Are Peripheral Purely Undifferentiated Pleomorphic Sarcomas With *MDM2* Amplification Dedifferentiated Liposarcomas?

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Abstract: Dedifferentiated liposarcoma (DDLPS) has been defined as a tumor composed of well-differentiated liposarcoma associated with a nonlipogenic undifferentiated sarcoma and is genetically characterized by a 12q13-15 amplicon with *MDM2* amplification. Some peripheral (extremities, trunk wall, head/neck) undifferentiated pleomorphic sarcomas (UPS) without areas of well-differentiated liposarcoma present an *MDM2* amplification. We addressed whether they are true DDLPS or not. We compared the clinical data, histologic data, MDM2 status (immunohistochemistry [IHC], fluorescence in situ hybridization [FISH]), genomic profile (array comparative genomic hybridization), and follow-up of 19 patients with peripheral UPS with *MDM2* amplification and 62 with peripheral conventional DDLPS retrieved from the French sarcoma network (RRePS) and the Conticabase (Connective Tissue Cancer Network database). For a control cohort, we described 153 patients from the Conticabase, with peripheral UPS without expression of MDM2 by IHC. By IHC, tumor cells were positive for MDM2 in 59 conventional DDLPS and in all UPS with *MDM2* amplification. FISH analysis and/or quantitative polymerase chain reaction showed amplification of *MDM2* in 54 conventional DDLPS and in all UPS with *MDM2* amplification. The 2-year overall survival rates of UPS with *MDM2* amplification, conventional DDLPS, and UPS without expression of MDM2 were 93.3%, 90.7%, and 73.9%, respectively. Such similarities in the clinical characteristics, morphology, genomic profile, and follow-up of peripheral UPS with *MDM2* amplification and peripheral conventional DDLPS strongly suggest that peripheral UPS with *MDM2* amplification are in fact DDLPS. Faced with histologic diagnosis of UPS, a systematic IHC evaluation of MDM2 allows a selection of cases for FISH analysis permitting the diagnosis of DDLPS.

Key Words: dedifferentiated liposarcoma, undifferentiated sarcoma, *MDM2*, FISH analysis, array-CGH

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Dedifferentiated liposarcoma (DDLPS) was a name given by Evans in 1979[1] to liposarcomas containing a mixture of well-differentiated and poorly differentiated areas. Histologically, DDLPS is defined by the association of atypical lipomatous tumor/well-differentiated liposarcoma (WDLPS) areas and a nonlipogenic sarcoma (usually resembling either high-grade pleomorphic sarcoma or fibrosarcoma) with amplification of the 12q13-15 region with *MDM2* amplification.[2–4] Most cases occur in the retroperitoneum of patients aged 40 years or more.[2,5] Several recent studies have reported that most sarcomas diagnosed as (inflammatory) poorly differentiated sarcomas and arising in the retroperitoneum are actually DDLPS[6,7] and can now be identified as such on the basis of their specific genomic profile even in the absence of WDLPS areas.[2,8] In peripheral locations (extremities, trunk wall, head, and neck), DDLPS are much less common, and identification of a well-differentiated component remains for many pathologists the most consensual way to assert diagnosis of

DDLPS. However, as such a well-differentiated component may not be identifiable in some DDLPS (mostly those in the retroperitoneum),[2,6] and as a few cases of peripheral undifferentiated pleomorphic sarcoma (UPS) without areas of WDLPS present an *MDM2* amplification,[8] the relationship between peripheral UPS with *MDM2* amplification and peripheral conventional DDLPS now needs investigating.

Accordingly, here we aimed to: (i) compare 19 tumors that were initially diagnosed as peripheral UPS but later shown by immunohistochemistry (IHC) to overexpress MDM2 and by fluorescence in situ hybridization (FISH) analysis the amplification of *MDM2*, with 62 peripheral conventional DDLPS, in terms of clinical data, histologic features, MDM2 status, genomic profile, and follow-up; and (ii) propose a simple diagnostic algorithm permitting the diagnosis of DDLPS (whatever its location, even outside the retroperitoneum) and which can be used in daily routine practice.

MATERIALS AND METHODS

Patient and Tumor Selection

Ethics approval from the appropriate committees was obtained. Nineteen cases of peripheral UPS with *MDM2* amplification were included: 10 presenting in 2010 and 2011 and retrieved from the RRePS (National prospective database on the systemic histologic review of every new sarcoma in France, https://rreps.sarcomabcb.org) and 9 from the Conticabase (European retrospective clinicopathologic database on sarcomas, https://conticabase.sarcomabcb.org). Only patients with a resection of the primary tumor, with local disease, and with adequate sampling (1 block for each centimeter of tumor) were included. Between January 1, 1980 and December 31, 2011, 198 consecutive adult patients with a peripheral conventional DDLPS and 944 consecutive adult patients with a peripheral UPS located in the extremities, trunk wall, or head and neck were treated in 22 participating cancer centers and entered in the Conticabase. We restricted our analysis to patients with: (i) resection of the primary tumor; (ii) deeply located tumor; (iii) local disease (metastasis at diagnosis clearly being an adverse prognostic factor); and (iiii) adequate sampling (the presence of a well-differentiated component of liposarcoma being required for diagnosis of DDLPS), to obtain a more homogenous patient population. On the basis of these criteria, 79 of the 198 patients with a peripheral conventional DDLPS and 481 of the 944 patients with peripheral UPS were selected. The peripheral UPS were used as the control cohort. To obtain a more robust control cohort, we selected only peripheral UPS tumors that either (i) did not express MDM2 by IHC or (ii) overexpressed MDM2 by IHC but without amplification of *MDM2* by FISH analysis. Of the 481 UPS tumors, 149 met these criteria. We named this cohort "peripheral UPS without MDM2 expression."

Histologic review with adequate sampling (1 block taken for each centimeter of tumor, Table 2) revealed no well-differentiated component of liposarcoma in any of the cases of peripheral UPS with *MDM2* amplification and in 5 of 67 cases of peripheral conventional DDLPS entered in this study. Among these 5 cases: 4 were reclassified as "peripheral UPS without MDM2 expression" as they showed no expression of MDM2 by IHC and no amplification of *MDM2* by FISH analysis, and the other was reclassified as "peripheral UPS with MDM2 amplification" as it showed overexpression of MDM2 by IHC, amplification of *MDM2* by FISH analysis, and amplification of the q13-15 region of chromosome 12 by array comparative genomic hybridization (array-CGH). Following this reclassification, our study was based on 19 cases of "peripheral UPS with *MDM2* amplification," 62 cases of "peripheral conventional DDLPS," and 153 "peripheral UPS without MDM2 expression."

