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

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Funding and conflict of interest disclosures: Dr. Chapel's work is supported by the Ovarian Cancer Research Alliance [Ann Schreiber Mentored Investigator Award; grant number 650320]. The authors have no other disclosures.

Data availability statement: Supporting data are available from the corresponding author on reasonable request

Running Title: Dedifferentiated uterine leiomyosarcoma

Word Count: 3405

Abstract

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 tumor 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 tumor of uncertain malignant potential (n=2). The dedifferentiated tumor component showed non-cohesive 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 vs 20 months; 5-year DSS, 46% vs 36%; P=0.04) and disease-free (median, 31 vs 8 months; 5-year DFS, 42% vs 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; 4 were alive with disease at 4, 12, 44, and 50 months; and 3 were alive with no evidence of disease at 56, 109, and 114 months.

Conclusions: Routine prospective recognition of dedifferentiated uterine LMS and distinction from mimics is advocated for accurate prognostication and for further characterization of these tumors.

MeSH Keywords: Leiomyosarcoma; Immunohistochemistry; Uterus; Fumarate hydratase

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 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 tumor, and LMS[8–11]—and heralds more aggressive clinical behavior. 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 hypothesized that dedifferentiation in uterine LMS is likely underrecognized, and that, when present, it heralds particularly aggressive behavior, 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 tumors, and
ii. immunophenotypic evidence of smooth muscle differentiation, defined as ≥5% tumor 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]

Tumors 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 tumor cells were termed "pleomorphic uterine LMS," in keeping with prior work.[14]

A search of the electronic medical record 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 tumors 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 hematoxylin and eosin (H&E)-stained slides (median 7; range, 1-24) were reviewed for 341 BWH/DFCI tumors and 83 tumors from a collaborating Italian consortium.

Thirteen BWH/DFCI and 3 Italian consortium tumors showed morphologic features of dedifferentiation, of which 9 and 2, respectively, were also negative for smooth muscle immunomarkers. (To permit 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, 5 DFCI referrals, 4 diagnostic consultations to one of the authors (M.R.N.), and 2 tumors 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 gynecological 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 **Supplemental Information 1**.

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 (400x) 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 characterized by a sharp demarcation between viable and non-viable tumor, most often showing an irregular geographic pattern. Lymphovascular invasion was defined by tumor protruding into or entirely within endothelial lined spaces outside of the main tumor 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 tumors for study inclusion. Additional immunostains were performed on 19 tumors with available tissue, including estrogen receptor (ER), progesterone receptor (PR), SMARCA4 (BRG1), SMARCB1 (INI1), retinoblastoma protein (Rb), p16, methylthioadenosine phosphorylase (MTAP), ATRX, and p53. Immunostaining specifications are in **Supplemental Table 1**. 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 tumor cells), and strong

diffuse (strong staining in ≥80% of tumor cells). (Note that although we have applied p53 staining thresholds used in high-grade serous carcinoma, we have 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 tumor 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 1 in-house surgery and 3 diagnostic consults). These tumors 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: 1) diagnosis of uterine LMS confirmed on morphologic and immunohistochemical review, 2) uterine corpus primary site, 3) in-house hysterectomy, or patient referred within 3 months of diagnosis with no interval disease progression, and 4) 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**. Median age at diagnosis was 57 (range, 42-90) years. All 23 dedifferentiated uterine LMS were primary to the uterine corpus. One tumor was stage IA, 12 stage IB, 4 stage II, 2 stage IIIB, and 4 stage IV. Surgical margins were negative in all 13 stage IA or IB tumors. Two (#15, #18) were surgically morcellated. Non-surgical treatment history was known for 13 patients: 5 (3 stage IV, 2 stage IB) received adjuvant therapy (4 chemotherapy, 1 radiation therapy) after hysterectomy, and an additional 5 patients received chemotherapy following recurrence.

Gross Pathology

Gross descriptions were available for 19 tumors. Median tumor size was 10 (range, 3-25) cm. Ten were infiltrative transmural masses replacing the uterine wall; 4, 2, and 1 were discrete intramural, subserosal, and submucosal masses, respectively; and 2 were polypoid in the endometrial cavity, including 1 presenting as myoma nascens. Tumors 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 hemorrhage in 10. Seven were grossly biphasic (**Figure 1**), with grossly discrete elements corresponding to differentiated and dedifferentiated morphology on microscopic examination.

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 tumors, with 7 diagnosed prospectively as dedifferentiated uterine LMS (all 7 between 2013-2020). The dedifferentiated component of the remaining 11 tumors 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 tumor. The tumor-myometrial interface was represented in 22 of 23 tumors and was circumscribed (n=3), microscopically infiltrative (n=3), or grossly infiltrative (n=16). Lymphovascular invasion was present in 12 tumors and included the differentiated component in 7 and the dedifferentiated component in 5.

Recurrent or metastatic tumor was biopsied in 5 cases, of which 2 comprised differentiated LMS only and 3 showed only dedifferentiated tumor (including 1 with osteosarcomatous differentiation).

