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Dedifferentiated leiomyosarcoma of the uterus: a clinicopathologic and immunohistochemical analysis of 23 cases

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Dedifferentiated leiomyosarcoma of the uterus: a clinicopathologic and immunohistochemical analysis of 23 cases

Aims: To morphologically and immunophenotypically characterize dedifferentiated uterine leiomyosarcoma (LMS).

Methods and results: We identified 23 dedifferentiated uterine LMS, defined as a malignant uterine smooth muscle tumour containing discrete differentiated and dedifferentiated components (i.e. with and without morphologic and immunophenotypic evidence of smooth muscle differentiation, respectively). The differentiated component was leiomyosarcoma in most cases (17/23), though some arose from a leiomyoma (n = 4) or smooth muscle tumour of uncertain malignant potential (n = 2). The dedifferentiated tumour component showed noncohesive polygonal cells with moderate to abundant cytoplasm, pleomorphic nuclei with coarse vesicular to smudged chromatin, one or more macronucleoli, frequent multinucleation, and atypical mitoses. Three cases showed heterologous osteosarcomatous or chondrosarcomatous differentiation. Immunohistochemistry revealed alterations characteristic of uterine LMS, including Rb loss (18/19); strong diffuse p16 (17/19); strong diffuse (9/19) or complete absence of (5/19) p53; and ATRX loss (6/16). Compared to a control cohort of uterine LMS without dedifferentiation, dedifferentiated uterine LMS showed significantly shorter disease-specific (median, 54 versus 20 months; 5-year DSS, 46% versus 36%; P = 0.04) and disease-free (median, 31 versus 8 months; 5-year DFS, 42% versus 8%; P = 0.002) survival. Of 19 dedifferentiated uterine LMS with follow-up, 12 had died of disease at median 14 (range, 2–73) months; four were alive with disease at 4, 12, 44, and 50 months; and three were alive with no evidence of disease at 56, 109, and 114 months.

Conclusion: Routine prospective recognition of dedifferentiated uterine LMS and distinction from mimics is advocated for accurate prognostication and for further characterisation of these tumours.

Keywords: fumarate hydratase, immunohistochemistry, leiomyosarcoma, uterus

Introduction

Leiomyosarcoma (LMS) accounts for 50–70% of uterine mesenchymal malignancies,[1-5] with an annual incidence of approximately 1 per 100,000 women in the United States.[2,5,6] Hysterectomy is the mainstay

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of treatment for resectable disease, with adjuvant chemotherapy and/or radiation for women with extrauterine spread (stage II–IV).[7]

Dedifferentiation has been described in several mesenchymal malignancies—including liposarcoma, chondrosarcoma, chordoma, solitary fibrous tumour, and LMS[8–11]—and heralds more aggressive clinical behaviour. Dedifferentiated LMS had been most often reported in soft-tissue sites.[12–15] In contrast, reports of dedifferentiated uterine LMS are limited.[12,16–21] We hypothesised that dedifferentiation in uterine LMS is likely underrecognised, and that, when present, it heralds particularly aggressive behaviour, warranting prospective recognition.

Materials & Methods

COHORT

This retrospective study was approved by the Institutional Review Board at Brigham and Women's Hospital (BWH) (2017P001291) with waiver of consent.

In keeping with prior publications of dedifferentiated soft-tissue LMS,[12] dedifferentiated uterine LMS was defined by two distinct components:

1. One or more *differentiated* smooth muscle components, showing
   i. morphological features of smooth muscle differentiation, including fascicular architecture, abundant eosinophilic cytoplasm, and cigar-shaped nuclei, or features characteristic of epithelioid or myxoid smooth muscle tumours, and
   ii. immunophenotypic evidence of smooth muscle differentiation, defined as ≥5% tumour cells staining for smooth muscle actin (SMA), desmin, or caldesmon.
2. A *dedifferentiated* component, without diagnostic smooth muscle morphology and with at most rare cells positive for any smooth muscle immunomarker. Heterologous differentiation was regarded as a form of dedifferentiation, in keeping with prior reports.[13,16,22]

Tumours with dedifferentiated morphology (i.e. a discrete component lacking morphologic features of smooth muscle differentiation, associated with a differentiated smooth muscle component) but with retained expression of at least one smooth muscle marker in >5% of tumour cells were termed "pleomorphic uterine LMS," in keeping with prior work.[14]

A search of the electronic medical records identified 1177 uterine LMS diagnosed between August 4, 1989 and June 15, 2020, including 157 in-house surgeries, 861 Dana-Farber Cancer Institute (DFCI) referrals, and 159 diagnostic consultations. Review of diagnostic reports identified 22 tumours with features of dedifferentiation noted at original diagnosis. Materials were available for review from 14 of these, of which 12 satisfied criteria for dedifferentiated uterine LMS.

Further, all available haematoxylin and eosin (H&E)-stained slides (median 7; range, 1–24) were reviewed for 341 BWH/DFCI tumours and 83 tumours from a collaborating Italian consortium. Thirteen BWH/DFCI and three Italian consortium tumours showed morphologic features of dedifferentiation, of which nine and two, respectively, were also negative for smooth muscle immunomarkers. (To permit a more accurate estimate of incidence, DFCI referrals were reviewed for inclusion only if the patient was referred within 3 months of original diagnosis and with no interval disease progression.)

The final cohort comprised 23 dedifferentiated uterine LMS, including 12 BWH in-house surgeries, five DFCI referrals, four diagnostic consultations to one of the authors (M.R.N.), and two tumours from the Italian consortium.

CLINICAL AND OUTCOMES PARAMETERS

Clinical and outcomes data were obtained from the electronic medical record, including age, stage, treatment history, date of diagnosis, date of first recurrence, and date of and clinical status at last follow-up.

