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Progressive loss of myogenic differentiation in leiomyosarcoma has prognostic value

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Aims: Well-differentiated leiomyosarcomas show morphologically recognizable smooth muscle differentiation, whereas poorly differentiated tumours may form a spectrum with a subset of undifferentiated pleomorphic sarcomas. The expression of certain muscle markers has been reported to have prognostic impact. We investigated the correlation between the morphological spectrum and the muscle marker expression profile of leiomyosarcoma, and the impact of these factors on patient outcomes.

Methods and results: Tissue microarrays including 202 non-uterine and 181 uterine leiomyosarcomas with a spectrum of tumour morphologies were evaluated for expression of immunohistochemical markers of muscle differentiation. Poorly differentiated tumours frequently lost one or more conventional smooth muscle markers [smooth muscle actin, desmin, h-caldesmon, and smooth muscle myosin ($P < 0.0001$)], as well as the more recently described markers SLMAP, MYLK, and ACTG2 ($P < 0.0001$). In primary tumours, both desmin and CFL2 expression predicted improved overall survival in multivariate analyses ($P = 0.0111$ and $P = 0.043$, respectively). Patients with muscle marker-enriched tumours (expressing all four conventional markers or any three of ACTG2, CFL2, CASQ2, MYLK, and SLMAP) had improved overall survival ($P < 0.05$) in univariate analyses.

Conclusions: Morphologically and immunohistochemically, poorly differentiated leiomyosarcomas can masquerade as undifferentiated pleomorphic sarcomas with progressive loss of muscle markers. The expression of muscle markers has prognostic significance in primary leiomyosarcomas independently of tumour morphology.

Keywords: desmin, differentiation, immunohistochemistry, leiomyosarcoma, prognosis

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Introduction

Leiomyosarcomas account for up to 20% of all adult soft tissue sarcomas,[1–7] and are characterized by an aggressive clinical course and relative insensitivity to cytotoxic chemotherapy.[4,5] Leiomyosarcomas show smooth muscle differentiation, and are typically subclassified for diagnostic and therapeutic purposes as being of uterine or non-uterine (somatic) origin. Uterine leiomyosarcomas are distinguished from the more common myometrial leiomyomas by a combination of mitotic activity of at least five per 10 high-power fields in the presence of necrosis and cellular atypia, or mitotic activity of >10 per 10 high-power fields in the absence of necrosis or atypia.[8–11] In somatic tissues outside of the dermis or gastrointestinal tract, leiomyomas are extremely rare. Thus, smooth muscle tumours with any mitotic activity are generally considered to be leiomyosarcomas, and are commonly graded according to the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) criteria.[6]

Well-differentiated leiomyosarcomas show a growth pattern of perpendicularly intersecting cellular fascicles composed of elongated spindle-shaped tumour cells with abundant, brightly eosinophilic, fibrillary cytoplasm. Nuclei generally have an elongated, blunt-ended oval 'cigar-like' shape. Variable numbers of markedly pleomorphic 'monster' cells may be seen. In poorly differentiated tumours, classic features may be focal or difficult to appreciate as the proportion of pleomorphic cells increases. Additional morphological variants include epithelioid and myxoid types, which are more commonly associated with a uterine origin. Although the majority of cases can be diagnosed on biopsy, on the basis of classic histological features on H&E-stained sections, morphological variants, poorly differentiated tumours and those with unusual features may be difficult to distinguish from other sarcomas, and may require additional immunohistochemical evaluation. In anaplastic variants, or extremely poorly differentiated tumours, myogenic markers may be lost.[12]

Commonly used markers of muscle differentiation in clinical practice include desmin, which is seen in all types of muscle, smooth muscle actin (SMA), which is most commonly present in smooth muscle or myofibroblasts, and h-caldesmon and smooth muscle myosin (SMMS), both of which are relatively specific for smooth muscle. More recently, expression profiling studies identified an additional set of muscle markers, including smooth muscle γ-actin (ACTG2, which is typically expressed in enteric smooth muscle), calsequestrin 2 (CASQ2, cardiac and skeletal muscle), human muscle cofilin 2 (CFL2, skeletal muscle), myosin light chain kinase (MYLK, smooth muscle) and sarcolemmal membrane-associated protein (SLMAP, all muscle types), as being associated with improved outcomes when three or more were coexpressed—so-called 'muscle-enriched' leiomyosarcomas.[13]

Few studies have investigated the utility of muscle marker expression as a more objective measure of differentiation status than histological appearance on H&E-stained sections. We therefore investigated how well muscle marker expression correlated with histological assessment of tumour differentiation, and whether muscle marker expression could independently predict tumour behaviour and survival outcomes in primary leiomyosarcoma.

Materials and methods

PATENTS AND TUMOUR TISSUES

Acquisition of tissue specimens and clinical information and subsequent analyses were approved by the Institutional Review Board (IRB) of The University of Texas MD Anderson Cancer Center (UTMDACC).

TISSUE MICROARRAY CONSTRUCTION

All available formalin-fixed, paraffin-embedded leiomyosarcoma specimens collected between 1993 and 2010 were retrieved from UTMDACC pathology archives. H&E-stained sections were reviewed to confirm the diagnosis, define areas of viable tumour, and select one or more areas (if there was morphological variability) for inclusion in tissue microarrays. Extremely poorly differentiated tumours or those that had heterologous elements were only included in the study if they had a prior documented history of leiomyosarcoma with at least focal typical immunohistochemical and/or morphological features.