Dedifferentiated Components

In all 23 tumors, the dedifferentiated component showed "malignant fibrous histiocytoma-like" morphology, characterized by large non-cohesive 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 tumors showed rhabdoid foci, characterized by abundant eosinophilic cytoplasm and an eccentrically placed nucleus. Multinucleated tumor giant cells were seen in 18 cases, with prominent nuclear pseudoinclusions in 5. Seven showed multiple tumor nuclei arranged in a peripheral ring (**Figures 2C, 3C**). Three tumors (#7, #12, #15) showed osteosarcomatous differentiation (**Figure 3A**), with 2 also showing chondrosarcomatous differentiation (**Figure 3B**). Mitoses ranged from 2-80 (median, 20) per 2.4 mm^2, with atypical mitoses in all 23 (**Figure 3C**) and coagulative necrosis in 19.

In 2 tumors, the dedifferentiated component was intimately admixed with the differentiated component, with cords of dedifferentiated tumor snaking through a differentiated background. In the remaining cases, the components were discrete (**Figure 4**). In 7, the dedifferentiated foci were confined to the luminal tumor aspect (abutting the endometrial cavity), with conspicuous osteoclast-like giant cells in 2 (**Figure 3D**) and abundant cholesterol clefts, foamy histiocytes, and hemosiderin deposition in 1 (**Figure 3E, 3F, 3G**).

Tumor vasculature in dedifferentiated foci was inconspicuous, principally comprising small arterioles, with 1 case each showing prominent hyalinized arteriolar walls, branching staghorn vessels, or sinusoidal vessels. Tumor stroma most often comprised scant collagenous fibers, though hyalinized bands separated individual tumor cells in 6 cases. Prominent

lymphohistiocytic inflammation was seen in 7, osteoclast-like giant cells in 4, eosinophilic infiltrate in 1, and neutrophilic infiltrate in 1.

Differentiated Components

The differentiated component of each tumor is detailed in **Table 1**. Among 17 tumors with a leiomyosarcomatous component, 11 showed severe (3+) and 6 showed moderate (2+) nuclear atypia, with coagulative necrosis in 14. Median mitotic count was 25 (range, 6-85) per 2.4 mm^2, with atypical mitoses in 9. On pairwise comparison, mitotic activity was greater in the dedifferentiated component in 9 tumors, greater in the differentiated leiomyosarcomatous component in 7, and equal in 1 (P=0.99, paired t-test).

Of 2 tumors with a STUMP component, 1 showed moderate (2+) atypia, indeterminate necrosis, and 2 mitoses per 2.4 mm^2 without atypical forms, and 1 showed focal coagulative necrosis and 6 mitoses per 2.4 mm^2 but no significant atypia. A leiomyomatous component was present in 6 tumors (4 cellular, 2 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 (median, 95%; range, 5-100%), and 21 were positive for caldesmon (median, 100%;

range, 1-100%). In 11 tumors, the dedifferentiated component was negative for SMA, desmin, and caldesmon. In 9 tumors, the dedifferentiated component showed rare cells positive for SMA, with 7 also showing rare desmin-positive cells. Two and 1 tumors showed rare cells positive only for desmin and caldesmon, respectively.

Additional Immunomarkers

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

The differentiated component was positive for ER in 11 tumors (median, 80%; range, 30-100%) and PR in 9 (median, 80%; range, 60-100%). In contrast, the dedifferentiated component was focally positive for ER in 3 (1-5%, weak) and for PR in 1 tumor (5%, weak).

Thirteen tumors showed loss of Rb expression in both tumor components (including 2 cases in which the differentiated component was cellular leiomyoma); 5 showed retained Rb in the differentiated component but loss in the dedifferentiated component; and 1 retained Rb in both tumor components.

Ten tumors showed strong diffuse p16 in both the differentiated and dedifferentiated components (the differentiated component was LMS in all 10); 7 showed patchy p16 in the differentiated component but strong diffuse staining in the dedifferentiated component; and 2 showed negative p16 in both components. One tumor 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 tumor with negative p16 and in both components of 9 additional tumors. Rb loss and diffuse p16 were strongly correlated: Of 18 dedifferentiated components

with Rb loss, 17 showed diffuse p16 (the single exception harboring possible chr 9p21 deletion), whereas p16 was negative in the sole dedifferentiated component with retained Rb.

Four tumors showed strong diffuse p53 and 5 showed complete absence of p53 staining in both tumor components (the differentiated component was LMS in all 9); 5 tumors showed heterogeneous staining in the differentiated component and strong diffuse staining in the dedifferentiated component; and 5 showed heterogeneous staining in both components.

ATRX immunohistochemistry was performed in 16 tumors. Five showed loss of ATRX expression in both tumor components (including 1 STUMP); 10 retained ATRX in both components; and 1 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 tumors.

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; 4 were alive with disease at 4, 12, 44, and 50 months; and 3 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 tumor cells were unrelated to survival.

Of 19 patients, only 1 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 3 patients' tumors, 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% vs. 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 **Supplemental Figure 1**.

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 vs 53 years; P=0.02), but there was no significant difference in stage distribution (P=0.79) or tumor 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 vs 54 months; 5-year DSS, 36% vs 46%; P=0.04) (**Supplemental**

Figure 1). Twelve patients with dedifferentiated uterine LMS and 242 with uterine LMS without dedifferentiation were operated 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 vs 31 months; 5-year DFS, 8% vs 42%; P=0.002).