Pathology Review

Histologic slides of all peripheral UPS with *MDM2* amplification and peripheral conventional DDLPS included in this study were reviewed by 1 of the 3 pathologists (J.-M.C., S.L.G., and A.-V.D.) from the pathology subcommittee of the French Sarcoma Group (GSF). One to 46 slides were reviewed for each tumor. Histologic review analyzed the presence of a well-differentiated component and the morphologic features of the dedifferentiated component. Histologic typing was based on the World Health Organization histologic typing of soft tissue tumors (World Health Organization 2013). Tumor grade was evaluated according to the previously established Fédération Nationale des Centres de Lutte Contre le Cancer scoring system on the basis of tumor differentiation, mitotic count, and necrosis.[9]

Data Collection

Data regarding patients' characteristics, tumor description, and outcome were obtained from a retrospective review of medical records. These records and histologic data were entered into a centralized database (https://conticabase.sarcomabcb.org). The following 11 variables were compared among the 3 cohorts (peripheral UPS with *MDM2* amplification, peripheral conventional DDLPS, and peripheral UPS without MDM2 expression): age at diagnosis, sex, tumor size, tumor location, tumor grade, number of paraffin blocks of tumor, expression of MDM2 by IHC, overall survival rate (OS), metastasis-free survival rate (MFS), and local recurrence-free survival rate (LRFS). Four other additional variables were compared between peripheral UPS with *MDM2* amplification and peripheral conventional DDLPS: tumor depth, morphologic features of the dedifferentiated component, expression of CDK4 by IHC, expression of HMGA2 by IHC, and *MDM2* gene status.

Immunohistochemistry

IHC analysis was performed in all cases of peripheral UPS with *MDM2* amplification and peripheral conventional DDLPS available as well as in all cases of peripheral UPS without MDM2 expression on

4-μm-thick serial sections from a representative paraffin wax block with MDM2 antibody (clone IF2, dilution 1:100; Invitrogen, Camarillo, CA). In all cases of peripheral UPS with *MDM2* amplification (18) and of peripheral conventional DDLPS (67), 2 supplementary antibodies were used: CDK4 (clone DCS-31, dilution 1:100; Invitrogen) and HMGA2 (dilution 1:500; Biocheck, Foster city, CA). In 32 cases, heterologous differentiation was suspected, and additional antibodies against desmin (clone D33, dilution 1:100; Dako, Glostrup, Denmark), h-caldesmon (clone h-CD, dilution 1:50; Dako), and myogenin (clone LO26, dilution 1:20; Novocastra, Newcastle-upon-Tyne, UK) were used. After microwave oven heating (20 min in 0.1 M citrate buffer at pH 6), sections were incubated with biotinylated link antibody, with peroxidase-labeled streptavidin (LSAB + Kit; Dako) and then with diaminobenzidine solution (Dako). Omitting the specific primary antibody was used as negative controls.

FISH and Quantitative Polymerase Chain Reaction

FISH analysis was performed in all cases of peripheral conventional DDLPS (67), in all cases of peripheral UPS with *MDM2* amplification, and in 12 cases of peripheral UPS without MDM2 expression showing overexpression of MDM2 by IHC. Two protocols were used: (i) FISH assay was performed using the Histology FISH accessory kit (Dako) as previously described according to the manufacturer's instructions.[10] *MDM2* and *CDK4* probes were prepared with BAC clones labeled using a Nick translation reagent kit according to the manufacturer's instructions (Abbott, North Chicago, IL). For the *MDM2* gene, 250 ng of RP11-775J10 and 250 ng of RP11-1024C4 BAC were labeled with spectrum green-dUTP, and for the *CDK4* gene, 250 ng of RP11-571M6 and 250 ng of CTD-2241M16 BAC were labeled using spectrum orange-dUTP (Abbott). Labeled BAC DNA were then precipitated with 25 μg of human Cot-1 DNA and resuspended in hybridization buffer (50% formamide, 2× SSC pH6.8, 1× Denhardt, 1% SDS, NaH_2PO_4 40 mM pH7, 10% dextran sulfate); (ii) FISH using the Zyto*Light* SPEC *MDM2*/CEN12 Dual-color Probe kit (ZytoVision GmbH, Bremerhaven, Germany) was performed on a full tissue section, according to the manufacturer's recommended protocol. The probe cocktail decorates the human chromosomal region *MDM2* with a green signal and the centromeric region of chromosome 12 (D12Z3 sequences) with a red signal. Green and red fluorescent signals were counted in regions of cellular tumor under a Nikon Eclipse80i fluorescent microscope with appropriate filters, and pictures were captured using a Hamamatsu C4742-95 CCD camera and analyzed with the Genikon software (Alphelys, Plaisir, France). A minimum of 100 nuclei per slide were visualized. For each nucleus analyzed, the number of fluorescent signals was evaluated. Amplification was defined as >12 fluorescent signals per cell. For each series of experiments, a control slide from a DDLPS case known to be positive for *MDM2* amplification was also examined. If at least 1 or 2 bright fluorescent spots per nucleus could not be seen on at least 80% of cells, the result was considered as uninterpretable, and *MDM2*/*CDK4* copy number was assessed by quantitative polymerase chain reaction (qPCR) as described previously.[11]

Genomic DNA Extraction and Array-CGH

Genomic DNA was extracted according to the protocol recommended by Agilent for DNA isolation on formalin-fixed paraffin-embedded tissue (http://www.chem-agilent.com/pdf/G4410-90020v3_1_CGH_ULS_Protocol.pdf) (Agilent Technologies, Santa Clara, CA), and array-CGH experiment was performed as previously described.[12] Briefly, arrays were scanned using an Agilent G2585CA DNA Microarray Scanner and images analyzed using the Feature-Extraction V10.1.1.1 software (Agilent Technologies). Data were normalized using the ranking-mode method available in the Feature-Extraction V10.1.1 software, with a default value for any parameter. Raw copy number ratio data were transferred to Genomic Workbench software (Agilent Technologies). The ADM-2 algorithm of Genomic Workbench software (Agilent Technologies) was used to identify 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 fell below -1.

Statistical Analysis

Tumor characteristics and histologic and molecular data were described using median and range for quantitative data and frequency and percentage for the qualitative data. Comparisons between the 2 histologies (peripheral UPS with *MDM2* amplification and peripheral conventional DDLPS) were made by the χ^2 test or Fisher exact test for qualitative variables and the Student *t* test or the Mann-Whitney *U* test for the quantitative data.

Survival data were summarized by the Kaplan-Meier method and presented by the 2-year survival rate with a 95% confidence interval (CI). Survival curves were plotted according to the Kaplan and Meier estimate. The log rank test was used to determine the significance of differences in survival distribution. To control potential selection bias, the model was adjusted for variables that show a statistically significant difference between 2 cohorts and could influence the survival. The statistical significance was set at 0.05. All analyses in the study were performed with the Stata Version 12.0 software (Stata, College Station, TX).

