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Atypical spindle cell/pleomorphic lipomatous tumour: a clinicopathologic, immunohistochemical and molecular study of 55 cases, highlighting *TP53* gene alterations as a genetic hallmark of atypical pleomorphic lipomatous tumour

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Atypical spindle cell/pleomorphic lipomatous tumour: a clinicopathologic, immunohistochemical and molecular study of 55 cases, highlighting *TP53* gene alterations as a genetic hallmark of atypical pleomorphic lipomatous tumour

Aims: Atypical spindle cell lipomatous tumour (ASLT) and atypical pleomorphic lipomatous tumour (APLT) have been grouped together under the umbrella designation atypical spindle cell/pleomorphic lipomatous tumour (ASPLT) in the 2020 edition of the World Health Organization (WHO) Classification of Soft Tissue and Bone Tumours. They are thought to exist on a morphologic spectrum and share similar clinicopathologic and biological characteristics. The aim of this study was to further explore the genetic background of ASLTs and APLTs by employing DNA-based next-generation sequencing and immunohistochemistry, with a specific focus on the *TP53* gene.

Methods and results: Using DNA-based NGS and immunohistochemistry, *TP53* alterations were identified in 20 out of 21 APLT cases (95%). This is in contrast to the ASLT cases, in which no *TP53* alterations could be observed. Among APLT cases with an abnormal p53 immunohistochemical profile and successful DNA NGS testing, 92% (12 of 13 cases) harboured a *TP53* alteration.

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Abbreviations: aCGH, array-based comparative genomic hybridization; ALT, atypical lipomatous tumour; APLT, atypical pleomorphic lipomatous tumour; ASLT, atypical spindle cell lipomatous tumour; ASPLT, atypical spindle cell/pleomorphic lipomatous tumour; FFPE, formalin-fixed paraffin-embedded; FISH, fluorescence-in-situ hybridization; IHC, immunohistochemistry; MLPA, multiplex ligation-dependent probe amplification; NGS, next-generation sequencing; PL, pleomorphic liposarcoma; sWGS, shallow-whole genome sequencing; VUS, variant of unknown significance; WDLS, well-differentiated liposarcoma; WHO, World Health Organization.

Conclusions: APLTs predominantly harbour a *TP53* alteration in contrast to ASLT cases. Our findings support the classification of APLT as a distinct (sub)entity within a spectrum that overlaps with ASLT, and it remains to be determined whether the broader term 'ASPLT' will hold up. Furthermore, p53 immunostaining proved to be a potentially valuable diagnostic tool, aiding pathologists in differentiating between ASLT and APLT.

Keywords: atypical pleomorphic lipomatous tumour, atypical spindle cell lipomatous tumour, atypical spindle cell/pleomorphic lipomatous tumour, molecular pathology, *RB1*-deleted soft tissue tumours, *TP53*

Introduction

Atypical spindle cell/pleomorphic lipomatous tumour (ASPLT) is a recently defined adipocytic neoplasm included in the 2020 edition of the World Health Organization (WHO) Classification of Soft Tissue and Bone Tumours. In the WHO classification, the terms atypical spindle cell lipomatous tumour (ASLT) and atypical pleomorphic lipomatous tumour (APLT) have been grouped together under the umbrella designation ASPLT, as they are thought to exist on a morphologic spectrum and share similar clinicopathologic and biological characteristics.[1] Also, cases with intermediate morphological features exist, displaying striking morphological overlap that prevents clear classification as either ASLT or APLT. Additionally, the wide range of histologic appearances observed at both the low-cellularity and high cellularity ends of this spectrum can make diagnosing ASPLT very challenging for pathologists, leading to the debate on more defining diagnostic criteria of ASPLTs.[2–12]

ASPLTs have shown to harbour chromosome 13q14 deletions, including the *RB1* gene, which can be detected through surrogate immunohistochemistry (IHC) or by various molecular techniques such as fluorescence-in-situ hybridization (FISH), copy number variation (CNV) analysis by array-based comparative genomic hybridization (aCGH) or shallow whole genome sequencing (sWGS), multiplex ligation-dependent probe amplification (MLPA) and targeted DNA sequencing.[3,9,13,14] ASPLTs therefore belong to the rapidly expanding group of so-called '*RB1*-deleted soft tissue tumours', alongside myofibroblastoma of soft tissue, cellular angiofibroma, acral fibromyxoma, pleomorphic fibroma, myxoid pleomorphic liposarcoma, spindle cell and pleomorphic lipoma, and pleomorphic liposarcoma, the three latter being the main differential diagnoses.[3,15] Importantly, ASPLTs are molecularly distinct from atypical lipomatous tumour/well-differentiated liposarcoma (ALT/WDLS) due to their lack of *MDM2* amplification.[2–5,16] *MDM2* encodes a ligase that binds to and inhibits p53, a crucial tumour suppressor protein.[17] Although *TP53* alterations are generally absent in ALTs/WDLSs, they were described in ALTs/WDLSs of young children with Li-Fraumeni syndrome in the absence of *MDM2* amplification.[18] Recently, Hammer *et al.* identified *TP53* alterations in a small series of 8 APLTs using IHC and/or next-generation sequencing (NGS).[19] This prompted us to further explore the pathogenesis of APLTs and ASLTs by employing targeted DNA-based NGS and IHC, with a specific focus on the *TP53* gene.

