Myogenic Differentiation and Histologic Grading Are Major Prognostic Determinants in Retroperitoneal Liposarcoma

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Abstract: The aim of the present work was to improve the understanding of the impact of malignancy grade and myogenic/rhabdomyoblastic differentiation on the natural course of retroperitoneal liposarcoma. All consecutive patients affected by primary well-differentiated (WD)/dedifferentiated (DD) retroperitoneal liposarcoma, surgically treated at our institution between January 2002 and December 2011, were retrospectively evaluated. Tumors were stained for mdm2 and 5 myogenic markers (smooth muscle actin-α, h-caldesmon, calponin, desmin, myogenin). The French National Federation of the Centers for the Fight Against Cancer (FNCLCC) grading system was applied. Overall survival, crude cumulative incidence of local recurrence, and distant metastases were calculated. Multivariable analyses were carried out. A total of 144 patients were identified. Median follow-up was 68 months (interquartile range: 46 to 104 mo). Fifty-two patients were affected by WD/G1 and 92 by DD liposarcoma. Among the latter, 60 were grade G2 and 32 G3. Myogenic differentiation was present in 54 cases (8/52 WD/G1, 27/60 DD/G2, 18/32 DD/G3). Seven cases had a rhabdomyoblastic DD component (1/60 DD/G2 and 6/32 DD/G3). Five-year overall survival rates were 93%, 57%, and 21% for WD/G1 liposarcoma, G2 DD, and G3 DD liposarcoma, respectively, and 75%, 42%, and 29% for liposarcoma without myogenic differentiation, with myogenic differentiation, with rhabdomyoblastic differentiation, respectively ($P < 0.001$). Of note, 5/6 patients affected by G3 DD liposarcoma with a rhabdomyoblastic component died within 8 months. FNCLCC grade and myogenic differentiation significantly predicted the outcome of retroperitoneal liposarcoma. These should be factored into treatment decision-making and possibly used to stratify patients in clinical trials.

Key Words: sarcoma, liposarcoma, retroperitoneal sarcoma, dedifferentiation, myogenic differentiation, rhabdomyoblastic differentiation, prognosis, survival

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Retroperitoneal liposarcomas are rare tumors with an expected incidence of 2 to 5 new cases per 1,000,000 inhabitants per year.[1]

Liposarcoma can be divided into 3 main clinicopathologic and genetic subgroups: myxoid/round cell liposarcoma, well-differentiated (WD)/dedifferentiated (DD) liposarcoma, and pleomorphic liposarcoma.[2] The vast majority of primary retroperitoneal liposarcomas are represented by the WD/DD subgroup, whereas others arise primarily in this site only occasionally.

Over time, many retroperitoneal undifferentiated pleomorphic sarcomas and pleomorphic leiomyosarcomas have been reclassified as examples of DD liposarcoma, sharing the same genetic aberrations (amplifications of *MDM2*, *CDK4*, and *HMGA2* genes in chromosome region 12q13-15) that typically characterize WD/DD liposarcomas.[3–5]

A recently published array comparative genome hybridization–based study provided a model describing the progression and dedifferentiation from WD to DD.[6] In this model, the classic 12q13-15 amplification encompassing *MDM2* and *CDK4* represents the initial, shared event, whereas the genomic events linked to dedifferentiation are mainly represented by losses, the most frequent being 11q23-24 and 19q13. Whereas 11q23-24 loss is associated with distinct morphology (undifferentiated pleomorphic sarcoma, myxofibrosarcoma), 19q13 loss seems to be associated with an aggressive phenotype and reduced expression of C/EBP-α, a marker of lipogenic differentiation.[6]

Clinically, WD liposarcoma is known to have a more indolent course than DD liposarcoma. DD liposarcoma represents the morphologic progression from

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WD to a mostly nonlipogenic sarcoma exhibiting histologic heterogeneity. Heterologous (most often myogenic) differentiation is rarely observed.[7] Interestingly, neither histologic grade nor type of heterologous differentiation has been consistently reported to predict outcome.[6–8]

Herein, using a large cohort of uniformly treated patients at a single institution, we intend to refine our preliminary data[8] by specifically testing the impact on outcome of morphologic features such as morphologic grade and type of divergent myogenic differentiation of the DD component.

PATIENTS AND METHODS

Patients

This series results from the collection of all consecutive patients affected by primary, localized, WD/DD retroperitoneal liposarcoma operated at our institution between January 2002 and December 2011. The very few primary myxoid/round cell liposarcomas were excluded.

Primary extended resection was uniformly offered to all patients, as previously described.[9]

Surgical resections were classified as macroscopically complete (R0 or R1) or incomplete (R2).

All adjuvant/neoadjuvant treatments were administered according to the decision of the multidisciplinary institutional sarcoma board. However, no prospectively selected criteria were used to this end.

Chemotherapy was given according to the standard regimens used at the time or within institutional/multiinstitutional clinical trials and included anthracycline often associated with ifosfamide. When administered, radiation therapy was delivered through external beams at doses ranging from 36 to 65 Gy (median 50 Gy).

All patients were followed up 1 month after discharge. Patients were then prospectively followed up by clinical examination and chest and abdomen computed tomography scan every 4 months for the first 2 years, then every 6 months for the following 3 years, and yearly thereafter.