An automated tissue microarray apparatus (ATA-27; Beecher Instruments, Sun Prairie, WI, USA) was used to obtain and format paired 1.2-mm punch samples from each case into recipient blocks.[14] H&E staining of 4-μm tissue microarray sections was used to verify all samples. Individual cores on the tissue microarray were screened by an experienced soft tissue pathologist, and classified by differentiation score as: (i) well differentiated; (ii) moderately differentiated; or (iii) poorly differentiated (Figure 1).[6] Tumours with myxoid or epithelioid morphology were considered to be moderately and poorly differentiated, respectively. Only the most poorly differentiated pair of cores from each case was considered in data analysis.

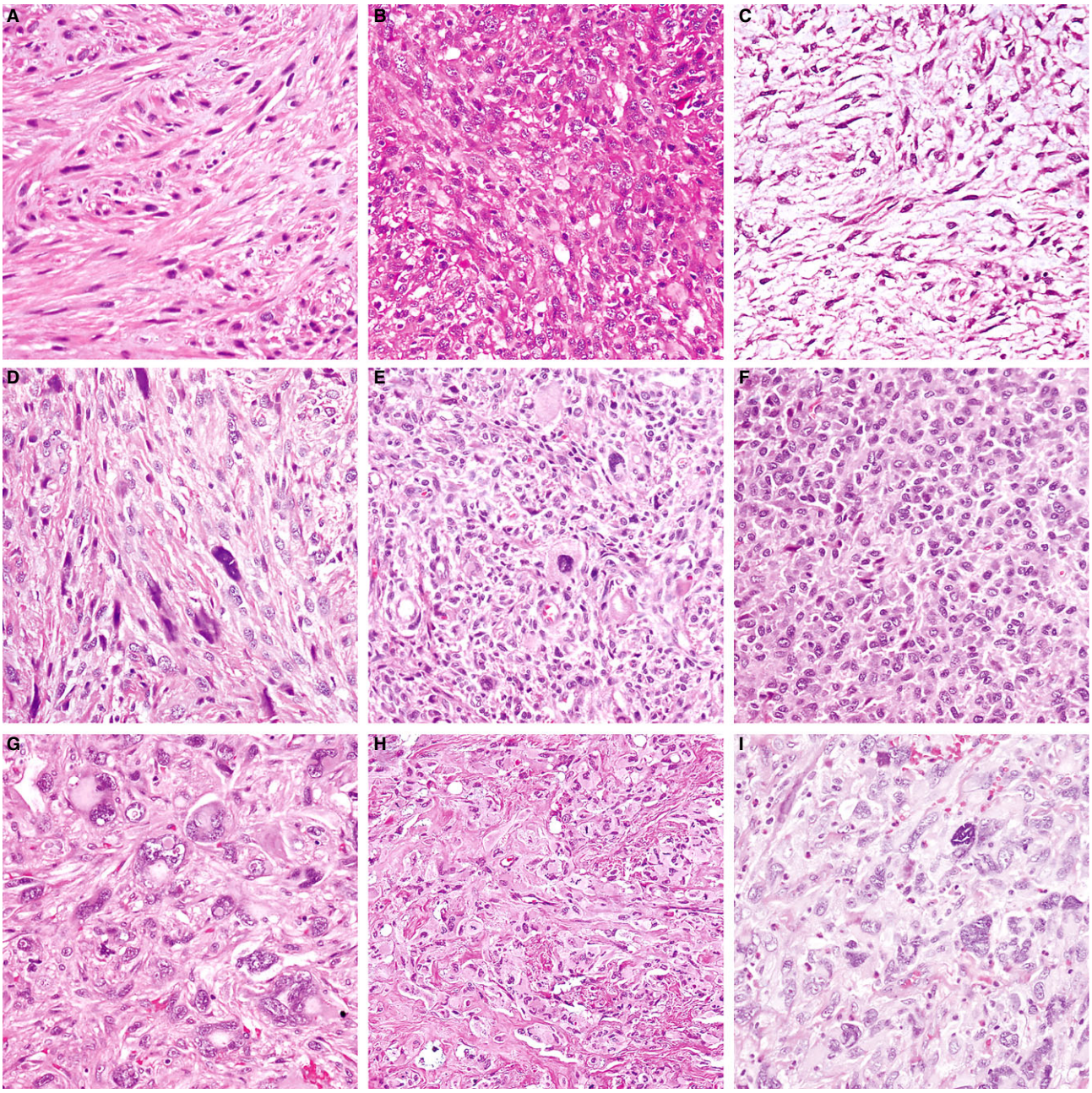

Figure 1. Morphological variation in leiomyosarcoma. **A**, Well-differentiated tumour showing intersecting fascicles of slightly atypical eosinophilic spindle cells. **B**, Moderately differentiated tumour with nuclear variability and increased disorganization of fascicles. **C**, Moderately differentiated myxoid tumour with bland spindle cells in a storiform to fascicular arrangement. **D**, Moderately differentiated tumour with scattered 'monster cells'. **E**, Poorly differentiated tumour with loss of fascicular architecture, and increased rounded to epithelioid cells. **F**, Poorly differentiated tumour with epithelioid features. **G**, Poorly differentiated tumour showing therapy effect and marked nuclear pleomorphism. **H**, Poorly differentiated tumour with pleomorphism, loss of architecture, and numerous mitoses. **I**, Poorly differentiated 'undifferentiated pleomorphic sarcoma-like' tumour showing storiform growth, marked pleomorphism, and scattered inflammatory infiltrate.