Materials and methods

STUDY POPULATION/CASE SELECTION

With approval from the ethical committee of Ghent University Hospital (EC B670201938578), formalin-fixed paraffin-embedded (FFPE) tissue blocks from 34 ASLT and 21 APLT cases, diagnosed between 2010 and 2024, were retrieved from the files of the Department of Pathology of Ghent University Hospital, as well as from the referral files of the authors (T.M., U.F., D.C.). The study was performed in accordance with the Code of Conduct of the Federation of Medical Scientific Societies in Belgium, the Netherlands and Germany. The study population consisted of samples of female and male patients that underwent resection of their lipomatous lesion. Clinical variables, including patient age, sex and tumour location, were recorded when available (Tables 1 and 2). All cases were evaluated by two certified pathologists with expertise in soft tissue tumour pathology (F.C., D.C.) to put them in a category of ASLT or APLT, using diagnostic criteria as described in previous studies by Mariño-Enriquez *et al.*, Creytens *et al.* and Anderson *et al.*[2–4] Furthermore, cases used in the previous study of Creytens *et al.*[3] were also included in the study population.

Table 1. Overview of the ASLT cases

Case no.	Sex/age	Location	Rb1 IHC	FISH *RB1* (loss in % of tumour cells)	p53 IHC	*TP53* status (NGS)
ASLT1	M/43	Shoulder	Deficient	16%	NE	*TP53* WT
ASLT2	M/82	Shoulder/neck	Deficient	66%, MA and M	Heterogeneous	*TP53* WT
ASLT3	M/63	Upper arm	Heterogeneous	6%	Heterogeneous	Failed
ASLT4	F/57	Upper leg	Deficient	14%	Heterogeneous	*TP53* WT
ASLT5	F/42	Thigh	Deficient	32%, MA and M	Heterogeneous	Failed
ASLT6	F/62	Knee	Heterogeneous	18%	Heterogeneous	*TP53* WT
ASLT7	M/67	Foot	Deficient	10%	Heterogeneous	*TP53* WT
ASLT8	F/76	Shoulder	Deficient	8%	Heterogeneous	*TP53* WT
ASLT9	F/70	Inguinal	Heterogeneous	33%, M	Heterogeneous	*TP53* WT
ASLT10	F/64	Inguinal	Deficient	16%	Heterogeneous	*TP53* WT
ASLT11	F/80	Upper arm	Intact	24%, M	Heterogeneous	*TP53* WT
ASLT12	M/53	Neck	Deficient	60%, MA and M	Heterogeneous	*TP53* WT
ASLT13	F/67	Knee	Deficient	14%	Heterogeneous	*TP53* WT
ASLT14	F/58	Webspace 1	Heterogeneous	10%	Heterogeneous	*TP53* WT
ASLT15	M/51	Upper arm	Heterogeneous	8%	Heterogeneous	*TP53* WT
ASLT16	F/58	Upper arm	Heterogeneous	38%, M	Heterogeneous	*TP53* WT
ASLT17	M/61	Neck	Deficient	40%, MA and M	Heterogeneous	Failed
ASLT18	M/66	Upper arm	Deficient	50%, M	Heterogeneous	*TP53* WT
ASLT19	M/55	Back	Deficient	60%, M	Heterogeneous	*TP53* WT
ASLT20	F/68	Hip	Deficient	50%, MA and M	Heterogeneous	*TP53* WT
ASLT21	M/59	Upper arm	Deficient	46%, MA and M	Heterogeneous	*TP53* WT
ASLT22	M/43	Hand	Deficient	44%, M	Heterogeneous	*TP53* WT
ASLT23	M/45	Back	Deficient	54%, M	Heterogeneous	*TP53* WT
ASLT24	F/76	Lower leg	Deficient	36%, M	NI	*TP53* WT
ASLT25	F/56	Upper arm	Intact	14%	Heterogeneous	*TP53* WT
ASLT26	M/68	Thorax	Deficient	40%, M	Heterogeneous	*TP53* WT
ASLT27	F/72	Gluteal region	Deficient	16%	Heterogeneous	*TP53* WT
ASLT28	F/60	Flank	Intact	38%, M	Heterogeneous	*TP53* WT
ASLT29	M/44	Shoulder	Deficient	26%, M	Heterogeneous	*TP53* WT
ASLT30	M/59	Upper arm	Deficient	46%, MA and M	Heterogeneous	*TP53* WT
ASLT31	M/75	hand	Deficient	42%, M	Heterogeneous	*TP53* WT
ASLT32	F/62	Axilla	Heterogeneous	42%, M	Heterogeneous	*TP53* WT
ASLT33	M/73	Thigh	Heterogeneous	8%	NI	Failed
ASLT34	F/66	Gluteal region	Heterogeneous	10%	Heterogeneous	*TP53* WT

M: Monosomy 13q; MA: Mono-allelic deletion; NE, Not executed; NI, not interpretable; WT, wild-type.