Examining Rare Cases

All 3 patients harboring heterologous differentiation (#7, #12, #15) presented with extrauterine disease (1 stage II, 2 stage IV), and all 3 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 4 of these patients. All 4 presented at stage IV. Three were dead of disease at 10, 10, and 16 months, and 1 was alive with disease at 23 months. Although this subcohort is small, these data suggest comparably aggressive behavior 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 behavior, even among patients

presenting with stage I disease, though prolonged DFS is possible, including after re-excision 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] recognized 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] though 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 totaling 90 tumors,[12–16] 74 (82%) dedifferentiated LMS had recurred or metastasized at median 8 months after initial diagnosis (range, 1-45 months), 49 (54%) had died of disease at median 11 months, and only 6 (7%) had greater than 60 months recurrence-free survival. In published series, dedifferentiated and pleomorphic LMS appear predisposed to comparably

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

Review of the literature identifies 11 primary dedifferentiated uterine LMS.[12,16–21] Follow-up was available in 6 cases, of whom 3 had died of disease at 4, 6, and 17 months,[16,17] 1 had died of other causes at 12 months,[12] and 2 were alive without disease at 12 and 28 months.[12,19] The literature also contains 8 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 behavior in both published reports and our cohort, our series also includes the first reports of favorable outcomes in dedifferentiated uterine LMS, including 2 women with prolonged (54- and 109-month) DFS after early recurrence and 1 with 114 months recurrence-free survival after hysterectomy.

At present, the management implications of dedifferentiation in uterine LMS are unclear.[26] However, some data suggest a role for immunotherapy in undifferentiated pleomorphic sarcomas of soft tissue,[27–29] 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,[30] though we found no consistent trigger for dedifferentiation. Second, we found longer survival among dedifferentiated uterine LMS with a non-leiomyosarcomatous versus 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, though we believe we have addressed this through a fair and rational approach, as discussed above.

Although not previously well characterized, 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 center referrals, which (despite the efforts described in our Methods) may have been enriched in aggressive and/or diagnostically challenging tumors, possibly including dedifferentiated uterine LMS.

To facilitate further study and to emphasize the potential for aggressive clinical behavior, 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 tumor or in recurrent or metastatic tumor[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.

Acknowledgements: The authors wish to thank Dr. Nafisa Wilkinson for contributing consultation material and clinical follow-up for this study.

Drs. Nucci, Quade, and Chapel conceived of and designed the study. All authors contributed cases for study and participated in morphologic evaluation. Drs. Nucci, Chapel, and Parra-Herran performed the consensus morphologic and immunohistochemical evaluation. Dr. Chapel performed statistical analyses and drafted the manuscript. All authors reviewed, edited, and approved of the final manuscript.

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Table legends

Table 1. Clinical, pathologic, and immunohistochemical findings for 23 dedifferentiated uterine leiomyosarcomas. "." 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 tumor of uncertain malignant potential.

Table 2. Differential diagnosis of dedifferentiated uterine leiomyosarcoma.

Figure legends.

Figure 1. Dedifferentiated uterine leiomyosarcoma. The tumor 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 few individual cells positive for desmin (E, right & inset) and strong diffuse p16 (F, right).

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

Figure 3. Morphologic features of dedifferentiated uterine leiomyosarcoma. This tumor showed dedifferentiation in the form of heterologous osteosarcomatous (A) and chondrosarcomatous (B) differentiation. C) Dedifferentiated uterine leiomyosarcoma with atypical mitoses and conspicuous multinucleated tumor giant cells. D) A dedifferentiated uterine leiomyosarcoma abutting the endometrial cavity, with prominent osteoclast-like giant cells. E) A dedifferentiated leiomyosarcoma LMS abutting the endometrial cavity with prominent cholesterol clefts and foamy histiocytes, with aberrant loss of MTAP (F) and Rb (G) expression in pleomorphic tumor cells.

Figure 4. Dedifferentiated uterine leiomyosarcoma. This tumor 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).

Figure 5. Dedifferentiated uterine leiomyosarcoma, immunohistochemistry. This tumor 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).

