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GLI1 Co-Amplification in Well-differentiated/Dedifferentiated Liposarcomas: Clinicopathologic and Molecular Analysis of 92 Cases

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Abstract

Background: *GLI1* (12q13.3) amplification is identified in a subset of mesenchymal neoplasms with a distinct nested round cell/epithelioid phenotype. *MDM2* and *CDK4* genes are situated along the oncogenic 12q13–15 segment, amplification of which defines well-differentiated (WDLPS)/dedifferentiated liposarcoma (DDLPS). The 12q amplicon can occasionally include *GLI1* – a gene in close proximity to *CDK4*. We hereby describe the first cohort of *GLI1/MDM2/CDK4* co-amplified WD/DDLPS.

Materials and Methods: The departmental database was queried retrospectively for all cases of WD/DDLPS having undergone next generation (IMPACT) sequencing with confirmed *MDM2*, *CDK4*, and *GLI1* co-amplification. Clinicopathologic data was obtained from review of the medical chart and available histologic material.

Results: 486 WD/DDLPS underwent DNA sequencing, 92 (19%) of which harbored amplification of the *GLI1* locus in addition to *MDM2* and *CDK4*. These included primary tumors (n=60), local recurrences (n=29), and metastases (n=3). Primary tumors were most frequently retroperitoneal (47/60,78%) mediastinal (4/60,7%), and paratesticular (3/60, 5%). Average age was 63 years with a male: female ratio of 3:2.

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The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

Ethics Approval / Consent to Participate

This study was approved by the Institutional Review Board at Memorial Sloan Kettering Cancer Center.

The cohort was comprised by DDLPS (86/92 [93%], 6 of which were comprised by WDLPS with early dedifferentiation), and WDLPS without any longitudinal evidence of dedifferentiation (6/92, 7%%). A fifth (13/86,17%) of DDLPS cases showed no evidence of a well-differentiated component in any of the primary, recurrent, or metastatic specimens. Dedifferentiated areas mostly showed high-grade undifferentiated pleomorphic sarcoma-like (26/86, 30%),) and high-grade myxofibrosarcoma-like (13/86, 16%)) morphology. A disproportionately increased incidence of meningothelial whorls with/without osseous metaplasia was observed as the predominant pattern in 16/86 (19%), and GLI1-altered morphology as described was identified in a total of 10/86 (12%) tumors.

JUN (1p32.1), also implicated in the pathogenesis of WD/DDLPS, was co-amplified with all three of *MDM2*, *CDK4*, and *GLI1* in 7/91 (8%) cases. Additional loci along chromosomal arms 1p and 6q, including *TNFAIP3*, *LATS1* and *ESR1*, were also amplified in a subset of cases.

Conclusions: In this large-scale cohort of *GLI1* co-amplified WD/DDLPS, we elucidate uniquely recurrent features including meningothelial whorls and GLI-altered morphology in dedifferentiated areas. Assessment of tumor location (retroperitoneal or mediastinal), identification of a well-differentiated liposarcoma component, and co-amplification of other spatially discrete genomic segments (1p, 6q) might aid in distinction from tumors with true driver *GLI1* alterations.

Keywords

GLI1 ; *MDM2* ; *CDK4* ; dedifferentiated liposarcoma; well-differentiated liposarcoma; 12q; amplification

Introduction

With the ongoing molecular interrogation of mesenchymal tumors, our understanding of neoplasms with *GLI1* aberrations has evolved significantly over the past decade. Originally conceptualized as group of pericyte-derived proliferations with underlying *GLI1::ACTB* fusions (1), this umbrella of '*GLI1*-altered mesenchymal tumors' now encompasses an array of entities with not only distinct clinicopathologic attributes but also different mechanisms of genomic tumorigenesis. Pathogenic fusions between *GLI1* and *MALAT1*, for example, give rise to both benign (plexiform fibromyxoma) and aggressive to malignant (gastroblastoma/'GNET') lesions of the gastric wall (2–4). Other fusion partners including *PTCH1*, *NCOR2*, *NEAT1, SYT, DDIT3,* and *AARS* are recently described in multiple case series, sourcing tumors with heterogenous demographics but a conspicuously recurrent histologic motif: multilobulated proliferations of monotonous round to epithelioid cells, arranged in nests with intervening rich arborescent capillary vasculature (5–10).

From a structural perspective, the *GLI1* gene is situated along an oncogenic expanse on chromosome 12q13.3 that is implicated in various soft tissue tumors, including *MDM2* (12q15), *CDK4* (12q14.1), *DDIT3* (12q13.3), *HMGA2* (12q14.3) and *STAT6* (12q13.3). In particular, amplification of *MDM2* and/or *CDK4* define the spectrum of atypical lipomatous tumor/well-differentiated (WDLPS)/dedifferentiated liposarcoma (DDLPS) in appropriate contexts. Due to their conglomerate spatial proximity, the driver *MDM2* and/or *CDK4* amplicon is associated with co-amplifications of these neighboring genes in a subset of

cases – a phenomenon which to date is not well-characterized in either clinicopathologic or genomic frameworks (11–14).

Prior investigation into an array of *GLI1*-amplified neoplasms revealed a considerable subset of DDLPS with evidence of concomitant *GLI1* (along with axiomatic *MDM2* and *CDK4)* amplification, comprising approximately a fifth of those archived in our institutional database. Remarkably, a minor proportion of these displayed an organoid arrangement of homogenous round/epithelioid cells evocative of underlying *GLI1* alteration within the dedifferentiated component, implying a potentially shared morphologic contribution of this specific molecular signature (15). In our practice, we also recently encountered a few enigmatic tumors challenging to characterize as DDLPS or *GLI1*-altered mesenchymal neoplasm, prompting further investigation into these specific lesions.

In this study we characterize the first cohort of *GLI1/MDM2/CDK4* co-amplified WD/DDLPS to elucidate any characteristic clinical, morphologic, or genomic patterns therein. The differential diagnoses and clinicopathologic implications of this subset of tumors are discussed.

Materials and Methods

Cohort selection

The cBioPortal platform at Memorial Sloan Kettering Cancer Center (a sequencing database including biopsies and resections from primary, recurrent, and metastatic in-house and consult specimens) was queried retrospectively for all cases of ‘well-differentiated liposarcoma,’ ‘dedifferentiated liposarcoma,’ and ‘sarcoma, not otherwise specified’ having undergone MSK-IMPACT from 2015 to the present (16, 17). Specimens with an initial diagnosis of well/dedifferentiated liposarcoma or nonspecific sarcoma without confirmatory *MDM2* and/or *CDK4* amplification were excluded. The resulting population was filtered for all cases with evidence of concurrent *GLI1, MDM2,* and *CDK4* amplification (including those with only one of either *MDM2* or *CDK4* amplification). Within the MSK-IMPACT platform, amplification is defined as a fold change of ≥2 for any gene; specimens under this threshold were excluded.

