a log 2 ratio > 0.25 and a copy number loss was defined as a log 2 ratio < -0.25 . A high-level gain or amplification was defined as a log 2 ratio > 1.5 and a homozygous deletion was suspected when the ratio was < -1 . The range for derivative log ratio spread cut-off was fixed to 0.50. Genomic index was calculated for each profile as follows: genomic index = A^2/C , where A is the total number of alterations (segmental gains and losses) and C is the number of involved chromosomes.^{19,17}

Statistical Analysis

Metastasis-free survival was calculated by the Kaplan-Meier method from the date of initial

diagnosis to the date of first metastasis or last follow-up. Overall survival, using the Kaplan-Meier method, was calculated from the date of diagnosis to death or last follow-up. Survival curves were compared with the log-rank test. Univariate survival analyses were performed by using the R software version 3.4.0 (R Development Core Team, Vienna, Austria, 2009) and the 'survival' package (A Package for Survival Analysis in S; Terry Therneau, February 2002; R package, version 2.40-1). To test whether gene alterations (losses or gains) are enriched in groups of tumors classified by the genomic index, Fisher's exact tests were performed. Multivariate survival analyses were performed using Cox regression with Firth's correction with the 'coxphf' package (Georg Heinze and Meinhard Ploner, 2016, version 1.12).

Results

Pathologic Features

After centralized pathological review, the series comprised 19 leiomyomas, 14 STUMP and 44 leiomyosarcomas. Morphologically, all leiomyomas but one case of bizarre nuclei leiomyoma, showed conventional spindle cell morphology. Spindle cell features were observed in the STUMP group (all 14 tumors).

Among the 44 leiomyosarcomas (43 patients), 29/44 tumors showed a spindle cell morphology, 10/44 epithelioid, 4/44 pleomorphic with giant osteoclastic cells (with negative stains for melanocytic markers) and 1/44 myxoid (Figure 1). The clinicopathological data are summarized in Table 1.

Genomic Data and Clinical Correlations

Genome complexity evaluation by genomic index assessment (quantitative approach) and prognostic value. Array-comparative genomic hybridization was analyzable in all 77 tumors. Follow-up data were available for all but two patients with leiomyomas (mean follow-up: 63.6 months, range: 9–232 months). The genomic profiling split the present series of uterine smooth muscle lesions in two groups according to the cut-off defined in our previous paper (genomic index = 10):¹⁷ a group with genomic index < 10 (19/74) and a second group with genomic index ≥ 10 (55/74; Figure 2a).

The first group (genomic index < 10) is characterized by a low level of chromosomal rearrangements (Figure 3a; mean genomic index: 2.3, range: 0–9.14) in contrast with the second group (genomic index ≥ 10) harboring complex genomic profiles (mean genomic index: 51.8, range: 11–180; Figure 3b).

The Kaplan-Meier analysis demonstrated a significant difference in clinical outcome with no recurrence in the group with genomic index < 10

