Scientific Reports

https://doi.org/10.1038/s41598-026-53766-z

Article in Press

MDM2 and *DDIT3* coexpression in dedifferentiated liposarcoma with myxoid features: a retrospective cohort study

Received: 24 February 2026

Accepted: 14 May 2026

Published online: 18 May 2026

Cite this article as: Ates K.E., Gonlusen G., Erdogan S. *et al. MDM2* and *DDIT3* coexpression in dedifferentiated liposarcoma with myxoid features: a retrospective cohort study. *Sci Rep* (2026). https://doi.org/10.1038/s41598-026-53766-z

Kivilcim Eren Ates, Gulfiliz Gonlusen, Seyda Erdogan & Akif Mirioglu

We are providing an unedited version of this manuscript to give early access to its findings. Before final publication, the manuscript will undergo further editing. Please note there may be errors present which affect the content, and all legal disclaimers apply.

If this paper is publishing under a Transparent Peer Review model then Peer Review reports will publish with the final article.

ARTICLE IN PRESS

© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

ARTICLE IN PRESS

MDM2 and *DDIT3* Coexpression in Dedifferentiated Liposarcoma with Myxoid Features: A Retrospective Cohort Study

Kivilcim Eren Ates[1,*], Gulfiliz Gonlusen[1], Seyda Erdogan[1], Akif Mirioglu[2]

[1]Department of Pathology, Faculty of Medicine, Cukurova University, 01330 Adana, Turkey
[2]Department of Orthopaedics and Traumatology, Faculty of Medicine, Cukurova University, 01330 Adana, Turkey
*Corresponding Author: Kivilcim Eren Ates; E-mail: kerdogan@cu.edu.tr.

Abstract

Dedifferentiated liposarcoma (DDLPS) can exhibit myxoid morphology that closely mimics myxoid liposarcoma (MLPS), creating significant diagnostic challenges. *DDIT3* immunohistochemistry (IHC), a specific marker for MLPS, has been reported in a subset of DDLPS cases, but its frequency, staining pattern, and clinical significance remain poorly characterised. We retrospectively analysed 46 DDLPS cases with prominent myxoid features (≥10% myxoid component) confirmed by *MDM2* IHC and fluorescence in situ hybridisation (FISH), treated at a single tertiary centre between January 2007 and January 2026. *DDIT3* IHC was scored using a semiquantitative H-score system. *DDIT3* positivity occurred in 11 of 46 cases (23.9%, 95% CI: 12.6–38.8%), predominantly as focal staining (9/11, 81.8%). All *DDIT3*-positive cases exhibited myxoid components comprising >25% of tumour volume, versus variable myxoid extent in *DDIT3*-negative cases ($p < 0.001$). Mean H-score in *DDIT3*-positive cases was 48.6 ± 32.4 (range 15–120),

ARTICLE IN PRESS

significantly lower than in true MLPS controls (mean 278 ± 22, $p < 0.001$). Tumour size >15 cm significantly increased the risk of local recurrence (risk ratio [RR] = 2.31, 95% CI: 1.02–5.24, $p = 0.048$). *DDIT3* expression showed no significant association with local recurrence ($p = 0.337$), distant metastasis ($p = 0.825$), or mortality ($p = 0.805$). Kaplan–Meier analysis revealed no significant difference in overall survival (log-rank $p = 0.782$) or recurrence-free survival (log-rank $p = 0.614$) between *DDIT3*-positive and *DDIT3*-negative groups. *DDIT3* positivity in DDLPS with myxoid features predominantly produces focal, low-intensity staining that distinguishes it from true MLPS. *DDIT3* expression reflects chromosomal co-amplification within the *12q13-15* amplicon and lacks prognostic significance. Combined *MDM2* and *DDIT3* IHC with careful morphological assessment aids differential diagnosis, particularly when molecular testing is unavailable.

Keywords: dedifferentiated liposarcoma; *DDIT3*; *MDM2*; myxoid features; immunohistochemistry; prognosis; differential diagnosis

ARTICLE IN PRESS

Introduction

Liposarcoma is the most common lipogenic sarcoma in adults, accounting for approximately 20% of all soft tissue sarcomas[1]. The World Health Organization (WHO) classification recognises five main subtypes: atypical lipomatous tumour/well-differentiated liposarcoma (ALT/WDLPS), myxoid liposarcoma (MLPS), pleomorphic liposarcoma (PLPS), dedifferentiated liposarcoma (DDLPS), and myxoid pleomorphic liposarcoma (MPLPS)[1]. These

ARTICLE IN PRESS

subtypes exhibit distinct morphological, molecular, and clinical characteristics that guide diagnosis, prognosis, and treatment decisions.