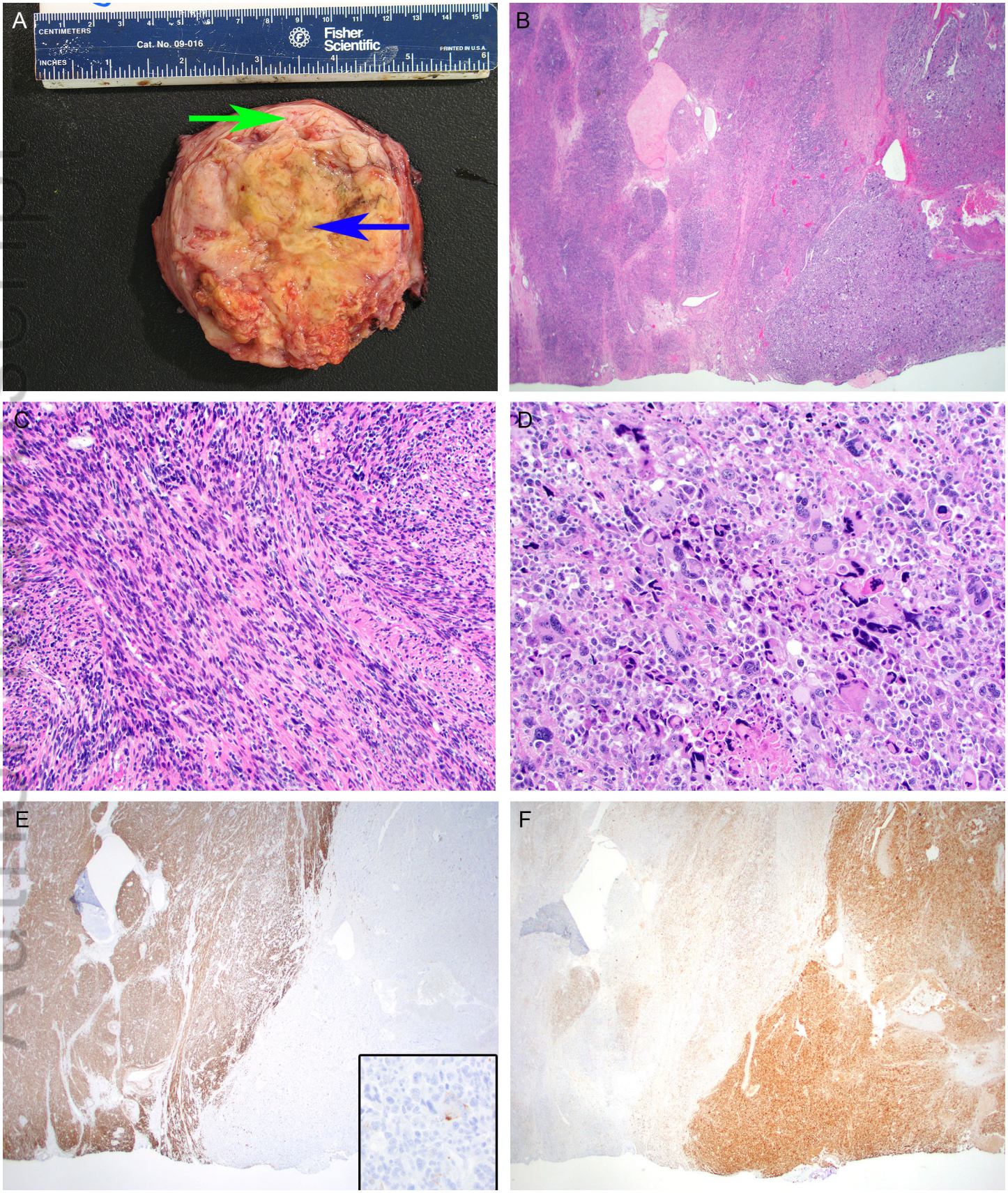
Figure 6. Pleomorphic uterine leiomyosarcoma. This tumor shows areas of conventional leiomyosarcoma (A) and a component morphologically compatible with dedifferentiation (C). However, both components show strong diffuse desmin expression (B, D), which per our criteria warrants diagnosis of pleomorphic leiomyosarcoma rather than dedifferentiated leiomyosarcoma.

Table 1

Case #	Age	Size (cm)	FIGO Stage (2018)	Outcome	DFS (months)	DSS (months)	Adjuvant therapy	Original diagnosis	Morp differentiat
1	88	.	IB	DOD	2	2	.	LMS, high grade	LMS,
2	66	6.5	IB	DOD	7	11	No know n therapy	LMS, high grade	LMS,
3	68	8.5	IB	DOD	3	20	No therapy at initial diagnosis; got chemotherapy after recurrence	LMS, high grade	LMS, spin leio
4	42	10	IB	DOD	.	23	No know n therapy	LMS, high grade (epithelioid)	Cellular
5	61	7	IB	DOD	.	5	.	LMS, high grade	LMS, e
6	57	12	IIIB	.	.	.	.	LMS, high grade, w ith pleomorphic features	LMS,
7	46	12.1	II	DOD	23	31	.	LMS, high grade	LMS,
8	54	8.2	IB	DOD	.	73	.	LMS, high grade	LMS,
9	59	6.8	IB	NED	114	114	Gemcitabine-paclitaxel at initial diagnosis	LMS, high grade, w ith areas of pleomorphic undifferentiated sarcoma	LMS,
10	50	3	IIIB	AWD	12	12	.	LMS	LMS,
11	53	4.5	IA	AWD	21	50	.	LMS	STUMP
12	63	11.7	IV	DOD	8	16	Gemcitabine-paclitaxel at initial diagnosis	LMS, high grade, w ith heterologous osteosarcomatous differentiation	LMS,
13	53	8	IV	DOD	n/a	15	Gemcitabine-paclitaxel at initial diagnosis	LMS	LMS,
14	54	25	IV	DOD	n/a	3	No know n therapy	LMS, high grade, w ith areas of pleomorphic undifferentiated sarcoma	LMS,
15	52	20	IV	DOD	n/a	10	Gemcitabine-paclitaxel at initial diagnosis	LMS, high grade, w ith heterologous osteosarcomatous and chondrosarcomatous differentiation	LMS,
16	57	9	IB	NED	4	109	No therapy at initial diagnosis; got chemotherapy after recurrence	Dedifferentiated leiomyosarcoma	Cellular
17	90	20	II	.	.	.	.	Dedifferentiated leiomyosarcoma	L
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
19	70	11	IB	AWD	15	44	Adjuvant radiation therapy at initial diagnosis	Dedifferentiated leiomyosarcoma	STUMP
20	75	6.1	IB	.	.	.	.	Dedifferentiated leiomyosarcoma	LMS,
21	55	10	IB	AWD	4	4	No therapy at initial diagnosis; got gemcitabine-paclitaxel after recurrence	Dedifferentiated leiomyosarcoma	LMS,
22	60	14.3	IB	DOD	8	13	No therapy at initial diagnosis; got doxorubicin after recurrence	Dedifferentiated leiomyosarcoma	LMS, spin leio
23	63	7.7	II	.	.	.	.	Dedifferentiated leiomyosarcoma	LMS,