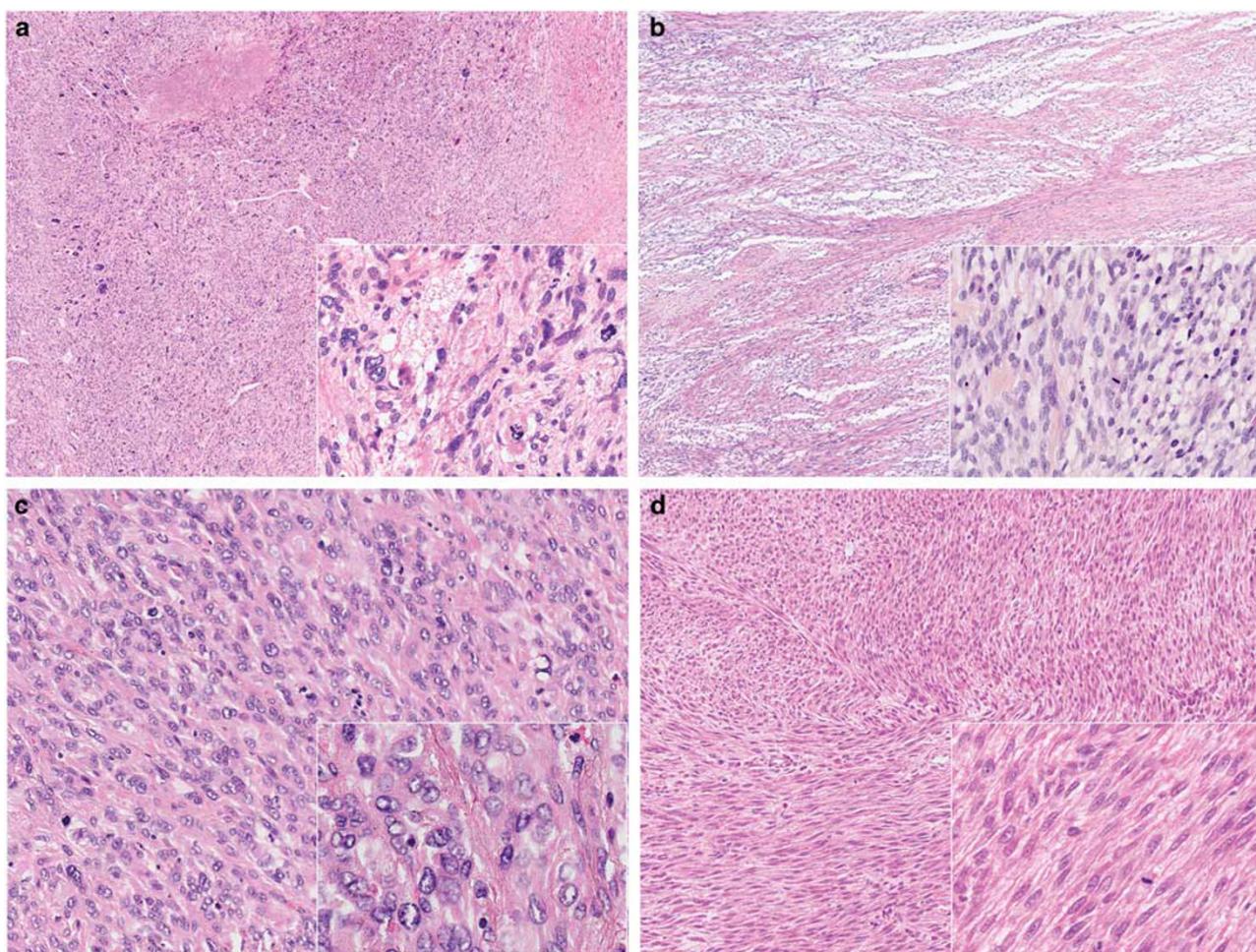


Figure 1 A representation of the morphological features of uterine smooth muscle lesions. (a) Uterine leiomyosarcoma with spindle cells, pleomorphic and osteoclastic features and atypical mitoses, stage FIGO IB and genomic index = 84. The patient developed lung metastases and died 32.4 months after the diagnosis (patient 68). (b) Myxoid leiomyosarcoma with infiltration of myometrial wall. Some areas are highly cellular with four mitoses, stage FIGO 2B and genomic index = 13.5 (patient 47). (c) Epithelioid leiomyosarcoma with genomic index = 27. The patient died of disease 26 months after the diagnosis with peritoneal metastases (patient 48). (d) Smooth muscle lesion with mild atypia, 20 mitoses (STUMP) with genomic index = 14.3. The patient had multiple peritoneal and retroperitoneal recurrences and she is alive after 103.2 months (patient 33).

and recurrences and deaths in the second group (Figure 2b), confirming our previous results.¹⁷

Morphologically, the first group included all leiomyomas, two STUMPs and no leiomyosarcomas. The second group included all leiomyosarcomas and 12 STUMPs. Only four patients with leiomyosarcomas did not recur (of note, one patient died of pulmonary embolism 9 days after surgery) and among the 12 STUMPs with genomic index ≥ 10 , seven recurred (Table 1).

All leiomyomas showed a flat or very simple profile (mean genomic index: 1.9, range: 0–9.1). The leiomyosarcomas group showed a complex, rearranged chromosome profile with numerous intra-chromosomal breaks (gains and losses). The mean genomic index in the leiomyosarcomas group was 55 (range: 13.5–180). The STUMP group was then split into two groups according to genomic index. Owing to the clinical aggressiveness and outcome of the

tumors with genomic index ≥ 10 , they were thereafter considered as leiomyosarcomas.

Therefore, the second step was to identify a death predictor in this newly defined group of leiomyosarcomas. Different genomic index cut-offs (five for each step) were tested and a cut-off of genomic index = 35 was identified as an efficient predictor of death ($P=0.035$, HR = 2.18 (1.04–4.58); Figure 2c).

In order to identify specific chromosomal alterations (qualitative approach) related to prognostic value, we analyzed differential penetrance plots among the patients alive, whose tumor had genomic index ≥ 10 (19/55) and patients who died of the disease (36/55). We identified five frequent alterations, in addition to genomic index ≥ 35 (Figure 2c), associated with overall survival: 5p gain (Figure 2d, Table 2a), 1p gain, 13q loss (including *RB1*) and 17p gain including *MYOCD* (Table 2a). Staging being the gold standard for clinical/pathological prognosis, all