Pathology

All surgical specimens were sampled as reported for sarcomas at other sites.[10]

Histologically, samples were categorized according to the 2013 WHO classification,[1] complemented by Evans'[11] refinements to the definition. Briefly, 3 WD liposarcoma variants were recognized: lipoma-like, inflammatory, and sclerosing. The cellular subvariant was considered to be part of the third variant. This subvariant is characterized by increased cellularity and the presence of atypical, floret-type giant cells coupled with a mitotic rate of < 5 mitotic figures per 10 high-power fields (HPF) (counting the number of mitotic figures in 4 sets of 10 consecutive HPF in the most active areas and accepting the highest count observed).[11] In cases in which the mitotic count was $\geq 5/10$ HPF, the tumoral component was considered DD and consistently graded as described below.

The DD component was graded according to the French National Federation of the Centers for the Fight Against Cancer (FNCLCC) grading system[12] on the available untreated tumor specimen (biopsy and/or surgical specimen).

In all cases, a panel of myogenic differentiation markers including smooth muscle actin-α (1A4), calponin, h-caldesmon, desmin, and myogenin in addition to mdm2 was applied. In cases showing rhabdomyoblastic differentiation, cdk4 and C/EBP-α antibodies were also applied. The antibodies, clones, dilutions, pretreatment conditions, and sources are listed in Table 1. Appropriate positive and negative controls were used. For a diagnosis of WD/DD liposarcoma, mdm2 positivity was required, along with morphologic features. Myogenic differentiation was recorded as "present" when at least 1 of the other 4 myogenic markers was expressed by at least 10% of neoplastic cells. Their positivity was scored as "focal" when $< 50\%$ of cells were reactive and "diffuse" when $\geq 50\%$ cells were reactive. Rhabdomyoblastic differentiation was considered as "present" when myogenin decorated any nucleus of any neoplastic cell. In 2 cases, the morphologic features and myogenic differentiation (marked expression of at least 2 myogenic markers, and possibly h-caldesmon, in the absence of any myogenin reactivity) were consistent with

TABLE 1. Antibodies, Clones, Dilutions, and Sources for the Immunophenotype

Marker	Clone	Dilution	Supplier	Antigen Retrieval	Detection System
Smooth muscle actin-α	1A4	1:800	Dako	PTLink Dako EDTA pH 8 15′	En Vision Flex + Dako
Calponin	Calp	1:1000	Bio-Genex	PTLink Dako Citrate pH 6 15′	En Vision Flex + Dako
h-caldesmon	h-CD	1:200	Dako	PTLink Dako Citrate pH 8 15′	En Vision Flex + Dako
Desmin	D 33	1:400	Dako	PTLink Dako EDTA pH 8 15′	En Vision Flex + Dako
Myogenin	F5D	1:200	Dako	PTLink Dako EDTA pH 8 15′	En Vision Flex + Dako
Mdm2	IF2	1:20	Calbiochem	PTLink Dako EDTA pH 8 30′	En Vision Flex + Dako
Cdk4	polyclonal	1:400	Santa Cruz	PTLink Dako Citrate pH 6 15′	En Vision Flex + Dako
C/EBP-α	8178	1:200	Cell Signaling	Extended CC1 buffer	Optiview DAB IHC

TABLE 2. Clinical, Morphologic, Immunohistochemical, and FISH Analyses of the 7 Cases of DD Liposarcoma With Divergent Rhabdomyosarcomatous Differentiation

				Immunohistochemical Findings						FISH Analyses			
				Rhabdomyosarcomatous DD Component			WD Component			Rhabdomyosarcomatous DD Component		WD Component	
Case	Age/Sex	Morphology	FNCLCC Grade	mdm2	cdk4	C/EBP-α	mdm2	cdk4	C/EBP-α	*MDM2*	*CDK4*	*MDM2*	*CDK4*
1	62/M	Rhabdomyoma-like	2	+	–	–	+	+	+	+	+	+	+
2	46/F	Pleomorphic round cell	3	+	–	–	+	+	+	+	+	+	+
3	40/M	Pleomorphic round cell	3	+	–	–	+	+	+	+	+	+	+
4	56/M	Pleomorphic round cell	3	+	–	–	+	+	+	+	+	+	+
5	69/F	Pleomorphic round cell	3	+	–	–	+	+	+	+	+	+	+
6	68/M	Spindle cell	3	+	–	–	+	+	+	+	+	+	+
7	54/F	Spindle cell	3	+	–	–	+	+	+	+	+	+	+

leiomyosarcomatous differentiation. Given their limited number, they were analyzed in the myogenic group.

Fluorescence in situ hybridization (FISH) analysis for *MDM2* was restricted to cases with equivocal morphology; moreover, FISH analysis for *MDM2* and *CDK4* was performed in all cases of liposarcoma showing rhabdomyoblastic dedifferentiation on both WD and DD components (Table 2). Paraffin sections were analyzed with BAC clones (kindly provided by M. Rocchi, University of Bari, Italy) for *MDM2* (Spectrum Red–labeled RP11-775J10) and *CDK4* (Spectrum Green–labeled RP11-571M6). Probe labeling, slide treatment, and hybridization were carried out according to standard procedures.

Statistical Analysis

The analyses were performed using SAS and R software.[13] Statistical tests were considered significant when the corresponding P value was $< 5\%$.

The main study outcomes were overall survival (OS), local recurrence (LR), and distant metastasis (DM). OS was defined as the time between surgery and death from any cause; time was censored at the date of last follow-up for patients remaining alive. Survival curves were estimated with the Kaplan-Meier method and statistically compared with the log-rank test. Crude cumulative incidence (CCI) curves of LR and DM were calculated in a competing risks framework.[14] In the LR (DM) analysis, deaths without evidence of disease and DM (LR), whichever occurred first, were regarded as competing events. CCI curves were compared by means of the Gray test.[15] Concomitant LRs and DMs were included in the estimation of the CCI curves for DM only.