RESULTS

Patient and Tumor Characteristics

Patient characteristics of the 3 cohorts are presented in Tables 1 and 2. Age at diagnosis for patients with "peripheral UPS with *MDM2* amplification" tended to be

TABLE 1. Clinical Data, Histologic Features, MDM2 Status, and Outcome Corresponding to Peripheral UPS With *MDM2* Amplification and Peripheral UPS Displaying No Expression of MDM2

	Peripheral UPS With *MDM2* Amplification	Peripheral UPS Without MDM2 Expression	*P*
N	19	153	
Age at diagnosis (median [range]) (y)	77 (45-90)	66 (16-92)	0.066
Sex (n [%])			0.528
Male	12 (63.2)	85 (556)	
Female	7 (36.8)	68 (44.4)	
Tumor location (n [%])			0.139
Extremities	10 (52.6)	112 (73.2)	
Trunk wall	9 (47.4)	39 (25.5)	
Head/neck	0	2 (1.3)	
Tumor size (median [range]) (cm)	11 (2.5-20)	9 (3-35)	0.337
Tumor grade (FNCLCC) (n [%])			**0.001**
Grade 2	11 (57.9)	33 (21.7)	
Grade 3	8 (42.1)	119 (78.3)	
No. mitoses (median [range])	12 (1-56)	21 (1-130)	**0.026**
MDM2 IHC status (n [%])			—
Positive	19 (100)	12 (7.8)	
Negative	0	141 (92.2)	
Uninterpretable	0	0	
Follow-up (median [95% CI]) (y)	23 (12-75)	22 (14-26)	—
Metastatic recurrence (n [%])	3 (15.8)	46 (30.3)	—
Local recurrence (n [%])	2 (10.5)	32 (21.1)	—
Death (n [%])	5 (26.3)	37 (24.2)	—
OS*	0.933	0.739	0.400†
MFS*	0.941	0.547	0.081†
LRFS*	0.848	0.609	0.060†

Bold values represent $P < 0.05$.
*2-year rate.
†After adjustment for grade and number of mitoses.

higher (median = 77, range = 45 to 90) as compared with "peripheral UPS without MDM2 expression" patients (median = 66, range = 16 to 92, $P = 0.066$). However, this trend was not statistically significant. The most common tumor location in the 3 cohorts was the thigh: 7 (36.7%) peripheral UPS with *MDM2* amplification, 32 (51.5%) peripheral conventional DDLPS, and 66 (43.2%) peripheral UPS without MDM2 expression. Peripheral UPS without MDM2 expression tended to be more often located at the extremities (112, 73.2%) as compared with peripheral UPS with *MDM2* amplification (10, 52.6%, $P = 0.139$). However, this trend was not statistically significant. The most common grade (Fédération Nationale des Centres de Lutte Contre le Cancer system) was 2 for the peripheral UPS with *MDM2* amplification (11, 57.9%) and peripheral conventional DDLPS (34, 54.8%), whereas it was 3 for the peripheral UPS without MDM2 expression (119, 78.3%), with a statistically significant difference between the peripheral UPS with *MDM2* amplification and the peripheral UPS without MDM2 expression ($P = 0.001$).

No statistically significant difference was found in terms of clinical data (patient's sex and age at diagnosis, and the location, depth, and size of the tumor) or with regard to tumor features (tumor grade, number of mitoses) between peripheral UPS with *MDM2* amplification and peripheral conventional DDLPS (Table 2).

Histologic and Molecular Data Of Peripheral UPS With *MDM2* Amplification and Peripheral Conventional DDLPS

Morphologic Features

The median number of paraffin blocks prepared was 14 (range, 3 to 23) for peripheral UPS with *MDM2* amplification and 13.5 (range, 1 to 46) for peripheral conventional DDLPS (Table 2). Most of the well-differentiated components of the peripheral conventional DDLPS were morphologically described as "sclerosing" in 58 (93.5%) (Fig. 1A), followed by "lipoma-like" in 21 (33.9%). The dedifferentiated component of the peripheral UPS with *MDM2* amplification and the peripheral conventional DDLPS, respectively, showed morphology of: undifferentiated sarcoma, 19 (100%) (Fig. 1B) and 41 (66.1%); fibrosarcoma, 5 (26.3%) and 14 (22.6%); myxofibrosarcoma, 3 (15.8%) (Fig. 1D) and 12 (19.4%); leiomyosarcoma, 1 (5.3%) and 5 (8.1%); osteosarcoma,

TABLE 2. Clinical Data, Histologic Features, MDM2 Status, and Outcome Corresponding to Peripheral UPS With *MDM2* Amplification and Peripheral Conventional DDLPS

	Peripheral UPS With *MDM2* Amplification	Peripheral Conventional DDLPS	*P*
N	19	62	
Age at diagnosis (median [range]) (y)	77 (45-90)	70.5 (23-93)	0.199
Sex (n [%])			0.613
Male	12 (63.2)	43 (69.4)	
Female	7 (36.8)	19 (30.6)	
Tumor location (n [%])			0.444
Extremities	10 (52.6)	38 (61.3)	
Thigh	7	32	
Arm	1	5	
Leg	2	1	
Trunk wall	9 (47.4)	20 (32.2)	
Buttock	1	8	
Thoracic wall	4	5	
Groin	2	2	
Armpit	2	2	
Abdominal wall	0	3	
Head/neck	0	4 (6.5)	
Depth (n [%])			0.787
Superficial	2 (10.5)	4 (6.6)	
Deep	17 (89.5)	57 (93.4)	
Unknown	0	1	
Tumor size (cm)			
Median (range)	11 (2.5-20)	12 (2.5-50)	0.272
≤ 10	9 (47.4)	29 (46.8)	0.964
> 10	10 (52.6)	33 (53.2)	
No. paraffin blocks (median [range])	14 (3-23)	13.5 (1-46)	0.709
Tumor size/number of paraffin blocks (median [range])	0.94 (0.45-1.5)	0.98 (0.21-30)	0.768
Tumor grade (FNCLCC) (n [%])			0.320
Grade 2	11 (57.9)	34 (54.8)	
Grade 3	8 (42.1)	28 (45.2)	
No. mitoses (n [%])			0.100
Median (range)	12 (1-56)	8 (1-58)	
≤ 9	6 (31.6)	33 (532)	
> 9 and ≤ 19	7 (36.8)	22 (35.3)	
> 19	6 (31.6)	7 (11.3)	
Necrosis			0.907
None	8 (42.1)	25 (40.3)	
$\leq 50\%$	10 (52.6)	35 (56.5)	
$> 50\%$	1 (5.3)	2 (3.2)	
OS*	0.933	0.907	0.437
MFS*	0.941	0.832	0.613
LRFS*	0.848	0.853	0.889

FNCLCC indicates Fédération Nationale des Centres de Lutte Contre le Cancer.
*2-year rate.

1 (5.3%) (Fig. 1F) and 5 (8.1%). Three (4.8%) peripheral conventional DDLPS showed morphologic features of rhabdomyosarcoma with expression of desmin and myogenin by IHC; 2 (3.2%) had giant cells (Fig. 1C) and the other (1.6%) meningothelial-like features (Fig. 1E).