Table 1

Patient	FU status	Clinical data						Genomic data											
		RFS		OS		FIGO stage	Size cm	Location	Type of surgery	Centralized diagnosis	Morphology	FH features		5p gain	1p gain	17p gain	17p loss	13 loss	GI
		Age months	months	Recurrence	Type of recurrence														
1	A-NOS	40	53.6	53.6	No		4	Uterus	Hysterectomy R0	LM	Spindle	No	0	0	0	0	0	0	
2	A-NOS	49	75.3	75.3	No		3.7	Uterus	Hysterectomy R0	LM	Spindle	No	0	0	0	0	0	1	
3	A-NED	50	68.6	68.6	No		5.5	Uterus	Hysterectomy R0	LM	Spindle	Yes	0	0	0	0	0	0	
4	A-NOS	63	37	37	No		1.4	Uterus	Hysterectomy R0	LM	Spindle	No	0	0	0	0	0	0	
5	Lost	45					3.5	Pelvis/ peritoneum	Total resection R0	LM	Spindle	No	0	0	0	0	0	4	
6	A-NED	46	58.4	58.4	No		4	Uterus	Total hysterectomy R0	LM	Spindle	Yes	0	0	0	0	0	2	
7	A-NED	48	53	53	No		15	Uterus	Total hysterectomy R0	LM	Spindle	Yes	0	0	0	0	0	2	
8	A-NED	49	72.9	72.9	No		6.5	Uterus	Total hysterectomy R0	LM	Spindle	Yes	0	0	0	0	0	0	
9	DOC	67	21.3	21.3	No		7	Large ligament	Total resection R0	LM	Spindle	No	0	0	0	0	0	2	
10	Lost	48					5.5	Uterus	Total hysterectomy R0	LM	Spindle cellular	Yes	0	0	0	0	0	5	
11	A-NED	32	101.5	101.5	No		6.5	Uterus	Myomectomy R0	LM	Spindle	Yes	0	0	0	0	0	0	
12	A-NED	44	102	102	No		4.5	Uterus	Total hysterectomy R0	LM	Spindle	No	0	0	0	0	0	0	
13	A-NED	67	102.1	102.1	No		1.5	Uterus	Total hysterectomy R0	LM	Spindle	No	0	0	0	0	0	0	
14	A-NED	68	102.2	102.2	No		1.2	Uterus	Total hysterectomy R0	LM	Spindle	Yes	0	0	0	0	0	0	
15	A-NED	40	137.8	137.8	No		4.5	Uterus	Total hysterectomy R0	LM	Spindle	Yes	0	0	0	0	0	1	
16	A-NED	36	137.2	137.2	No		15	Uterus	Myomectomy R0	LM	Spindle	Yes	0	0	0	0	0	1	
17	A-NED	44	175.2	175.2	No		4.5	Uterus	Total hysterectomy R0	LM	Spindle	Yes	0	0	0	0	0	1	
19	A-NED	80	93.8	93.8	No	Residual disease	ND	15	Pelvis/ uterus	STUMP	Spindle	Yes	0	0	0	0	0	3	
20	A-NED	36	84.2	84.2	No		7	Uterus	Total hysterectomy R0	LM	BN-LM	Yes	0	0	0	-2 TP53	-2 RB1	9	
21	A-NED	34	128.9	128.9	No		ND	NA	Uterus	Myomectomy R0	STUMP	Spindle	Yes	0	0	0	0	0	8.3
22	A-NED	47	99.9	99.9	No		6	Uterus	Total hysterectomy R0	LM	Spindle	No	0	0	0	0	0	9.14	
23	A-NED	63	51.6	51.6	No		IB	7	Uterus	Total hysterectomy	STUMP	Spindle	Yes	0	0	0	0	0	56.81
24	A-NED	42	NA	61.2	No		IB	8	Uterus	Subtotal hysterectomy	STUMP	Spindle	No	0	0	0	0	0	32.6
25	A-NED	47	NA	99.6	No		IB	7	Uterus	Total hysterectomy	STUMP	Spindle	Yes	0	0	0	0	0	14.28
26	AWD	48	36	162.0	Yes	Bladder, rectum, omentum, para-aortic LN, lung	IB	7	Uterus	Total hysterectomy	STUMP	Spindle	Yes	0	0	0	0	-1RB1	16.9

Table 1 (Continued)