Clinicopathologic analysis

Demographic information (age, sex, tumor size, sites of primary/recurrent/metastatic tumor) and survival parameters (date of diagnosis and primary resections, interval to recurrence, interval to death, cause of death) were tabulated from the pathology database and electronic medical record.

At our institution, dedifferentiated liposarcoma or ‘dedifferentiation’ of a well-differentiated liposarcoma is defined per the Weiss et al. criteria of at least a single low-power field lacking evidence of lipogenic differentiation, regardless of mitotic activity and in some cases regardless of cellularity (18, 19). Therein, ‘high-grade’ and ‘low-grade’ dedifferentiation are qualified by an integrated assessment of degree of cellularity, cytonuclear atypia/pleomorphism, and mitotic activity - similar to the conventional two-tiered appraisal of other soft tissue sarcomas. Well-differentiated liposarcoma with ‘early dedifferentiation’ is defined

as well-differentiated liposarcoma with foci of cellular non-lipogenic/non-myxoid tissue comprising <5% of the overall appropriately sampled tumor volume (20, 21). As per prior publications, so-called '*GLI1*-altered morphology' features a proliferation of monomorphic round to epithelioid cells with scant pale cytoplasm, partitioned into symmetric small nests/clusters by an anastomotic capillary network (5–10, 15).

Histologic features were recorded upon review of all available digital slides. These included the presence or absence of WDLPS, and the histologic subtypes represented therein (sclerosing, lipoma-like, inflammatory). Prevailing and secondary morphologies of the dedifferentiated component (spindle cell sarcoma, high-grade or low-grade myxofibrosarcoma-like, high-grade undifferentiated pleomorphic sarcoma-like, meningothelial whorls with or without osseous metaplasia, heterologous e.g., osteosarcomatous or rhabdomyosarcomatous differentiation, *GLI1*-altered morphology) were qualitatively tabulated. Non-*GLI1*-amplified DDLPS sequenced during the same interval served as an internal control cohort for the morphologic analysis.

Molecular analysis

All samples were selected per the above parameters after having undergone MSK-IMPACT sequencing for either diagnostic or therapeutic purposes (22). (Of note, for technical reasons this platform does not appraise copy number changes in the *DDIT3* locus.) Amplification fold changes were recorded for each of *MDM2, CDK4, GLI1*, and *JUN*. Copy number alterations and pathogenic mutations in accessory loci were recorded if present, in particular those involving the 1p or 6q chromosomal arms due to their documentation as spatially discrete segments of co-amplification in DDLPS (23–26).

Statistical analysis

Recurrence-free survival (RFS) as an event was defined as the interval of time between primary resection and either radiologic or histologic recurrence/metastasis; death from disease was censored. (27) Disease-specific survival (DSS) was defined as the interval of time between primary resection and date of death due to WD/DDLPS; death from other causes were not censored. Statistical comparisons and survival analyses were performed using Graphpad Prism® and XLMiner.

Results

Demographic features

Clinicopathologic and genomic features of the entire cohort are summarized in Table 1. A total of 486 WD/DDLPS underwent sequencing via MSK-IMPACT between the years 2015 to the current time. 92 of these tumors (19%) harbored amplification of the *GLI1* locus in addition to *MDM2* and/or *CDK4*. This core cohort was comprised by 55 males and 37 females (male to female ratio of 3:2) with an average age of 63 years (median 64, range 31–86). Individual specimens from these patients previously selected for MSK-IMPACT were represented by primary tumors (n=60), local recurrences (n=29), and metastases (n=3). Primary tumors were most frequently designated as retroperitoneal (47/60, 78%) but also mediastinal (4/60, 7%), paratesticular (3/60, 5%), neck (1/57, 2%), duodenal (1/57. 2%),

groin (1/57, 2%) and musculoskeletal tissues of the trunk or extremities (3/57, 5%). Sites of sequenced locally recurrent tumors were also predominated by the retroperitoneum (23/29, 80%), followed by the abdominal cavity (4/29, 10%) and mediastinum (2/29, 7%). All sequenced metastatic sites sourced from the lung (3/3), primary sites for which were the retroperitoneum (1/3), soft tissues of posterior neck (1/3), and paratesticular region (1/3).

Morphologic features

The cohort of sequenced tumors (primary, recurrent, and metastatic) was comprised by dedifferentiated liposarcoma (86/92, 94%, 6 of which were comprised by WDLS with early dedifferentiation) and well-differentiated liposarcoma without any longitudinal evidence of dedifferentiation (6/92, 7%). Of the sequenced DDLPS, 69/86 (80%) were associated with a WDLPS component in the same specimen. In 4/86 (5%) cases, a well-differentiated component was only appreciable in the subsequent recurrent specimen. A portion of the dedifferentiated samples (13/86, 15%) showed no evidence of a well-differentiated component in any of the primary, recurrent, or metastatic specimens.

Of the sequenced WDLPS, 6/9 (67%) never recurred and 3/9 (33%) had evidence of dedifferentiation in the subsequent recurrence (as above rendering 6 cases without any longitudinal evidence of dedifferentiation). Of the sequenced WDLPS with early dedifferentiation, 3/6 (50%) recurred with only 1 of 3 with evidence of dedifferentiation in the recurrence.

The tumors displayed a wide morphologic spectrum in regard to both well-differentiated and dedifferentiated components, frequently with multiple (2–3) discrete patterns within the same lesion. Of the sequenced tumors with an appreciable well-differentiated component with or without association with a dedifferentiated component (n=75), 10/75 showed a scant amount of well-differentiated tumor not amenable to further classification. The prevailing histology of WDLPS in the remaining tumors by proportion of tumor volume was lipoma-like (55/65, 85%), followed by the sclerosing subtype (9/65, 14%, Figure 1). Secondary and tertiary morphologies were comprised by the sclerosing subtype (26/65, 40% - frequently in tumors with a predominant lipoma-like morphology), and the inflammatory variant (3/65, 5%).