CLINICOPATHOLOGICAL DATA COLLECTION

Patient and tumour variables, including age, gender, tumour site, tumour size, and disease status, were recorded. Sites of primary tumours were categorized as follows: uterine; vascular (apparently arising from or extensively invading a large-calibre vein); retroperitoneal/pelvic (not clearly arising in association

with a specific intra-abdominal organ or vascular structure); extremity (leg or arm, including dermal tumours); trunk (chest wall, superficial abdominal wall, back, and paraspinal); or other miscellaneous sites (including viscera, bone, and head and neck).

Outcome data, including survival and disease recurrence, were tabulated for primary tumours only. Complete FNCLCC grading criteria (mitotic figures per 10 high-power fields and percentage necrosis) were not tabulated, because these were not equivalently applicable in primary uterine leiomyosarcoma.

IMMUNOHISTOCHEMICAL ANALYSIS

Immunohistochemical staining was performed on 4-μm-thick tissue microarray sections using an automated stainer (Dako, Carpinteria, CA, USA), according to the manufacturer's instructions, and commercially available antibodies (Table 1). Positive and negative controls were run in parallel. Sections were counterstained with haematoxylin. SMA, desmin, SMMS and h-caldesmon were scored as absent (0), focal (<10%), or diffuse (≥10%). ACTG2, CASQ2, CFL2, MYLK and SLMAP were scored as completely absent (0), or by intensity, if any staining was present, as weak (1) or strong (2). For the purposes of outcome analysis, only diffuse/strong staining was considered to be positive. Muscle-enriched tumours were defined as those expressing three or more of ACTG2, CASQ2, CFL2, MYLK, and SLMAP.

STATISTICAL METHODS

Associations between histopathological features and immunophenotype were examined using Fisher's exact test or chi square test, with an alpha of $P \leq 0.05$ (adjusted to $P \leq 0.025$ for multiple comparisons) considered to be significant. For primary tumours only, the method of Kaplan and Meier was used to assess outcomes. Univariable and multivariable Cox proportional hazards regression models were used to evaluate the association of histopathological features or biomarker expression with overall survival (OS), disease-specific survival (DSS), and time to first metastasis, with alpha of 0.05 considered to be significant.

Results

LEIOMYOSARCOMA CHARACTERISTICS

Tumours included 383 leiomyosarcoma from 257 patients, comprising 198 women and 59 men. Primary sites of origin included the uterus (41%), retroperitoneum/pelvis (19%), large vessels (16%), extremities (11%), trunk (3%), and miscellaneous sites (9%). Vascular leiomyosarcomas predominantly arose in the inferior vena cava and renal vessels, with a few cases originating from other deep veins. Tumours in the head and neck arose in the nasal cavity, sinuses, and mandible, whereas organs giving rise to leiomyosarcomas included the lung/bronchus, bowel, bladder, liver, and penis. Extremity tumours included four leiomyosarcomas of dermal origin. There were 98 primary leiomyosarcomas, 93 local recurrences (including both those recurring within a prior surgical site and intra-abdominal spread), and 192 distant metastases. The median size of primary leiomyosarcomas was 80 mm (range, 7–300 mm).

When tumours were classified by their microarray core with the least-differentiated histology, there were

Table 1. Antibodies used for immunohistochemistry

Marker	Catalog no.	Company	Dilution
Smooth muscle actin (SMA)	A2547	Sigma, St Louis, MO, USA	1:80 000
Desmin	M0760	Sigma	1:200
Caldesmon	M3557	Dako, Carpenteria, CA, USA	1:50
Smooth muscle myosin (SMMS)	IR066	Dako	1:200
Smooth muscle γ-actin (AC TG2)	H00000072-A01	Novus Biologicals, Littleton CO, USA	1:2000
Calsequestrin 2 (CASQ2)	GTX90833	GeneTex, Irvine, CA, USA	1:100
Human muscle cofilin 2 (CFL2)	GTX92818	GeneTex	1:100
Myosin light chain kinase (MYLK)	GTX91044	GeneTex	1:25
Sarcolemmal associated protein (SLMAP)	GTX94163	GeneTex	1:2500

131 (34%) well-differentiated leiomyosarcomas, 170 (45%) moderately differentiated leiomyosarcomas, and 82 (21%) poorly differentiated leiomyosarcomas. Loss of differentiation correlated with disease progression ($P < 0.0001$), with primary tumours being more frequently well differentiated (53%) than either recurrent tumours (30%, $P = 0.0013$) or metastatic tumours (26%, $P < 0.0001$) (Figure 2). There was no significant further loss of differentiation between recurrent and metastatic tumours.