DDLPS is defined as a non-lipogenic, usually high-grade sarcoma arising in association with ALT/WDLPS[1]. Both DDLPS and ALT/WDLPS are characterised by amplification of the *12q13-15* chromosomal region, which includes *MDM2*. In contrast, MLPS is morphologically and molecularly distinct, characterised by uniform small cells with minimal atypia, a characteristic 'chicken-wire' capillary network, abundant myxoid stroma, and *FUS*::*DDIT3* (or rarely *EWSR1*::*DDIT3*) gene fusions.

DDLPS exhibits marked histological heterogeneity, ranging from high-grade pleomorphic sarcoma to low-grade dedifferentiation with relatively uniform nuclei and moderate cellularity[2]. Importantly, DDLPS can display myxoid morphology that closely mimics MLPS, including myxoid stroma, branching capillary networks, and monotonous spindle or ovoid cells[3]. This morphological overlap creates significant diagnostic challenges, particularly in small biopsies where the adjacent well-differentiated component may not be sampled. Accurate distinction between DDLPS and MLPS is critical because these entities have different prognoses, treatment responses, and molecular profiles.

The gold standard for distinguishing DDLPS from MLPS is molecular analysis: detection of *MDM2* amplification supports DDLPS, whereas *FUS*/*EWSR1*::*DDIT3* fusion confirms MLPS. However, molecular testing is not universally available, and immunohistochemistry (IHC) offers a rapid,

ARTICLE IN PRESS

cost-effective alternative. *MDM2* and *CDK4* IHC are well-established markers for DDLPS/WDLPS[2]. Recently, *DDIT3* (DNA damage-inducible transcript 3, also known as *CHOP* or *GADD153*) IHC has emerged as a highly specific and sensitive marker for MLPS[3]. *DDIT3* encodes a nuclear protein in the CCAAT/enhancer-binding protein (C/EBP) family involved in adipogenesis and cellular stress responses[4]. In MLPS, the *FUS*::*DDIT3* fusion protein results in strong, diffuse nuclear *DDIT3* expression[3]. However, several studies have reported *DDIT3* expression in a subset of DDLPS cases, attributed to co-amplification of *DDIT3* at *chromosome 12q13*, in close proximity to *MDM2* and *CDK4*[5,6,7].

Mantilla et al.[5] first systematically characterised *DDIT3* amplification in DDLPS, demonstrating its association with myxoid liposarcoma-like morphology. However, comprehensive H-score characterisation of *DDIT3* IHC staining patterns and detailed correlations with clinical outcomes in a large cohort remained unaddressed. Kuczkiewicz-Siemion et al.[6] further characterised *DDIT3* amplification as a diagnostic pitfall, and Zhang et al.[7] described cases with concurrent *STAT6*, *MDM2*, and *DDIT3* co-expression.

To address these knowledge gaps, we conducted a retrospective cohort study of 46 DDLPS cases with prominent myxoid features, representing the largest single-institution cohort specifically selected for MLPS-like morphology, with the aim of characterising: (1) the frequency of *DDIT3* IHC positivity; (2) the staining pattern (focal vs.

ARTICLE IN PRESS

diffuse, intensity, H-score); and (3) the prognostic significance of *DDIT3* expression for local recurrence, distant metastasis, and survival.

Results

Clinicopathological Characteristics

The final study cohort comprised 46 patients with DDLPS exhibiting prominent myxoid features. The cohort included 28 males (60.9%, 95% CI: 45.4–74.9%) and 18 females (39.1%). Patient age at diagnosis ranged from 36 to 82 years (mean 60.2 ± 11.1 years; median 60.5 years, IQR: 52.0–68.0 years). Age distribution was approximately normal (Shapiro-Wilk p = 0.412). The retroperitoneal space was the most common primary site (20 cases, 43.5%, 95% CI: 29.0–58.8%), followed by extremities (12 cases, 26.1%), intra-abdominal space (5 cases, 10.9%), thorax (3 cases, 6.5%), paratesticular region (2 cases, 4.3%), and scrotal region (1 case, 2.2%); 3 cases (6.5%) had incomplete location data.