Immunohistochemistry

In all cases (19) of peripheral UPS with *MDM2* amplification and in 59 cases of peripheral conventional DDLPS, tumor cells were positive for MDM2 (Figs. 1G, H). IHC gave no interpretable results for 3 peripheral conventional DDLPS (Bouin fixed tissues). No statistically significant difference was found in terms of protein expression (CDK4 and HMGA2) between peripheral UPS with *MDM2* amplification and peripheral conventional DDLPS (Table 3). For these 3 markers, the positivity was nuclear and was visible in 10% to 100% of tumor cells (Figs. 1G–J).

FISH Analysis

In all cases (19) of peripheral UPS with *MDM2* amplification and in 50 cases of 62 peripheral conventional DDLPS analyzed, high-level amplification (> 12 copies per cell and clustered) of the *MDM2* gene was detected (Figs. 2A, B). FISH analysis gave no interpretable result in 12 peripheral conventional DDLPS (Bouin fixed tissues). Quantitative PCR showed amplification of *MDM2* in 4 of these 12 cases. The level of amplification

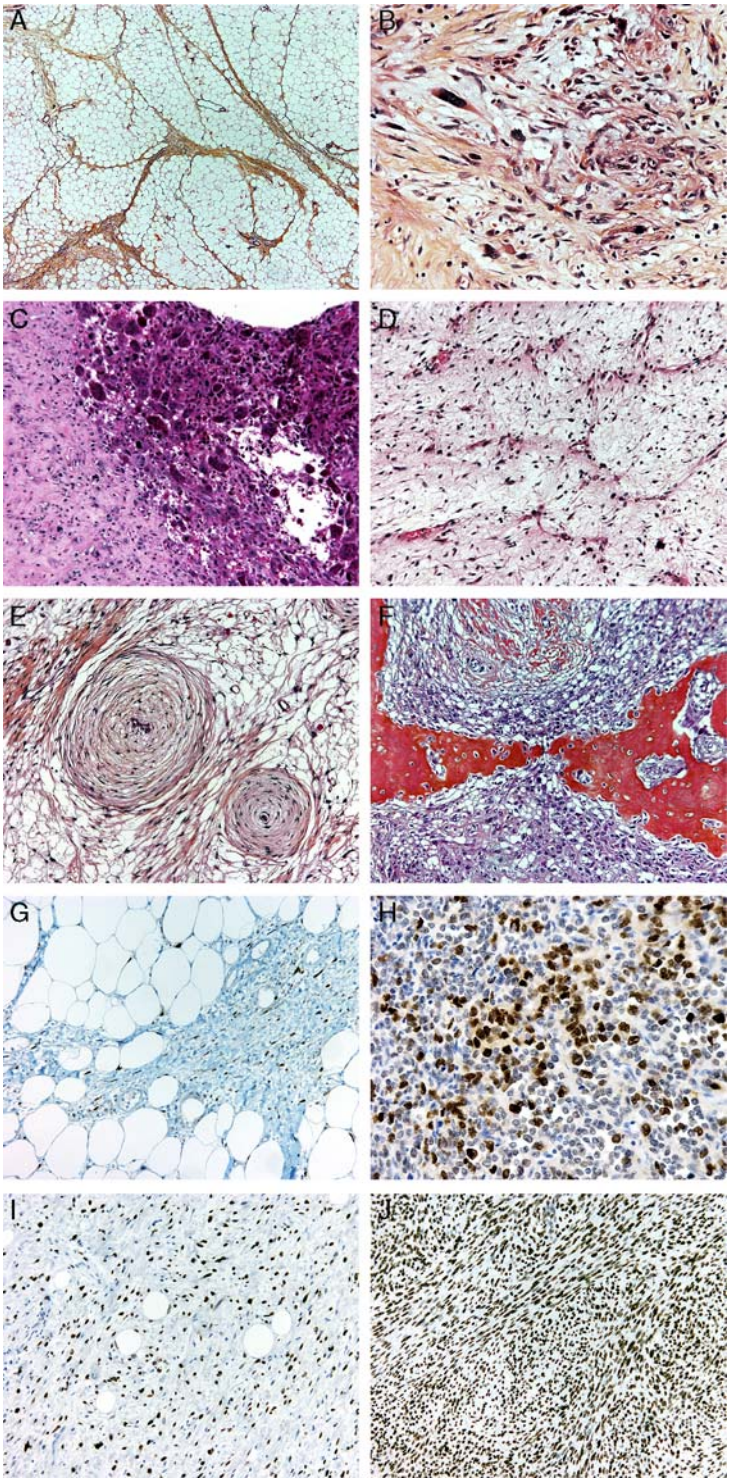

FIGURE 1. Histologic data. A–F, Histology. A, WDLPS of peripheral conventional DDLPS with sclerosing features. Dedifferentiated component of peripheral conventional DDLPS with giant cells (C), with meningothelial-like features (E). Peripheral UPS with *MDM2* amplification with undifferentiated features (B), with myxofibrosarcoma features (D) and with osteosarcoma features (F). G–J, IHC. G, Well-differentiated component of peripheral conventional DDLPS is positive for MDM2. H, Undifferentiated features of peripheral UPS with *MDM2* amplification show nuclear expression of MDM2. I, Peripheral conventional DDLPS with fibrosarcoma features overexpress HMGA2 (nuclear staining). J, Peripheral UPS with *MDM2* amplification with leiomyosarcoma features show nuclear expression for HMGA2.

TABLE 3. Histologic Features, MDM2, CDK4, and HMGA2 Status in Peripheral UPS With *MDM2* Amplification and Peripheral Conventional DDLPS

	Peripheral UPS With *MDM2* Amplification	Peripheral Conventional DDLPS	*P*
N	19	62	
Percentage of WD component (median [range])	0	20 (2-97)	—
Morphologic appearance of WD component (n [%])			—
Lipoma-like	—	21 (33.9)	
Sclerosing	—	58 (93.5)	
Inflammatory	—	1 (0.02)	
Percentage of DD component (median [range])	100 (100-100)	80 (3-98)	—
Morphologic appearance of DD component (n [%])			
Undifferentiated	19 (100)	41 (66.1)	**0.002**
Fibrosarcoma	5 (26.3)	14 (22.6)	0.761
Myxofibrosarcoma	3 (15.8)	12 (19.4)	1.00
Leiomyosarcoma	1 (5.3)	5 (8.1)	1.00
Rhabdomyosarcoma	0	3 (4.8)	1.00
Osteosarcoma	1 (5.3)	5 (8.1)	1.00
Chondrosarcoma	0	3 (4.8)	1.00
Hemangiopericytoma-like	0	1 (1.6)	1.00
Meningothelial-like	0	1 (1.6)	1.00
Desmoid-like	0	2 (3.2)	1.00
Giant cells	0	2 (3.2)	1.00
Inflammatory MFH–like	0	3 (4.8)	1.00
Pleomorph liposarcoma-like	0	1 (1.6)	1.00
Interface between WD and DD component			—
Abrupt	—	38 (61.3)	
Gradual	—	24 (38.7)	
MDM2 IHC status (n [%])			—
Positive	19 (100)	59 (100)	
Negative	0	0	
Uninterpretable	0	3	
CDK4 IHC status (n [%])			0.063
Positive	8 (42.1)	39 (66.1)	
Negative	11 (57.9)	20 (33.9)	
Uninterpretable	0	3	
HMGA2 IHC status (n [%])			1.00
Positive	14 (77.8)	45 (76.3)	
Negative	5 (22.2)	13 (23.7)	
Uninterpretable	0	3	
MDM2 gene status (n [%])			—
Amplified	19 (100)	54 (100)	
Nonamplified	0	0	
Uninterpretable	0	8	

Bold values represent $P < 0.05$.