Table 2. Overview of the APLT cases

Case no.	Sex/ age	Location	Rb1 IHC	FISH *RB1* (loss in % of tumour cells)	p53 IHC	*TP53* status (NGS)
APLT1	M/82	Abdominal wall	Heterogeneous	44%, MA and M	Heterogeneous	*TP53* c.326 T>C p.(Phe109Ser)—VUS
APLT2	F/60	Upper leg	Deficient	56%, MA	Loss	*TP53* c.824G>A p.(Cys275Tyr)—likely pathogenic variant
APLT3	M/66	Lower arm	Deficient	24%, MA and M	Loss	*TP53* deletion based on coverage analysis
APLT4	M/55	Thigh	Deficient	30%, MA and M	Loss	Failed
APLT5	M/48	Shoulder	NE	50%	Loss	*TP53* deletion based on coverage analysis
APLT6	M/68	Lower arm	NE	60%	Loss	*TP53* deletion based on coverage analysis
APLT7	M/76	Thigh	Deficient	64%	Loss	*TP53* c.673-1G>T p.?—likely pathogenic variant
APLT8	M/73	Back/neck	Deficient	18%	Loss	*TP53* c.80del p.(Pro27Leufs*17)—likely pathogenic variant
APLT9	M/83	Neck	Deficient	NI	Loss	Failed
APLT10	F/48	Thigh	Deficient	32%	Loss	*TP53* WT
APLT11	F/54	Back	Deficient	44%	Loss	Failed
APLT12	M/79	Upper arm	Deficient	18%	Heterogeneous	*TP53* c.581T>A p.(Leu194His)—VUS
APLT13	F/63	Upper arm	Deficient	30%	Loss	Failed
APLT14	F/54	Upper arm	Deficient	36%	Loss	*TP53* deletion based on coverage analysis
APLT15	M/54	Neck	Deficient	NI	Overexpression	*TP53* c.832C>G p.(Pro278Ala)—likely pathogenic variant
APLT16	M/68	Gluteal	Deficient	38%	Heterogeneous	Failed
APLT17	F/38	Scapula	Deficient	4%	NI	*TP53* c.782_782 + 18del p.?—likely pathogenic variant
APLT18	M/52	Hand	Deficient	50%, MA and M	Loss	*TP53* deletion based on coverage analysis
APLT19	M/66	Neck	Deficient	36%, MA and M	Loss	*TP53* deletion based on coverage analysis
APLT20	M/67	Neck	Deficient	46%, MA and M	Loss	*TP53* c.532del p.(His178Thrfs*69)—likely pathogenic variant
APLT21	M/67	Thorax	Heterogeneous	20%	Overexpression	*TP53* c.247G>A p.(Ala83Thr)—VUS

M, Monosomy 13q; MA, Mono-allelic deletion; NE, Not executed; NI, Not interpretable; VUS, Variant of unknown significance; WT, Wild-type.

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In each case, 4-μm thick sections from a representative FFPE block were used for immunohistochemical analysis. Immunohistochemistry was performed using a Benchmark XT immunostainer (Ventana Medical Systems). Sections were stained with primary monoclonal antibodies against Rb1 (1:50; G3-245; BD Pharmingen, San Diego, CA, USA) and p53 (prediluted; DO-7; Ventana Medical Systems, Roche, Indianapolis, IL, USA). Nuclear staining for Rb1 and p53 was scored by two of the authors (F.C. and D.C.). P53 nuclear staining was assessed as follows: loss (no staining of tumour cells, p53 null-pattern), heterogeneous (staining in <80% of tumour cells, p53 wild-type pattern) and overexpression (staining in ≥80% of tumour cells, p53 overexpression mutation pattern).[20–22] Rb1 nuclear immunoreactivity was

classified as: deficient (<10% of tumour cells with nuclear staining), heterogeneous/equivocal (nuclear staining in 10%–80% of tumour cells) or intact (>80% of tumour cells with nuclear staining).[3,9] Appropriate positive and negative controls were used throughout the study.

MOLECULAR ANALYSIS

FISH was performed on FFPE tissue using the ZytoLight SPEC *RB1*/13q12 detection kit, containing a mixture of an orange fluorochrome probe specific for the *RB1* gene in the chromosomal region 13q14.2 and a green fluorochrome probe specific for chromosomal region 13q12, which served as a control. Fifty non-overlapping nuclei were examined. Possible deletion patterns are mono-allelic deletion (1 orange/2 green signals), biallelic deletion (2 green signals) and monosomy of chromosome 13q (1 orange/1 green signal). The FISH results were interpreted according to the criteria of Agaimy *et al.*[13] Furthermore, FISH for *MDM2* was performed in all cases to exclude ALT/WDLS.