Multivariable analyses were based on cause-specific hazards and were therefore carried out using Cox regression models. Patient's age and tumor size were modeled as continuous variables using 3-knot restricted cubic splines,[16] whereas the other categorical variables used dummy variables. To adjust for overfitting due to the model's high dimensionality, penalized maximum likelihood estimation methods[17] were applied as implemented in the R package *Penalized*. The proportional hazard assumption of the Cox model was checked and verified by relying on statistical tests based on scaled Schoenfeld residuals.[18]

RESULTS

Pathology

A total of 144 patients were identified. In 52 patients findings were consistent with WD/G1 liposarcoma and in 92 patients with DD liposarcoma. The 52 WD/G1 liposarcomas displayed rather heterogenous morphologic features. The most frequent subtype was the lipoma-like variant (50 cases), followed by the sclerosing variant (43 cases). The 2 aspects were combined in 40/52 cases (80%). Inflammatory and cellular features combined with the first 2 were present in 14 and 9 cases, respectively. Myxoid changes were detected in 15 of 52 cases.[19]

Myogenic differentiation was found in 8/52 WD/G1 cases (15.3%). Desmin reactivity occurred in all these 8 cases, diffuse in 7 cases and focal in 1 case. Smooth muscle actin-α (1A4) was present as diffuse in 2/52 cases (4%), calponin in 2/52 cases (diffuse in 1 case and focal in another case), and h-caldesmon focal in 1/52 case (2%). Of note, myogenin was not present in any WD case.

Among the 92 DD liposarcomas, the amount of DD component ranged from 5% to 95% (median 55%). In 60 patients the DD component was G2 and in 32 G3. Myogenic differentiation was found in 45 patients (48%) affected by DD liposarcoma: 27/60 (45%) G2 and 18/32 (56%) G3. Smooth muscle actin-α was present in 90% of these cases, in 95% of G2 (diffuse in 50% of cases) and in 86% of G3 (diffuse in 61%). Desmin was present in 48%, in 37% of G2 (diffuse in 15% of cases) and in 63% of G3 (diffuse in 75% of cases). Calponin was present in 60% of cases, in no case as the only myogenic marker. h-caldesmon was present in 16% of cases, in no case as the only

myogenic marker. All myogenin-positive cases showed coexpression of desmin.

In 7 (8%) cases the immunoreactivity for both desmin and myogenin was consistent with rhabdomyosarcomatous differentiation, of which 1/60 (1.7%) were in the G2 and 6/32 (19%) in the G3 category. The only G2 case exhibited a morphology consistent with a rhabdomyoma-like rhabdomyosarcoma[20] (Fig. 1). Four cases