DD indicates dedifferentiated; FNCLCC, Fédération Nationale des Centres de Lutte Contre le Cancer; MFH, malignant fibrous histiocytoma; WD, well-differentiated.

(copy number of target gene *MDM2*/copy number of reference gene [*ALB*]) varied from 30.3 to 84.3. In 8 cases of peripheral conventional DDLPS, qPCR was not contributive (Table 3).

Array-CGH. In all cases (19) of peripheral UPS with *MDM2* amplification and in the 20 analyzed cases of peripheral conventional DDLPS, a gain or amplification of the q13-15 region of chromosome 12 (containing *CDK4* and *MDM2* genes), known to be distinctive of well-differentiated and DDLPS, was detected (Figs. 2C, D). Figure 3 shows the penetrance plot (ie, frequencies of imbalances across samples) of 13 cases of peripheral UPS with *MDM2* amplification and 9 cases of peripheral conventional DDLPS. Both are associated with 100% of *MDM2* amplification and exhibit common main alterations such as chromosome 13, 16q, and 9p losses and the chromosome 17p, 19q, and 22 gains. Even if peripheral UPS with *MDM2* amplification genomes are slightly more complex, profiles of peripheral UPS with *MDM2* amplification and peripheral conventional DDLPS appear very similar.

Outcome

Median follow-up was 22.9 months (95% CI, 12.32-76.64) for peripheral UPS with *MDM2* amplification, 28.8 months (95% CI, 15.08-44.74) for peripheral conventional DDLPS, and 21.8 months (95% CI, 14.32-25.89) for peripheral UPS without MDM2 expression.

Recurrence

At the end of the follow-up period, metastatic recurrence was observed in 3 (15.8%) patients with

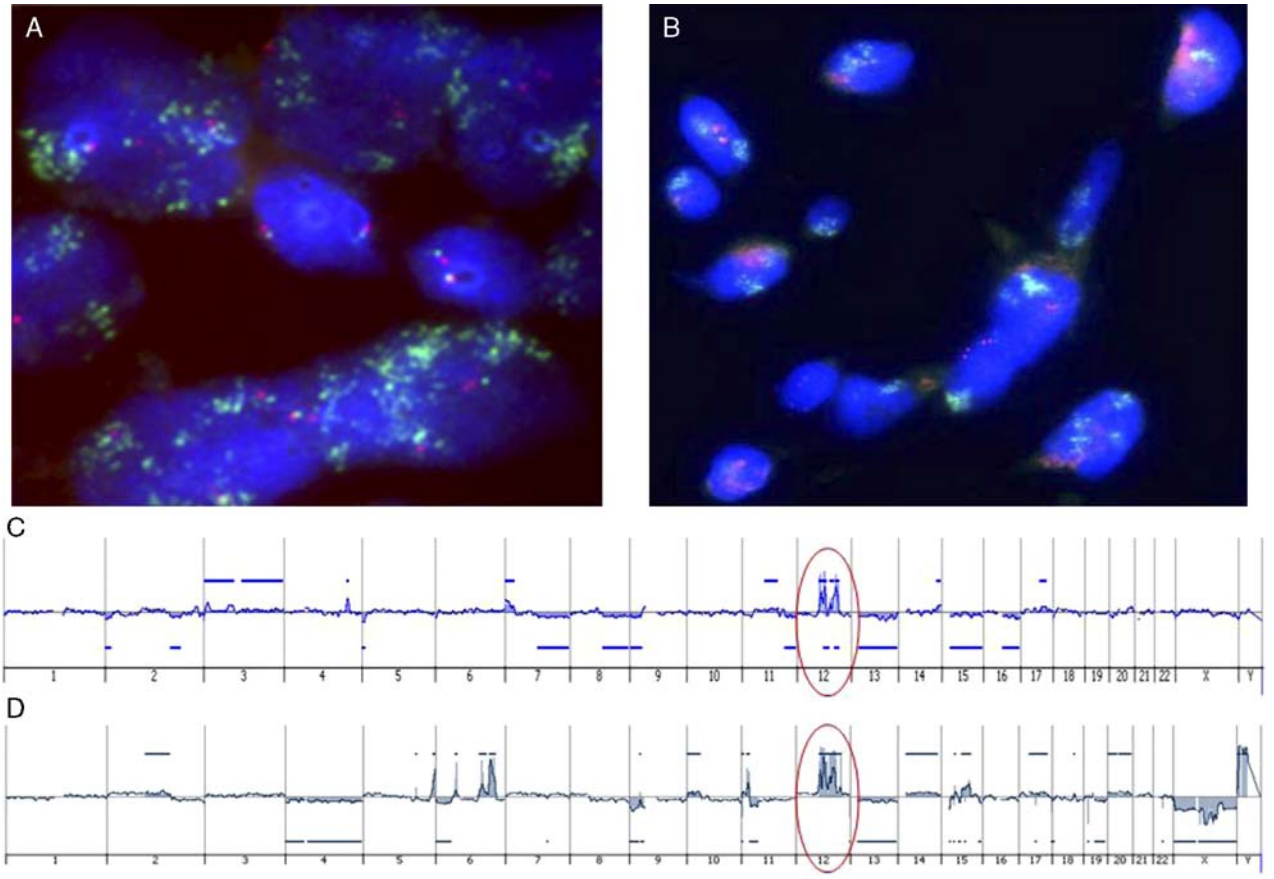

FIGURE 2. Molecular data. A, FISH analysis: showed an *MDM2* green signal (>12) present in a cluster in most tumor cell nuclei (red: centromere of chromosome 12) in peripheral conventional DDLPS. B, FISH analysis showed an *MDM2* green signal and a *CDK4* red signal (>12) present in a cluster in most tumor cell nuclei in peripheral UPS with *MDM2* amplification. Array-CGH showed a 12q13-15 amplification in peripheral conventional DDLPS (C) and in peripheral UPS with *MDM2* amplification (D) (circled in red).

peripheral UPS with *MDM2* amplification, in 9 (15.3%) with peripheral conventional DDLPS, and in 46 (30.3%) with peripheral UPS without MDM2 expression. Two patients (10.5%) with peripheral UPS with *MDM2* amplification, 7 (11.9%) with peripheral conventional DDLPS, and 32 (22.1%) with peripheral UPS without MDM2 expression had a local recurrence.