PATHOLOGIC PARAMETERS

Gross findings were documented from pathology reports. All H&E-stained slides for the final cohort were reviewed at a two-headed microscope by two gynaecological pathologists (D.B.C., M.R.N.). The histologic classification of the differentiated smooth muscle component(s) was determined by WHO criteria.[23] Morphologic parameters were annotated separately for the differentiated and dedifferentiated components and are detailed in Appendix S1. In brief, nuclear atypia was classified by the four-tier Broders system: no to mild atypia (1+), moderate atypia without pleomorphism (2+), scattered pleomorphic cells (3+), and diffuse pleomorphism (4+).[24] Mitoses were counted per 2.4 mm^2 (10 high-power [400×] fields with diameter 0.55 mm) in foci of greatest mitotic activity. Atypical mitoses were defined by tripolar or multipolar mitotic spindles. Coagulative necrosis was

characterised by a sharp demarcation between viable and nonviable tumour, most often showing an irregular geographic pattern. Lymphovascular invasion was defined by the tumour protruding into or entirely within the endothelial lined spaces outside of the main tumour mass. The amount of the dedifferentiated component was recorded as the percentage of the entire tumoral mass. Within the dedifferentiated component, heterologous elements and tumoral inflammation were also documented.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry for SMA, desmin, and caldesmon was performed on all tumours for study inclusion. Additional immunostains were performed on 19 tumours with available tissue, including oestrogen receptor (ER), progesterone receptor (PR), SMARCA4 (BRG1), SMARCB1 (INI1), retinoblastoma protein (Rb), p16, methylthioadenosine phosphorylase (MTAP), ATRX, and p53. Immunostaining specifications are in Table S1. For ER and PR, extent and intensity of staining were noted. SMARCA4, SMARCB1, Rb, ATRX, and MTAP were scored as retained (normal) or lost (aberrant), with stromal and inflammatory cells as positive internal controls. P16 was scored as negative (no identifiable staining), patchy, or strong diffuse ("block positivity"). P53 staining profiles were divided into three groups: complete absence of p53 staining, heterogeneous (staining of variable intensity in <80% of tumour cells), and strong diffuse (strong staining in ≥80% of tumour cells). (Note that although we applied p53 staining thresholds used in high-grade serous carcinoma, we used descriptive terms for each staining group, as high-quality modern studies validating the correlation between p53 immunohistochemistry and *TP53* molecular alterations have not been performed in uterine LMS.) All immunostains were scored separately in dedifferentiated and differentiated tumour components.

STATISTICAL ANALYSES

All statistical analyses were performed in SAS 9.4 (SAS Institute, Cary, NC, USA). Statistical significance was defined by $P < 0.05$, and all P values were two-sided. Categorical variables were compared by the chi-squared test, and continuous variables by the Mann–Whitney U test.

Survival data were available for 19 patients with dedifferentiated uterine LMS (unavailable for one in-house surgery and three diagnostic consults). These tumours were compared to a control cohort of 345 uterine LMS without dedifferentiation (i.e. conventional spindled, epithelioid, or myxoid uterine LMS) meeting the following criteria: (i) diagnosis of uterine LMS confirmed on morphologic and immunohistochemical review, (ii) uterine corpus primary site, (iii) in-house hysterectomy, or patient referred within 3 months of diagnosis with no interval disease progression, and (iv) clinical follow-up available.

Survival curves were plotted using the Kaplan–Meier method and compared by the log-rank test. The principal outcome was disease-specific survival (DSS), defined by the interval between first pathologic diagnosis and death from uterine LMS. For patients surgically resected to no evidence of disease, disease-free survival (DFS) was also calculated, defined by the interval between surgery and first clinical, radiographic, or pathologic evidence of recurrence or death from disease.

Results

CLINICAL PARAMETERS

Clinicopathologic details for each case are in Table 1. The median age at diagnosis was 57 (range, 42–90) years. All 23 dedifferentiated uterine LMS were primary to the uterine corpus. One tumour was stage IA, 12 stage IB, four stage II, two stage IIIB, and four stage IV. Surgical margins were negative in all 13 stage IA or IB tumours. Two (#15, #18) were surgically morcellated. Nonsurgical treatment history was known for 13 patients: five (three stage IV, two stage IB) received adjuvant therapy (four chemotherapy, one radiation therapy) after hysterectomy, and an additional five patients received chemotherapy following recurrence.

GROSS PATHOLOGY

Gross descriptions were available for 19 tumours. Median tumour size was 10 (range, 3–25) cm. Ten were infiltrative transmural masses replacing the uterine wall; four, two, and one were discrete intramural, subserosal, and submucosal masses, respectively; and two were polypoid in the endometrial cavity, including one presenting as myoma nascens. Tumours were grossly infiltrative ($n = 16$) or circumscribed ($n = 3$), pink-tan to yellow to orange-brown, and soft to fleshy, with gross necrosis in 13 and gross haemorrhage in 10. Seven were grossly biphasic (Figure 1), with grossly discrete elements corresponding to differentiated and dedifferentiated morphology on microscopic examination.

Table 1. Clinical, pathologic, and immunohistochemical findings for 23 dedifferentiated uterine leiomyosarcomas

Case #	Age	Size (cm)	FIGO stage (2018)	Outcome	DFS (months)	DSS (months)	Adjuvant therapy	Original diagnosis	Morphology, differentiated component	% Dediff	Rb		p16		MTAP		p53		ATRX	
											Diff	Dediff	Diff	Dediff	Diff	Dediff	Diff	Dediff	Diff	Dediff
1	88	.	IB	DOD	2	2	.	LMS, high grade	LMS, spindle	30	Ret	Los	+/−	+++	Ret	Ret	+++	+++	Ret	Ret
2	66	6.5	IB	DOD	7	11	No known therapy	LMS, high grade	LMS, spindle	20	.	.	.	.	.	.	.	.	.	.
3	68	8.5	IB	DOD	3	20	No therapy at initial diagnosis; got chemotherapy after recurrence	LMS, high grade	LMS, spindle + cellular leiomyoma	40	Los	Los	+++	+++	Ret	Ret	het	het	Ret	Ret
4	42	10	IB	DOD	.	23	No known therapy	LMS, high grade (epithelioid)	Cellular leiomyoma	50	.	.	.	.	.	.	.	.	.	.
5	61	7	IB	DOD	.	5	.	LMS, high grade	LMS, epithelioid	5	Los	Los	+++	+++	.	.	+++	+++	Los	Los
6	57	12	IIIB	.	.	.	.	LMS, high grade, with pleomorphic features	LMS, spindle	15	Ret	Ret	0	0	Ret	Ret	0	0	Los	Los
7	46	12.1	II	DOD	23	31	.	LMS, high grade	LMS, spindle	20	Los	Los	+++	+++	Ret	Ret	het	het	Los	Los
8	54	8.2	IB	DOD	.	73	.	LMS, high grade	LMS, spindle	10	Los	Los	+++	+++	.	.	0	0	.	.
9	59	6.8	IB	NED	114	114	Gemcitabine-paclitaxel at initial diagnosis	LMS, high grade, with areas of pleomorphic undifferentiated sarcoma	LMS, spindle	40	Los	Los	+++	+++	.	.	0	0	Ret	Ret
10	50	3	IIIB	AWD	12	12	.	LMS	LMS, spindle	50	.	.	.	.	.	.	.	.	.	.
11	53	4.5	IA	AWD	21	50	.	LMS	STUMP, spindle	30	Los	Los	+/−	+++	.	.	het	het	Los	Los
12	63	11.7	IV	DOD	8	16	Gemcitabine-paclitaxel at initial diagnosis	LMS, high grade, with heterologous osteosarcomatous differentiation	LMS, spindle	10	.	.	.	.	.	.	.	.	.	.
13	53	8	IV	DOD	n/a	15	Gemcitabine-paclitaxel at initial diagnosis	LMS	LMS, spindle	25	Los	Los	+++	+++	Ret	Ret	0	0	Ret	Ret
14	54	25	IV	DOD	n/a	3	No known therapy	LMS, high grade, with areas of pleomorphic undifferentiated sarcoma	LMS, spindle	20	Los	Los	+++	+++	Ret	Ret	+++	+++	Los	Los
15	52	20	IV	DOD	n/a	10	Gemcitabine-paclitaxel at initial diagnosis	LMS, high grade, with heterologous osteosarcomatous and chondrosarcomatous differentiation	LMS, spindle	70	Los	Los	+++	+++	.	.	het	+++	.	.