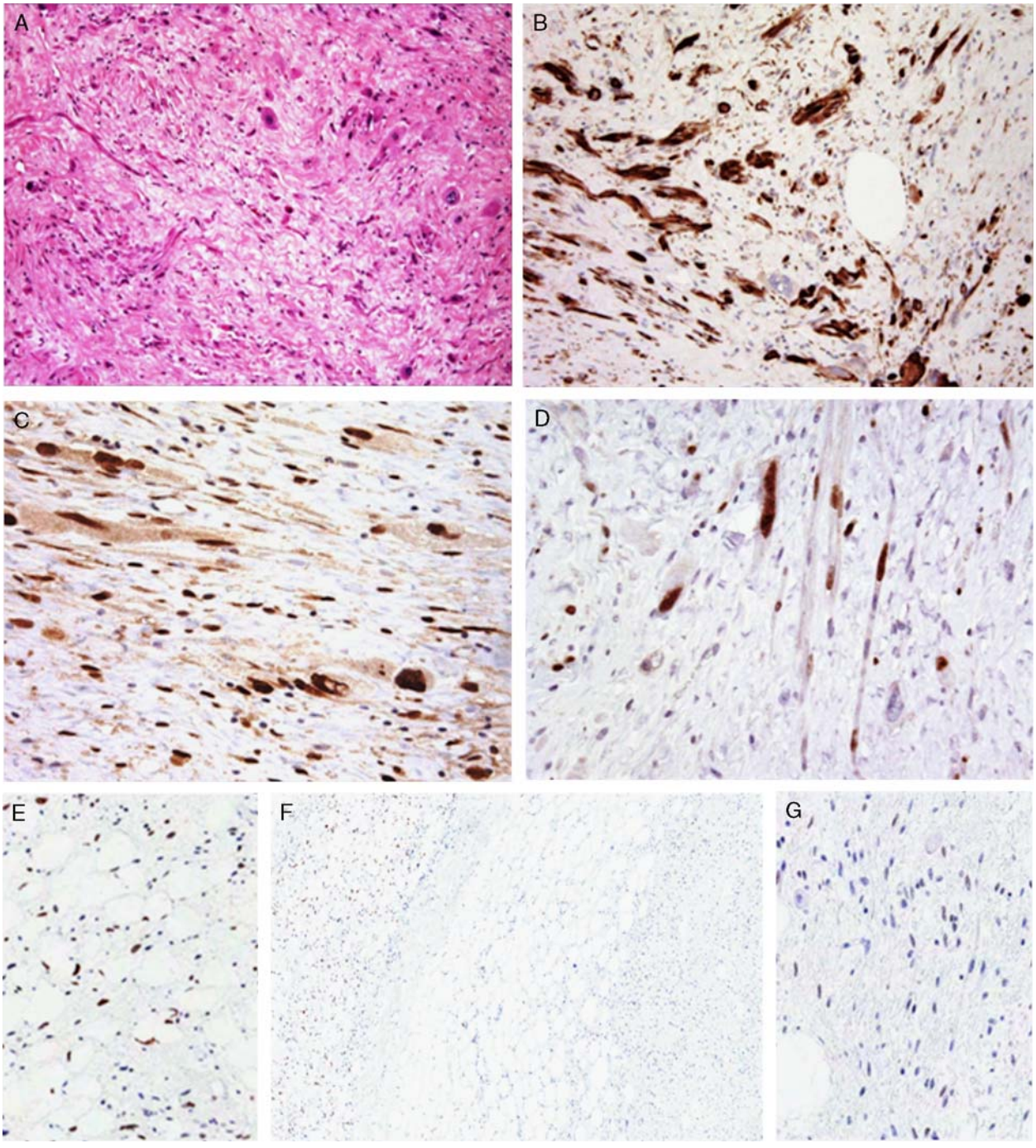

FIGURE 1. Case 1 in Table 2: A, Grade 2 DD liposarcoma with divergent rhabdomyoma-like rhabdomyosarcomatous differentiation (hematoxylin and eosin). Tumoral cells exhibit cytoplasmic expression of desmin (B), nuclear expression of myogenin (C), and nuclear expression of mdm2 (D). Low magnification of a sample showing both WD (left) and DD (right) components (F) in which C/EBP-α nuclear immunostaining was retained in the former (F [left] and E [higher magnification]) and lost in the latter (F [right] and G).

featured pleomorphic, round cell morphology (Fig. 2), whereas spindle and giant cells, also called anaplastic spindle cells,[20] were observed in the remaining 2 cases (Fig. 3).

As shown in Table 2, in these 7 cases mdm2 immunohistochemical profile fitted with that of FISH analyses, whereas cdk4 expression was restricted to the WD component in all cases.

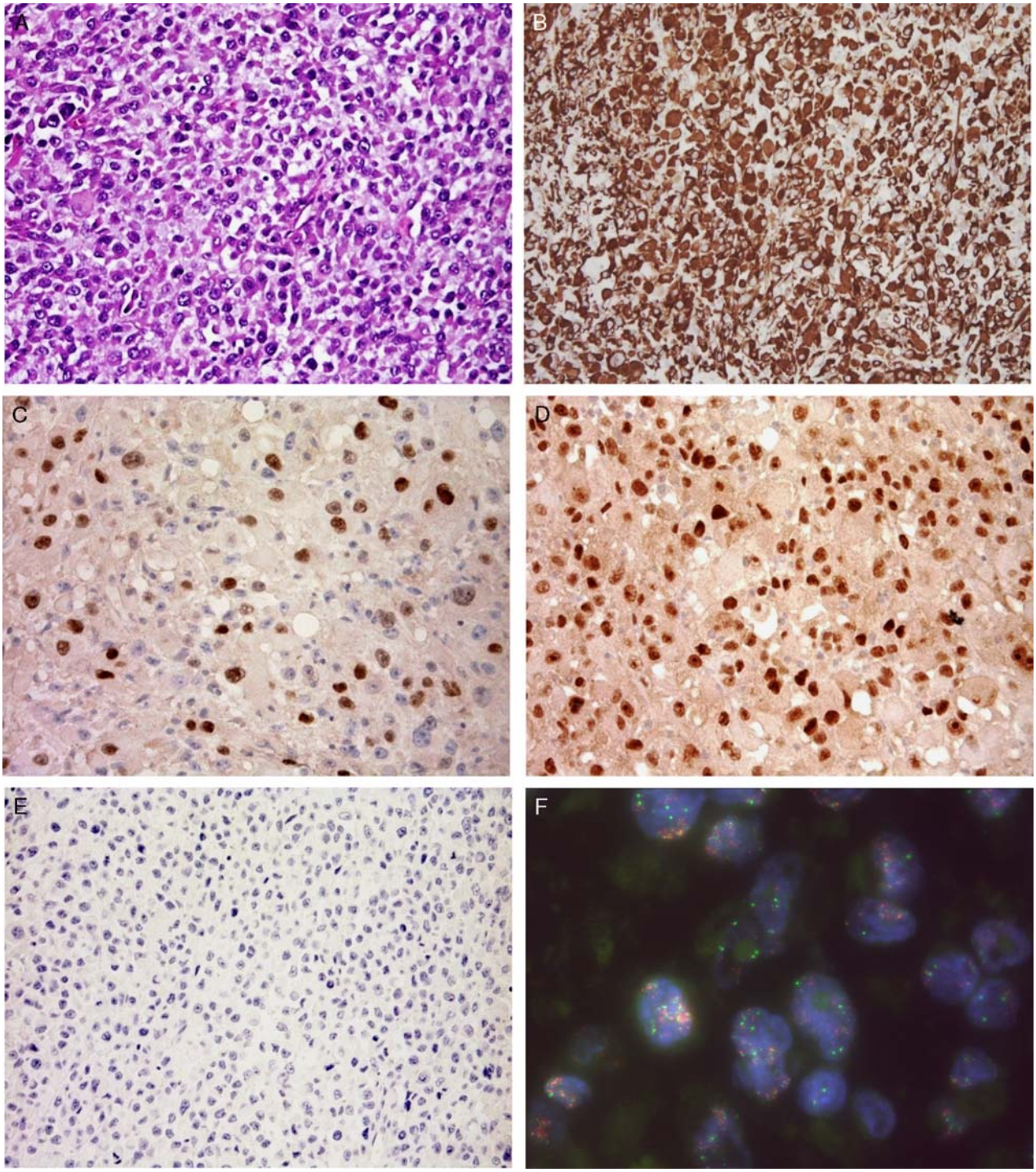

FIGURE 2. Case 2 in Table 2: A, Grade 3 DD liposarcoma with divergent pleomorphic rhabdomyosarcomatous differentiation (hematoxylin and eosin). Tumor cells are immunoreactive for desmin (B), myogenin (C), and mdm2 (D) and negative for C/EBP-α (E). F, FISH shows *MDM2* (Spectrum Red) and *CDK4* (Spectrum Green) coamplification.