DNA-based NGS was performed on FFPE tissue, The DNA was extracted with the QIAamp DNA FFPE Tissue kit (QIAGEN) followed by DNA quality assessment using the DIN value of the Genomic DNA ScreenTape assay (TapeStation, Agilent). Molecular analysis was performed at the gDNA level using targeted sequencing with a capture-based technology (Roche) using a custom panel of 73 genes including *AKT1, ALK, APC, AR, ARID1A, ATM, BAP1, BRAF, BRCA1, BRCA2, CCND1, CDK12, CDK4, CDK6, CDKN2A, CDKN2B, CTNNB1, DICER1, DPYD, EGFR, ERBB2, ERBB3, ESR1, FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, FOXL2, FRK, GATA3, GNA11, GNAQ, GNAS, H3-3A, H3-3B, H3C2, H3C3, HNF1A, HRAS, IDH1, IDH2, IL6ST, JAK1, JAK2, KEAP1, KIT, KRAS, MAP2K1, MET, MYOD1, NRAS, NTRK1, NTRK2, NTRK3, PDGFRA, PDGFRB, PIK3CA, PIK3R1, POLE, PTEN, RB1, RET, RNF43, ROS1, SMAD4, SMO, SPOP, STAT3, STK11, TERT* promoter, *TP53* and *VHL* followed by Illumina's sequencing by synthesis (SBS) technology on a NextSeq2000 or NovaSeq6000 sequencer. The sequencing reads are aligned to the reference sequence (Genome build 38) using an in-house Bcbio workflow with the vardict variant caller. Deviations regarding the reference sequence or variants are annotated using VEP (Ensembl). Variant nomenclature is according to HGVS (www.hgvs.org): A of ATG start codon is nucleotide +1.

Results

CLINICAL FEATURES

Our study population included 32 male and 23 female patients with an age range of 38–83 years (mean age of 62.2 years; Tables 1 and 2). The 34 patients with ASLT had a mean age of 61.8 years (median 62 years), and the 21 patients with APLT had a mean age of 62.9 years (median 66 years). ASLTs were predominantly located in the soft tissue of the upper extremities ($n = 12$), followed by the lower extremities ($n = 8$), shoulder/neck ($n = 6$), trunk/back ($n = 4$), inguinal region ($n = 2$) and gluteal region ($n = 2$). APLTs were also predominantly located in the upper extremities ($n = 6$) and shoulder/neck region ($n = 6$), followed by the lower extremities ($n = 4$), trunk/back ($n = 3$), abdominal wall ($n = 1$) and gluteal region ($n = 1$). All cases underwent surgical excision—12 of which were preceded by an open biopsy—except for 2 cases where only an open biopsy was performed. Clinical follow-up data were available for 30 cases, with a mean follow-up duration of 24 months. Local recurrence was observed in two ASLT cases (ASLT22 and ASLT23) and two APLT cases (APLT6 and APLT14), occurring at 15, 20, 24 and 48 months, respectively, all following incomplete resection.

MICROSCOPIC FEATURES

The study included ASPLTs representing the whole morphological spectrum from low- to high cellularity. ASLTs exhibited characteristic morphological features consistent with diagnostic criteria, including adipocytic differentiation, variable presence of scattered atypical spindle cells with hyperchromatic nuclei, lipoblasts and a collagenous or myxoid background with ropey collagen and mast cells (Figure 1).[2,3,5,9] In contrast, APLTs were distinguished by the presence of more bizarre, pleomorphic (multinucleated) and hyperchromatic cells throughout the lesion, together with multinucleated floret-like cells and/or pleomorphic lipoblasts (Figure 2).[3,4] The APLTs demonstrated higher cellularity than typically seen in pleomorphic lipomas. Notably, none of the cases exhibited tumour necrosis, sheet-like growth or high mitotic activity. Furthermore, none of the cases showed dedifferentiation.

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The results for Rb1 and p53 IHC are summarized in Tables 1 and 2 for ASLT and APLT cases,