Dedifferentiated areas (analyzed across all sequenced cases with evidence of dedifferentiation in either the primary, recurrent or metastatic lesion; n=86) were predominated by high-grade undifferentiated pleomorphic sarcoma-like (26/86, 30%) and high-grade myxofibrosarcoma-like (13/86, 15%) histologies, both frequently present in tandem (Figure 1). Other salient patterns included meningothelial whorls with or without osseous metaplasia (12/86 [14%] and 4/86 [5%], respectively), high-grade spindle cell sarcoma not otherwise specified (14/86 [16%], 2/14 of which showed storiform morphology), low-grade myxofibrosarcoma-like (8/86, 8%), low-grade spindle cell sarcoma not otherwise specified (1/86, 1%), inflammatory myofibroblastic tumor-like (1/86, 1%), heterologous osteosarcomatous differentiation (1/86, 1%), and heterologous rhabdomyosarcomatous differentiation (1/86, 1%). (Figures 1 and 2). *GLI1*-altered morphology, as described, was identified as the prevailing pattern in 5/86 (6%), as the secondary pattern in 3/86 (4%), and as the tertiary pattern in 2/86 (2%) tumors.

Secondary morphologies by proportion of tumor volume were again predominated by high-grade myxofibrosarcoma-like morphology (14/86, 16%), low-grade spindle cell sarcoma not otherwise specified (7/86, 8%), high-grade undifferentiated pleomorphic sarcoma-like (4/86, 5%), low-grade myxofibrosarcoma-like (2/86, 2%), round cell sarcoma not otherwise specified (1/86, 1%), meningothelial whorls (3/86, 3%), low-grade myxoid liposarcoma-like (1/86, 1%), metaplastic ossification (1/86, 1%), desmoid fibromatosis-like (1/86, 1%), heterologous osteosarcomatous differentiation (1/86, 1%), and heterologous rhabdomyosarcomatous differentiation (1/86, 1%).

A control group of 255 cases of non-*GLI1* co-amplified DDLPS was assessed by pathology reports for morphologic cross-comparison. 182/255 cases (71%) showed undifferentiated pleomorphic sarcoma-like, 62/255 (24%) showed myxofibrosarcoma-like, and 11/255 (5%) showed divergent osteosarcomatous (8) or rhabdomyosarcomatous (3) morphologies. No cases with meningothelial whorls with or without osseous metaplasia or *GLI1*-altered morphology were identified in this group.

Molecular features

All tumors showed co-amplification of *MDM2, CDK4*, and *GLI1*, save one which showed only *CDK4* and *GLI1* co-amplification (i.e., all 92 cases were *CDK4* co-amplified, and 91 cases were *MDM2* co-amplified). Average fold change for *MDM2, CDK4*, and *GLI1* was 13.2 (median 12.6, range 2.3–33.7), 11.0 (median 10.0, range 2.6–32.1), and 6.7 (median 5.8, range 2–21.2), respectively. As absolute comparison of copy number alterations across different tumors is unreliable due to individual disparities in tumor purity and volume, *GLI1:MDM2* and *GLI1:CDK4* ratios within each individual tumor were instead assessed (Table 1). Average *MDM2:CDK4* ratio was 1.4 (median 1.1, range 0.07–3.8), average *GLI1:MDM2* ratio was 0.67 (median 0.5, range 0.07–2.2), and average *GLI1:CDK4* ratio was 0.7 (median 0.68, range 0.3–5.5). One-way ANOVA with post-hoc Tukey test demonstrated significant differences between the average fold changes of *MDM2* and *GLI1* as well as between *CDK4* and *GLI1*, but not between those of *MDM2* and *CDK4*.

In addition to amplification, 3 tumors harbored additional structural and sequence abnormalities in the *GLI1* locus. Two demonstrated gene rearrangements – one a *GLI1::YEATS4* fusion of indeterminate functional significance, which histologically appeared as a lipoma-like WDLPS with early dedifferentiation into low-grade myxofibrosarcoma-like morphology. The other harbored a *GLI1::ZFAND3* fusion, also of indeterminate significance and possibly structurally related to the concomitant *GLI1* amplification. All specimens from this patient lacked evidence of a juxtaposed well-differentiated component, and histologically were composed entirely of low-grade spindle cell sarcoma-like DDLPS. The third showed a pathogenic *GLI1* mutation (exon12 p.P869A [c.2605C>G]); all specimens from this patient also lacked evidence of a juxtaposed WDLPS, and histologically appeared as a low-grade spindle cell sarcoma and desmoid fibromatosis-like DDLPS. *GLI1* morphology was conspicuously absent from any specimen in these three patients.

The *JUN* gene (located on 1p32.1) was co-amplified with all three of *MDM2, CDK4*, and *GLI1* in 7/91 cases (8%, not including the *MDM2* non-amplified), with an average fold

change of 11.3 (median 9, range 2.5–30). Genes along chromosomal arms 1p and 6q were also amplified in a fraction of all tumors, including most commonly *TNFAIP3* (11/92, 11%), followed by *LATS1* (5/92, 5%), *ESR1* (5/92, 5%), *IFNGR1* (2/92, 2%), *FYN* (2/92, 2%), *ARID1B* (2/92, 2%), and *NOTCH2* (1/92, 1%).

Analysis of *MDM2, CDK4* and *GLI1* fold changes in the 8 tumors displaying any proportion of '*GLI1* morphology' revealed no correlation between magnitude of amplification nor ratio of fold change between each of *GLI:MDM2* and *GLI1:CDK4*.

Survival metrics

Follow-up was available for most patients (83/92, 90%), an advantage of longitudinal treatment and surveillance at a dedicated referral cancer center (Table 1). Average length of follow-up was 65 months (median 52, range 1–240), during which 67% (56/83) of patients experienced a local recurrence or distant metastasis within an average of 37 months (range 4–156), and 30% (25/83) of patients died of disease within an average of 82 months (range 4–132). 33% (27/83) of patients remained alive with persistent disease at the time of last follow-up, and 39% (31/80) patients are alive without evidence of disease at last follow-up. 12% (10/83) of patients developed distant metastatic disease, occasionally multiple sites of which included liver (3/10), lung (5/10), thoracic vertebrae (2/10), humeral bone (1/10), and soft tissues of the fourth metacarpal ray (1/10).

Kaplan-Meier curves for the core cohort are provided in Figures 3A (recurrence-free survival, RFS) and 3B (disease-specific survival, DSS). Median RFS was 36 months, with 3-year recurrence-free survival at 48%, and 5-year recurrence-free survival at 30%. Median DSS was 141 months, with 5-year DSS at 80% and 10-year DSS at 60%.

Subset analysis of *GLI1* co-amplified dedifferentiated liposarcoma with no well-differentiated component.