FISH analysis showed amplification of both *MDM2* and *CDK4* genes in the WD and DD components of all 7 cases. Furthermore, the DD component of each of these cases showed loss of C/EBP-α immunoreactivity.

Outcome

The median follow-up was 68 months (interquartile range 46 to 104 mo). Patients and treatment characteristics are listed in Table 3.

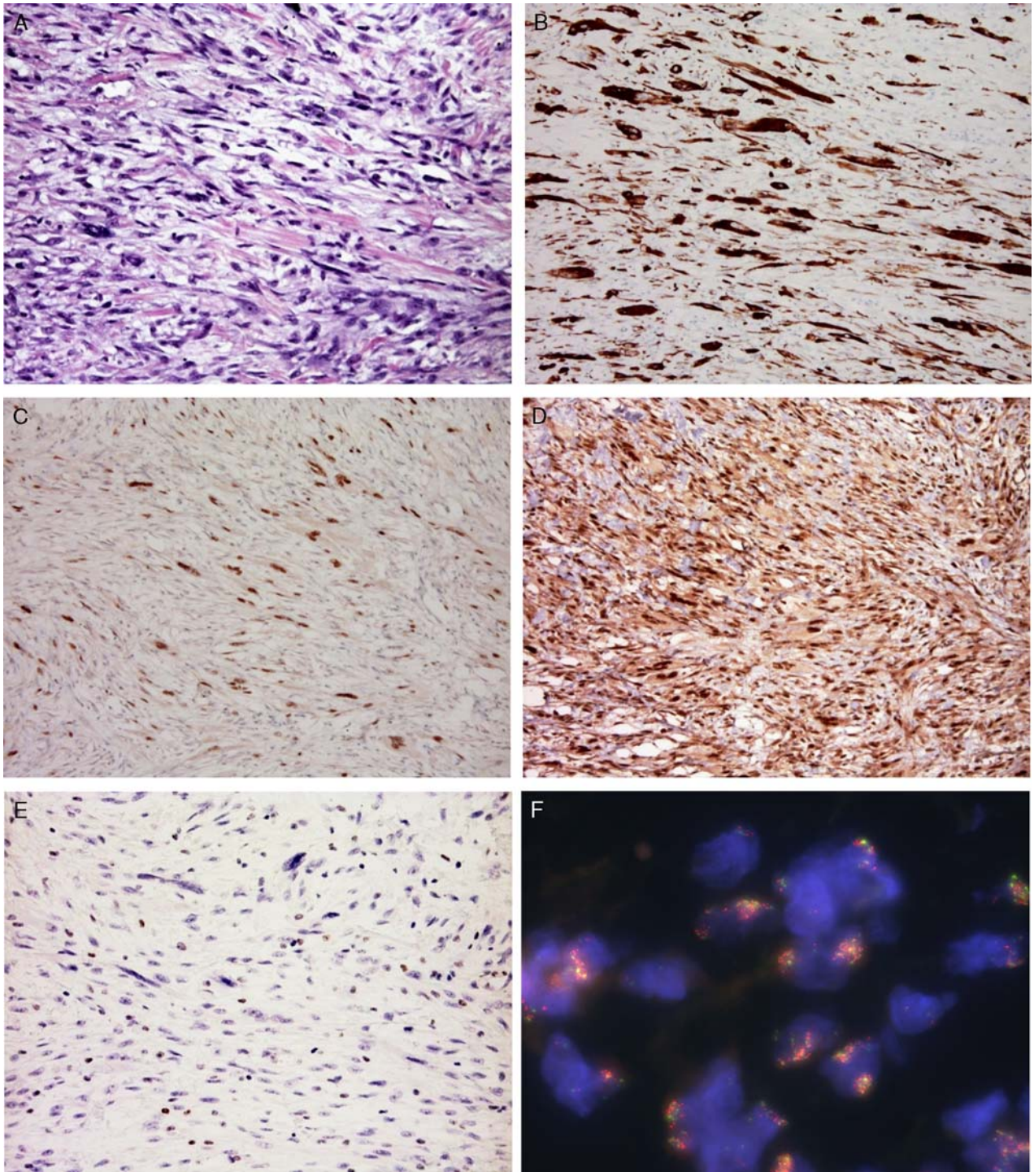

FIGURE 3. Case 6 in Table 2: A, Grade 3 DD liposarcoma with divergent spindle/giant cell rhabdomyosarcomatous differentiation (hematoxylin and eosin). Tumor cells show desmin (B), myogenin (C), and mdm2 (D) immunodecoration and are negative for C/EBP-α (E). F, FISH shows *MDM2* (Spectrum Red) and *CDK4* (Spectrum Green) coamplification.

TABLE 3. Demographic, Clinical, and Pathologic Characteristics of the Series

	N (%)
Sex	
Female	61 (42.4)
Male	83 (57.6)
Patient's age (median [IQR]) (y)	61 (53-68)
Tumor size (median [IQR]) (cm)	23 (16-32)
FNCLCC grade	
WD/G1	52 (36.1)
DD G2	60 (41.7)
DD G3	32 (22.2)
Differentiation	
No myogenic differentiation	83 (57.6)
Myogenic differentiation	54 (37.5)
Rhabdomyoblastic differentiation	7 (4.9)
Adjuvant/neoadjuvant CT	
Done	46 (31.9)
Not done	98 (68.1)
Adjuvant/neoadjuvant RT	
Done	27 (18.8)
Not done	117 (81.2)

CT indicates chemotherapy; IQR, interquartile range; RT, radiation therapy.