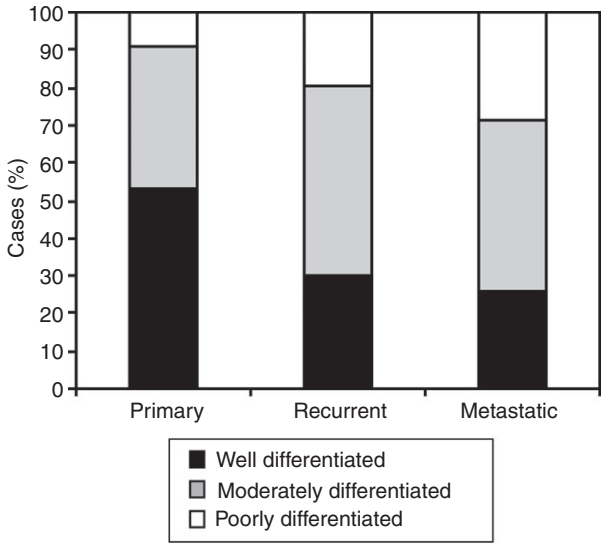

Figure 2. Histological features of leiomyosarcoma by stage. Tumour status correlates with differentiation. Primary tumours are more frequently well differentiated (53%) than recurrences or metastases (respectively: 30%, $P = 0.0013$; and 26%, $P < 0.0001$). Poorly differentiated tumours are more common among metastases (29%) than recurrences (19%, $P = 0.087$) and primaries (9%, $P = 0.0001$).

MUSCLE MARKER EXPRESSION

The smooth muscle markers SMA and ACTG2 were most frequently expressed (91% and 90% of cases, respectively), and the striated muscle marker CASQ2 was least frequently expressed (18% of cases) (Table 2). In sum, 235 of 377 (62%) tumours expressed all four conventional muscle markers, 292 (77%) expressed three or more, and 56 (15%) expressed only one or none. The muscle-enriched phenotype was seen in 298 of 357 cases (83%).

Expression of muscle markers correlated with morphological differentiation (Figure 3). Expression of all four conventional muscle markers decreased from 82% (106/129) in well-differentiated tumours, to 60% (100/167) in moderately differentiated tumours, to 36% (24/81) in poorly differentiated tumours ($P < 0.0001$). The most dramatic loss of expression was seen for SMMS, which was expressed in 118 of 130 (91%) well-differentiated tumours, 133 of 169 (79%) moderately differentiated tumours, and only 38 of 81 (47%) poorly differentiated tumours; SMA was retained at the highest levels, being expressed by 129 of 130 (99%) of well-differentiated tumours, 158 of 170 (93%) moderately differentiated tumours, and 60 of 82 (73%) poorly differentiated tumours. Statistically significant loss of expression of SLMAP, ACTG2,

Table 2. Strong/diffuse muscle marker expression by differentiation status

	Total ($n = 383$), %	Well differentiated ($n = 131$), %	Moderately differentiated ($n = 170$), %	Moderately versus well differentiated (*P*-value)	Poorly differentiated ($n = 82$), %	Poorly versus well differentiated (*P*-value)	Poorly versus moderately differentiated (*P*-value)
SMA	91	99	93	0.0082	73	<0.0001	<0.0001
Desmin	74	89	75	0.0045	50	<0.0001	<0.0001
SMMS	76	91	79	0.0065	47	<0.0001	<0.0001
Caldesmon	78	94	78	0.00013	51	<0.0001	<0.0001
SLMAP	83	93	84	0.028	63	<0.0001	0.003
CASQ2	18	23	17	0.24	12	0.044	0.34
ACTG2	90	99	94	0.047	66	<0.0001	<0.0001
CFL2	69	77	64	0.0196	64	0.0537	1
MYLK	82	95	77	<0.0001	71	<0.0001	0.42