In as much as one diagnostic hinge of DDLPS in the setting of *GLI1* co-amplification is the identification of a well-differentiated component, the clinicopathologic and molecular features of tumors in our cohort lacking the latter are also summarized briefly. A total of 13 DDLPS without evidence of a well-differentiated component in any of the primary, recurrent, or metastatic tumors were identified within the core cohort, as above. These were composed of 7 males and 6 females (male to female ratio of 1:1) with an average age of 59 years (median 61, range 40–86). Anatomic sites were represented by retroperitoneal soft tissues (7/13, 54%), mediastinum (4/13, 31%), gluteus muscle (1/13, 8%), and abdominal cavity (1/13, 8%). In contrast to the core cohort, tumors in this particular subdivision were more likely to feature only a single homogeneous pattern of dedifferentiated morphology (observed in 12/13, 92%): high-grade undifferentiated pleomorphic sarcoma (4/13, 30%), high-grade spindle cell sarcoma not otherwise specified (2/13, 15%), meningothelial whorls with osseous metaplasia (3/13, 2%), high-grade myxofibrosarcoma-like (1/13, 8%), and low-grade spindle cell sarcoma not otherwise specified (1/13, 8%). In a proportion similar to that of the overall cohort, *GLI1*-altered morphology was observed as the dominant component by overall tumor volume in 2/13 (15%) cases and the minor component in 1/13 (8%, as above).

Average fold change for *MDM2, CDK4*, and *GLI1* was 9.9 (median 12.7, range 3.2–16.4), 10.3 (median 10.2, range 4.4–22.8), and 9.1 (median 8, range 4–21.2). *JUN* was co-amplified with all three of *MDM2*, *CDK4*, and *GLI1* in 2/13 (15%) of cases, with fold changes of 14.1 and 9.1. Genes along 1p and 6q were additionally amplified in a subset of tumors, including most commonly TNFAIP3 (1/13, 8%), followed by LATS1 (1/13, 8%), and ESR1 (1/13, 8%).

Discussion

As with any tumor harboring a recurrent molecular signature, the morphologic heterogeneity of the WD/ DDLPS spectrum stands in contrast to its relatively invariable underlying genetic driver (28–37). Expansion of our technical capabilities to an unprecedented degree of genomic granularity, along with the ongoing classification of other molecularly defined mesenchymal entities has refined our understanding of their histology and clinical trajectory. An essentially relentless locally recurrent disease with a risk of metastatic potential much lower than that of undifferentiated pleomorphic sarcoma, high-grade myxofibrosarcoma, and pleomorphic liposarcoma, this subtype of liposarcoma is typically a surgical disease with repetitive radical debulking as a primary modality of treatment (38–41). Ongoing clinical and basket trials with targeted MDM2 and CDK4 inhibitors have shown promise in adjuvant and advanced-stage settings, but their efficacy and impact on survival are still to be established (42–46).

The topography of chromosome 12q, with coding segments for not only *MDM2* and *CDK4* but also oncogenes such as *DDIT3* and *STAT6* in close juxtaposition, renders its derivative tumors susceptible to copy alteration ‘carryover’ (i.e., secondary co-amplification likely ascribed to this structural coincidence). The ensuing morphologic and immunohistochemical overlap can be misleading, especially when a diagnostic body of evidence credibly aligns. For example, pathogenic fusions involving *DDIT3* and *STAT6* are definitional of myxoid/ round cell liposarcoma and solitary fibrous tumor, respectively; nuclear upregulation of translated protein products can be exploited immunohistochemically towards their diagnosis. Indeed, early studies assessing the evidently excellent specificity of the STAT6 antibody demonstrated its exceptionally rare expression in WD/DDLPS as a potent pitfall, alluding perhaps inadvertently to this particular genomic phenomenon (14, 47). Similarly, myxoid portions of a WDLPS or DDLPS can show DDIT3 immunoreactivity so to misconstrue myxoid liposarcoma, which represents a diagnostic challenge in plausible clinicopathologic circumstances (12, 33).

The *GLI1* locus is positioned within the oncogenic 12q segment and hence also geographically involved in this event. An abbreviation of ‘glioma-associated oncogene,’ it encodes a zinc finger transcription factor activated by the canonical sonic hedgehog signaling cascade (Shh), dyscrasias of which are fundamental to certain malignancies of the brain, breast, and melanocytes. (48–53) Unlike the *DDIT3* and *STAT6* genes, which appear relatively specific in their tumorigenic potential, *GLI1* has demonstrated remarkable and categorical plasticity. Whether by rearrangement or amplification (occasionally concurrently), *GLI1* aberrations are documented to source tumors of as yet ambiguous lineage – hypothesized over recent years to represent the gamut of myoepithelial, neural

crest, myopericytic, and/or fibroblastic/myofibroblastic derivation due to their inconsistent and noncontextual expression of SMA, S100, and cytokeratin cocktails. Histologic features of *GLI1*-altered tumors are also commensurate with their genomic diversity, characterized by dichotomy even within the same location. In the stomach, a single pathogenic *MALAT1::GLI1* fusion propagates two diametrically different tumors in young patients (identical structural and functional fusion properties across both) – one a benign plexiform growth comprised by bland spindle cells in richly vascularized myxoid matrix (plexiform fibromyxoma), and the other a primitive and highly aggressive biphasic spindle and epithelial/epithelioid neoplasm (gastroblastoma/'GNET') (2, 4). In the submucosa of the small bowel, another biphasic but putatively indolent neoplasm with tubular epithelial differentiation, diffuse cytokeratin expression, and consistent negativity for S100, has also just emerged (52). In the head and neck with a peculiar predilection for the tongue, these neoplasms range from SMA-positive spindled perivasculocentric proliferations (so-called 't[7:12] pericytoma'), to S100-positive monomorphic round/ovoid-appearing cells partitioned into nests by a rich arborizing vasculature. The latter morphology was subsequently recapitulated in three larger series of *GLI1*-altered tumors accompanied by tentative labels of '*GLI*-altered mesenchymal neoplasm,' and 'nested glomoid neoplasm,' - an appearance which is now a recognized suggestion of underlying *GLI1* influence (6, 8, 15) (54, 55).

We recently examined ten retrospectively identified specimens unified by this nested round/ovoid cell morphology and evidence of *GLI1* amplification confirmed by fluorescent *in situ* hybridization (FISH) (15). When queried for fold changes in these neighboring loci, a substantial proportion did show concurrent amplifications in *DDIT3, HMGA2*, *STAT6* and – of particular relevance here – *MDM2* and *CDK4.* A tangential inquiry into the incidence of *GLI1* co-amplification in WD/DDLPS resulted in a substantial subset of patients from the MSK-IMPACT database by which to address the diagnostic corollaries of this observation. This is relevant from both histomorphologic and therapeutic standpoints insofar as evolving investigations into sonic hedgehog pathway inhibition (of which *GLI1* is a member) might render another effective target in the trajectory of this primarily surgical disease. (56–59)

A brief overview of our data reveals that approximately a fifth of malignancies called as WD/DDLPS harbor underlying *GLI1/MDM2/CDK4* co-amplification. The demographic profile of this subgroup was comparable to that of WD/DDLPS as documented in the literature, presenting in the retroperitoneal or mediastinal soft tissues of older adults. Importantly, many of the dedifferentiated tumors were associated with a well-differentiated component, either adjacent to or evident within the recurrence or metastasis. These two features, namely tumor location and association with a morphologically well-differentiated lipogenic sarcoma, are conceptually central to the classification of these *GLI1* co-amplified tumors, especially given what we now understand about the extent of their overlap.