Table 1. (*Continued*)

Case #	Age	Size (cm)	FIGO stage (2018)	Outcome	DFS (months)	DSS (months)	Adjuvant therapy	Original diagnosis	Morphology, differentiated component	% Dediff	Rb		p16		MTAP		p53		ATRX	
											Diff	Dediff	Diff	Dediff	Diff	Dediff	Diff	Dediff	Diff	Dediff
16	57	9	IB	NED	4	109	No therapy at initial diagnosis; got chemotherapy after recurrence	Dedifferentiated leiomyosarcoma	Cellular leiomyoma	50	Los	Los	+/−	+++	.	.	het	het	Ret	Los
17	90	20	II	.	.	.	.	Dedifferentiated leiomyosarcoma	LMBN	50	Ret	Los	+/−	+++	Ret	Ret	het	+++	Ret	Ret
18	54	14.5	II	NED	2	56	No therapy at initial diagnosis; got chemotherapy and radiation after recurrence	Pleomorphic sarcoma, arising in a cellular leiomyoma	Cellular leiomyoma	20	Los	Los	0	+++	Ret	Ret	het	+++	Ret	Ret
19	70	11	IB	AWD	15	44	Adjuvant radiation therapy at initial diagnosis	Dedifferentiated leiomyosarcoma	STUMP, spindle	40	Ret	Los	+/−	+++	Ret	Ret	het	+++	Ret	Ret
20	75	6.1	IB	.	.	.	.	Dedifferentiated leiomyosarcoma	LMS, spindle	70	Ret	Los	0	0	Ret	Los	het	+++	Ret	Ret
21	55	10	IB	AWD	4	4	No therapy at initial diagnosis; got gemcitabine-paclitaxel after recurrence	Dedifferentiated leiomyosarcoma	LMS, spindle	40	Los	Los	+++	+++	Ret	Ret	+++	+++	Ret	Ret
22	60	14.3	IB	DOD	8	13	No therapy at initial diagnosis; got doxorubicin after recurrence	Dedifferentiated leiomyosarcoma	LMS, spindle + LMBN + leiomyoma	10	Ret	Los	+/−	+++	Ret	Ret	het	het	Ret	Ret
23	63	7.7	II	.	.	.	.	Dedifferentiated leiomyosarcoma	LMS, spindle	70	Los	Los	+/−	+++	.	.	0	0	.	.

Abbreviations: "." indicates unknown or missing data point; +++, strong diffuse expression; +/−, patchy expression; 0, complete absence of staining; AWD, alive with disease; Dediff, dedifferentiated component; DFS, disease-free survival; Diff, differentiated component; DOD, dead of disease; DSS, disease-specific survival; het, heterogeneous staining; LMS, leiomyosarcoma; Los, loss of expression; n/a, not applicable (patient not operated to no evidence of disease); NED, alive with no evidence of disease; Ret, retained expression; STUMP, smooth muscle tumour of uncertain malignant potential.

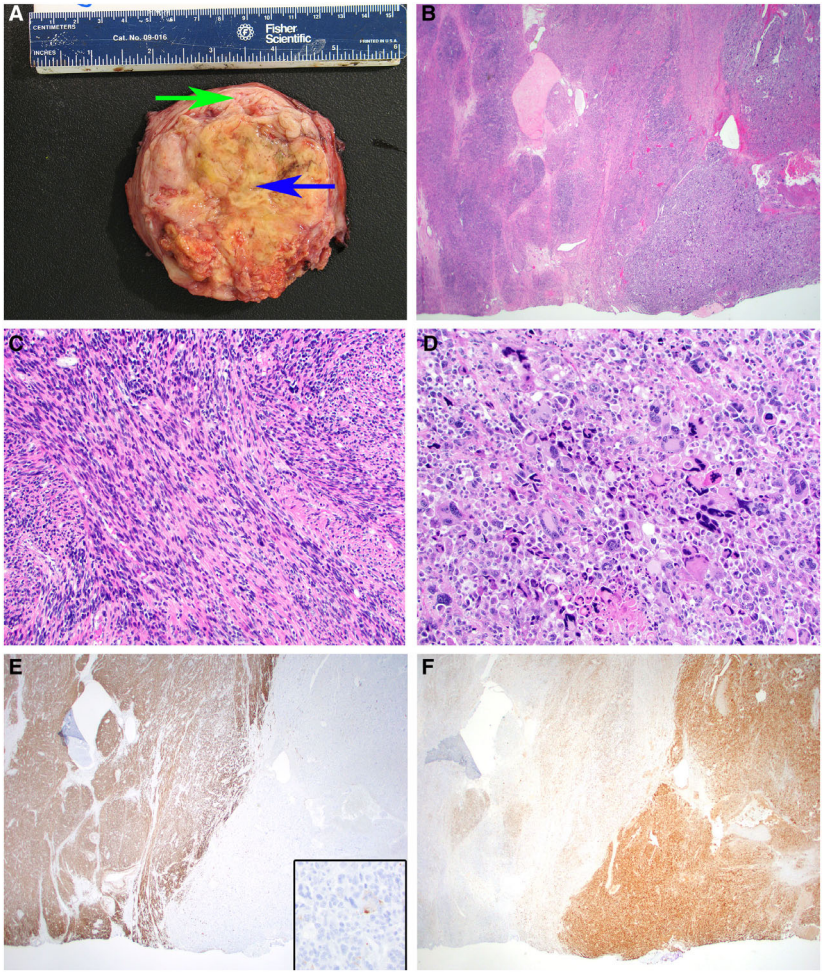

Figure 1. Dedifferentiated uterine leiomyosarcoma. The tumour is grossly biphasic, with tan-white cellular leiomyomatous (**A**, green arrow) and yellow dedifferentiated (**A**, blue arrow) components, corresponding to discrete components of cellular leiomyoma (**B**, left; **C**) and dedifferentiated leiomyosarcoma (**B**, right; **D**). The cellular leiomyoma component is diffusely positive for desmin (**E**, left) and shows patchy p16 (**F**, left), whereas the dedifferentiated leiomyosarcoma component shows only a few individual cells positive for desmin (**E**, right & inset) and strong diffuse p16 (**F**, right).