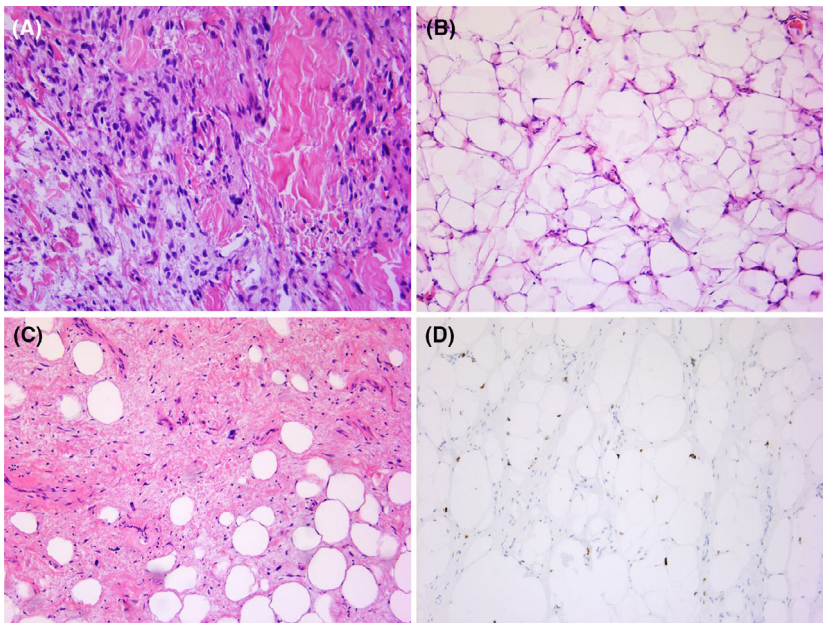

Figure 1. Morphological and immunohistochemical spectrum of atypical spindle cell lipomatous tumours (ASLT). A high-cellular ASLT case predominantly shows scattered atypical hyperchromatic spindle cells in a collagenous stroma with ropey collagen (**A**, spindle cell-rich subtype, HE original magnification 200×). In contrast, low-cellular ASLTs show variable proportions of adipocytes and atypical spindle cells (**B** and **C**, spindle cell-poor subtype, HE original magnification 100×). Immunohistochemistry for p53 showed a heterogeneous staining pattern (**D**).

respectively. In 74% of ASPLTs (39 of 53 cases with interpretable IHC results), nuclear Rb1 expression was lost in the atypical spindled cells (deficient pattern). This Rb1 loss was observed in 65% of ASLTs (22 of 34 cases) and 89% of APLTs (17 of 19 cases). Heterogeneous staining (equivocal pattern) of Rb1 was observed in 11 cases, comprising 26% of ASLTs (9 of 34 cases) and 11% of APLTs (2 of 19 cases). In 3 ASLTs, nuclear Rb1 expression remained (intact pattern).

P53 IHC was performed on 54 of 55 ASPLT cases, yielding interpretable results in 31 ASLT and 20 APLT cases (non-interpretable cases lacked positive controls with absence of staining in lymphocytes and/or endothelial cells). In all ASLTs (31 of 31 cases), a heterogeneous staining pattern of p53 was observed (Figure 1D). In contrast, only 15% of APLTs (3 of 20 cases) showed a heterogeneous p53 staining pattern, while 75% (15 of 20 cases) showed loss of p53 expression, and 10% of APLTs (2 of 20 cases) showed p53 overexpression (Figure 2).

MOLECULAR FEATURES

The *RB1* FISH results are summarized in Tables 1 and 2. All ASPLT cases demonstrated *RB1* deletion with an average loss of 33% of tumour cells (range: 4% to 66%). This *RB1* (13q14.2) deletion often coincided with the co-loss of the corresponding 13q12 signal, indicating monosomy at the 13q region and/or mono-allelic deletion of 13q12. No *MDM2* amplification was detected by FISH in any of the cases. Furthermore, no case showed *CDK4* amplification by DNA NGS.

DNA-based NGS yielded interpretable data in 30/34 ASLT and 16/21 APLT cases. We observed in 94% of APLTs (15 of 16 cases) *TP53* alterations (Table 2), whereas no *TP53* alterations were found in ASLT cases (Table 1). Notably, 20 out of 21 APLT cases exhibited a *TP53* abnormality detected by IHC and/or DNA NGS. One case, which displayed a heterogeneous p53 staining pattern, failed NGS testing. APLT1 and APLT12 also exhibited a heterogeneous (wild-type) p53 staining pattern yet harboured a *TP53* variant of unknown significance (VUS). Among APLT cases with an abnormal p53 immunohistochemical profile and successful DNA NGS testing, 92% (12 of 13 cases) harboured a *TP53* alteration, suggesting a genetic basis for the abnormal staining pattern. One case (APLT10) showed discordant results, exhibiting a null-pattern on p53 IHC but no detectable *TP53* alteration by DNA NGS.