When evaluating *GLI1/MDM2/CDK4* co-amplified neoplasms (especially referencing those with accompanying eponymous *GLI1* morphology as in our cohort), the issue of parsing liposarcomas with driving *MDM2/CDK4* amplification and incidental *GLI1* amplification from tumors with true 'primary' oncogenic *GLI1* alteration becomes especially germane. Indeed - *MDM2* and *CDK4* amplification is pathogenic of other sarcomas such as intimal

sarcoma, low-grade central osteosarcoma, parosteal osteosarcoma, and rhabdomyosarcoma. It has even been reported incidentally in other soft tissue malignancies including malignant peripheral nerve sheath tumor, endometrial stromal sarcoma, and leiomyosarcoma, which are distinguished from liposarcoma via antecedent factors such as demography, site/radiology, histology, and immunoprofile. As such, assignment of a sarcomatoid or malignant epithelioid neoplasm as a DDLPS frequently and circumstantially relies on the detection of a proximal well-differentiated lipogenic sarcoma in addition to this characteristic molecular aberration – a common diagnostic scenario in which lineage immunohistochemistry is almost categorically inconclusive. It goes without saying that extensive gross sampling of any peripheral or intralesional fatty tissue is a simple but powerful tool applied to this conundrum. Careful histologic examination of these areas might establish adipocytic lineage, frequently indiscernible within swathes of completely dedifferentiated lesional material. Admittedly, absence of an unequivocal WDLPS does not exclude the diagnosis of DDLPS, as the latter may occur completely *de novo* or represent covertly unsampled tumor. It does provoke speculation that a few of the 13 tumors in our cohort lacking this feature in the setting of an oncogenic 'hit' at the *GLI1* locus - might actually represent primary *GLI1*-altered neoplasms; however, this is unlikely given the classic clinical presentations and additional genomic co-amplifications characteristic of true DDLPS.

Primary tumor location is also directional towards this distinction, in that WD/DDLPS typically have epicenters in the retroperitoneum, mediastinum, or deep somatic soft tissues. In contrast, *GLI1*-altered neoplasms rarely derive from viscera (case reports in the stomach, small bowel, uterus, ovary, as above) or retroperitoneum/mediastinal soft tissues; they are encountered more often in the head and neck (particularly the tongue) and trunk, but also long and flat bones and both deep and superficial soft tissues of the extremities. (6–10) (54, 55, 60–62). For example, one patient in our cohort presented with a soft tissue lesion in the ring finger with classic *GLI1* morphology; the possibility of DDLPS was, quite reasonably, not entertained given both the unconventional location and histology. Only after resection of a paratesticular mass which revealed a *MDM2/CDK4/GLI1* co-amplified WD/DDLPS was a metastasis of the former to the finger postulated.

Morphologically, the dedifferentiated components in our cohort were usually variegated with a combination of high-grade undifferentiated pleomorphic sarcoma-like and high-grade myxofibrosarcoma-like features being common. Notably, we observed a salient proportion of the otherwise quite rare meningothelial whorls/osseous metaplasia pattern identified as a primary/secondary/tertiary element in 22% (19/86) of tumors with dedifferentiated morphology (32, 37, 63, 64). Ironically, this was recorded as nigh commensurate with the characteristic high-grade myxofibrosarcoma-like pattern (present in 30% [26/86] of cases), and more commonly than the nested *GLI1*-altered pattern (present in 12% [10/86] of cases). In contrast, review of a control group of 255 non-GLI1 coamplified DDLPS, there were no cases with meningothelial whorls/osseous metaplasia or GLI1-altered pattern - a novel finding meriting further investigation. Conceptually, recognizing either histologic variant – especially in an isolated core biopsy with minimal peripheral tissue for context – can be helpful to postulate a DDLPS when the appearances would otherwise prompt workup for a nonspecific small round cell tumor, carcinoma, extraskeletal osteosarcoma, follicular dendritic cell sarcoma, or even a true *GLI1*-altered tumor. Somewhat counterintuitively,

the presence or absence of either *GLI1* morphology or meningothelial whorls/osseous metaplasia did not correlate with the magnitude (either absolute or relative) of *GLI1* amplification, nor were these patterns observed exclusively in tumors lacking a WDLPS.

From an objective genomic standpoint, a wide array of comparative fold changes in *MDM2*, *CDK4*, and *GLI1* was observed between each individual specimen. Although the comparison of fold changes across tumors is technically unsound as their derivative calculations are multifactorial and contingent in part upon tumor quantity and purity - in any single tumor, *MDM2* and *CDK4* were duplicated in generally similar magnitudes (i.e., *MDM2:CDK4* fold ratio of approximately ~1), whereas the degree of *GLI1* amplification was about 60–70% of these two.

A subset of these *GLI1*-amplified liposarcomas also showed co-amplification of other regions previously described in DDLPS, namely those along the 1p and 6q chromosomal arms. In contrast to the structural contiguity of 12q, the precise mechanism of this phenomenon is not entirely clear but can be exploited as another ancillary tool towards this diagnosis - especially when clinicopathologic circumstances are equivocal and comprehensive sequencing data is available. For example, in a neoplasm that defies classification (due to either noncommittal histology, lack of a lipogenic component, and/or peculiar site/demographic as above) with documented underlying *GLI1/MDM2/CDK4* co-amplification, the presence of concurrent copy number gains along these axes would align most closely with the molecular profile of a DDLPS. In our cohort, the presence of any of *JUN*, *TNFAIP3*, *ESR1*, and/or *IFNGR1* co-amplifications (among others) was observed in 21/92 (23%) of tumors, and in 3/13 (24%) of tumors comprehensively lacking a well-differentiated liposarcomatous component. Admittedly the specificity of 1p/6q amplification is not well-established across the spectrum of soft tissue sarcomas, but in the background of *GLI1/MDM2/CDK4* co-amplification is likely indicative of WD/DDLPS.