Table 2

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 tumor cells
PEComa	
	Nuclear pleomorphism typically (though not always) non-diffuse
	Sinusoidal vessels and/or dense hyalinization characteristic
	Cathepsin K diffusely positive (sensitive but non-specific 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 carcinona	
	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



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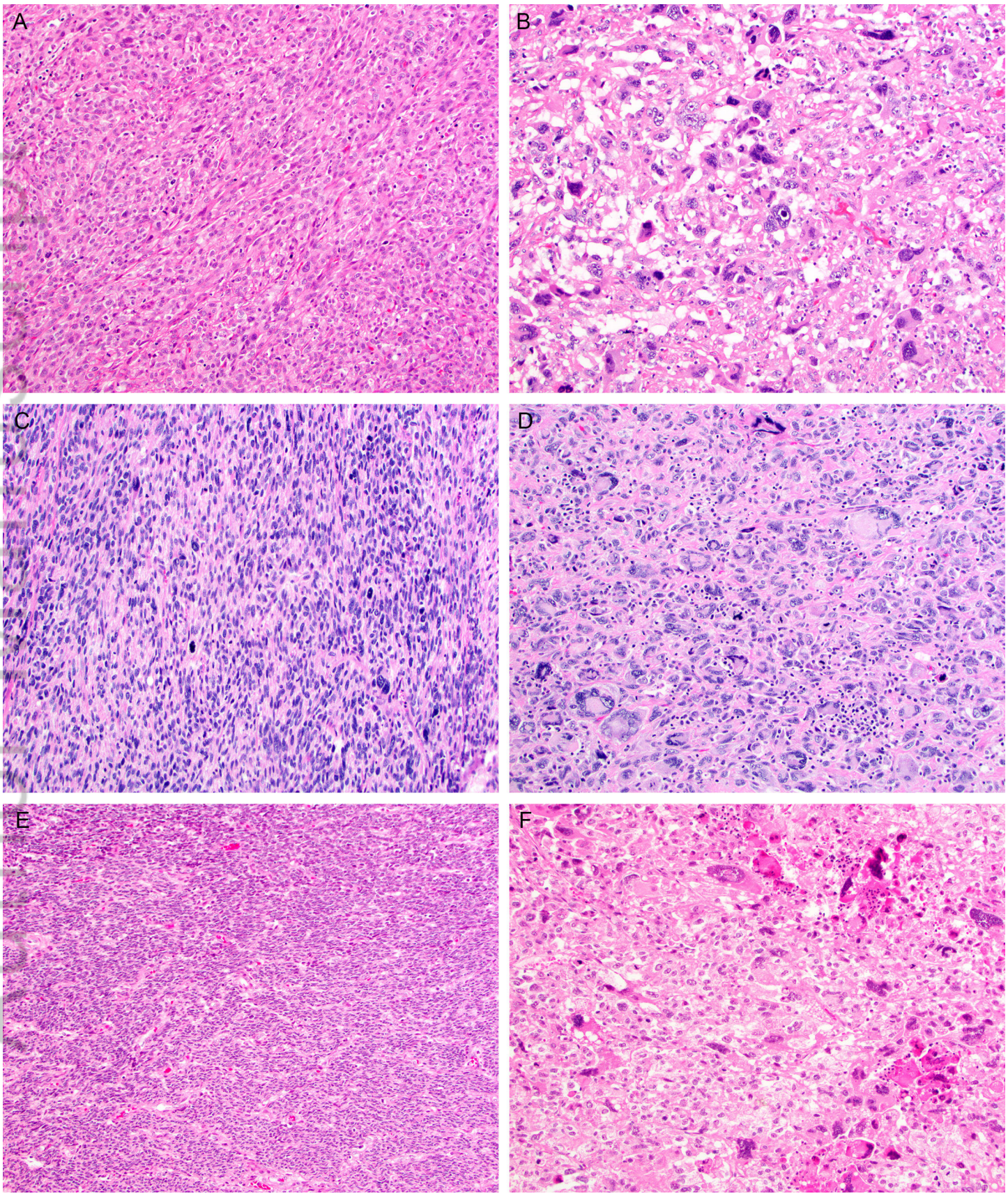