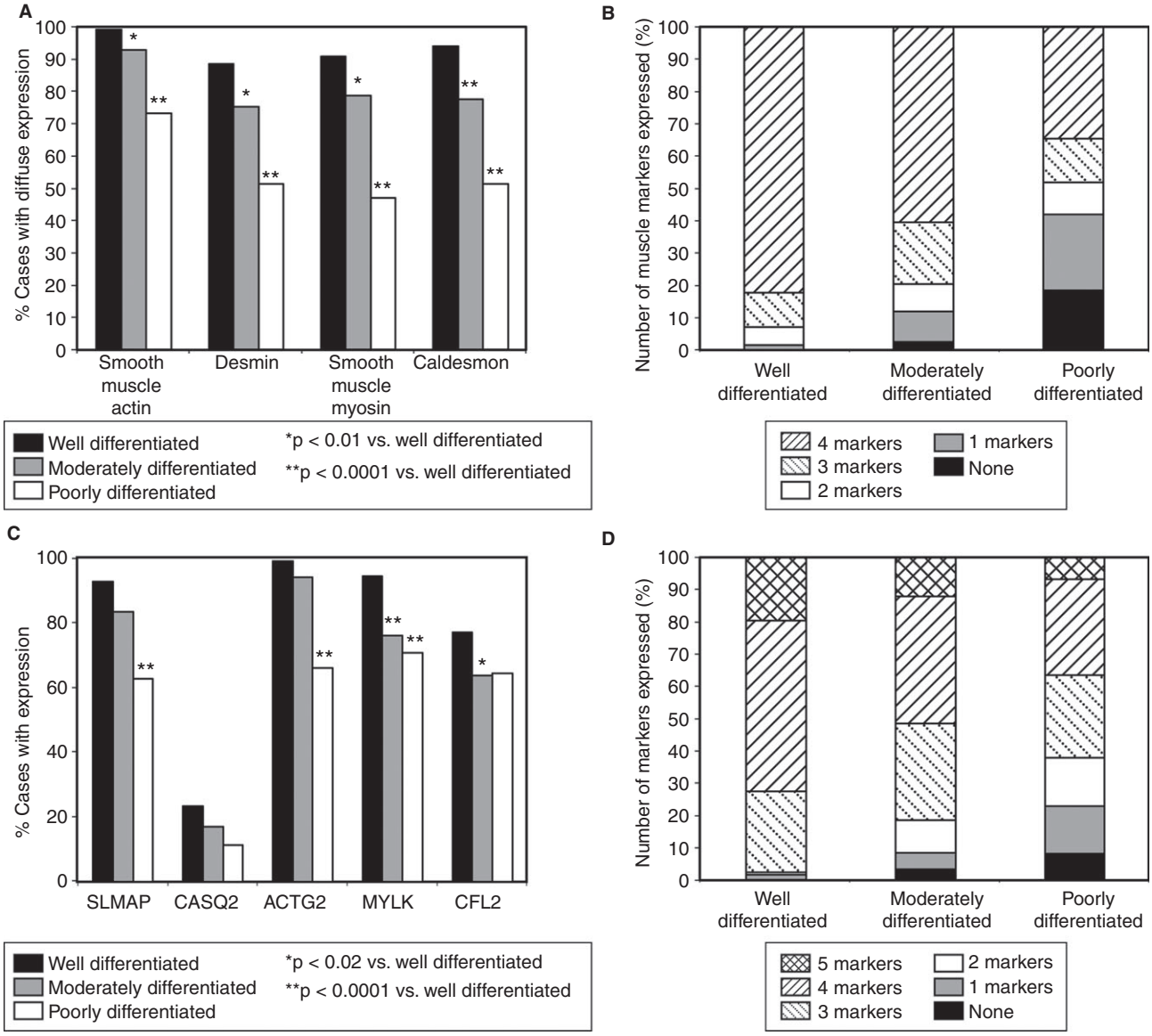

Figure 3. Expression of markers of smooth muscle differentiation in leiomyosarcoma. **A**, Diffuse expression of smooth muscle actin, desmin, smooth muscle myosin and caldesmon is reduced in less well-differentiated tumours. **B**, Total number of conventional muscle markers with diffuse expression is reduced in more poorly differentiated tumours ($P < 0.0001$). **C**, Strong expression of SLMAP, CASQ2, ACTG2, MYLK and CFL2 is less frequent in more poorly differentiated tumours. **D**, The muscle-enriched phenotype (strong expression of three or more of SLMAP, CASQ2, ACTG2, MYLK, and CFL2) is less frequent in more poorly differentiated tumours ($P = 0.0089$).

MYLK and COFL2 was also seen as tumours became more poorly differentiated.

PROGNOSTIC UTILITY OF DIFFERENTIATION MARKERS IN PRIMARY LEIOMYOSARCOMA

Clinical follow-up was available for 97 primary tumours, and ranged from 2.6 to 258 months (median, 50 months). Five-year and 10-year OS were 54% and 33%, respectively, and 5-year and 10-year DSS were 60% and 41%, respectively (Figure 4A). Four patients had distant metastases at the time of diagnosis, and 52 of 97 patients developed metastases during the course of follow-up. For these 52 patients, the median time to development of the first metastasis after primary resection was 15 months (range, 8–167 months).

There was no difference in outcome by sex or site (uterine versus somatic). A primary tumour size of >50 mm was associated with poor OS and DSS, but