A recent investigation illustrated the utility of a GLI1 antibody in the distinction of ‘primary’ *GLI1*-altered (both rearranged and amplified) neoplasms from histologic analogs but also its positivity in a subset of tumors with molecularly confirmed, but presumed ‘secondary,’ *GLI1* amplification (2/5 DDLPS, 1/1 alveolar rhabdomyosarcoma, and 1/2 unclassifiable uterine sarcomas). (65) While GLI1 positivity by immunohistochemistry may be helpful, it should also be deployed in an appropriate clinicopathologic context, and thereafter assessed with caution rather than serve as a diagnostic endpoint. As the literature and our findings imply, *GLI1* morphology is identified in tumors with both primary and secondary underlying *GLI1* abnormality - as would translate to GLI1 immunoreactivity in most instances - but the presence of either should consequently prompt both additional molecular and histologic evaluation to clarify its active or passive role in oncogenesis. Similarly, the DDIT3 antibody in these circumstances can function not only as a diagnostic aid but also red herring, as the *DDIT3* and *GLI1* loci are more closely apposed than any other paired coding regions of relevance along this 12q13–15 stretch. Immunohistochemical or select molecular techniques exploiting amplification or protein product upregulation of either gene will also – inadvertently or otherwise - straddle the other. (5, 66–68) Employing already established DDIT3 immunohistochemistry as an inexpensive predictive screening test in a neoplasm with standard *GLI1* morphology (wherein positivity would extrapolate

to potential *GLI1* alteration) could adjudicate further molecular workup (but should not be misinterpreted as evidence of a high-grade round cell myxoid liposarcoma - an unlikely but sometimes credible morphologic mimic). This concept requires validation within a molecular platform that can definitively characterize the *DDIT3* locus, which is another limitation of this study.

It may be that details innate to the cohort we enumerate above become academic in the overarching context of longitudinal tumor behavior. Unfortunately, the fact that most basket survival analyses of retroperitoneal and somatic soft tissue sarcomas neglect to perform sub-stratification of only WD/DDLPS, along with widely discrepant use of survival metrics and definitions, renders comparison to prior studies quite challenging (69–73). In the future, statistical cross-comparison to an internal group of *GLI1*-normal 'control' DDLPS will be necessary to elucidate any outcome metric discrepancies.

In conclusion, we present the seminal large-scale characterization of *GLI1* co-amplified WD/DDLPS in a cohort benefitted by longitudinal oncologic follow-up from a single cancer center. Distinguishing these neoplasms from morphologic mimics – including true primary *GLI1*-altered neoplasms – is important towards treatment and prognostication. This entails careful examination for a well-differentiated lipogenic component, assessment of primary tumor location, and integration of any coincident genomic co-amplifications, in particular those along chromosomal arms 1p/6q. While many clinicopathologic aspects mirror those of conventional DDLPS, certain attributes including a predilection for variant meningothelial whorls/osseous metaplastic and eponymous nested round cell *GLI1* morphology can alert the practicing pathologist to this important consideration.

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Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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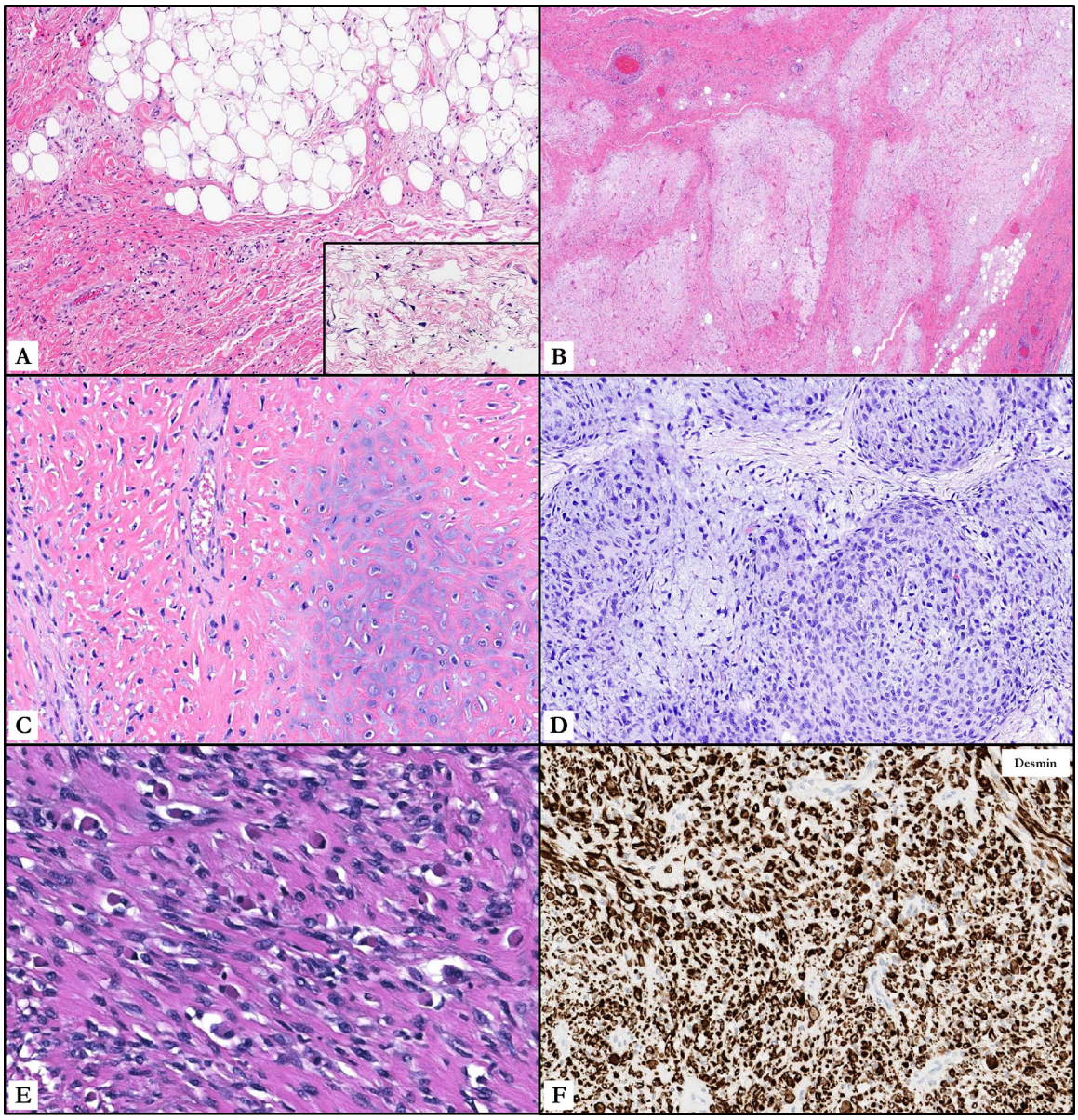

Figure 1.