Patient	FU status	Clinical data										Genomic data										S Croce et al		
		RFS		OS		Recurrence	Type of recurrence	FIGO stage	Size		Type of surgery	Centralized diagnosis	Morphology	FH features	5p gain		1p gain		17p gain		17p loss		GI	
		Age months	months						cm	Location					0	0	0	0	0	0	-1	no TP53	-1	RB1
27	AWD	50	57	104.4	Yes	Paravertebral soft tissue, bone	I	NA	Uterus	Total hysterectomy	STUMP	Epithelioid	No	0	0	0	0	0	-1	no TP53	-1	RB1	22.23	
28	A-NOS	77	17	25.2	Yes	Peritoneum, ileum, vagina, pelvis	IB	11	Uterus	Total hysterectomy	STUMP	Spindle	No	0	1	0	0	0	-1	no TP53	-2	RB1	48	
29	A-NED	60	NA	42.0	No		IA	2	Uterus	Myomectomy	STUMP	Spindle	No	0	1	2 ampl MYOC	0	0	-2	RB1	52			
30	DOD	66	55	55.2	Yes	Vagina	IB	8	Uterus	Total hysterectomy	STUMP	Spindle	Yes	0	0	0	0	0	-1	no TP53	-1	RB1	21.33	
31	A-NED	85	NA	106.8	No		IB	11	Uterus	Total hysterectomy	STUMP	Epithelioid	No	0	1	0	0	0	-1	no TP53	-1	RB1	94.1	
32	DOD	43	10	15.6	Yes	Bone	IB	20	Uterus	Total hysterectomy	STUMP	Spindle	Yes	0	1	2 ampl MYOC	-1	TP53	-1	RB1	100			
33	A-NED	46	35	103.2	Yes	Peritoneum, right ovary, retroperitoneum	IA	5	Uterus	Total hysterectomy	STUMP	Spindle	Yes	0	0	0	0	0	0	0	0	0	14.3	
34	A-NED	48	12	147.6	Yes	Uterine cervix, peritoneum, soft tissue (leg and arm)	I	NA	Uterus	Total hysterectomy	STUMP	Spindle	Yes	0	0	0	0	0	0	0	0	0	11	
35	DOD	55	26.4	56.4	Yes	Pelvis, peritoneum	III	NA	Uterus	Total hysterectomy	LMS	Spindle	Yes	0	0	0	0	0	0	0	0	0	44.64	
36	DOD	51	26.4	61.2	Yes	Soft tissue, bone, lung	IB	9	Uterus	Total hysterectomy	LMS	Epithelioid	Yes	0	0	0	0	0	-2	RB1	156			
37	DOD	56	136.8	171.6	Yes	Lung	IA	1.8	Uterus	Total hysterectomy	LMS	Epithelioid	No	0	1	0	-1	TP53	-2	RB1	18			
38	DOD	50	19.2	57.6	Yes	Lung	IB	7	Uterus	Total hysterectomy	LMS	Spindle	Yes	0	0	0	-2	TP53	-2	RB1	22			
39	DOD	59	21.6	48	Yes	Lung	IA	3	Uterus	Total hysterectomy	LMS	Spindle	Yes	0	0	1	MYOCD	-1	TP53	-2	RB1	52.26		
40	AWD	40	72	110.4	Yes	Clavicular LN, lung, pancreas	IB	19	Uterus	Total hysterectomy	LMS	Spindle	Yes	0	0	1	MYOCD	-2	TP53	-1	RB1	25		
40	AWD	45	NA	NA	—		NA	NA	Uterus	Total hysterectomy	LMS	Spindle	No	0	0	1	MYOCD	-2	TP53	-1	RB1	69		
41	DOD	40	0	13.2	Yes	Lung	IVB	8	Uterus	Total hysterectomy	LMS	Spindle	Yes	0	1	0	-1	TP53	-1	RB1	28.9			
42	DOD	63	8.5	27.6	Yes	Lung	IB	7	Uterus	Total hysterectomy	LMS	Spindle	No	0	1	0	0	0	-2	RB1	19.6			
43	DOD	53	9	27.6	Yes	Lung	III	2.8	Uterus	Total hysterectomy	LMS	Spindle	No	1	1	1	MYOCD	-1	TP53	-1	RB1	69		
44	DOD	62	1.6	34.8	Yes		IA	4.5	Uterus	Total hysterectomy	LMS	Spindle	Yes	1	0	2	MYOCD	-1	TP53	-2	RB1	40		
45	AWD	63	1.2	84	Yes	Lung	IB	10	Uterus	Total hysterectomy	LMS	Spindle	No	0	0	0	0	0	-2	RB1	15.12			
46	DOD	68	ND	6	ND	ND	IIB	12	Uterus	Total hysterectomy	LMS	Epithelioid	Yes	0	1	1	MYOCD	0	0	-1	RB1	46		
47	DOC pulmonary embolism	62	NA	0	9 days	No	IIB	8	Uterus	Total hysterectomy	LMS	Myxoid	No	0	0	0	0	0	0	0	0	13.5		
48	DOD	71	13.2	26	Yes	Peritoneum	III	12	Uterus	Total hysterectomy	LMS	Epithelioid	No	0	0	2 ampl MYOC	-2	TP53	-1	RB1	27			

Table 1 (Continued)