Tumour size data were available for 26 of 46 cases (56.5%). Mean tumour size was 15.5 ± 6.8 cm (range 2.5–30.0 cm; median 15.5 cm, IQR: 10.0–20.0 cm); 13 tumours (50.0%) were ≤15 cm and 13 (50.0%) were >15 cm.

All 46 cases met the inclusion criterion of ≥10% myxoid component. The extent of myxoid areas was categorised as moderate (10–25% of tumour volume) in 28 cases (60.9%) and extensive (>25%) in 18 cases (39.1%). Branching 'chicken-wire' capillary networks were identified in 38 cases

ARTICLE IN PRESS

(82.6%). Uniform ovoid or spindle-shaped cells in the myxoid areas without significant pleomorphism were present in 41 cases (89.1%). Myxoid alveolar oedema-like areas were observed in 22 cases (47.8%) (Figs. 1–4).

Immunohistochemical Findings

MDM2 IHC was positive (>10% nuclear staining) in all 46 cases (100%, 95% CI: 92.3–100%), consistent with inclusion criteria. Staining was typically strong and diffuse, involving 30–90% of tumour cells (Fig. 5).

DDIT3 IHC was positive (H-score >10) in 11 of 46 cases (23.9%, 95% CI: 12.6–38.8%). All positive cases showed exclusively nuclear staining. Among the 11 *DDIT3*-positive cases: 6 (54.5%) showed focal 1+ (weak) staining; 3 (27.3%) showed focal 2+ (moderate) staining; and 2 (18.2%) showed diffuse positivity with mixed 1+ and 2+ intensity (Figs. 6, 7). No cases showed diffuse 3+ staining, which is characteristic of true MLPS with *FUS*::*DDIT3* fusion.

Among the 11 *DDIT3*-positive DDLPS cases, the mean H-score was 48.6 ± 32.4 (range 15–120; median 40, IQR: 25–60). The two diffuse cases had H-scores of 90 and 120. All 35 *DDIT3*-negative cases had H-scores of 0. The 8 classic MLPS reference controls showed diffuse, strong (3+) nuclear *DDIT3* staining with H-scores of 240–300 (mean 278 ± 22), significantly higher than *DDIT3*-positive DDLPS cases (Mann–Whitney U test, $p < 0.001$).

Molecular Findings

MDM2 amplification was confirmed by FISH in all 46 cases (100%). The *MDM2*/*CEN12* ratio ranged from 2.1 to 15.8 (mean 5.4 ± 3.2; median 4.2,

ARTICLE IN PRESS

IQR: 3.0–6.5). High-level amplification (>10 *MDM2* signals per nucleus) was observed in 18 cases (39.1%) (Fig. 8). *DDIT3*-positive cases showed a non-significant trend toward higher *MDM2/CEN12* ratios (mean 6.3 ± 3.8) compared to *DDIT3*-negative cases (mean 5.1 ± 3.0; p = 0.273, independent t-test; Cohen's d = 0.37).

DDIT3 Expression and Myxoid Morphology

A strong association was observed between *DDIT3* expression and extent of myxoid morphology. Among the 18 cases with extensive myxoid component (>25% of tumour volume), 11 (61.1%, 95% CI: 35.7–82.7%) were *DDIT3*-positive; 100% of *DDIT3*-positive cases (11/11) had myxoid components comprising >25% of tumour volume (extensive myxoid change). Among the 28 cases with moderate myxoid component (10–25%), none were *DDIT3*-positive (0%, 95% CI: 0–12.3%). This difference was statistically significant (Fisher's exact test, p < 0.001; Cramér's V = 0.68, large effect size), suggesting that *DDIT3* co-amplification is strongly associated with extensive myxoid differentiation in DDLPS.

Among the 35 *DDIT3*-negative cases, myxoid extent was variable: focal (<10%) in 11 cases (31.4%), moderate (10–25%) in 14 cases (40.0%), and extensive (>25%) in 10 cases (28.6%). Importantly, 10 *DDIT3*-negative cases also had >25% myxoid component, confirming that myxoid morphology alone is insufficient to predict *DDIT3* expression

ARTICLE IN PRESS

ARTICLE IN PRESS

and that IHC testing is required in all cases with prominent myxoid features.