Histomorphologic features of *GLI1*-amplified well-differentiated/dedifferentiated liposarcomas. **A.** Lipoma-like and sclerosing subtypes comprised the most common variants of well-differentiated liposarcoma, identification of which is crucial in the context of a *GLI1* co-amplified tumor. **B.** Area of 'early dedifferentiation' showing a predominantly non-lipogenic low-grade myxofibrosarcoma-like expanse. **C.** Heterogeneous morphology of dedifferentiated liposarcoma with malignant osteocartilaginous differentiation. **D.** Dedifferentiated liposarcoma with high-grade myxofibrosarcoma-like morphology. **E.** Dedifferentiated liposarcoma with heterologous rhabdomyoblastic differentiation. **F.** Immunohistochemical stain for desmin, which is diffusely positive in areas of rhabdomyosarcomatous differentiation.

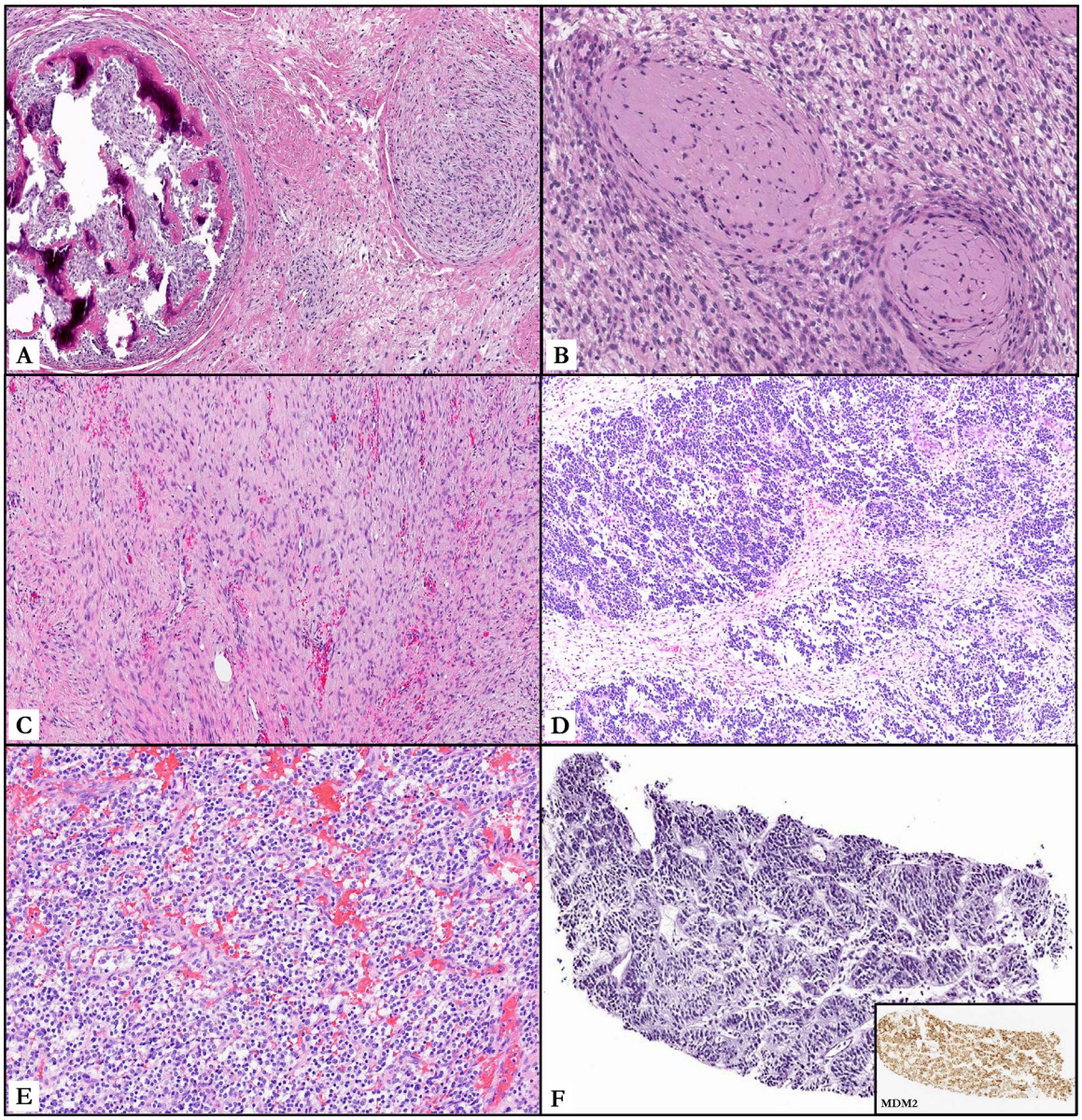

Figure 2.

Rare morphologic patterns of dedifferentiation observed in a subset of *GLI1* co-amplified well-differentiated/dedifferentiated liposarcomas. **A.** Osseous metaplasia (left) accompanied by a meningothelial-like whorl (right); a rare dyad observed in the context of dedifferentiated liposarcoma. **B.** Multiple meningothelial-like nodules of swirling spindle cells. **C.** Dedifferentiated component composed of a low-grade spindle cell sarcoma with sweeping fascicles reminiscent of desmoid-type fibromatosis. **D.** '*GLI1*-altered morphology' showing a distinctly nested architecture of monotonous round cells as a morphologic pattern of dedifferentiation. **E**. Characteristic *GLI1*-altered morphology of epithelioid cells in a vaguely organoid pattern imparted by a delicate arborizing capillary network. **F.** A richly vascularized and nested proliferation of monotonous undifferentiated round cells

should raise the differential diagnosis of a *GLI1*-altered tumor or a *GLI1* co-amplified dedifferentiated liposarcoma, the latter corroborated by an *MDM2* immunostain (inset).