Patient	FU status	Clinical data						Genomic data											
		RFS Age months	OS months	Recurrence	Type of recurrence	FIGO stage	Size cm	Location	Type of surgery	Centralized diagnosis	Morphology	FH features	5p gain	1p gain	17p gain	17p loss	13 loss	GI	
49	DOD	57	19.2	23	Yes	Lung	IB	7.5	Uterus	Total hysterectomy	LMS	Spindle	No	0	0	0	0	-1RB1	36
50	DOD	38	27.6	ND	Yes	Lung	IB	7.5	Uterus	Total hysterectomy	LMS	Spindle	No	0	0	1MYOCD	0	-1RB1	41.66
51	DOD	47	12	32	Yes	Vagina, bladder, rectum	I	NA	Uterus	Total hysterectomy	LMS	Spindle	No	0	0	1MYOCD	0	0	44
52	DOD	59	0	3	Yes	Lung	IVB	23	Uterus	Total hysterectomy	LMS	Spindle	Yes	0	0	0	0	0	33
53	DOD	87	1.6	3.2	Yes	Peritoneum, ileum	IIB	11	Uterus	Total hysterectomy	LMS	Epithelioid	No	1	1	1MYOCD	0	-2 RB1	38.4
54	DOD	58	0	2.9	Yes	Lung	IVB	6	Uterus	Total hysterectomy	LMS	Spindle	No	1	1	1 no MYOCD	0	-1RB1	24.9
55	DOD	26	25.2	48	Yes	Soft tissue, lung	IA	4	Uterus	Total hysterectomy	LMS	Spindle	No	1	1	1MYOCD	-1 TP53	-2 RB1	141
56	DOD	60	0	ND	Yes	Peritoneum, omentum	III	15	Uterus	Total hysterectomy	LMS	Pleomorphic osteoclastic	No	1	1	0	0	-1RB1	35
57	DOD	63	2.8	8.2	Yes	Lung, peritoneum, brain	IB	10	Uterus	Total hysterectomy	LMS	Pleomorphic osteoclastic	Yes	0	1	0	0	-1RB1	24.9
58	A-NED	76	NA	104.4	No		IA	2	Uterus	Total hysterectomy	LMS	Epithelioid	No	0	0	0	0	0	18.7
59	DOD	67	7	12	Yes	Peritoneum	III	20	Uterus	Total hysterectomy	LMS	Spindle	No	0	0	0	0	-1 RB1	17.28
60	DOD	27	12	19.2	Yes	Peritoneum	IIB	8	Uterus	Total hysterectomy	LMS	Spindle	Yes	0	0	1MYOCD	-2 TP53	-2RB	39.7
61	DOD	44	0	13.2	Yes	Lung, peritoneum	IVB	16	Uterus	Total hysterectomy	LMS	Spindle	No	1	1	1 no MYOCD	-1 TP53	-1 RB1	88.47
62	DOD	54	73.2	102	Yes	Lung	IB	7.5	Uterus	Total hysterectomy	LMS	Spindle	No	0	0	0	-1 TP53	-1 RB1	60
63	AWD	59	14.4	75.6	Yes	Lung, liver, peritoneum	IVA	6	Uterus	Total hysterectomy	LMS	Spindle	Yes	0	0	1 no MYOCD	-2 TP53	-2 RB1	115
64	DOD	58	50.4	232.8	Yes	Lung	I	NA	Uterus	Total hysterectomy	LMS	Spindle	Yes	0	0	1 no MYOCD	0	0	48
65	DOD	36	2.9	9.4	Yes	Vagina, peritoneum	IB	6	Uterus	Total hysterectomy	LMS	Spindle	No	1	0	2 MYOCD	-2 TP53	-2RB	82
66	A-NED	53	NA	57.6	No		IB	6	Uterus	Total hysterectomy	LMS	Spindle	No	0	0	0	-1 TP53	-2RB	45
67	DOD	63	10.6	18	Yes	Lung, bone, pelvis	IB	10	Uterus	Total hysterectomy	LMS	Spindle	No	1	0	1MYOCD	0	-2 RB1	101
68	DOD	60	1.1	32.4	Yes	Lung	IB	9	Uterus	Total hysterectomy	LMS	Pleomorphic osteoclastic	No	1	1	1MYOCD	0	-1 RB1	84
69	A-NED	43	NA	42	No		IB	6.5	Uterus	Total hysterectomy	LMS	Epithelioid	Yes	0	0	0	0	0	32
70	DOD	58	1.1	3.4	Yes	Peritoneum	IVA	14	Uterus	Total hysterectomy	LMS	Epithelioid	Yes	1	1	1MYOCD	0	-1 RB1	72.2
71	DOD	76	2.3	2.6	Yes	Lung, peritoneum	III	7	Uterus	Total hysterectomy	LMS	Spindle	Yes	1	1	1MYOCD	-1 TP53	-2RB	64.69
72	A-NED	69	4.5	96	Yes	Lung	IB	30	Uterus	Total hysterectomy	LMS	Epithelioid	Yes	0	0	1MYOCD	0	-1 RB1	180
73	AWD	69	21.6	58.8	Yes	Lung	III	13	Uterus	Total hysterectomy	LMS	Spindle	No	0	0	0	0	-1 RB1	33.9

Table 1 (Continued)

Clinical data										Genomic data								
Patient FU status	RFS	OS	Age months	months	Recurrence	Type of recurrence	FIGO stage	Size cm	Location	Type of surgery	Centralized diagnosis	Morphology	FH features	5p gain	17p gain	17p loss	13 loss	GI
74 DOD	80	1.3	2.6	Yes	Peritoneum, bone, lung	III	14	Uterus	Total hysterectomy	LMS	Epithelioid	Yes	1	1	1 no	-1 TP53-1 RB1	114.33	
75 DOD	55	NA	39.6	ND	ND	IB	10	Uterus	Total hysterectomy	LMS	Spindle	Yes	0	0	0	-2 RB1	56.81	
76 DOD	57	48	78.0	Yes	Pelvis, peritoneum, uterus	IB	7	Uterus	Total hysterectomy	LMS	Spindle	Yes	0	0	1 MYOCD	0	-1 RB1 42.88	
77 DOD	53	8.1	57.6	Yes	Peritoneum, liver, lung	IB	13	Uterus	Total hysterectomy	LMS	Pleomorphic	Yes	1	0	1 MYOCD	0	-1 RB1 60.84	