MORPHOLOGY

General

Original pathologic diagnoses are in Table 1. Dedifferentiated, undifferentiated, pleomorphic, or heterologous morphology was mentioned in the original diagnostic report in 12 of 23 tumours, with seven diagnosed prospectively as dedifferentiated uterine LMS (all seven between 2013–2020). The dedifferentiated component of the remaining 11 tumours was discovered on retrospective morphologic review. In all cases, dedifferentiation was identified in the hysterectomy.

On microscopic examination, the dedifferentiated component constituted 5–70% (median, 30%) of the tumour. The tumour–myometrial interface was represented in 22 of 23 tumours and was circumscribed (n = 3), microscopically infiltrative (n = 3), or grossly infiltrative (n = 16). Lymphovascular invasion was present in 12 tumours and included the differentiated component in seven and the dedifferentiated component in five.

Recurrent or metastatic tumour was biopsied in five cases, of which two comprised differentiated LMS only and three showed only dedifferentiated tumour (including one with osteosarcomatous differentiation).

Dedifferentiated components

In all 23 tumours, the dedifferentiated component showed “malignant fibrous histiocytoma-like” morphology, characterised by large noncohesive polygonal cells with moderate to abundant pale eosinophilic

to amphophilic foamy cytoplasm; large pleomorphic nuclei with coarse vesicular to smudged chromatin; and one or more macronucleoli (Figure 2). Eighteen tumours showed rhabdoid foci, characterised by abundant eosinophilic cytoplasm and an eccentrically placed nucleus. Multinucleated tumour giant cells were seen in 18 cases, with prominent nuclear pseudoinclusions in five. Seven showed multiple tumour nuclei arranged in a peripheral ring (Figures 2C and 3C). Three tumours (#7, #12, #15) showed osteosarcomatous differentiation (Figure 3A), with two also showing chondrosarcomatous differentiation (Figure 3B). Mitoses ranged from 2 to 80 (median, 20) per 2.4 mm^2, with atypical mitoses in all 23 (Figure 3C) and coagulative necrosis in 19.

In two tumours, the dedifferentiated component was intimately admixed with the differentiated component, with cords of dedifferentiated tumour snaking through a differentiated background. In the remaining cases, the components were discrete (Figure 4). In seven, the dedifferentiated foci were confined to the luminal tumour aspect (abutting the endometrial cavity), with conspicuous osteoclast-like giant cells in two (Figure 3D) and abundant cholesterol clefts, foamy histiocytes, and hemosiderin deposition in one (Figure 3E–G).

Tumour vasculature in dedifferentiated foci was inconspicuous, principally comprising small arterioles, with one case each showing prominent hyalinised arteriolar walls, branching staghorn vessels, or sinusoidal vessels. Tumour stroma most often comprised scant collagenous fibres, though hyalinised bands separated individual tumour cells in six cases. Prominent lymphohistiocytic inflammation was seen in seven,

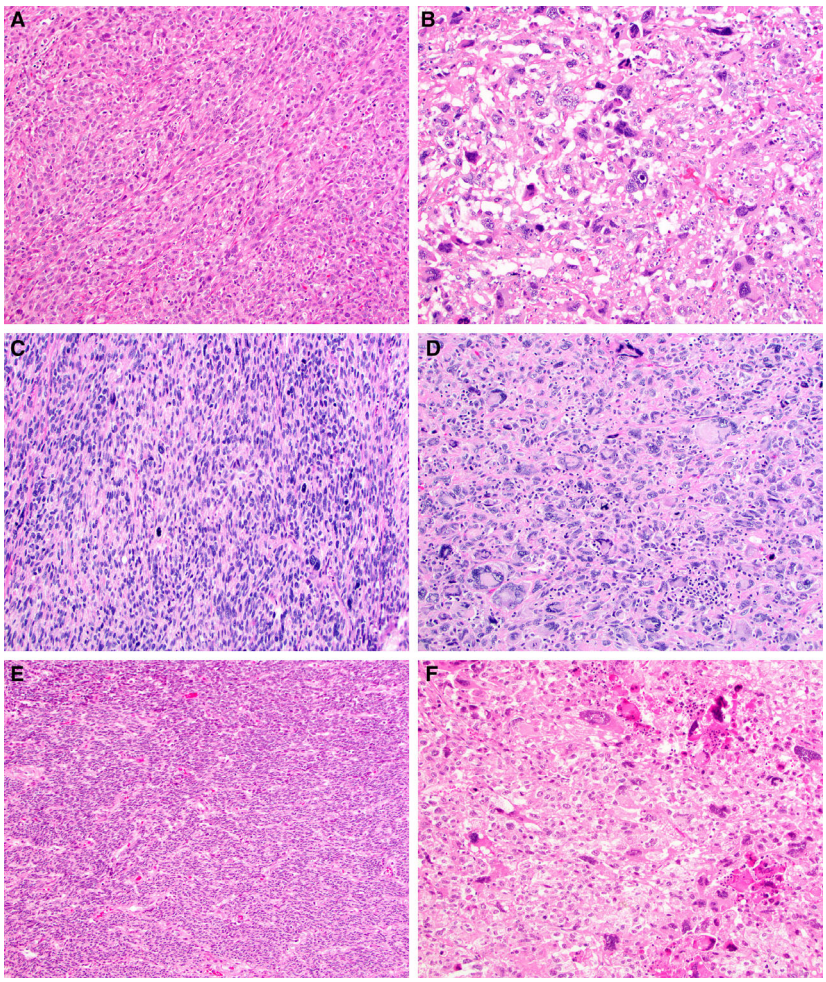

Figure 2. Dedifferentiated uterine leiomyosarcoma, three examples. (**A**,**B**) This tumour has a component of conventional leiomyosarcoma (**A**) and a dedifferentiated component with malignant fibrous histiocytoma-like morphology (**B**). (**C**,**D**) A second tumour shows a component of conventional leiomyosarcoma (**C**) and a dedifferentiated component with prominent multinucleated tumour giant cells (**D**). A third tumour comprises cellular leiomyomatous (**E**) and dedifferentiated (**F**) components.