Overall Survival

At the time of the present analysis, 60/144 patients died. Death was the first event in 9 patients, whereas it followed LR in 31 and DM in 20. The 5-year overall OS was 61.2 (95% confidence interval [CI]: 53.2%-70.5%).

Six of the 52 patients with WD/G1 liposarcoma, 29/60 patients with G2 DD liposarcoma and 25/32 patients with G3 DD liposarcoma died. The corresponding 5-year OS figures were 92.9% (95% CI: 85.4%-100.0%), 56.5% (95% CI: 44.5%-71.7%), and 21.2% (95% CI: 10.2%-43.7%), respectively ($P < 0.0001$) (Fig. 4A).

Twenty-five of the 83 patients without myogenic differentiation, 30/54 patients with myogenic differentiation, and 5/7 patients with a rhabdomyoblastic component died. The corresponding 5-year OS figures were 75.4% (95% CI: 66.2%-85.8%), 42.4% (95% CI: 29.6%-60.8%), and 28.6% (95% CI: 8.9%-92.2%), respectively ($P < 0.0001$) (Fig. 4B).

Besides age, the only significant determinants of survival at multivariable analysis were malignancy grade and presence of myogenic differentiation (Table 4).

Local Recurrence

Fifty-eight of 144 patients (40.3%) developed LR after surgery at our institution. Forty-seven developed LR as their first event. The 5-year CCI of LR was 28.3% (95% CI: 21.6%-37.1%).

Eleven of the 52 patients with WD/G1 liposarcoma, 24/60 patients with G2 DD liposarcoma, and 12/32 patients with G3 DD liposarcoma developed LR as their first event. The corresponding 5-year CCIs of LR were 14.1% (95% CI: 7.0%-28.5%), 36.7% (95% CI: 25.8%-51.9%), and 34.4% (95% CI: 21.1%-56.0%), respectively ($P = 0.093$) (Fig. 4C).

Twenty-six of the 83 patients with liposarcoma without myogenic differentiation, 19/54 patients with liposarcoma with myogenic differentiation, and 2/7 patients with liposarcoma with a rhabdomyoblastic component developed LR as their first event.

The corresponding 5-year CCIs of LR were 25.6% (95% CI: 17.4%-37.8%), 32.1% (95% CI: 21.4%-47.9%), and 28.6% (95% CI: 8.0%-100.0%; last LR at 4 mo), respectively ($P = 0.814$) (Fig. 4D).

At multivariable analysis only tumor size and grade yielded a P value as low as about 10% (Table 4).

Distant Metastases

Twenty-five/144 patients (17.4%) developed DM (8 to the lung, 3 to the liver, 1 to the bone, 13 to other sites including a combination of the above). Twenty-three patients developed DM as the primary event. The corresponding CCI of DM at 5 years was 16.2% (95% CI: 11.1%-23.8%).

None of the 52 patients with WD/G1 liposarcoma, 8/60 patients with G2 DD liposarcoma, and 15/32 patients with G3 DD liposarcoma developed DM as their first event. The corresponding 5-year CCIs of DM were 0%, 13.3% (95% CI: 6.9%-25.6%; last DM at 29 mo), and 46.9% (95% CI: 32.1%-68.5%; last DM at 45 mo), respectively ($P < 0.0001$) (Fig. 4E)

Four of the 83 patients with liposarcoma without myogenic differentiation, 16/54 patients with liposarcoma with myogenic differentiation, and 3/7 patients with liposarcoma with a divergent rhabdomyoblastic component developed DM as their first event. The corresponding 5-year CCIs of DM were 4.8% (95% CI: 1.8%-12.7%; last DM at 29 mo), 30.6% (95% CI: 20.1%-46.5%; last DM at 45 mo), and 42.9% (95% CI: 16.7%-100.0%; last DM at 4 mo), respectively ($P < 0.0001$) (Fig. 4F).

Malignancy grade and presence of myogenic differentiation were the only significant determinants of DM at multivariable analysis (Table 4).

DISCUSSION

In this series of 144 patients affected by primary retroperitoneal liposarcoma, consecutively treated at our institution over a 10-year time span, 5-year OS was 61.2%, whereas CCI of LR and DM were 28.3% and 16.2%, respectively. Histologic grade evaluated according to the FNCLCC system proved to be the strongest prognosticator of outcome. In addition, the presence of myogenic differentiation significantly impacted OS and DM, with a particularly dismal prognosis when a rhabdomyosarcomatous component was evident.