Abbreviations: A-NED: alive not evidence of disease; A-NOS: alive not otherwise specified; AWD: alive with disease; DOD: dead of disease; DOC: dead of other disease; GI: genomic index; MYOCD: myocardin gene; OS: overall survival; RFS: relapse-free survival; -1 heterozygous loss; -2 homozygous loss; +1 gain; +2 amplification.

markers (including genomic index ≥ 35) were tested for independency against the stage in a multivariate analysis. Stage and 5p gain were shown to be statistically independent prognostic factors at multivariate analysis (stage: $P < 0.001$, HR = 4.36 (1.82–10.42), 5p gain: $P = 0.04$, HR = 3.24 (1.04–10.04); Table 2a). Given that stage I tumors can have a risk of metastasis, we then further refined staging by testing molecular prognostic factors within stage. Among the tumors with genomic index ≥ 10 and stage I (37/55 patients), four overall survival prognostic factors were still significant: 5p gain ($P < 0.001$, HR = 4.88 (1.74–13.7); Figure 2e), genomic index at the cut-off of 35 ($P = 0.0193$, HR = 3.2 (1.15–8.92); Figure 2f), 13 chromosome loss including *RB1* ($P = 0.0096$, HR = 9.04 (1.2–67.81)) (Supplementary Figure 1A) and 17p gain including *MYOCD* ($P = 0.0425$, HR = 2.45 (1–5.97)) (Supplementary Figure 1B).

Among the other clinicomorphological parameters tested, the presence of moderate and marked atypia (Fisher's $P = 0.043$), the presence of tumor necrosis (Fisher's $P = 0.001$) and a mitotic index (cut-off ≥ 20 ; *t*-test $P < 0.001$) were poor prognostic markers for overall survival (Table 2b, Supplementary Figure 1C). For these parameters, multivariate analysis showed that genomic index ≥ 35 remained significantly independent ($P = 0.0333$; Table 2c).

Correlation Between Chromosomal Alterations and Morphology

No correlation was found between genomic index and morphology of the tumor (spindle, epithelioid or myxoid). No correlation was observed between any specific genomic alteration (5p gain, 17p loss, 13 loss, 17p gain) and tumor morphology (spindle vs epithelioid).

Discussion

In the last 5 years, many analyses based on whole-genome approaches have improved our knowledge of uterine smooth muscle lesion biology.^{20–22} Nevertheless, the routine diagnostic practice lacks complementary diagnostic tools. For uterine leiomyosarcomas, despite very aggressive clinical features,⁵ it is difficult to predict the outcome, especially when the diagnosis is made at stage I (tumor confined to the uterus).

According to the literature,^{8,10,23} adjuvant treatment of a stage I uterine leiomyosarcomas is an option without evidence of benefit. There is a need for clinical trials to highlight the benefit of chemotherapy in terms of overall survival and relapse-free survival. In absence of clinical prognostic markers, the identification of new genomic prognostic markers appears critical for setting up clinical trials aiming to evaluate new treatments in uterine leiomyosarcomas. Genomic prognostic markers