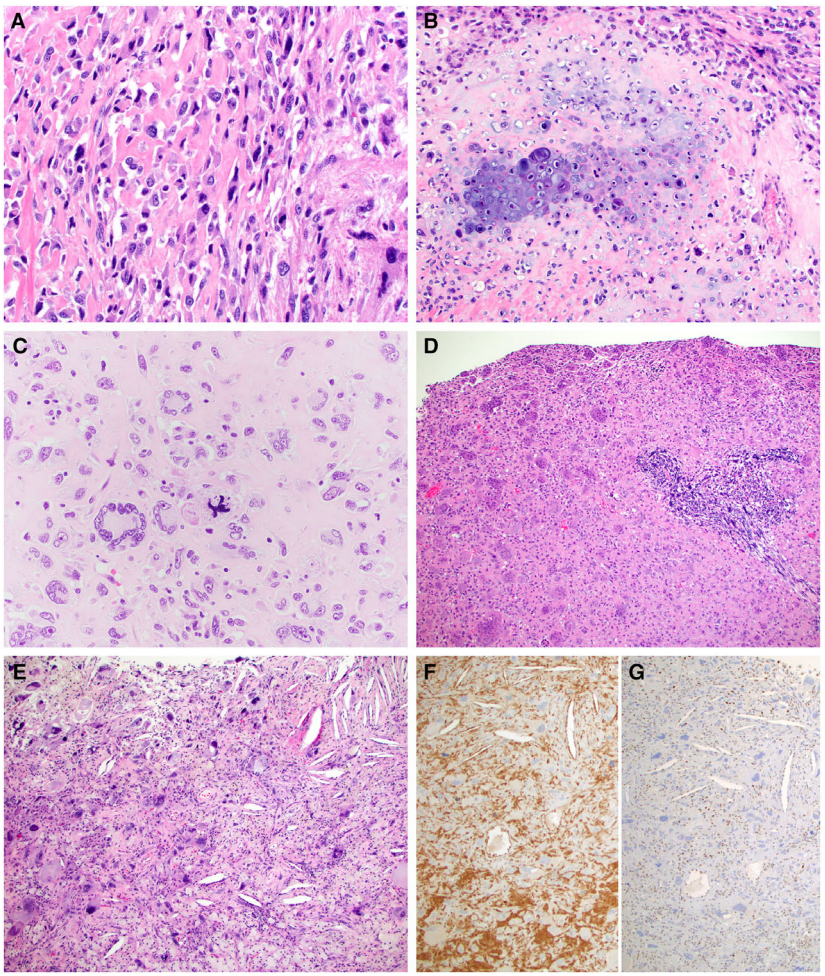

Figure 3. Morphologic features of dedifferentiated uterine leiomyosarcoma. This tumour showed dedifferentiation in the form of heterologous osteosarcomatous (**A**) and chondrosarcomatous (**B**) differentiation. (**C**) Dedifferentiated uterine leiomyosarcoma with atypical mitoses and conspicuous multinucleated tumour giant cells. (**D**) A dedifferentiated uterine leiomyosarcoma abutting the endometrial cavity, with prominent osteoclast-like giant cells. (**E**) A dedifferentiated leiomyosarcoma abutting the endometrial cavity with prominent cholesterol clefts and foamy histiocytes, with aberrant loss of MTAP (**F**) and Rb (**G**) expression in pleomorphic tumour cells. [Colour figure can be viewed at wileyonlinelibrary.com]

osteoclast-like giant cells in four, eosinophilic infiltrate in one, and neutrophilic infiltrate in one.

Differentiated components

The differentiated component of each tumour is detailed in Table 1. Among 17 tumours with a leiomyosarcomatous component, 11 showed severe (3+) and six showed moderate (2+) nuclear atypia, with coagulative necrosis in 14. The median mitotic count was 25 (range, 6–85) per 2.4 mm^2, with atypical mitoses in nine. On pairwise comparison, mitotic activity was greater in the dedifferentiated component in nine tumours, greater in the differentiated leiomyosarcomatous component in seven, and equal in one ($P = 0.99$, paired t test).

Of two tumours with a STUMP component, one showed moderate (2+) atypia, indeterminate necrosis, and two mitoses per 2.4 mm^2 without atypical forms, and one showed focal coagulative necrosis and six mitoses per 2.4 mm^2 but no significant atypia. A leiomyomatous component was present in six tumours (four cellular, two with bizarre nuclei), with median 0 (range 0–2) mitoses per 2.4 mm^2 and no atypical mitoses or coagulative necrosis.

IMMUNOHISTOCHEMICAL PARAMETERS

Smooth muscle markers

All 23 differentiated components were positive for SMA (median, 100%; range, 10–100%) and desmin

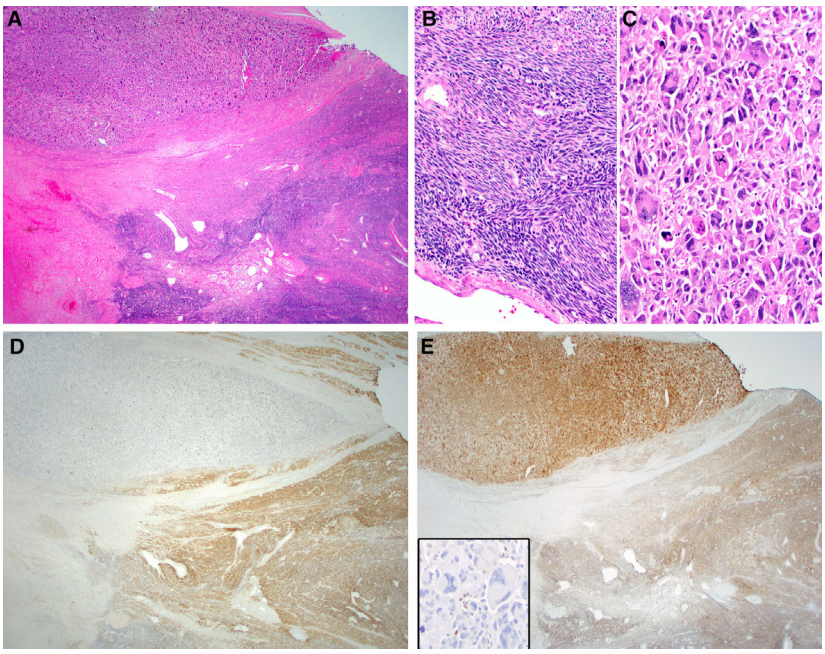

Figure 4. Dedifferentiated uterine leiomyosarcoma. This tumour comprises discrete components of cellular leiomyoma (**A**, lower right; **B**) and dedifferentiated leiomyosarcoma (**A**, upper left; **C**). The cellular leiomyoma component is diffusely positive for desmin (**D**, lower right) and shows diffuse p16 (**E**, lower right), while the dedifferentiated leiomyosarcoma component is negative for desmin (**D**, upper left) and shows strong diffuse p16 (**E**, upper left). Both components showed aberrant loss of Rb expression (**E**, inset). [Colour figure can be viewed at wileyonlinelibrary.com]

(median, 95%; range, 5–100%), and 21 were positive for caldesmon (median, 100%; range, 1–100%). In 11 tumours, the dedifferentiated component was negative for SMA, desmin, and caldesmon. In nine tumours, the dedifferentiated component showed rare cells positive for SMA, with seven also showing rare desmin-positive cells. Two and one tumours showed rare cells positive only for desmin and caldesmon, respectively.