Retroperitoneal liposarcoma represents a challenging disease, marked by a high risk of death even when biologically indolent, because of inoperable locoregional recurrences.[2,21] Historically, they were split into 2 entities: WD liposarcoma, with a natural history characterized by multiple locoregional recurrences and long initial disease-free intervals, and DD liposarcoma, with a natural history characterized by faster locoregional recurrences and—in a limited proportion of patients—by the occurrence of DM.[1]

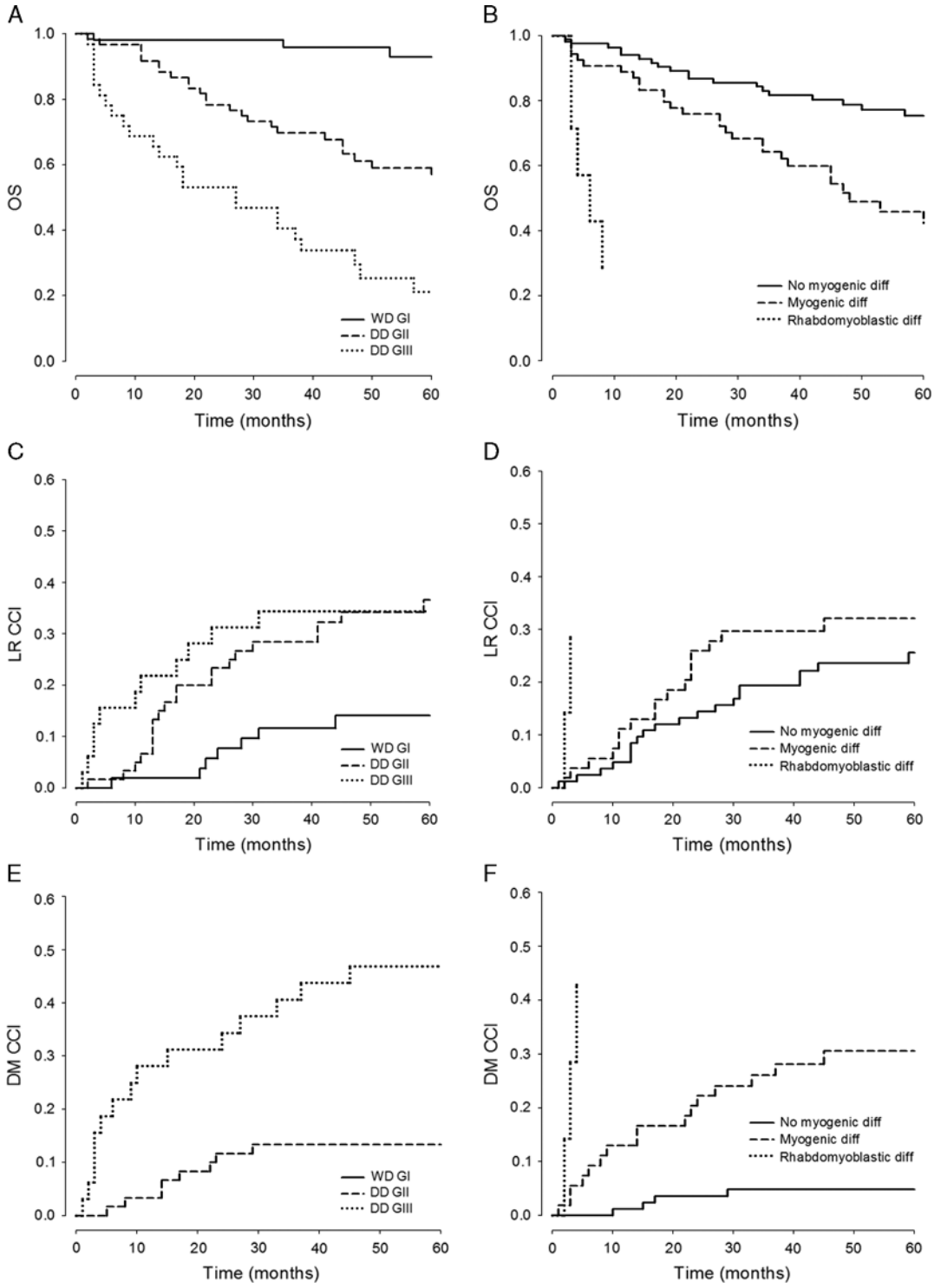

FIGURE 4. A and B, 5-year OS by histologic grade of aggressiveness (A) and myogenic differentiation (B). C and D, 5-year CCI of LR by histologic grade of aggressiveness (C) and myogenic differentiation (D). E and F, 5-year CCI of DM by histologic grade of aggressiveness (E) and myogenic differentiation (F).

There is a remarkable association between FNCLCC malignancy grade and outcome in soft tissue sarcomas.[22] It is based on morphology and requires assessment of mitotic count, differentiation, and presence of necrosis. Its applicability to retroperitoneal liposarcoma has been challenged over the years, and it is still not recommended by the current WHO classification.[1]

We already showed some years ago, in a limited series of historical patients, how grading the DD component of retroperitoneal liposarcoma had prognostic meaning, but the retrospective nature of the analysis and the limited number of patients in that series, spanning over a long time period, prevented us from drawing any definitive conclusions.[8] More recently, we collected a multi-institutional series of retroperitoneal sarcomas and built a nomogram to predict OS and disease-free survival, on the basis of independent prognostic factors.[23] Malignancy grade was one of these factors, indirectly confirming its independent association with the prognosis of retroperitoneal liposarcoma.

Notably, the present series is composed of patients uniformly treated with extended primary resection by a dedicated sarcoma surgical team. We previously offered evidence on how this approach was associated with better local control and possibly better survival, especially for low-intermediate grade retroperitoneal sarcoma.[24–27] It may therefore be that by optimizing local therapy against this tumor, we have reduced the number of surgical failures, making the prognosis more clearly dependent on the biology of the disease. This may have helped to highlight the importance of grading the DD component in its natural history.

The present findings underscore the higher incidence of DM as compared with the historical series.[2,21,28,29] This may be due to a different case mix, better technical expertise, and therefore the inclusion of more advanced/aggressive tumors, but we cannot rule out the possible role of better local control. This higher risk was predominantly associated with high-grade DD liposarcoma, which had more than a 40% chance of developing DM at 5 years. This incidence clearly prompts the need for systemic therapy to complement the best local approach.