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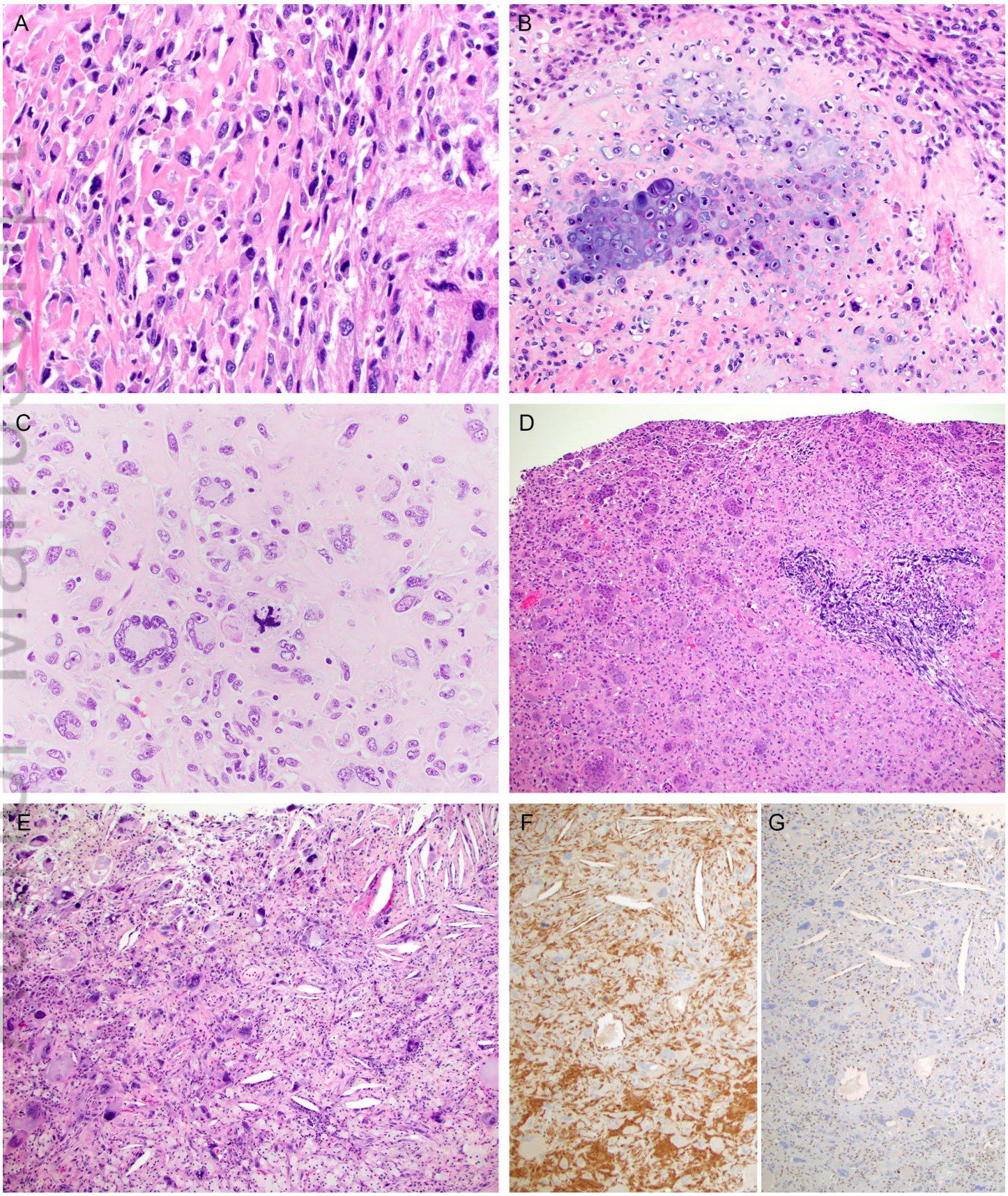