Additional immunomarkers

Additional tissue sections were available for further immunoprofiling in 19 tumours (Figure 5).

The differentiated component was positive for ER in 11 tumours (median, 80%; range, 30–100%) and PR in nine (median, 80%; range, 60–100%). In contrast, the dedifferentiated component was focally positive for ER in three (1–5%, weak) and for PR in one tumour (5%, weak).

Thirteen tumours showed loss of Rb expression in both tumour components (including two cases in which the differentiated component was cellular leiomyoma); five showed retained Rb in the differentiated component but loss in the dedifferentiated component; and one retained Rb in both tumour components.

Ten tumours showed strong diffuse p16 in both the differentiated and dedifferentiated components (the differentiated component was LMS in all 10); seven showed patchy p16 in the differentiated component but strong diffuse staining in the dedifferentiated component; and two showed negative p16 in both components. One tumour with negative p16 showed loss of MTAP expression in the dedifferentiated component, suggesting possible chr 9p21 deletion. MTAP was retained in both components of the second tumour with negative p16 and in both components of nine additional tumours. Rb loss and diffuse p16 were strongly correlated: Of 18 dedifferentiated components with Rb loss, 17 showed diffuse p16 (the single exception harbouring possible chr 9p21 deletion), whereas p16 was negative in the sole dedifferentiated component with retained Rb.

Four tumours showed strong diffuse p53 and five showed complete absence of p53 staining in both tumour components (the differentiated component was LMS in all nine); five tumours showed heterogeneous staining in the differentiated component and strong diffuse staining in the dedifferentiated component; and five showed heterogeneous staining in both components.

ATRX immunohistochemistry was performed in 16 tumours. Five showed loss of ATRX expression in both tumour components (including one STUMP); 10

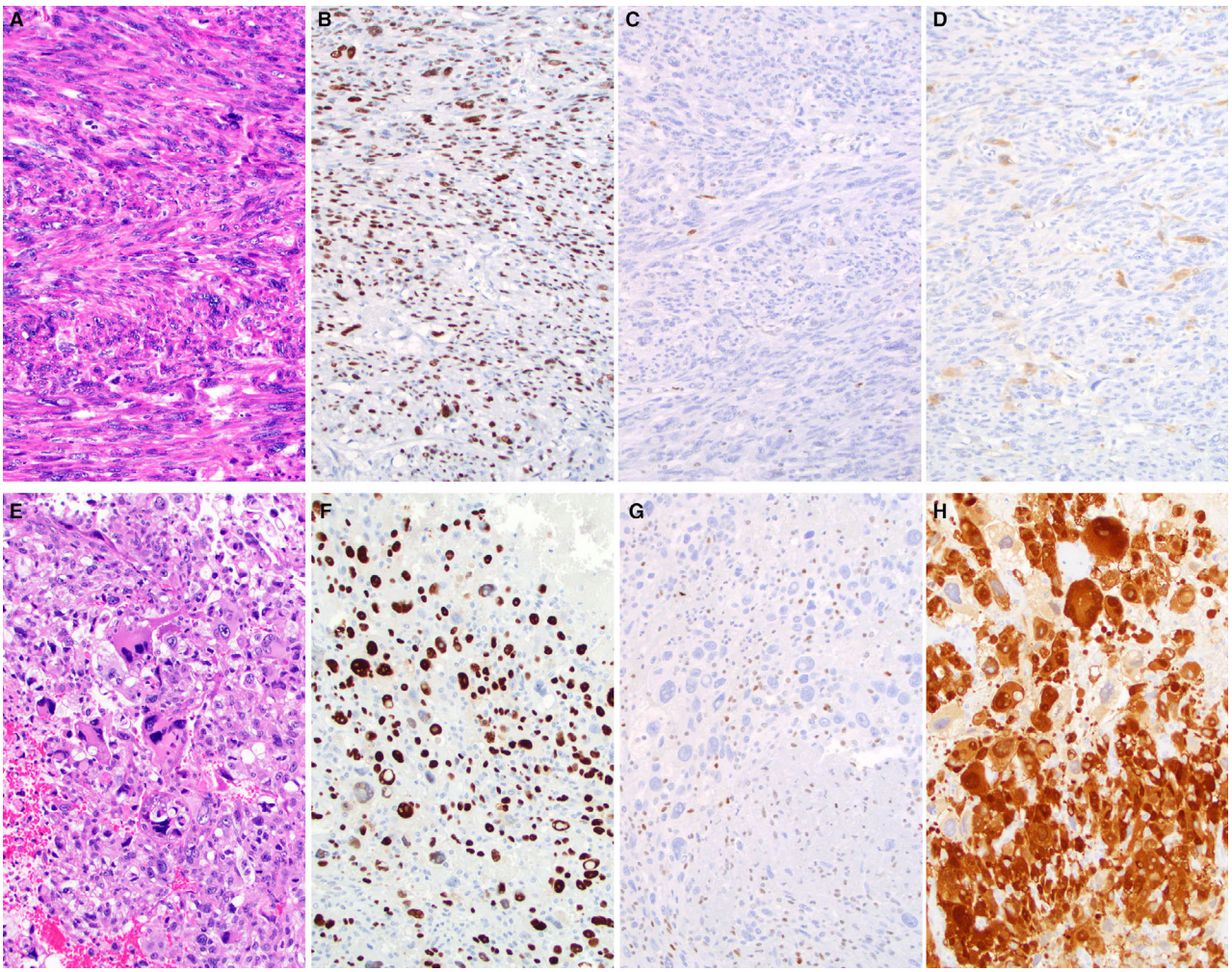

Figure 5. Dedifferentiated uterine leiomyosarcoma, immunohistochemistry. This tumour comprises components of conventional (**A**) and dedifferentiated leiomyosarcoma (**E**). The conventional leiomyosarcoma shows strong diffuse p53 (**B**), focal Rb (**C**), and patchy p16 (**D**), while the dedifferentiated leiomyosarcoma shows strong diffuse p53 (**F**), aberrant loss of Rb (**G**), and strong diffuse p16 (**H**). [Colour figure can be viewed at wileyonlinelibrary.com]

retained ATRX in both components; and one retained ATRX in the differentiated component but showed loss in the dedifferentiated component.