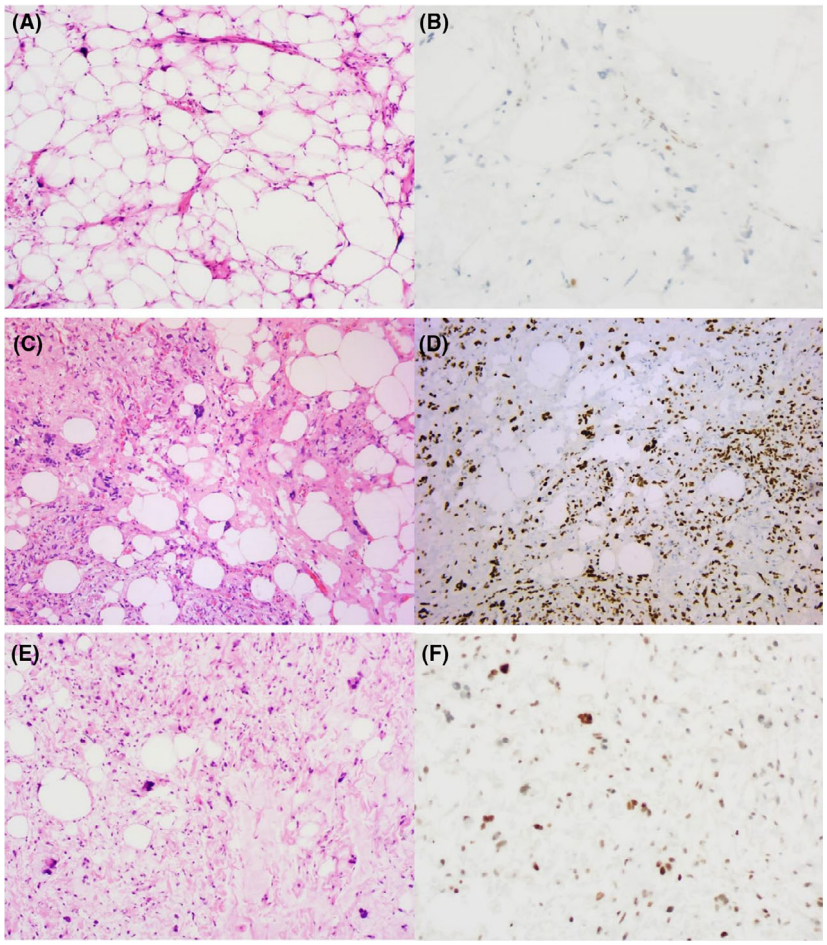

Figure 2. Morphological and immunohistochemical spectrum of atypical pleomorphic cell lipomatous tumours (APLT). APLT showing bizarre, pleomorphic (multinucleated) and hyperchromatic cells (**A**, HE original magnification 40×), with loss of p53 expression (with preserved nuclear staining of endothelial cells as internal control) (**B**). An APLT showing multinucleated floret-like cells, pleomorphic lipoblasts and atypical hyperchromatic spindle cells in a collagenous stroma with ropey collagen (**C**, HE, original magnification 100×). This case showed p53 overexpression (**D**). APLT1 harbouring a *BRAF* mutation (**E**, HE, original magnification 40×), with p53 heterogeneous (wild-type) staining pattern (**F**).

Furthermore, one APLT (APLT1) showed a *BRAF* c.1397G>A p.(Gly466Glu) likely pathogenic variant (Figure 2E,F). *RB1* alterations were detected in three ASLTs (ASLT18, 21 and 30).

Discussion

To the best of our knowledge, this is the first study investigating *TP53* gene alterations in a large cohort of ASPLTs, including both APLTs and ASLTs, using IHC and DNA-based NGS. APLT and ASLT are currently considered part of a biological continuum, as both entities exhibit significant histological and molecular overlap. As a result, they are classified together under the designation 'ASPLT' in the 2020 WHO Classification of Soft Tissue and Bone Tumours.[1,3]

In this study, ASLT and APLT cases also showed clinical overlap, such as predominantly occurring in older patients (mean age ~62 years) and primarily affecting the upper extremities. However, ASLT cases were occasionally found in the inguinal region, while one APLT case involved the abdominal wall, as already described in the literature.[2,3,5] Both ASLTs and APLTs were characterized by *RB1* abnormalities, as confirmed by IHC and/or FISH.

Importantly, we observed that APLTs frequently harboured *TP53* alterations (with 20 out of 21 APLT cases exhibiting a *TP53* abnormality detected by IHC and/or DNA NGS), while no such *TP53* alterations were detected in ASLT cases. The presence of *TP53*

alterations has already been described in a small series of APLTs and 3 recently published ASPLT cases with sarcomatous differentiation, in which a *TP53* alteration was present in both the low-grade APLT and high-grade sarcomatous components, showing that dedifferentiation is based on additional genetic aberrations.[19,23]

However, our current study is the first to investigate *TP53* alterations in ASLTs and to compare these with a large series of APLTs. The absence of *TP53* alterations in ASLT cases raises the possibility that these lesions may be more distinct from APLTs than previously assumed. In this context, ASLTs could be considered more closely related to spindle cell lipomas, as suggested by Mentzel *et al.* and Creytens *et al.*,[9,16] but with more pronounced cytonuclear atypia. Conversely, APLTs may align more closely with pleomorphic liposarcomas (PLs), as distinguishing between highly cellular APLTs and PLs can be challenging based on morphology.[3] Additionally, APLTs have been shown to rarely undergo sarcomatous differentiation.[23,24] Furthermore, both APLTs and PLs can harbour *RB1* deletions and *TP53* alterations, making these molecular markers insufficient for distinguishing between them. Therefore, differentiation between APLT and PL relies heavily on a careful assessment of morphological features. Tumour necrosis, sheet-like growth, high cellularity and increased mitotic activity are indicative features suggestive of malignancy.[3,4] Moreover, PLs tend to exhibit more complex genetic alterations, characterized by a greater number of losses and gains than APLTs.[3] However, we acknowledge that cases with intermediate morphological features exist, exhibiting striking morphological overlap that prevents clear classification as either ASLT or APLT, suggesting a biological spectrum.