Survival Analysis

OS, MFS, and LRFS of the 3 cohorts is depicted in Figures 4A–C. The OS rate at 2 years of patients with peripheral UPS with *MDM2* amplification, peripheral conventional DDLPS, and peripheral UPS without MDM2 expression were 93.3%, 90.7%, and 73.9%, respectively. To control a potential selection bias, the model was adjusted for the grade and the number of mitoses. No statistically significant difference between OS of peripheral UPS with *MDM2* amplification and peripheral conventional DDLPS was found ($P = 0.437$). The MFS rate at 2 years of patients with peripheral UPS without MDM2 expression tended to be much worse as compared with that for patients with peripheral UPS with *MDM2* amplification (54.7% vs. 94.1%, hazard ratio 2.3 [95% CI, 0.9-5.8], $P = 0.081$). However, this trend was not statistically significant. In contrast, no statistically significant difference between the MFS of patients with peripheral UPS with *MDM2* amplification and peripheral conventional DDLPS (83.2%; 95% CI, 70.1-90.9) was found ($P = 0.613$). The LRFS rate at 2 years of patients with peripheral UPS without MDM2 expression tended to be much worse as compared with that for patients with peripheral UPS with *MDM2* amplification (60.9% vs. 84.8%, hazard ratio 2.5 [95% CI, 0.96-6.3], $P = 0.06$). However, this trend was not statistically significant. In contrast, no statistically significant difference between MFS of patients with peripheral UPS with *MDM2* amplification and peripheral conventional DDLPS (85.3%; 95% CI, 72.7-92.4) was found ($P = 0.889$). At the last follow-up visit, 14 (73.7%) patients with peripheral UPS with *MDM2* amplification, 46 (74.2%) with peripheral conventional DDLPS, and 86 (56.2%) with peripheral UPS without MDM2 expression were alive and tumor

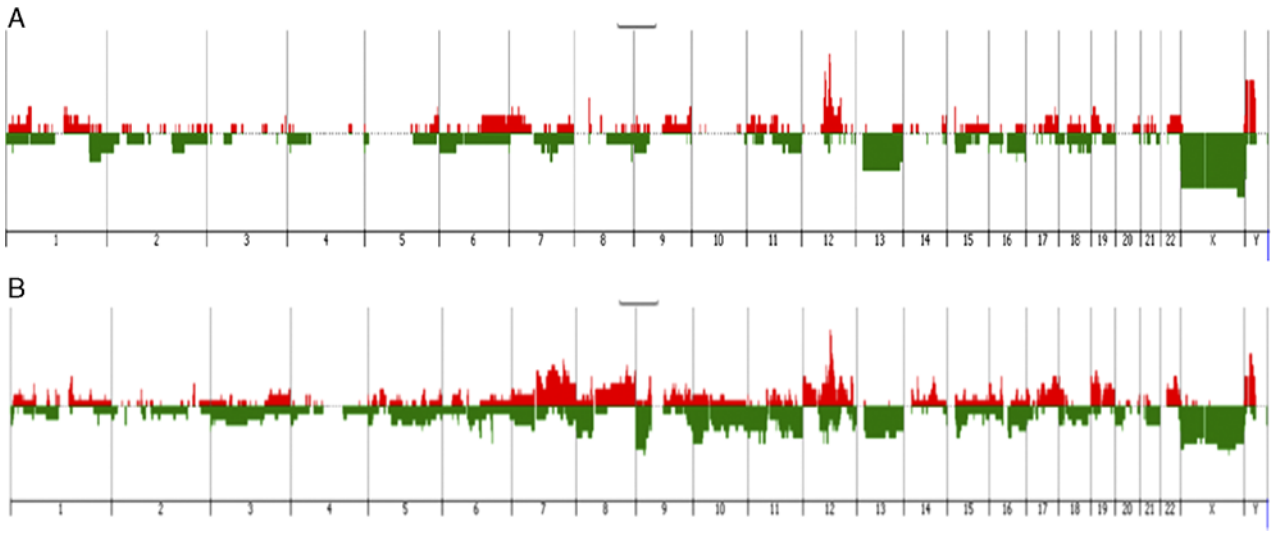

FIGURE 3. Overview of the copy number abnormalities identified in peripheral UPS with *MDM2* amplification and peripheral conventional DDLPS. Penetrance plot summarizing the copy number imbalances per chromosome. Red blocks represent chromosome gains; green blocks represent chromosome losses. The amplitude of each abnormality corresponds to its prevalence. A, Penetrance plot of peripheral conventional DDLPS. B, Penetrance plot of peripheral UPS with *MDM2* amplification.

free; 4 (8%) patients with peripheral conventional DDLPS and 28 (24.3%) with peripheral UPS without MDM2 expression were alive with metastatic sarcoma. Five (26.3%) patients with peripheral UPS with *MDM2* amplification, 12 (19.4%) with peripheral conventional DDLPS and 37 (24.2%) with peripheral UPS without MDM2 expression had died at the time of analysis. Of the patients who had died, 2 (10.5%) with peripheral UPS with *MDM2* amplification, 3 (4.8%) with peripheral conventional DDLPS, and 6 (3.9%) with peripheral UPS without MDM2 expression had died from a cause unrelated to the treated sarcoma; death was related to the sarcoma in 3 (15.8%) patients with peripheral UPS with *MDM2* amplification, 9 (14.5%) with peripheral conventional DDLPS, and 31 (20.2%) with peripheral UPS without MDM2 expression. Three (4.8%) patients with peripheral conventional DDLPS and 4 (2.6%) with peripheral UPS without MDM2 expression were lost to follow-up.

DISCUSSION

In this study, we have shown that peripheral UPS without areas of WDLPS but with *MDM2* amplification are similar to peripheral conventional DDLPS in terms of patient age and sex, tumor location and size, histologic features, tumor grade, MDM2 status (IHC, FISH analysis, or qPCR), genomic profile (array-CGH), and follow-up. In 19 cases initially diagnosed as peripheral UPS, in which no areas of WDLPS could be identified with adequate sampling (1 block taken for each centimeter of tumor), we were able to demonstrate that the IHC and genomic investigations were in accordance with a DDLPS pattern: by IHC, all cases overexpressed MDM2, 8 (42.1%) overexpressed CDK4, and 14 (77.8%) HMGA2, whereas FISH analysis demonstrated genuine amplification (high level and clustering of the signal) of *MDM2* in all of these 19 tumors. Moreover, in all 19 cases, array-CGH analysis showed amplification of the 12q13-15 region, typical of that described in DDLPS.[13–15] Both types of tumor are associated with genetics based on amplifications, and even if peripheral UPS with *MDM2* amplification appear more rearranged, genomic profiles of peripheral UPS with *MDM2* amplification and peripheral conventional DDLPS are quite congruent. Moreover, we found no differences in terms of clinical and morphologic data or sampling technique between these 19 tumors and peripheral conventional DDLPS. From a prognosis point of view, data from our series revealed no statistically significant difference in OS, MFS, and LRFS between these 2 cohorts; in contrast, the control cohort (peripheral UPS without MDM2 expression) showed a far worse prognosis in accordance with data in the literature.[2–4,16] Altogether, these data strongly suggest that peripheral UPS without areas of WDLPS but with *MDM2* amplification are actually peripheral DDLPS.