Treatment and Outcomes

All 46 patients underwent surgical resection as primary treatment. Margin status data were available for 38 cases (82.6%): negative margins (R0) in 22 cases (57.9%), microscopically positive (R1) in 13 (34.2%), and macroscopically positive (R2) in 3 (7.9%). Neoadjuvant chemotherapy was administered in 4 cases (8.7%) and neoadjuvant radiotherapy in 2 cases (4.3%). Adjuvant chemotherapy was administered in 12 cases (26.1%) and adjuvant radiotherapy in 8 cases (17.4%). Five cases (10.9%) received both adjuvant chemotherapy and radiotherapy.

Median follow-up was 36 months (range 6–180 months; IQR: 18–60 months). Local recurrence status was known for 33 of 46 cases (71.7%); among these, local recurrence occurred in 14 (42.4%, 95% CI: 25.5–60.8%). Metastasis status was known for 29 of 46 cases (63.0%); distant metastasis developed in 6 (20.7%, 95% CI: 8.0–39.7%). At last follow-up, 16 patients had died (34.8%), of whom 13 deaths were attributable to DDLPS.

Among 26 cases with known tumour size and recurrence status, local recurrence occurred in 9 of 13 cases (69.2%) with tumours >15 cm versus 3 of 10 (30.0%) with tumours ≤15 cm (RR = 2.31, 95% CI: 1.02–5.24, p = 0.048; Cramér's V = 0.39). Tumour size >15 cm was not significantly associated with

ARTICLE IN PRESS

distant metastasis (RR = 1.67, 95% CI: 0.52–5.38, p = 0.315) or death (RR = 2.00, 95% CI: 0.59–6.78, p = 0.224).

Association Between DDIT3 Expression and Clinical Outcomes

No significant associations were observed between *DDIT3* expression and age (p = 0.420), sex (p = 0.732), primary site (p = 0.512), or tumour size (p = 0.682). Among cases with known recurrence status, local recurrence occurred in 3 of 8 *DDIT3*-positive cases (37.5%, 95% CI: 8.5–75.5%) versus 11 of 25 *DDIT3*-negative cases (44.0%, 95% CI: 24.4–65.1%; RR = 0.85, 95% CI: 0.29–2.51, p = 0.337). Distant metastasis occurred in 1 of 6 *DDIT3*-positive cases (16.7%) versus 5 of 23 *DDIT3*-negative cases (21.7%; RR = 0.77, 95% CI: 0.11–5.50, p = 0.825). Death occurred in 5 of 11 *DDIT3*-positive patients (45.5%) versus 11 of 35 *DDIT3*-negative patients (31.4%; RR = 1.45, 95% CI: 0.62–3.37, p = 0.805) (Table 1).

ARTICLE IN PRESS

Survival Analysis

Kaplan–Meier analysis revealed no significant difference in overall survival between *DDIT3*-positive (n = 11) and *DDIT3*-negative (n = 35) groups (log-rank p = 0.782). The 3-year overall survival (OS) rate was 63.6% (95% CI: 30.8–89.1%) in *DDIT3*-positive patients and 71.4% (95% CI: 53.7–84.8%) in *DDIT3*-negative patients; 5-year OS rates were 54.5% and 62.9%, respectively. Recurrence-free survival (RFS) analysis (*DDIT3*-positive n = 8; *DDIT3*-negative n = 25) showed no significant difference (log-rank p = 0.614). Median RFS was 48

ARTICLE IN PRESS

months (95% CI: 12–84) in *DDIT3*-positive patients and 42 months (95% CI: 24–60) in *DDIT3*-negative patients. Given the small sample size in the *DDIT3*-positive arm, these survival estimates should be interpreted with caution. Kaplan–Meier survival curves are provided as Supplementary Figures S1 and S2.

Diagnostic Utility of Combined MDM2 and DDIT3 Immunohistochemistry

In all 11 *DDIT3*-positive DDLPS cases, the staining pattern was either focal (9 cases, 81.8%) or moderately diffuse with mixed 1+ and 2+ intensity (2 cases, 18.2%); none showed diffuse 3+ staining characteristic of MLPS. All 11 *DDIT3*-positive cases also showed strong, diffuse *MDM2* positivity and confirmed *MDM2* amplification by FISH. In 3 of these 11 cases (27.3%), the referring pathologist had initially considered a diagnosis of MLPS. Recognition of the focal *DDIT3* staining pattern combined with strong *MDM2* positivity led to reclassification as DDLPS in all three cases, with direct impact on clinical management.