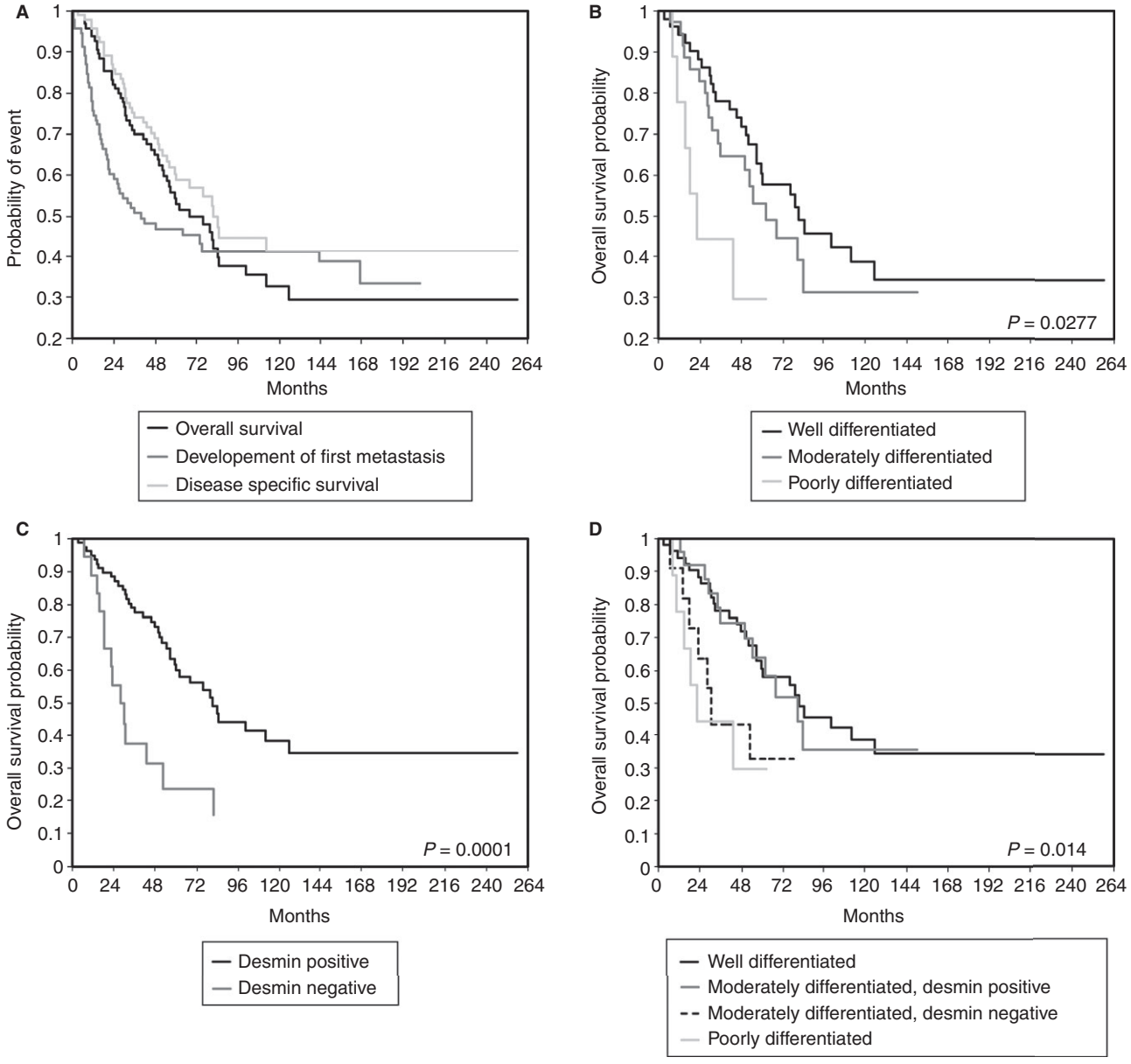

Figure 4. Survival outcomes in primary leiomyosarcoma. **A**, Overall survival, disease-specific survival and time to development of first metastasis for the entire cohort. **B**, Patients with well-differentiated leiomyosarcomas have better overall survival than those with poorly differentiated tumours. **C**, Expression of desmin correlates with improved overall survival. **D**, Patients with moderately differentiated tumours expressing desmin have identical survival to that of patients with well-differentiated tumours, whereas moderately differentiated tumours without desmin expression behave like poorly differentiated tumours.

not with time to first metastasis (Tables 3–5). Histopathological differentiation status alone was a modest predictor of clinical outcome (Figure 4B). Poorly differentiated tumours were associated with a worse OS than well-differentiated tumours [HR 3.281, 95% confidence interval (CI) 1.311–8.212, $P = 0.0112$), and were associated with a shorter time to first metastasis ($P = 0.0587$), but were not predictive of DSS ($P = 0.0897$). There was no significant difference in outcome between well-differentiated and moderately differentiated tumours.

Muscle-enriched primary leiomyosarcoma was associated with improved OS in univariate analysis (HR 0.4177, 95% CI 0.1763–0.9897, $P = 0.0473$), as was expression of all four conventional smooth muscle markers (HR 0.4887, 95% CI 0.2711–0.8809,

Table 3. Overall survival, univariate and multivariate analyses

Variable	Level	*N*	No. of all-cause deaths	Hazard ratio	95% CI, lower	95% CI, upper	*P*-value
Univariate analyses							
Actin, smooth muscle	≥10% positive	96	51	1.2191	0.1673	8.8841	0.845
Caldesmon	≥10% positive	97	52	0.4154	0.2011	0.8583	0.0177
Desmin	≥10% positive	97	52	0.3058	0.1617	0.5783	0.0003
Myosin, smooth muscle	≥10% positive	95	51	0.7951	0.3867	1.635	0.5331
Standard muscle markers	All four markers expressed	94	50	0.4887	0.2711	0.8809	0.0172
ACTG2	Strong, diffuse	96	51	1.8255	0.2507	13.2914	0.5524
CASQ2	Strong, diffuse	96	51	0.8104	0.4433	1.4816	0.4946
CFL2	Strong, diffuse	91	48	0.4191	0.2288	0.7676	0.0049
MYLK	Strong, diffuse	95	50	0.5227	0.2046	1.3356	0.1753
SLMAP	Strong, diffuse	97	52	0.6123	0.2981	1.2576	0.1816
Muscle-enriched phenotype	Any 3 of 5	94	49	0.4177	0.1763	0.9897	0.0473
Differentiation score	Moderate	97	52	1.4259	0.7843	2.5923	0.2447
	Poor			3.2807	1.3107	8.2119	0.0112
Gender (male/female)	Male	97	52	1.068	0.6086	1.8742	0.8186
Site	Uterine versus all other	97	52	1.2194	0.5489	2.709	0.6262
Size	>50 mm	97	52	2.7263	1.1624	6.3944	0.0211
Multivariate analyses							
Differentiation	Moderate	91	48	0.9084	0.4373	1.8869	0.7967
	Poor			3.5215	1.2365	10.0286	0.0184
Desmin	≥10% positive	91	48	0.3077	0.1216	0.7788	0.0129
CFL2	Strong, diffuse	91	48	0.4943	0.2615	0.9343	0.0301
Size	>50 mm	91	48	4.5457	1.7251	11.9782	0.0022

$P = 0.0172$). As individual markers, CFL2, desmin and h-caldesmon were all associated with improved survival ($P = 0.0049$, $P = 0.0003$, and $P = 0.0177$, respectively) (Table 3). The small sample size limited multivariate analyses of OS, and only the two known risk factors (tumour size and differentiation score) and two muscle markers (desmin and CFL2) were included in the model. All four retained independent prognostic significance.