First introduced in 1979 by Evans,[1] DDLPS is today defined as the association of atypical lipomatous tumor/WDLPS areas and a nonlipogenic sarcoma of variable grade with amplification of the 12q13-15 region and *MDM2* expression.[2–4] Dedifferentiated areas exhibit a variable histologic picture; most frequently they resemble UPS, fibrosarcoma, or intermediate-grade to high-grade myxofibrosarcoma.[2] In about 5% to 10% of cases, DDLPS may show heterologous differentiation featuring myogenic, osteochondromatous elements, or rarely a "meningothelial-like" whorling pattern.[17] These data are similar to those observed in the peripheral UPS with *MDM2* amplification and peripheral conventional

DDLPS cohorts (Fig. 4). In terms of genomics, DDLPS display 1 or 2 supernumerary rings or giant rod chromosomes, which always contain amplified sequences of the region q14-15 of chromosome 12.[18] Concordantly, we found a consistent amplification of the *MDM2* gene, which is located at 12q15 and could be considered as the target gene of this amplicon. Exons 1 and 2 of *HMGA2*, a gene located at 12q14.3, were more recently found to be consistently coamplified with *MDM2*.[19] In our series, nuclear expression of *HMGA2* was found in 14 (77.8%) peripheral UPS with *MDM2* amplification and in 45 (76.3%) peripheral conventional DDLPS. This specific genomic profile displayed by DDLPS has allowed its identification in the retroperitoneum in the absence of a WDLPS component.[8] Moreover, sarcomas initially diagnosed as poorly differentiated sarcoma (or pleomorphic rhabdomyosarcoma) and arising in the retroperitoneum/internal trunk have been shown to be actually DDLPS (with divergent differentiation).[6,20] The absence of the WDLPS component may be explained by inappropriate sampling, the disappearance of the WDLPS, or even by the absence of the well-differentiated component in the primary tumor. The genomic abnormalities displayed by DDLPS are evidence in favor of it corresponding to a malignant adipocytic tumor progressing from WDLPS to nonlipogenic sarcoma of variable aspect.[8,21,22]

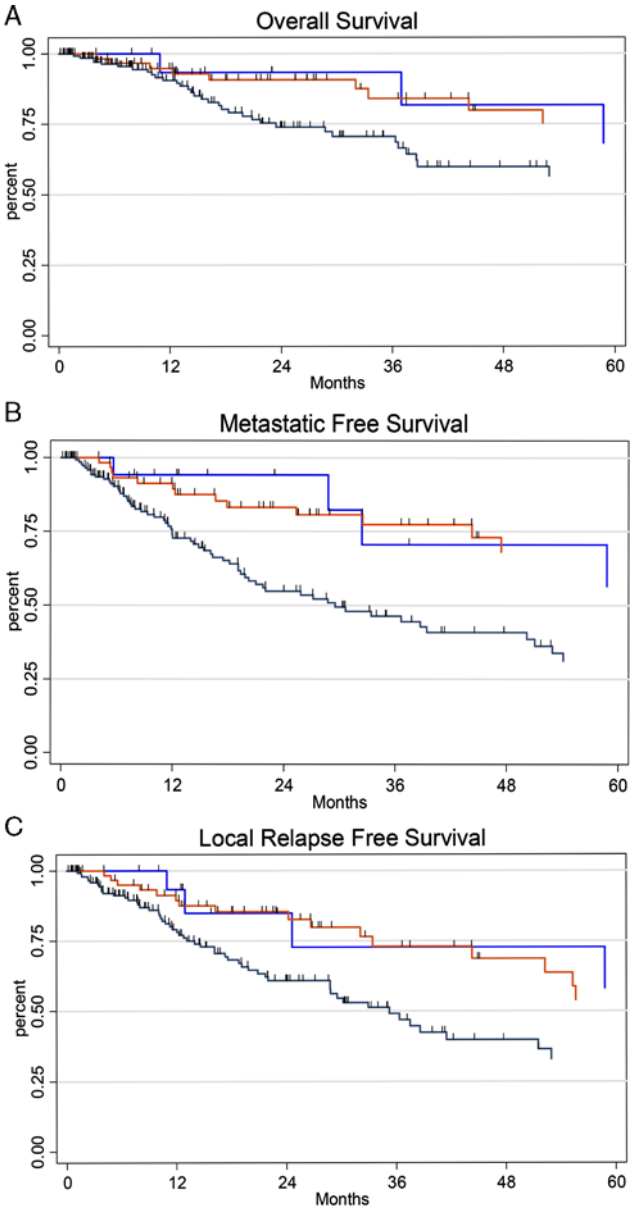

FIGURE 4. Follow-up. A, OS of the 19 patients with peripheral UPS with *MDM2* amplification (in blue), the 62 with peripheral conventional DDLPS (in red), and the 153 with peripheral UPS without MDM2 expression (in black). B, MFS of the 19 patients with peripheral UPS with *MDM2* amplification (in blue), the 62 with peripheral conventional DDLPS (in red), and the 153 with peripheral UPS without MDM2 expression (in black). C, LRFS of the 19 patients with peripheral UPS with *MDM2* amplification (in blue), the 62 with peripheral conventional DDLPS (in red), and the 153 with peripheral UPS without MDM2 expression (in black).

DDLPS occurs in late adult life with no sex predilection, and most commonly in the retroperitoneum (80% of cases according to the literature).[2] From the RRePS database, 475 cases of DDLPS were identified who presented between January 1, 2010 and December 31, 2011). Of these 475, 120 (25.3%) occurred in the peripheral location (extremities, trunk, head and neck), in accordance with previous data.[4] The most important prognostic factor in the behavior of DDLPS is anatomic location, with retroperitoneal tumors exhibiting a worse clinical prognosis than those peripherally located.[3,4,16,23,24] In our study, the 2-year OS and LRFS rates of peripheral conventional DDLPS were 90.7% and 85.3%, respectively, which is consistent with literature data.[3,4,16,17] OS and LRFS at 2 years of patients with peripheral UPS with *MDM2* amplification were similar (93.3% and 84.8%, respectively) to those of DDLPS. In contrast, the outcome of undifferentiated sarcoma (peripheral UPS without MDM2 expression) was relatively poor with an OS at 2 years of 73.9% and an LRFS of 60.9%; these data are comparable to previously published data.[2]

Concerning clinical outcome, the presence of a heterologous differentiation in DDLPS does not appear to have a negative impact, whereas leiomyosarcoma or rhabdomyosarcoma have a much more aggressive clinical course.[2,25,26] Similarly, DDLPS exhibits a less aggressive clinical course than other types of high-grade UPS.[2–4,16,17,26] These data emphasize the need to correctly identify DDLPS in peripheral locations in terms of prognosis and treatment strategy.