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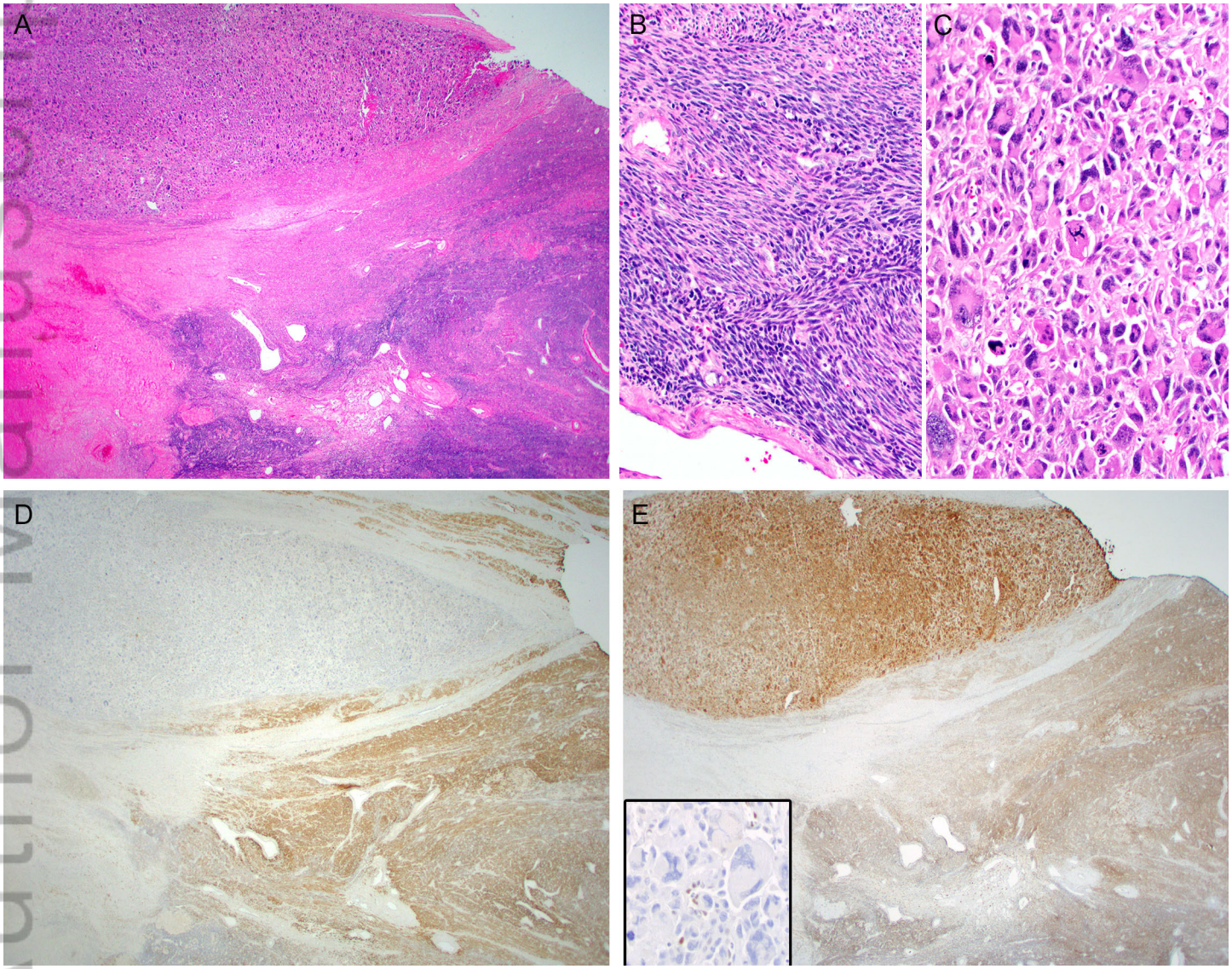

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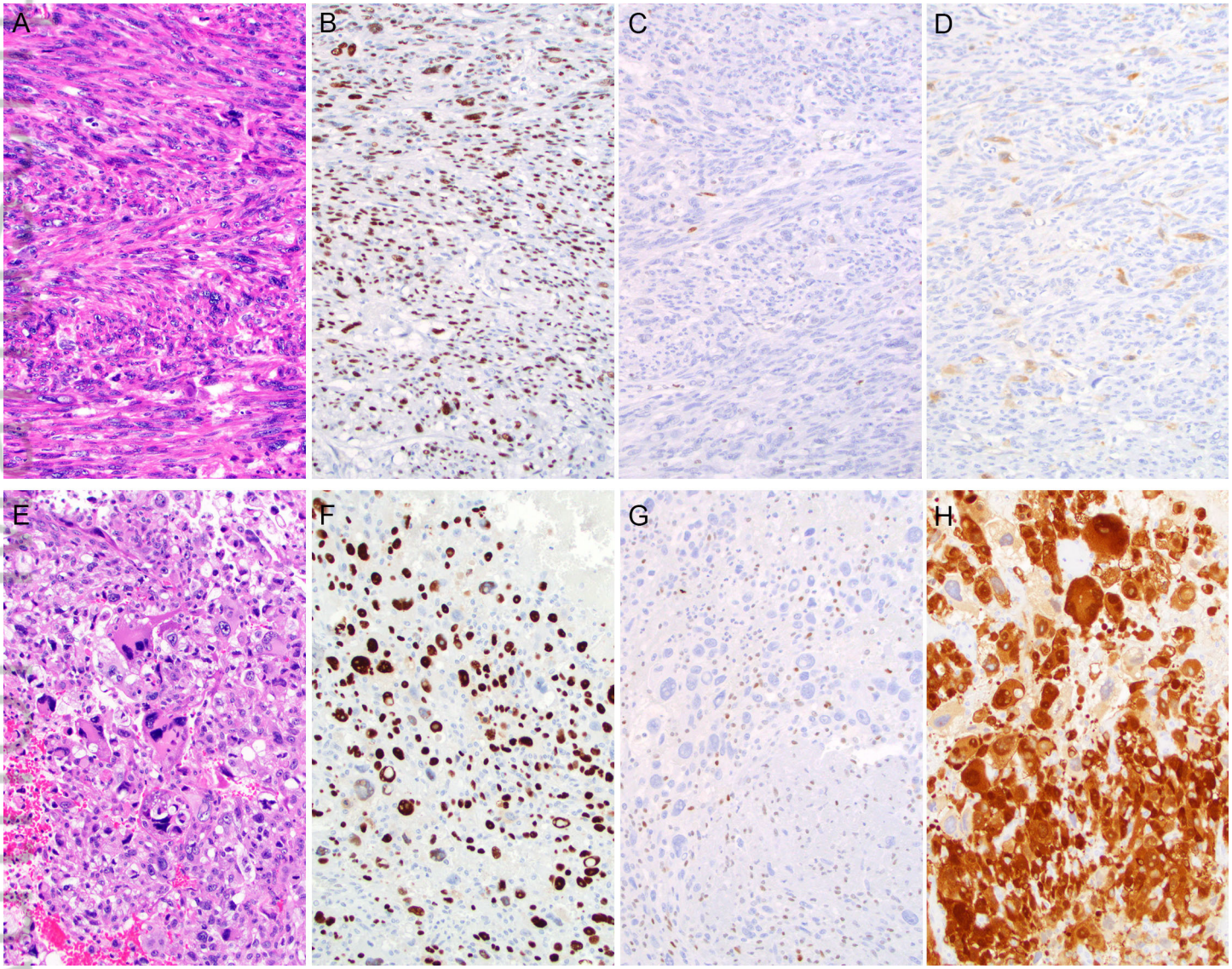