Interestingly, our study demonstrated a good overall correlation between p53 IHC (loss of expression or overexpression) and *TP53* alterations in APLT cases. This implies that p53 IHC could be used as a potential discriminating tool to distinguish between ASLTs and APLTs in cases where morphological features overlap. Specifically, loss of p53 expression on IHC correlated well with *TP53* alterations detected by a targeted DNA-based NGS panel that includes *TP53* deletions based on coverage analysis. In APLT cases showing p53 loss on IHC, *TP53* deletions suggested biallelic inactivation, detectable through targeted DNA-based coverage analysis.[19] The *TP53* variants identified in APLT1, APLT12 and APLT21 were classified as VUS. The heterogeneous p53 IHC staining in APLT1 and APLT12 suggests that these *TP53* variants do not affect p53 expression. In contrast, the p53 overexpression observed in APLT21 implies that its variant may represent an activating alteration. One discordant case was identified in our cohort: APLT10 exhibited a null pattern on IHC, yet no *TP53* alteration was detected by DNA NGS. This finding could reflect low proliferative activity in a *TP53* wild-type tumour, where p53 expression is naturally low or absent despite an intact gene. Alternatively, the result may represent a false negative, as the targeted NGS panel reliably detects only biallelic *TP53* losses, potentially missing more subtle deletions. CNV sequencing or a SNP-based targeted CNV analysis would be more suitable approaches to uncover such alterations. Alternatively, epigenetic factors or alterations in other pathways could suppress *TP53* function even in the absence of detectable *TP53* alterations.

Distinguishing between ASLT and APLT may become more clinically relevant if further observations confirm that APLTs have the potential to transform or dedifferentiate—though current data remain inconclusive. In such a scenario, p53 IHC and DNA NGS testing could emerge as valuable screening or prognostic tools, allowing for more rigorous follow-up of p53-positive cases. Future research, including CNV analysis and methylation profiling, may provide deeper insights into the classification of ASPLT.

Conclusion

In this study, we compared a large cohort of ASLT and APLT cases using IHC and DNA-based NGS. Our findings revealed that APLTs frequently harbour *TP53* alterations, in contrast to ASLTs, where no such alterations were detected. This observation suggests that APLTs may be more biologically distinct from ASLTs than previously assumed. Furthermore, p53 immunostaining proved to be a potentially valuable diagnostic tool, aiding pathologists in differentiating between ASLTs and APLTs. These findings support the classification of APLT as a distinct (sub) entity within the broader ASPLT spectrum. However, it remains to be determined whether the broader term 'atypical spindle cell/pleomorphic lipomatous tumour' will continue to be favoured or if further refinements to this classification will be proposed in the future.

Author contributions

FC and TK performed the writing of the paper. TM, UF, JvG, LF, SVB, SL, JVdM, JVD and AHGC

performed the review of the paper. FC and DC performed the study concept, design and writing of the paper. All authors read and approved the final paper.

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Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Data availability statement

Data are available on request from the authors.