Currently, the best criterion to determine the diagnosis of DDLPS when faced with UPS is the presence of an atypical lipomatous tumor/WDLPS area. The key is therefore the careful sampling and rigorous examination of the surrounding adipocytic tissue. However, in some resected primary tumor cases and on core needle biopsy (the present gold standard in sarcoma care),[1] this well-differentiated component may not be identifiable.[1] In

light of these data, the morphologic diagnosis of undifferentiated sarcoma or myxofibrosarcoma should lead to the use of a diagnostic flow chart to identify cases of DDLPS. Thereby, whatever the location, within or outside the retroperitoneum, we performed a careful pathology review. If an area of WDLPS was identified by histology, the diagnosis of DDLPS was made; if no WDLPS could be demonstrated, we executed an IHC study of MDM2. If this IHC study was negative, we classified this case as undifferentiated/unclassified sarcoma, not otherwise specified or myxofibrosarcoma. However, if pleomorphic cells showed nuclear positivity for MDM2, we performed FISH analysis to identify *MDM2* amplification. If FISH analyses were uninterpretable, we performed qPCR to reveal *MDM2*/*CDK4* copy numbers, and if they showed no *MDM2* amplification (or showed aneuploidy/polysomy) we classified this case as undifferentiated/unclassified sarcoma, not otherwise specified. If a genuine *MDM2* amplification was found, the diagnosis of DDLPS was made. In this study, array-CGH confirmed FISH analysis data; however, in daily routine practice, FISH analysis (showing true *MDM2* amplification) is sufficient for the diagnosis of DDLPS. Array-CGH should be reserved for clarifying doubtful cases.

This diagnostic algorithm (Fig. 5) has been applied since January 1, 2010 by all pathologists of the RRePS network (French Sarcoma Group) and has enabled the identification of 120 cases of peripheral DDLPS over a period of 2 years (2010 and 2011). For 65 (54.2%) of these 120 cases, the identification of a WDLPS by careful pathology review allowed the diagnosis of DDLPS. For the 55 (45.8%) cases in which no area of WDLPS could be identified, 10 (8.3%) cases were diagnosed on surgical excision and 45 (37.5%) on core needle biopsies (the current gold standard in sarcoma care).[1] The diagnosis of DDLPS on core needle biopsies seems all the more relevant and interesting now that new MDM2 targeting treatments (such as nutlin, a small-molecule inhibitor of the p53-MDM2 interaction) are being tested in clinical trials.

In conclusion, in this series, peripheral UPS without areas of WDLPS but with *MDM2* amplification are similar to peripheral conventional DDLPS in terms of clinical, histopathologic, molecular, and follow-up data and, therefore, represent the existence of true DDLPS without a WDLPS area.

A systematic IHC evaluation of MDM2 should be performed when dealing with a UPS whatever its location and even outside the retroperitoneum. This strategy allows a selection of cases for FISH analysis permitting the diagnosis of DDLPS (especially on core needle biopsies), which exhibits a less aggressive clinical course than other

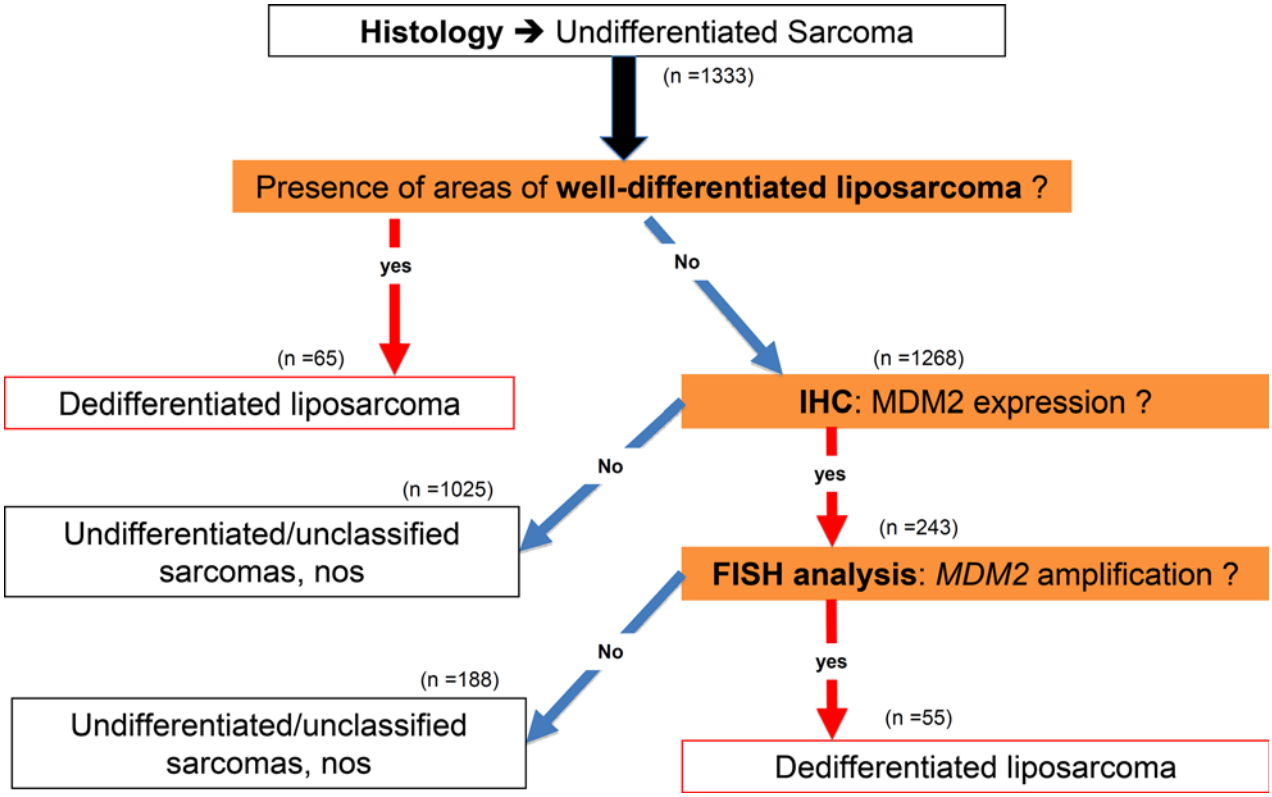

FIGURE 5. Diagnostic algorithm. Algorithm applied when faced with a morphologic diagnosis of undifferentiated sarcoma and/or undifferentiated sarcoma with divergent differentiation. In parentheses: number of patients meeting the criteria at each step of the flow chart over the 2 years after its introduction (2010 and 2011) and use by all pathologists of the RRePS network (French Sarcoma Group) for peripherally located tumors (extremities, trunk wall, head, and neck). Nos indicates not otherwise specified.

types of high-grade UPS and for which therapies targeting MDM2 could be an additional therapeutic approach.

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