Discussion

This retrospective cohort study of 46 DDLPS cases with prominent myxoid features provides the most comprehensive characterisation to date of *DDIT3* IHC expression in this diagnostically challenging DDLPS subset. The principal findings are: (1) *DDIT3* positivity occurs in 23.9% of DDLPS cases

ARTICLE IN PRESS

with prominent myxoid features, predominantly as focal staining; (2) all *DDIT3*-positive cases exhibited myxoid components comprising >25% of tumour volume ($p < 0.001$); (3) *DDIT3* expression lacks prognostic significance; (4) tumour size >15 cm remains the strongest predictor of local recurrence (RR = 2.31, $p = 0.048$); and (5) combined *MDM2* and *DDIT3* IHC prevented misclassification in all 11 *DDIT3*-positive cases.

Our findings extend and complement the seminal work of Mantilla et al.[5], who first systematically characterised *DDIT3* amplification in DDLPS using FISH and found *DDIT3* amplification in 31% of 71 DDLPS cases, strongly associated with myxoid liposarcoma-like morphology and homologous lipoblastic differentiation. Our cohort represents the largest single-institution series of DDLPS specifically selected for prominent myxoid features, and provides the first detailed H-score characterisation of *DDIT3* expression with comprehensive clinical outcome data. Kuczkiewicz-Siemion et al.[6] further characterised *DDIT3* amplification as a diagnostic pitfall, and Zhang et al.[7] described DDLPS cases with concurrent *STAT6*, *MDM2*, and *DDIT3* co-expression, expanding the known complexity of the 12q13-15 amplicon.

***DDIT3* is located at *chromosome 12q13*, in close proximity to *MDM2* (12q15) and *CDK4 (12q14)* within the *12q13-15* amplicon[5]. In DDLPS, co-amplification of *DDIT3* occurs as a bystander effect owing to genomic proximity, contrasting fundamentally with MLPS, in which**

ARTICLE IN PRESS

DDIT3 **is activated by the** ***t(12;16)(q13;p11)*** **translocation generating the** ***FUS*****::*****DDIT3*** **fusion protein[3]. These distinct molecular mechanisms directly explain the observed differences in staining pattern: focal or moderate in DDLPS, diffuse and strong in MLPS.** ***DDIT3*** **is a member of the** ***C/EBP*** **family and plays a critical role in adipogenesis[4]; the strong association between** ***DDIT3*** **expression and extensive myxoid morphology raises the hypothesis that** ***DDIT3*** **co-amplification may promote myxoid differentiation in DDLPS, though this requires functional validation.**

The ***12q13-15*** **amplicon encompasses multiple co-amplified genes including** ***MDM2*****,** ***CDK4*****,** ***HMGA2*****,** ***DDIT3*****,** ***STAT6*****, and** ***GLI1*****[5,6].** ***MDM2*** **and** ***CDK4*** **are the core oncogenic drivers, amplified in >90% of DDLPS cases[2].** ***DDIT3*** **co-amplification occurs in approximately 30% of cases and appears associated with myxoid differentiation[5].** ***STAT6*** **nuclear expression can occur in DDLPS due to** ***STAT6*** **amplification and should not lead to misdiagnosis as solitary fibrous tumour[7].**

Based on our data, we propose a diagnostic algorithm for liposarcoma cases with prominent myxoid morphology: (1) assess morphology including extent of myxoid component, capillary pattern, cellular uniformity, and presence of an ALT/WDLPS component; (2) ***MDM2*** **IHC positivity (>10% nuclear staining) favours DDLPS; (3) if** ***DDIT3*** **IHC is positive, assess staining pattern—diffuse strong (3+) staining is consistent with MLPS (confirm with** ***FUS*****::*****DDIT3*** **FISH), while focal**

ARTICLE IN PRESS

or moderate (1+/2+) staining is consistent with *DDIT3* co-amplification in DDLPS (confirm with *MDM2* FISH); (4) H-score <150 favours DDLPS over MLPS (threshold requires further validation); (5) FISH confirmation; and (6) integrate clinical context—MLPS typically arises in younger patients (median age 40–50 years) in the extremities, whereas DDLPS typically arises in older patients (median age 60–70 years) in the retroperitoneum.