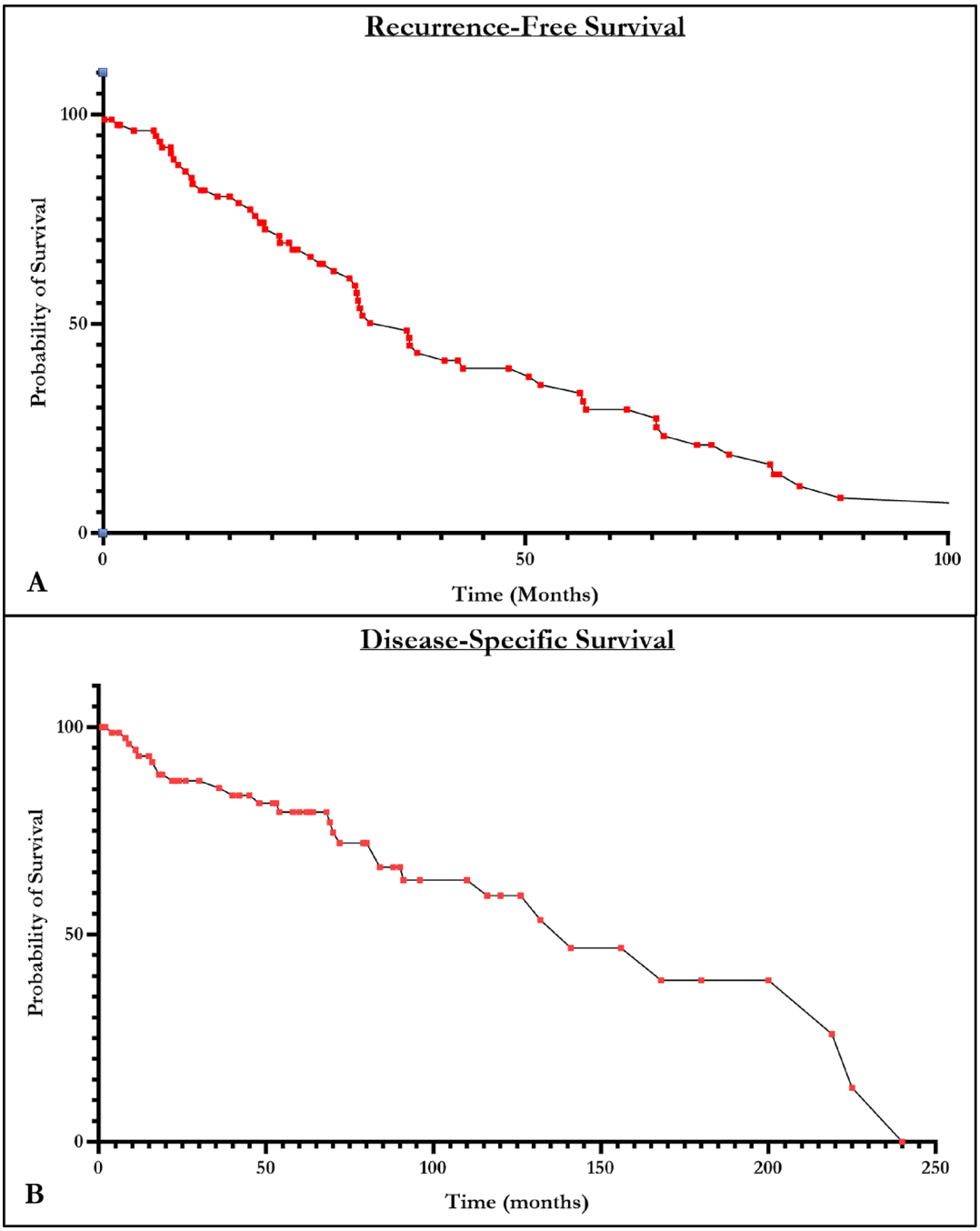

Figure 3.

Kaplan-Meier curves for the core cohort: **A.** shows recurrence-free survival (RFS). Median RFS was 36 months, with 3-year recurrence-free survival at 48%, and 5-year recurrence-free survival at 30%. **B**. shows disease-specific survival (DSS). Median DSS was 141 months, with 5-year DSS at 80% and 10-year DSS at 60%.

Table 1:

Clinicopathologic and molecular features of the cohort of *GLI1* co-amplified well-differentiated/dedifferentiated liposarcomas.

Total patients	**92**
Age (years)	63 (range 31–86)
Gender	
Male	55/92 (60%)
Female	37/92 (40%)
Site of primary tumor	
Retroperitoneum	71/92 (77%)
Mediastinum	6/92 (7%)
Paratesticular/spermatic cord	4/92 (4%)
Abdominal cavity	5/92 (6%)
Trunk/extremities	6/92 (7%)
Diagnosis (sequenced tumors)	
Well-differentiated liposarcoma (without any dedifferentiation)	6/92 (7%)
Well-differentiated liposarcoma with early dedifferentiation	6/92 (7%)
Dedifferentiated liposarcoma…	**86/92 (94%)**
...with accompanying WDLPS *	73/86 (94%)
...without accompanying WDLPS *	13/86 (17%)
Dedifferentiated morphology (predominant pattern)	**86** #
High-grade undifferentiated pleomorphic sarcoma	26/86 (30%)
Meningothelial whorls with/without osseous metaplasia	**16/86 (19%)**
High-grade spindle cell sarcoma, not otherwise specified	14/86 (17%)
High-grade myxofibrosarcoma	13/86 (16%)
Low-grade myxofibrosarcoma	8/86 (8%)
***GLI1* morphology**	**5/86 (6%)**
Low-grade spindle cell sarcoma, not otherwise specified	1/86 (1%)
Osteosarcomatous differentiation	1/86 (1%)
Rhabdomyoblastic differentiation	1/86 (1%)
Inflammatory myofibroblastic tumor	1/86 (1%)
GLI1 morphology (any proportion)	10/86 (12%)
Meningothelial whorls with/without osseous metaplasia (any proportion)	19/86 (86%)
Follow-up available	**83/92 (90%)**
Local recurrence	56/83 (67%)
Distant metastasis	10/83 (13%)
Alive with no evidence of disease	31/83 (37%)
Alive with disease	27/83 (33%)
Dead of disease	25/83 (31%)

Average *MDM2* fold change	13.2 (range 2.3–33.7)
Average *MDM2:CDK4* fold change ratio	1.4 (range 0.07–3.8)
Average *CDK4* fold change	11 (range 2.6–32.1)
Average *GLI1* fold change	6.7 (range 2–21.2)
Average *GLI1:MDM2* fold change ratio	0.67 (range 0.07–2.2)
Average *GLI1:CDK4* fold change ratio	0.7 (range 0.3–5.5)
1p co-amplification	
JUN	7/91 (8%)
NOTCH2	1/91 (1%)
6p co-amplification	
TNFAIP3	11/92 (12%)
LATS1	5/92 (5%)
ESR1	5/92 (6%)
IFNG1	2/92 (2%)
FYN	2/92 (2%)
ARID1B	2/92 (2%)

[*] WDLPS: Well-differentiated liposarcoma;

[#] 86 cases with dedifferentiation includes cases a) with early dedifferentiation (6), b) with associated WDLPS (73), and c) without associated WDLPS (13).