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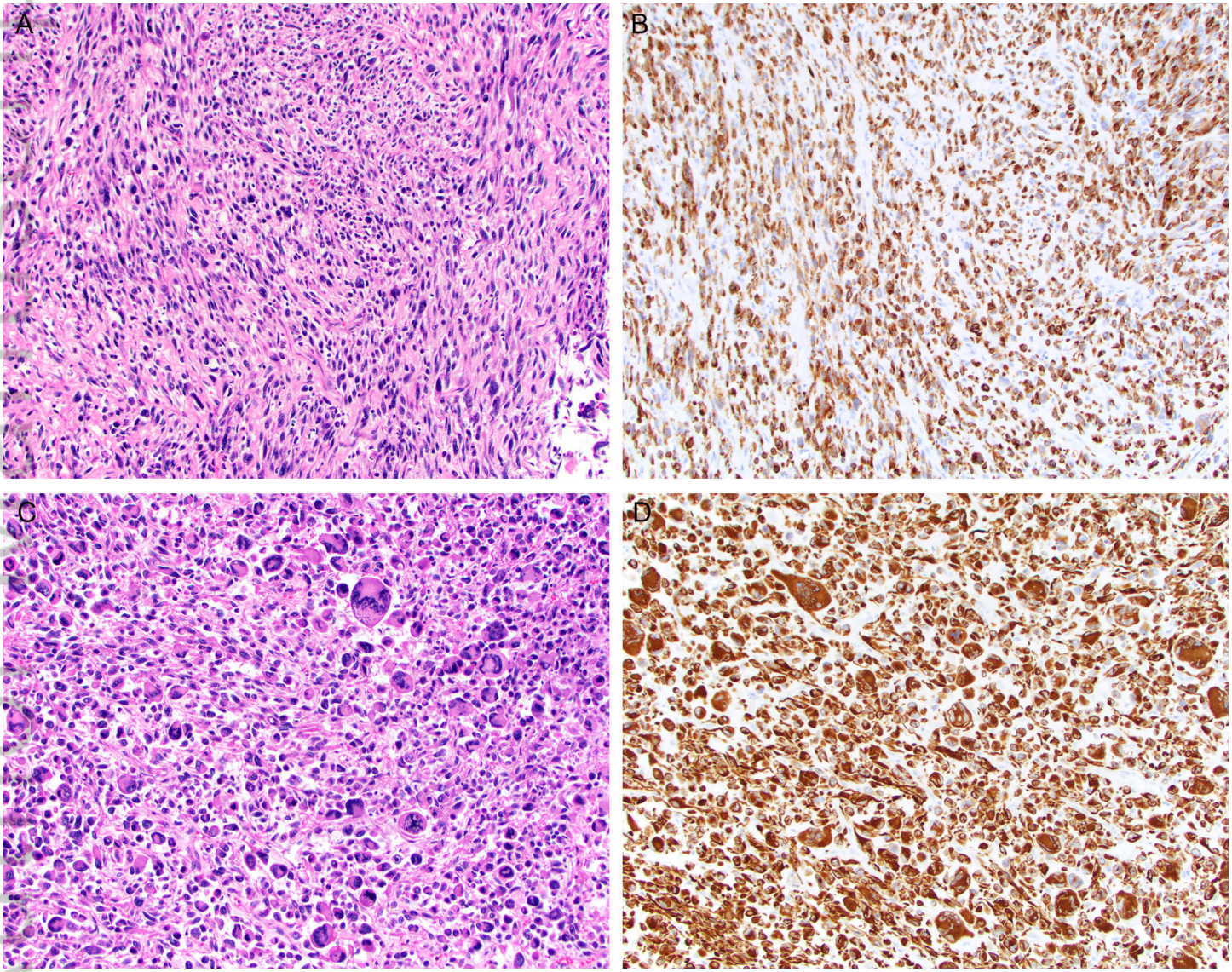

HIS_14870_Figure 6.jpg