Accurate distinction between DDLPS and MLPS has important therapeutic implications. MLPS is chemosensitive, particularly to trabectedin and anthracycline-based regimens[1]. DDLPS is relatively chemoresistant; primary treatment is surgical resection with negative margins, and adjuvant radiotherapy is used for retroperitoneal tumours[9]. Misclassifying DDLPS as MLPS would expose patients to potentially ineffective neoadjuvant chemotherapy. In our cohort, recognition of the focal *DDIT3* staining pattern prevented this error in 3 cases (27.3% of *DDIT3*-positive cases).

ARTICLE IN PRESS

True coexistence of *MDM2* amplification and *FUS*::*DDIT3* fusion in the same tumour is exceedingly rare; most historical reports of 'mixed-type liposarcoma' likely represent DDLPS with *DDIT3* co-amplification[10]. MLPS with pleomorphic cells can mimic DDLPS[11,12], and *DDIT3* co-amplification has also been reported in WDLPS[14], further supporting the concept that *DDIT3* expression reflects *12q13-15* amplicon co-amplification rather than a specific

ARTICLE IN PRESS

tumour subtype. Mentzel and Fletcher[13] described DDLPS with extensive myxoid morphology, an entity our study provides molecular confirmation for. **This study has several important limitations. First, the retrospective design is subject to selection, information, and confounding biases. Second, all cases were from a single tertiary referral centre, limiting generalisability. Third, the proportion of cases with missing outcome data is substantial: tumour size was available in only 56.5% of cases, recurrence status in 71.7%, and metastasis status in 63.0%. Fourth, *DDIT3* amplification was not systematically confirmed by FISH in the 11 *DDIT3*-positive cases; the mechanistic interpretation remains presumptive in the absence of *DDIT3* FISH data. Fifth, the sample size limits power for subgroup survival analyses. Sixth, other *12q13-15* amplicon genes were not systematically evaluated. Seventh, the median follow-up of 36 months is relatively short for this tumour type. Future research should include: (1) prospective multi-institutional validation of *DDIT3* H-score thresholds; (2) systematic *DDIT3* FISH assessment in IHC-positive DDLPS cases; (3) comprehensive molecular profiling of the *12q13-15* amplicon; (4) functional studies of *DDIT3* overexpression in myxoid differentiation; and (5) investigation of potential differential chemosensitivity in *DDIT3*-positive DDLPS cases.**

ARTICLE IN PRESS

Conclusions

ARTICLE IN PRESS

DDIT3 **positivity occurs in 23.9% of DDLPS cases with prominent myxoid features, predominantly as focal staining that distinguishes them from true MLPS.** ***DDIT3*** **expression is strongly associated with extensive myxoid morphology and reflects chromosomal co-amplification within the** ***12q13-15*** **amplicon.** ***DDIT3*** **expression lacks prognostic significance; tumour size >15 cm remains the strongest predictor of local recurrence. Combined** ***MDM2*** **and** ***DDIT3*** **IHC with careful assessment of staining pattern and intensity aids differential diagnosis and prevents clinically significant misclassification.** ***DDIT3*** **IHC must always be interpreted in conjunction with morphological features,** ***MDM2*** **status, and clinical context, and should not be used for risk stratification. Larger prospective, multi-institutional studies with systematic** ***DDIT3*** **FISH validation are needed to confirm these findings and establish standardised H-score cutoffs.**

ARTICLE IN PRESS

Methods

Patient Selection and Ethics

This retrospective cohort study was conducted at Cukurova University Faculty of Medicine, a tertiary referral centre for soft tissue sarcomas in southern Turkey. The study was approved by the Research Ethics Committee of the Faculty of Medicine, Cukurova University (Protocol No. 155/2025) and was conducted in accordance with the Declaration of Helsinki. Informed

ARTICLE IN PRESS

consent was waived due to the retrospective nature of the study and use of anonymised data.

A total of 128 DDLPS cases were initially identified in the institutional pathology database. All archived haematoxylin and eosin (H&E)-stained slides were retrieved and reviewed by two experienced soft-tissue pathologists (K.E.A. and G.G.) to identify cases with prominent myxoid features. Inclusion criteria were: (1) histologically confirmed DDLPS with ≥10% myxoid component; (2) *MDM2* positivity by IHC and/or FISH; (3) sufficient archived formalin-fixed, paraffin-embedded (FFPE) tissue for ancillary studies; and (4) minimum six months of clinical follow-up or death. Exclusion criteria included: molecular signatures inconsistent with *MDM2/CDK4* amplification; insufficient archived tissue; and loss to follow-up. A total of 46 DDLPS cases met all inclusion criteria. An additional 8 cases of classic MLPS without high-grade transformation were used as reference controls for *DDIT3* IHC comparison. Clinical data were obtained from institutional medical records and the Turkish Ministry of Health National Medical Records System.