Significant prognostic biomarkers for DSS were similar to those for overall survival (Table 4). Loss of any one of the conventional smooth muscle markers increased risk (HR 2.5031, 95% CI 1.3078–4.7916, $P = 0.0056$), whereas muscle-enriched tumours were associated with improved DSS ($P = 0.0535$). Both CFL2 and desmin were associated with decreased risk ($P = 0.0076$ and $P < 0.0001$), and expression of h-caldesmon showed a trend towards being protective ($P = 0.061$), in univariate analyses. In multivariate analysis (including size, expression of all four conventional muscle markers, CFL2 and desmin in the model), only size >50 mm retained prognostic significance.

Desmin was the only significant predictor of time to first metastasis in univariate analysis (HR 0.46343, 95% CI 0.2407–0.8957, $P = 0.0221$).

Table 4. Disease-specific survival, univariate and multivariate analyses

Variable	Level	*N*	No. of all-cause deaths	Hazard ratio	95% CI, lower	95% CI, upper	*P*-value
Univariate analyses							
Actin, smooth muscle	≥10% positive	96	40	0.9083	0.1239	6.658	0.9246
Caldesmon	≥10% positive	97	41	0.4042	0.1567	1.0426	0.061
Desmin	≥10% positive	97	41	0.2398	0.121	0.4753	<0.0001
Myosin, smooth muscle	≥10% positive	95	40	0.8018	0.3544	1.8145	0.5961
Standard muscle markers	All four markers expressed	94	39	0.3995	0.2087	0.7646	0.0056
ACTG2	Strong, diffuse	96	40	1.457	0.199	10.6664	0.711
CASQ2	Strong, diffuse	96	40	0.6548	0.3087	1.3887	0.2696
CFL2	Strong, diffuse	91	37	0.3978	0.2022	0.7829	0.0076
MYLK	Strong, diffuse	95	39	0.503	0.1761	1.4368	0.1994
SLMAP	Strong, diffuse	97	41	0.5184	0.2391	1.1239	0.0961
Muscle-enriched phenotype	Any 3 of 5	94	38	0.3928	0.1521	1.0141	0.0535
Differentiation score	Moderate	97	41	1.3427	0.6902	2.6119	0.3854
	Poor			2.5765	0.8634	7.6884	0.0897
Gender (male/female)	Male	97	41	1.2493	0.6692	2.3325	0.4847
Site	Uterine versus all other	97	41	0.8502	0.3025	2.3895	0.7583
Size	>50 mm	97	41	2.561	1.0028	6.5405	0.0493
Multivariate analyses							
Standard muscle markers	All four markers expressed	91	37	0.6245	0.139	2.8055	0.5391
Size	>50 mm	91	37	4.2788	1.4224	12.8719	0.0097
Desmin	≥10% positive	91	37	0.2378	0.0474	1.1923	0.0707
CFL2	Strong, diffuse	91	37	0.5996	0.2769	1.2986	0.1946

Discussion

Although immunohistochemistry is frequently used to support a diagnosis of leiomyosarcoma, few studies have specifically addressed the correlation between morphological evidence of smooth muscle differentiation and immunohistochemical markers. We examined muscle marker expression in a diverse array of leiomyosarcomas to better understand the patterns of marker loss over the course of disease progression (from well-differentiated to poorly differentiated tumours, and from primary to metastatic disease). Of particular interest was whether immunohistochemical markers of muscle differentiation could provide a more objective measure of tumour differentiation than morphology in predicting patient outcomes.

When we reviewed the leiomyosarcoma tissue cored on our tissue microarray, we identified a number of poorly differentiated cores that showed few, if any, classic features of leiomyosarcoma, and that could easily have been mistaken for undifferentiated pleomorphic sarcoma if examined out of context. These cores showed a significant loss of markers of smooth muscle differentiation as compared with well-differentiated and moderately differentiated cores. Whereas Carvalho *et al.*[15] reported no correlation of muscle marker

Table 5. Time to first metastasis, univariate analyses

Variable	Level	*N*	No. with metastases	Hazard ratio	95% CI, lower	95% CI, upper	*P*-value
Actin, smooth muscle	≥10% positive	92	51	0.9286	0.1276	6.7582	0.9417
Caldesmon	≥10% positive	93	52	0.6418	0.288	1.4304	0.2781
Desmin	≥10% positive	93	52	0.4643	0.2407	0.8957	0.0221
Myosin, smooth muscle	≥10% positive	91	50	0.8576	0.4156	1.7696	0.6777
Standard muscle markers	All four markers expressed	90	49	0.5986	0.3255	1.101	0.0988
ACTG2	Strong, diffuse	92	51	1.789	0.2462	13.0021	0.5654
CASQ2	Strong, diffuse	92	51	0.6913	0.367	1.302	0.253
CFL2	Strong, diffuse	87	46	0.559	0.2876	1.0868	0.0864
MYLK	Strong, diffuse	91	50	0.9674	0.3463	2.7026	0.9496
SLMAP	Strong, diffuse	93	52	0.8091	0.3794	1.7254	0.5835
Muscle-enriched phenotype	Any 3 of 5	90	49	0.8023	0.2872	2.2412	0.6743
Differentiation score	Moderate	93	52	1.104	0.6043	2.0171	0.7476
	Poor			2.4072	0.9684	5.9836	0.0587
Gender (male/female)	Male	93	52	1.324	0.766	2.2886	0.3148
Site (U/S)	Uterine versus all other	93	52	0.5359	0.1927	1.4903	0.2319
Size	>50 mm	93	52	1.7609	0.881	3.5196	0.1093