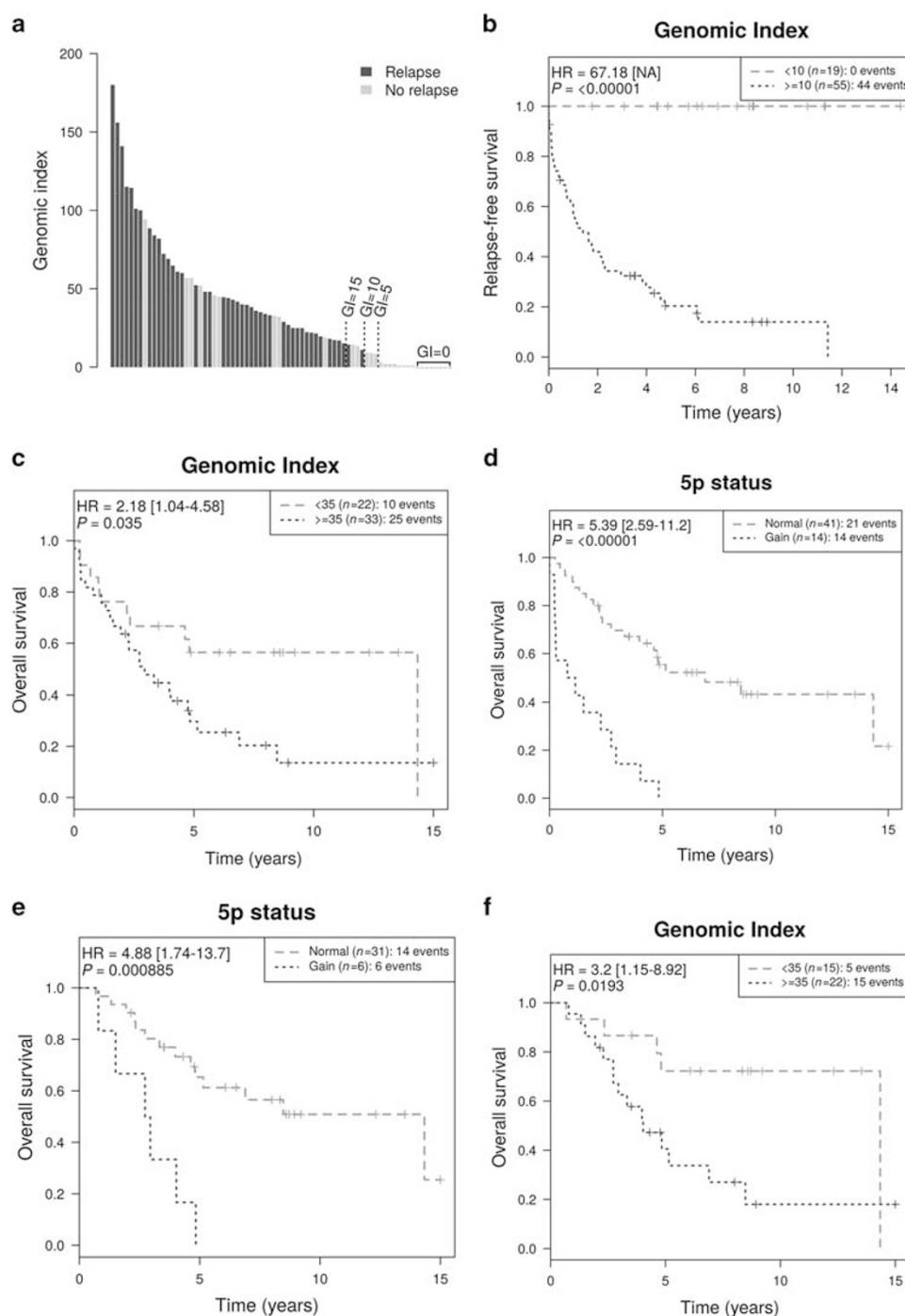


Figure 2 Genomic index and genomic alterations and clinical outcome. (a) Histogram. The number of patients with relapse (black bar) and without relapse (gray bar) shown in x axis; the genomic index level in y axis. The genomic index at the cut-off of 10 splits the population of 74 patients with uterine smooth muscle lesion in a group of 19 patients without metastases and a group of 55 patients with 44 metastatic events. (b) Kaplan-Meier relapse-free survival analysis of 74 uterine smooth muscle tumors according the genomic index at the cut-off of 10. (c) Kaplan-Meier for overall survival according to genomic index at the cut-off of 35 in the subgroup with genomic index ≥ 10 (genomic leiomyosarcomas). (d) Kaplan-Meier analysis of overall survival in the group with genomic index ≥ 10 (genomic leiomyosarcomas) with 5p gain. (e) Kaplan-Meier analysis of overall survival in the group with genomic index ≥ 10 (genomic leiomyosarcomas) with 5p gain with tumors limited to the uterus (stage I). (f) Kaplan-Meier analysis of overall survival in the group with genomic index ≥ 10 (genomic leiomyosarcomas) with tumors limited to the uterus (stage I) according to genomic index at the cut-off of 35.

could have an essential role in deciding on adjuvant chemotherapy for stage I uterine leiomyosarcomas. In our series of 37 molecular leiomyosarcomas (genomic index ≥ 10) at stage I, four genomic

prognostic markers correctly separated the outcomes for patients alive or dead: 5p gain, chromosome 13 loss including *RB1*, proximal 17p gain with *MYOCD* and genomic index ≥ 35.



Figure 3 Penetrance plots of the different subgroups classified according to genomic index. (a) Penetrance plot of the tumors with genomic index < 10 (benign tumors: all leiomyomas and two STUMPs). (b) Penetrance plot of tumors with genomic index > 10 (malignant tumors: all leiomyosarcomas and 12 STUMPs). (c) Penetrance plot of tumors with genomic index > 10 (malignant tumors: all leiomyosarcomas and 12 STUMPs) of patients alive. (d) Penetrance plot of tumors with genomic index > 10 (malignant tumors: all leiomyosarcomas and 12 STUMPs) of patients dead of disease.

Further analyses are required to understand whether this is due to a chromosomal mechanism (specific or general) or due to genes located in these regions that are specifically overexpressed as a consequence of a chromosomal gain. The 5p gain was previously reported in extra-uterine²⁴ and uterine leiomyosarcomas²⁵ but no association with outcome was observed. The 17p proximal gain, including the *MYOCD* gene, was previously reported in literature in soft tissue leiomyosarcomas^{24,26-28} and in uterine leiomyosarcomas.²⁵ *MYOCD* gene induces smooth muscle differentiation and promotes

cell migration.²⁷ In a human uterine leiomyosarcoma cell line, *MYOCD* induced a phenotypic cell switch from a dedifferentiated to a differentiated smooth muscle phenotype.²⁹ *MYOCD* expression level controls smooth muscle differentiation protein expression and has an impact on cell migration in soft tissue leiomyosarcomas.²⁷ Furthermore, it confers aggressive outcome in soft tissue sarcomas.³⁰

*Hu et al*²⁵ found a gain of 17p in 38% of the uterine leiomyosarcomas and interestingly, no 17p gain was found in alive patients (4/19). Chromosome 13 was lost in 80% of the leiomyosarcomas in our

Table 2 Statistical data: univariate and multivariate analyses for OS of the subgroup with GI > 10 (malignant tumors)