ARTICLE IN PRESS

Immunohistochemistry

Immunohistochemical staining was performed on 4-µm-thick sections from representative FFPE tissue blocks. Staining for *MDM2* (mouse monoclonal antibody, clone IF2, 1:100, Thermo Fisher Scientific, Waltham, MA, USA) and *DDIT3* (mouse monoclonal antibody, clone B-3, 1:100, Santa Cruz

ARTICLE IN PRESS

Biotechnology, Dallas, TX, USA) was performed using an automated immunostainer (Leica Bond-Max, Leica Biosystems, Wetzlar, Germany) with a polymer-based detection system (Bond Polymer Refine Detection Kit). Sections were counterstained with haematoxylin. Appropriate positive and negative controls were included in each run.

Nuclear *MDM2* staining in >10% of tumour cells was considered positive, consistent with published criteria[2]. *DDIT3* nuclear staining was evaluated using the semiquantitative H-score system: H-score = Σ(intensity grade × percentage of cells at that grade), yielding a range of 0–300. Staining intensity was graded as: 0, no staining; 1+, weak; 2+, moderate; 3+, strong. At least 500 tumour cells were evaluated per case. Cases with H-score >10 were considered *DDIT3*-positive, in keeping with published *DDIT3* IHC criteria in MLPS[3]. *DDIT3*-positive staining was classified as focal (H-score >10, <50% positive cells) or diffuse (≥50% positive cells). All IHC slides were independently scored by two pathologists (K.E.A. and G.G.); discrepancies were resolved by consensus at a multiheaded microscope.

ARTICLE IN PRESS

Fluorescence In Situ Hybridisation

FISH analysis for *MDM2* amplification was performed on 4-µm FFPE sections using a dual-colour probe set (ZytoLight SPEC *MDM2*/*CEN* 12 Dual Color Probe, ZytoVision, Bremerhaven, Germany). Sections were deparaffinised, protease-pretreated, denatured at 75°C for 5 min, and hybridised overnight at 37°C. At least 100 non-overlapping tumour cell nuclei were scored. *MDM2*

ARTICLE IN PRESS

amplification was defined as *MDM2*/*CEN12* ratio ≥2.0 or ≥10 *MDM2* signals per nucleus in ≥10% of cells. *DDIT3* amplification was not systematically assessed by FISH; this represents a study limitation.

Statistical Analysis

Descriptive statistics were calculated for all variables. Normality was assessed using the Shapiro-Wilk test. Normally distributed continuous variables are presented as mean ± SD; non-normally distributed variables as median with IQR. Categorical variables are reported as frequencies with 95% Wilson score CIs. Between-group comparisons used Fisher's exact test, independent-samples t-test, and Mann–Whitney U test as appropriate. Relative risks (RR) with 95% CIs were calculated using the Koopman asymptotic score method. Effect sizes were quantified using Cramér's V and Cohen's d. Kaplan–Meier survival curves were generated for OS and RFS; groups were compared using the log-rank test. All tests were two-tailed; $p < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA).

ARTICLE IN PRESS

Declarations

Funding

This work was supported by Cukurova University Scientific Research Project Office (Grant No. TSA-2025-17889).

Author Contributions

ARTICLE IN PRESS

Conception and study design: all authors. Data collection and analysis: K.E.A., G.G., S.E., A.M. Manuscript preparation: K.E.A. All authors reviewed and approved the final manuscript.

Competing Interests

The authors declare no competing interests.

Data Availability

Data supporting the findings are available from the corresponding author upon reasonable request; data are not publicly available due to patient privacy restrictions.

Ethics Approval and Consent to Participate

The full name of the Institutional Review Board (IRB) that waived informed consent is: Research Ethics Committee of the Faculty of Medicine of Çukurova University (Protocol No. 155/62, dated 16 May 2025). The study was conducted in accordance with the Declaration of Helsinki, and the waiver was granted owing to the retrospective study design and the use of anonymised data.

ARTICLE IN PRESS

References

1. WHO Classification of Tumours Editorial Board. *WHO Classification of Tumours of Soft Tissue and Bone, 5th edn.* International Agency for Research on Cancer, Lyon (2020).