A recent report from the Dana Farber group is in accordance with our findings.[30]

We therefore believe that grading the DD component of retroperitoneal liposarcoma should become standard practice, because it increases the accuracy of prognostic risk prediction and may be included in patient stratification criteria for clinical trials.

The other intriguing finding of our analysis is the independent impact of myogenic differentiation on distant spread and survival. The DD component of retroperitoneal liposarcoma may indeed exhibit divergent differentiation. According to the literature, heterologous differentiation accounts for 5% to 10% of cases, mainly involving differentiation toward myogenic lineage.[7] We found myogenic non rhabdomyoblastic differentiation in 15% and 48% of WD and DD liposarcoma, respectively, and pure rhabdomyoblastic differentiation in 8% of DD retroperitoneal liposarcoma. The negative prognostic impact of myogenic differentiation in undifferentiated pleomorphic sarcomas was shown a decade ago,[31] but the same impact was not observed in DD liposarcoma.[7] Our findings indicate that the presence of myogenic differentiation in retroperitoneal liposarcoma was independently associated with a higher risk for DM and death. This was even more true in the presence of a rhabdomyoblastic component. Notably, 5 of 6 patients who had G3 DD liposarcoma with rhabdomyoblastic differentiation developed widespread metastatic disease shortly after surgery of the primary tumor and died within 8 months. The only case with G2 DD liposarcoma with divergent rhabdomyoblastic differentiation (Fig. 1) was an expected exception corresponding to a very rare subtype, which presents a less aggressive course, despite sharing histologic features with rhabdomyosarcoma.[20,32] To detect myogenic differentiation we recommend the application of smooth muscle actin-α (1A4) and desmin immunohistochemistry, complemented by myogenin in desmin-positive cases.

The high occurrence of heterologous myogenic differentiation supports the notion that adipose tissue, similarly to bone marrow, represents a source of adult stem cells capable of differentiating toward many mesodermal lineages,[33,34] most notably myogenic.[33,34] In addition,

TABLE 4. Results From the Cox Proportional Hazards Models on the 3 Endpoints Analyzed

	OS			LR			DM		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Patient's age (y)			0.028			0.336			0.401
68 vs. 52*	1.71	1.09-2.66		1.19	0.77-1.84		1.44	0.85-2.44	
Tumor size (cm)			0.467			0.089			0.651
32 vs. 16*	1.23	0.78-1.95		1.66	1.03-2.70		1.21	0.65-2.28	
FNCLCC grade			< 0.001			0.099			0.003
DD G2 vs. G1 WD	2.10	1.20-3.68		1.61	1.01-2.57		1.68	0.80-3.53	
DD G3 vs. G1 WD	3.43	1.89-6.20		1.46	0.85-2.51		3.73	1.74-8.02	
Differentiation			0.015			0.375			0.023
Myogenic vs. no myogenic	1.77	1.08-2.90		1.37	0.87-2.16		2.43	1.24-4.76	
Rhabdomyoblastic vs. no myogenic	3.44	1.37-8.62		1.54	0.68-3.50		3.44	1.11-10.65	
Adjuvant/neoadjuvant CT			0.968			0.422			0.739
Yes vs. no	0.99	0.57-1.73		1.22	0.75-2.01		1.13	0.54-2.35	
Adjuvant/neoadjuvant RT			0.327			0.246			0.479
Yes vs. no	0.72	0.38-1.39		0.72	0.41-1.25		0.74	0.32-1.70	

*The 2 values are, respectively, the third and first quartiles of the variable distribution.
CT indicates chemotherapy; HR, hazard ratio; *P*, *P* value at Wald test; RT, radiation therapy.

recently reported array comparative genome hybridization results demonstrated that progression from WD toward DD is marked by a striking correlation between loss of genes that are downregulated during progression and dedifferentiation.[6] Altogether these findings suggest that dedifferentiation implies genetic reprogramming, determined at least in part by the loss of genes required to maintain the WD phenotype. In keeping with it, all the 7 cases with rhabdomyoblastic differentiation resulted null for C/EBP-α. We cannot of course rule out the alternative hypothesis that the initiating cell could be a differentiated adipocyte derailed into muscle lineage by an activated oncogene. In support of this, adipocyte-restricted activation of sonic hedgehog signaling has been reported to give rise to aggressive rhabdomyosarcomas in mice.[35] Further analyses are needed to decipher this phenomenon.

Regarding the FISH results, the *MDM2*/*CDK4* profile observed in the DD liposarcoma with pure rhabdomyoblastic differentiation overlaps with that of the WD/DD liposarcoma. This is in stark contrast with the level of amplifications observed in rhabdomyosarcoma, which displays only copy gains in multiple chromosome loci.[36]

Amplification of *MDM2* in the DD component by FISH in the absence of cdk4 immunoreactivity is likely to be linked to tumor differentiation. It could be postulated that the acquisition of new genetic lesions removes the tumor's dependence on *CDK4* and that this loss might also be part of the global reprogramming of the tumor. Unfortunately, no whole-genome profiling studies for liposarcoma with rhabdomyosarcomatous dedifferentiation are yet available to help us identify such genetic lesions.

In conclusion, our data prove that retroperitoneal liposarcoma can be broken down into different risk categories, on the basis of malignancy grade and expression of myogenic markers. This should be factored into clinical decision-making and possibly used to stratify patients in clinical trials. A subset of retroperitoneal liposarcomas featuring rhabdomyoblastic differentiation seems to be highly aggressive and may warrant the use of systemic therapies.

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