References

1. Creytens D, Marino-Enriquez A. *Atypical Spindle Cell/Pleomorphic Lipomatous Tumour. World Health Organization classification of soft tissue and bone Tumours*. 5th ed. Lyon: IARC Press, 2020.
2. Marino-Enriquez A, Nascimento AF, Ligon AH, Liang C, Fletcher CD. Atypical spindle cell lipomatous tumor: clinicopathologic characterization of 232 cases demonstrating a morphologic Spectrum. *Am. J. Surg. Pathol.* 2017; **41**; 234–244.
3. Creytens D, Mentzel T, Ferdinande L *et al*. "Atypical" pleomorphic lipomatous tumor: a clinicopathologic, immunohistochemical and molecular study of 21 cases, emphasizing its relationship to atypical spindle cell lipomatous tumor and suggesting a morphologic spectrum (atypical spindle cell/pleomorphic lipomatous tumor). *Am. J. Surg. Pathol.* 2017; **41**; 1443–1455.
4. Anderson WJ, Fletcher CDM, Jo VY. Atypical pleomorphic lipomatous tumor: Expanding our current understanding in a clinicopathologic analysis of 64 cases. *Am. J. Surg. Pathol.* 2021; **45**; 1282–1292.
5. Bahadir B, Behzatoglu K, Hacihasanoglu E, Koca SB, Sigirci BB, Tokat F. Atypical spindle cell/pleomorphic lipomatous tumor: a clinicopathologic, immunohistochemical, and molecular study of 20 cases. *Pathol. Int.* 2018; **68**; 550–556.
6. Lecoutere E, Creytens D. Atypical spindle cell/pleomorphic lipomatous tumor. *Histol. Histopathol.* 2020; **35**; 769–778.
7. Creytens D. What's new in adipocytic neoplasia? *Virchows Arch.* 2020; **476**; 29–39.
8. Creytens D. A contemporary review of myxoid adipocytic tumors. *Semin. Diagn. Pathol.* 2019; **36**; 129–141.
9. Creytens D, van Gorp J, Savola S, Ferdinande L, Mentzel T, Libbrecht L. Atypical spindle cell lipoma: a clinicopathologic, immunohistochemical, and molecular study emphasizing its relationship to classical spindle cell lipoma. *Virchows Arch.* 2014; **465**; 97–108.
10. Creytens D, Mentzel T, Ferdinande L, van Gorp J, Van Dorpe J, Flucke U. Fat-rich (spindle cell-poor) variants of atypical spindle cell lipomatous tumors show similar morphologic, immunohistochemical and molecular features as "dysplastic lipomas": are they related lesions? Comment on Michal et al (2018). *Am. J. Surg. Pathol.* 2019; **43**; 288–289.
11. Qorbani A, Horvai A. Atypical spindle cell/pleomorphic lipomatous tumor. *Surg. Pathol. Clin.* 2024; **17**; 97–104.
12. Nishio J, Nakayama S, Chijiiwa Y, Koga M, Aoki M. Atypical spindle cell/pleomorphic lipomatous tumor: a review and update. *Cancer* 2024; **16**; 3146.
13. Agaimy A, Michal M, Giedl J, Hadravsky L, Michal M. Superficial acral fibromyxoma: clinicopathological, immunohistochemical, and molecular study of 11 cases highlighting frequent Rb1 loss/deletions. *Hum. Pathol.* 2017; **60**; 192–198.
14. Chen BJ, Marino-Enriquez A, Fletcher CD, Hornick JL. Loss of retinoblastoma protein expression in spindle cell/pleomorphic lipomas and cytogenetically related tumors: an immunohistochemical study with diagnostic implications. *Am. J. Surg. Pathol.* 2012; **36**; 1119–1128.
15. Libbrecht S, Van Dorpe J, Creytens D. The rapidly expanding group of RB1-deleted soft tissue tumors: an updated review. *Diagnostics (Basel)* 2021; **11**; 430.
16. Mentzel T, Palmedo G, Kuhnen C. Well-differentiated spindle cell liposarcoma ('atypical spindle cell lipomatous tumor') does not belong to the spectrum of atypical lipomatous tumor but has a close relationship to spindle cell lipoma: clinicopathologic, immunohistochemical, and molecular analysis of six cases. *Mod. Pathol.* 2010; **23**; 729–736.
17. Somaiah N, Tap W. MDM2-p53 in liposarcoma: the need for targeted therapies with novel mechanisms of action. *Cancer Treat. Rev.* 2024; **122**; 102668.
18. Debelenko LV, Perez-Atayde AR, Dubois SG *et al*. p53+/mdm2-atypical lipomatous tumor/well-differentiated liposarcoma in young children: an early expression of Li-Fraumeni syndrome. *Pediatr. Dev. Pathol.* 2010; **13**; 218–224.
19. Hammer PM, Kunder CA, Howitt BE, Charville GW. Well-differentiated lipomatous neoplasms with p53 alterations: a clinicopathological and molecular study of eight cases with features of atypical pleomorphic lipomatous tumour. *Histopathology* 2022; **80**; 656–664.
20. Redston M, Noffsinger A, Kim A *et al*. Abnormal TP53 predicts risk of progression in patients with Barrett's esophagus regardless of a diagnosis of dysplasia. *Gastroenterology* 2022; **162**; 468–481.
21. Osakabe M, Yamada N, Sugimoto R *et al*. The pattern-based interpretation of p53 immunohistochemical expression as a surrogate marker for TP53 mutations in colorectal cancer. *Virchows Arch.* 2025; **486**; 333–341.
22. Mohammad N, Stewart CJR, Chiang S *et al*. p53 immunohistochemical analysis of fusion-positive uterine sarcomas. *Histopathology* 2021; **78**; 805–813.
23. Perret R, Charville GW, Alame M *et al*. Atypical spindle cell/pleomorphic lipomatous tumor with Sarcomatous transformation: clinicopathologic and molecular analysis of 4 cases. *Mod. Pathol.* 2024; **37**; 100454.
24. Sugita S, Sugawara T, Emori M *et al*. Atypical spindle cell/pleomorphic lipomatous tumor with a sarcomatous component shoxing high mitotic activity and Ki67-labeling index: report of a unique case mimicking dedifferentiated liposarcoma. *Med. Mol. Morphol.* 2022; **55**; 323–328.