SMARCB1 and SMARCA4 expression was retained in both components of all 19 tumours.

OUTCOMES

Survival data were available for 19 patients with dedifferentiated uterine LMS. Twelve had died of disease at median 14 (range, 2–73) months after diagnosis; four were alive with disease at 4, 12, 44, and 50 months; and three were alive with no evidence of disease at 56, 109, and 114 months. The percent dedifferentiation and the finding of rare smooth muscle marker immunoreactivity in dedifferentiated tumour cells were unrelated to survival.

Of 19 patients, only one experienced recurrence-free survival >24 months: a 59-year-old woman (#9) at stage IB who received adjuvant gemcitabine-paclitaxel and was alive without disease 114 months after hysterectomy. Two additional patients sustained early recurrence followed by >24 months DFS after reoperation. The first (#16) presented at stage IB, but a lung metastasis was discovered 4 months later. Following metastatectomy and chemotherapy, the patient was alive and disease-free 109 months after hysterectomy. The second patient (#18) presented at stage II and had a peritoneal recurrence 2 months after hysterectomy. After repeat surgery and

chemoradiation, the patient was alive with no evidence of disease 56 months after diagnosis. Immunohistochemistry did not suggest anything unusual about these three patients' tumours, and each showed multiple alterations characteristic of uterine LMS (see Table 1).

Dedifferentiated uterine LMS with only a non-leiomyosarcomatous differentiated component (n = 6) showed significantly longer DSS than dedifferentiated uterine LMS with a leiomyosarcomatous differentiated component (5-year DSS, 80% versus. 18%; P = 0.02). There was no difference in DFS (P = 0.65) or stage distribution (P = 0.33) between these groups. Kaplan–Meier curves are in Figure S1.

Comparison to LMS without dedifferentiation (control cohort)

Compared with a control cohort of 345 uterine LMS without dedifferentiation, women with dedifferentiated uterine LMS were slightly older (median, 57 versus 53 years; P = 0.02), but there was no significant difference in stage distribution (P = 0.79) or tumour size (P = 0.58) between dedifferentiated uterine LMS and uterine LMS without dedifferentiation.

DSS was significantly shorter for dedifferentiated uterine LMS than for uterine LMS without dedifferentiation (median, 20 versus 54 months; 5-year DSS, 36% versus 46%; P = 0.04) (Figure S1). Twelve patients with dedifferentiated uterine LMS and 242 with uterine LMS without dedifferentiation were operated on to no evidence of disease and thus included in calculations of DFS. DFS was significantly shorter for dedifferentiated uterine LMS than for uterine LMS without dedifferentiation (median, 8 versus 31 months; 5-year DFS, 8% versus 42%; P = 0.002).

Examining rare cases

All three patients harbouring heterologous differentiation (#7, #12, #15) presented with extrauterine disease (one stage II, two stage IV), and all three died of disease at 11, 16, and 31 months.

Five patients initially considered for study inclusion on the basis of morphologic criteria were ultimately classified as "pleomorphic uterine LMS" due to >5% smooth muscle marker expression (Figure 6). Survival data were available for four of these patients. All four presented at stage IV. Three were dead of disease at 10, 10, and 16 months, and one was alive with disease at 23 months. Although this subcohort is small, these data suggest comparably aggressive behaviour in pleomorphic and dedifferentiated uterine LMS.

Discussion

Although dedifferentiated LMS across all visceral and soft-tissue sites has been the subject of five case series,[12–16] our series represents the first comprehensive evaluation of dedifferentiated LMS in the uterus. Our data indicate almost invariably aggressive behaviour, even among patients presenting with stage I disease, although prolonged DFS is possible, including after reexcision of early local or distant disease recurrence.

This study addresses two important and related questions: Is dedifferentiated leiomyosarcoma a distinct diagnostic entity, and is its prospective recognition clinically important? Regarding classification, earlier series regarded dedifferentiation as a morphologic phenomenon, and thus grouped pleomorphic and dedifferentiated leiomyosarcoma.[15] In 2001, Oda *et al.*[14] recognised that some LMS with pleomorphic foci retained, while others lost, smooth muscle marker expression, and suggested that the latter might be most rigorously regarded as dedifferentiated LMS. Two subsequent series adopted this model,[12,13] although there has been some disagreement as to the precise immunophenotypic delimitation between pleomorphic and dedifferentiated LMS—some authors explicitly[14] or implicitly[12] permit rare smooth muscle marker-positive cells in dedifferentiated LMS, while others[13] exclude such cases. Overall, though, a consensus appears across series that dedifferentiated LMS shows undifferentiated morphology and no or at most very focal smooth muscle marker expression. In our study, we permitted rare cells positive for smooth muscle markers, based on prior work,[14] and the concept that only rare cells weakly positive for a muscle marker are not *per se* diagnostic of leiomyosarcoma in a morphologically undifferentiated neoplasm.

Published series indicate that prospective recognition of dedifferentiated LMS is prognostically relevant, due to significantly shorter survival than LMS without dedifferentiation.[14] Across five series totalling 90 tumours,[12–16] 74 (82%) dedifferentiated LMS had recurred or metastasised at median 8 months after initial diagnosis (range, 1–45 months), 49 (54%) had died of disease at median 11 months, and only six (7%) had greater than 60 months recurrence-free survival. In published series, dedifferentiated and pleomorphic LMS appear predisposed to comparably aggressive behaviour.[12–14] Our data indicate that these tenets also apply to dedifferentiated uterine LMS. We found significantly shorter DFS and DSS for dedifferentiated uterine LMS than for uterine LMS without dedifferentiation, while four pleomorphic