expression (SMA, desmin, caldesmon, calponin, and myosin) with histological differentiation in a series of 78 cases, other studies reported similar findings to ours, including retention of diffuse SMA expression in poorly differentiated tumours, and loss of both desmin and caldesmon (59% versus 83%, and 19% versus 68%, respectively in poorly differentiated tumors compared to well/moderately differentiated).[16–18] Loss of smooth muscle marker expression in the less differentiated areas of pleomorphic and 'dedifferentiated' variants of leiomyosarcoma was also reported.[12,19,20]

Not only were individual muscle markers frequently lost in poorly differentiated tumours in our series, but the overall total number of muscle markers was reduced, with less than half of poorly differentiated tumours expressing three or more of SMA, desmin, h-caldesmon, and SMMS, as compared with >90% of well-differentiated tumours. This is consistent with reports that myosin and caldesmon are frequently coexpressed.[15] Similarly to Mills *et al.*,[21] we found that poorly differentiated 'undifferentiated pleomorphic sarcoma-like' leiomyosarcomas less frequently showed a muscle-enriched phenotype, i.e. strong positivity for three or more of CASQ2, SLMAP, CFL2, MYLK, and ACTG2, with only 62% of poorly differentiated tumours falling into this category, as compared with 98% of well-differentiated tumours.

A significant proportion of so-called undifferentiated pleomorphic sarcomas (UPS, formerly termed malignant fibrous histiocytoma) are probably related to leiomyosarcoma, and, indeed, may represent anaplastic 'dedifferentiated' leiomyosarcoma. In multiple studies using protein expression analyses,[22–26] gene expression analysis,[27–30] and/or comparative genomic hybridization,[31] a subset of UPS consistently clustered with leiomyosarcoma, whereas up to 5% of UPS showed a muscle-enriched phenotype.[21] These studies suggest that a subset of UPS represent a form of tumour progression from leiomyosarcoma.[26,27,31,32] Effectively differentiating between the two classes may have implications for prognostication and therapeutic selection as we more fully understand the disease biology.

In our series, desmin and CFL2 were associated with improved OS, independently of histological differentiation or tumour size. Further analysis of desmin expression demonstrated that the impact of this

marker in predicting outcome appeared to rest mainly on its ability to segregate moderately differentiated tumours into two groups—those that behaved like well-differentiated tumours, and those that behaved like poorly differentiated tumours (Figure 4C,D). We also confirmed, in univariate analyses, the reported association of the muscle-enriched phenotype with improved outcome. It is of note that our study was underpowered for robust analysis of DSS, owing to limited available clinical follow-up. However, in general, prognosticators of OS showed a trend towards predicting DSS, implying that tumour-related deaths may have been more frequent in our cohort than we were able to confirm.

Whereas we found that increased muscle marker expression predicted improved outcomes in leiomyosarcomas, other studies have found that UPS with expression of muscle markers behave more aggressively than those without.[22,33–35] Thus, we are left with the seeming paradox that evidence of myoid differentiation is a positive predictor in leiomyosarcomas, and a negative one in true UPS. This contradiction merits further study, and reinforces the need to carefully evaluate biomarkers only in context with morphology.

In summary, we have assessed the diagnostic and prognostic utility of an array of muscle markers in one of the largest cohorts of uterine and non-uterine leiomyosarcomas to date. We have demonstrated that morphologically less well-differentiated leiomyosarcomas are associated with loss of markers of muscle differentiation, thereby reducing the diagnostic value of immunohistochemistry in biopsies of such cases. Moreover, our findings are congruent with the theory that a subset of UPS represent pleomorphic or very poorly differentiated variants of leiomyosarcoma. Finally, we have identified desmin as an independent predictor of survival that seems to be of most value in moderately differentiated tumours. Taken together, our findings help to more clearly delineate patterns of muscle expression in leiomyosarcoma, which may help to improve diagnostic algorithms, and provide evidence for the overlooked prognostic significance of everyday immunohistochemical assays.

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Author contributions

E. G. Demicco, K. E. Torres, D. Lev, W.-L. Wang and A. J. Lazar designed the study. G. M. Boland, K. J. Brewer Savannah, K. Lusby, K. L. Watson and M. Bailey assembled the clinical database. E. D. Young, D. Ingram, X. Guo, J. L. Hornick and M. van de Rijn performed the immunohistochemistry. E. G. Demicco analysed the data and wrote the paper. W.-L. Wang, A. J. Lazar, J. L. Hornick and M. van de Rijn provided manuscript critiques. All authors approved the final version.

Conflicts of interest

The authors have no conflicts of interest to declare.

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