OS	Univariate		Multivariate	
	P-value	HR	P-value	HR
Stage	< 0.001	4.32 (2.03–9.2)	< 0.001	4.36 (1.82–10.42)
5p gain	< 0.001	5.39 (2.59–11.2)	0.0042	3.24 (1.04–10.04)
1p gain	< 0.001	3.3 (1.66–6.56)	0.2989	1.61 (0.66–3.96)
GI ≥ 35	0.0349	2.18 (1.04–4.58)	0.8419	1.10 (0.44–2.71)
17p loss TP53	0.1119	1.72 (0.87–3.36)	0.4807	0.75 (0.33–1.68)
13 loss RB1	0.0103	4.19 (1.28–13.77)	0.1860	2.44 (0.65–9.19)
17p gain MYOCD	0.0054	2.57 (1.29–5.13)	0.6188	1.29 (0.48–3.47)

Abbreviations: GI: genomic index; HR: hazard ratio; OS: overall survival.
Bold values signify the significant results.

Table 2B Univariate analysis for RFS and OS of the subgroup with GI > 10 (malignant tumors)

	RFS	OS
Atypia	P = 0.04918	P = 0.04345
Mitoses cut-off 20	P < 0.001	P < 0.001
Necrosis	P = 0.0963	P = 0.001

Abbreviations: GI: genomic index; OS: overall survival; RFS: relapse-free survival.
Bold values signify the significant results.

Table 2C Multivariate analysis for OS for stage I LMS

OS	P-value	HR
GI ≥ 35	0.0333	3.10 (1.09–10.78)
Atypia	0.0837	10.89 (0.77–>100)
Mitoses cut-off 20	0.128	0.48 (0.09–1.63)
Necrosis	0.263	0.48 (0.09–1.63)

Abbreviation: OS: overall survival.
Bold values signify the significant results.

series and is the most common genomic event in uterine (76%)²⁵ and extra-uterine leiomyosarcomas (ranging from 54%^{28,24} to 71%²⁶) and in the majority of the cases, correlation between this event and follow-up was not established.

The morphological analysis based on the presence of atypia, mitotic count and tumor cell necrosis correlated to a poor outcome in our series. The prognostic value of cytological atypia,^{31,32} as well the mitotic count^{5,33,34} was reported in previous publications. However, atypia and mitoses could be difficult to assess and there is only a moderate inter-observer agreement on tumor cell necrosis in uterine smooth muscle lesions among gynecological pathologists.³⁵ Genomic index assessment could be a useful tool, as highlighted in our multivariate analysis (Table 2c), to avoid such diagnostic discrepancies.

In our series, the genomic index cut-off of 10 splits the STUMP group into two: a flat or very simple genomic profile group akin to leiomyomas and a group of tumors with complex genomic profile similar to

leiomyosarcomas (with recurrences and deaths) thereby erasing the STUMP category. In the benign lesions group with genomic index < 10 (all leiomyomas and two STUMP), there were no chromosomal alterations such as RB1 and TP53 loss (Table 1). One exception is the bizarre nuclei leiomyoma case, with a borderline genomic index = 9, which showed chromosome 13 loss including the RB1 gene and chromosome 17p loss including the TP53 gene. These alterations have already been reported in these benign lesions.^{36–39} In fact, some bizarre nuclei leiomyomas inexplicably show rearranged profiles (in our experience lower than leiomyosarcomas) and a good outcome. Furthermore, the origin of this subtype of leiomyoma is not clear either.

Genomic profiling by array-comparative genomic hybridization on formalin-fixed and paraffin-embedded samples is a useful, easy and accessible tool complementary to the morphological approach. It is a diagnostic tool that splits the STUMP category into benign (leiomyoma) and malignant (leiomyosarcomas) tumors. It is a prognostic marker and a predictor of overall survival in stage I uterine leiomyosarcomas. Indeed, all the comparative genomic hybridization analyses on our series were performed on formalin-fixed and paraffin-embedded and -extracted DNA, with 100% feasibility. Genomic profiling could be used even on a limited amount of material such as pre-operative biopsies in order to guide surgical intervention (hysterectomy vs myomectomy or a minimally invasive surgery).

In conclusion, we have demonstrated that STUMP classification could be overcome by utilizing genomic index at the cut-off of 10. The 5p gain as genomic event and the stage as clinical parameter are poor overall survival prognostic factors in uterine leiomyosarcomas. In stage I tumors, the 5p gain, 17p gain, chromosome 13 loss and genomic index at the cut-off of 35 are poor prognostic factors of overall survival and therefore they could be potential parameters for randomization in prospective clinical trials. This approach opens the way to new insights into uterine and other gynecological smooth muscle lesions and would allow reclassification of lesions

according to genomic complexity. In fact, there is a continuum gradient of genomic complexity and instability correlating with tumor aggressiveness. As genomic profiling by array-comparative genomic hybridization on formalin fixed and paraffin embedded is feasible and accessible in hospital laboratories, this approach could be used in routine practice as a complement to histology.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Modern Pathology website (<http://www.nature.com/modpathol>)