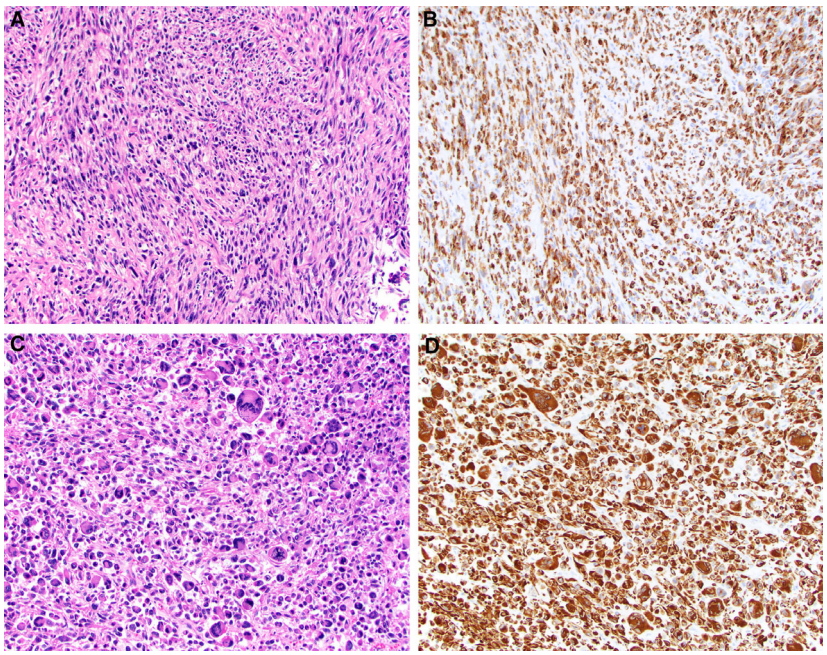

Figure 6. Pleomorphic uterine leiomyosarcoma. This tumour shows areas of conventional leiomyosarcoma (**A**) and a component morphologically compatible with dedifferentiation (**C**). However, both components show strong diffuse desmin expression (**B,D**), which per our criteria warrants diagnosis of pleomorphic leiomyosarcoma rather than dedifferentiated leiomyosarcoma. [Colour figure can be viewed at wileyonlinelibrary.com]

uterine LMS in our cohort behaved as aggressively as the dedifferentiated tumours. On the basis of this latter finding, it does not appear clinically necessary at present to distinguish pleomorphic from dedifferentiated uterine LMS, although this distinction may be noted in cases where smooth muscle immunostains have been performed for diagnostic purposes.

Review of the literature identified 11 primary dedifferentiated uterine LMS.[12,16–21] Follow-up was available in six cases, of whom three had died of disease at 4, 6, and 17 months,[16,17] one had died of other causes at 12 months,[12] and two were alive without disease at 12 and 28 months.[12,19] The literature also contains eight additional cases of primary conventional uterine LMS with subsequent dedifferentiation at metastatic sites,[16,21] with death from disease at median 7 months (range 1–18 months) after dedifferentiation. Despite near-universally aggressive behaviour in both published reports and our cohort, our series also includes the first reports of favourable outcomes in dedifferentiated uterine LMS, including two women with prolonged (54- and 109-month) DFS after early recurrence and one with 114 months recurrence-free survival after hysterectomy.

At present, the management implications of dedifferentiation in uterine LMS are unclear.[25] However, some data suggest a role for immunotherapy in undifferentiated pleomorphic sarcomas of soft tissue,[26–28] which could suggest therapeutic significance for prospective identification of dedifferentiated uterine LMS in the future.

Our series offers some initial (albeit limited) insight into the pathogenesis of dedifferentiation in uterine LMS. First, our immunohistochemical data suggest that dedifferentiation is accompanied by accumulation of pathogenic molecular alterations common in uterine LMS,[29] though we found no consistent trigger for dedifferentiation. Second, we found longer survival among dedifferentiated uterine LMS with a nonleiomyosarcomatous versus a leiomyosarcomatous differentiated component, suggesting a pathogenesis that, in at least some cases, may be distinct from conventional uterine LMS. Comparative molecular studies would be of interest to further clarify these phenomena.

This study has certain limitations. First, our retrospective cohort review focused on hysterectomy specimens, precluding detection of dedifferentiation arising only in recurrent or metastatic disease. Second, comparative molecular analysis was beyond the scope of this study. Finally, the slightly divergent criteria used to define dedifferentiated LMS in prior studies were noted, although we believe we have addressed this through a fair and rational approach, as discussed above.

Although not previously well characterised, dedifferentiation in uterine LMS does not appear to be an exceptionally rare phenomenon. In the two uterine LMS cohorts (Italian and BWH/DFCI) examined for this study, the prevalence of dedifferentiation was 2.4% and 5.0%, respectively. The former may be an underestimate, as only one slide per case was available for review from the Italian cohort, whereas the latter figure may be an overestimate, as the BWH cohort included cancer centre referrals, which (despite the efforts described in our Methods) may have been enriched in aggressive and/or diagnostically challenging tumours, possibly including dedifferentiated uterine LMS.

Table 2. Differential diagnosis of dedifferentiated uterine leiomyosarcoma

Differential diagnosis
Undifferentiated uterine sarcoma
Thorough sampling reveals no associated differentiated smooth muscle component
Epithelioid leiomyosarcoma
Diffuse nuclear pleomorphism uncommon
Malignant heterologous differentiation absent (considered dedifferentiation, by definition)
Positive smooth muscle immunostains in >5% of tumour cells
PEComa
Nuclear pleomorphism typically (though not always) nondiffuse
Sinusoidal vessels and/or dense hyalinisation characteristic
Cathepsin K diffusely positive (sensitive but nonspecific in this differential)
Melanocytic markers (HMB45, Melan-A, PNL2) at least focally positive
TSC1, *TSC2*, *FLCN* mutations, or *TFE3* fusions
TP53, *RB1*, *ATRX*, and *CDKN2A* alterations not specific in this differential
Undifferentiated endometrial carcinoma
Nuclear pleomorphism in 25% of cases, though typically less diffuse and without truly bizarre nuclei
Thorough sampling may reveal associated differentiated carcinoma component ("dedifferentiated carcinoma")
Thorough sampling reveals no associated differentiated smooth muscle component
Mismatch repair deficiency in 50%
SMARCA4 or SMARCB1 loss in 20%
PTEN, *PIK3CA*, *ARID1A*, or *CTNNB1* mutations frequent

To facilitate further study and to emphasize the potential for aggressive clinical behaviour, we advocate prospective diagnosis of dedifferentiated uterine LMS when diagnostic morphologic and immunophenotypic findings, as defined herein, are present. Dedifferentiation may occur in the primary uterine tumour or in recurrent or metastatic tumour[15,16,22] and heralds a dismal prognosis.[16] Awareness of the capacity for dedifferentiation in uterine LMS will facilitate distinction from mimics with undifferentiated and undifferentiated-like components (Table 2). Our findings suggest that a subset of undifferentiated uterine sarcomas (UUS) may represent total or near-total dedifferentiation of uterine LMS, and thorough sampling of any apparent UUS is indicated to exclude an associated differentiated smooth muscle component.

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Conflict of interest

The authors have no other disclosures.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Definitions of morphologic parameters.
Table S1. Immunohistochemical assay specifications.
Figure S1. Survival outcomes in dedifferentiated uterine leiomyosarcoma (uLMS).