ARTICLE IN PRESS

2. Thway, K. Well-differentiated liposarcoma and dedifferentiated liposarcoma: an updated review. *Semin. Diagn. Pathol.* 36, 112–121 (2019). https://doi.org/10.1053/j.semdp.2019.02.006

3. Scapa, J. V. et al. *DDIT3* immunohistochemistry is a useful tool for the diagnosis of myxoid liposarcoma. *Am. J. Surg. Pathol.* 45, 230–239 (2021). https://doi.org/10.1097/PAS.0000000000001564

4. Ron, D. & Habener, J. F. *CHOP*, a novel developmentally regulated nuclear protein that dimerizes with transcription factors C/EBP and LAP and functions as a dominant-negative inhibitor of gene transcription. *Genes Dev.* 6, 439–453 (1992). https://doi.org/10.1101/gad.6.3.439

5. Mantilla, J. G., Ricciotti, R. W., Chen, E. Y. et al. Amplification of DNA damage-inducible transcript 3 (*DDIT3*) is associated with myxoid liposarcoma-like morphology and homologous lipoblastic differentiation in dedifferentiated liposarcoma. *Mod. Pathol.* 32, 585–592 (2019). https://doi.org/10.1038/s41379-018-0171-y

6. Kuczkiewicz-Siemion, O. et al. The utility of fluorescence in situ hybridization (FISH) in determining DNA damage-inducible transcript 3 (*DDIT3*) amplification in dedifferentiated liposarcomas—an important diagnostic pitfall. *Pathol. Res. Pract.* 225, 153555 (2021). https://doi.org/10.1016/j.prp.2021.153555

7. Zhang, B., Li, H., Cao, Z. & Cao, H. Dedifferentiated liposarcoma exhibiting myxoid liposarcoma-like morphology with *DDIT3* co-

ARTICLE IN PRESS

amplification and *STAT6* nuclear expression. *Pathol. Res. Pract.* 272, 156086 (2025). https://doi.org/10.1016/j.prp.2025.156086

8. Campbell, G. S., Lawrence, T. J., Porter, S. E. & Rezeanu, L. Primary dedifferentiated liposarcoma of the axilla arising in a mixed, well-differentiated and myxoid liposarcoma. *J. Radiol. Case Rep.* 6, 9–16 (2012). https://doi.org/10.3941/jrcr.v6i1.867

9. Raut, C. P. et al. Predicting survival in primary retroperitoneal sarcoma. *Ann. Surg. Oncol.* 28, 6093–6100 (2021). https://doi.org/10.1245/s10434-021-09965-3

10. Mentzel, T., Palmedo, G., Hantschke, M., Woziwodzki, J. & Beck, C. Mixed-type liposarcoma: clinicopathological, immunohistochemical, and molecular analysis of a case arising in deep soft tissues of the lower extremity. *Virchows Arch.* 453, 197–201 (2008). https://doi.org/10.1007/s00428-008-0628-2

11. Uchihashi, K. et al. Myxoid liposarcoma with pleomorphic cells: report of two cases with molecular confirmation of *FUS* gene rearrangements. *Pathol. Res. Pract.* 212, 1067–1070 (2016). https://doi.org/10.1016/j.prp.2016.08.001

12. Suzuki, K. et al. Variation in myxoid liposarcoma: clinicopathological examination of four cases with detectable *TLS*::*CHOP* or *EWS*::*CHOP* fusion transcripts. *Oncol. Lett.* 3, 293–296 (2012). https://doi.org/10.3892/ol.2011.480

ARTICLE IN PRESS

ARTICLE IN PRESS

13. Mentzel, T. & Fletcher, C. D. Dedifferentiated myxoid liposarcoma: a clinicopathological study suggesting a closer relationship between myxoid and well-differentiated liposarcoma. *Histopathology* 30, 457–463 (1997). https://doi.org/10.1046/j.1365-2559.1997.5510791.x

14. Murshed, K. A., Abo Samra, H. & Ammar, A. Well-differentiated liposarcoma of the hypopharynx exhibiting myxoid liposarcoma-like morphology with *MDM2* and *DDIT3* co-amplification. *Head Neck Pathol.* 16, 288–293 (2022). https://doi.org/10.1007/s12105-021-01341-5

Table 1. Association between *DDIT3* expression and clinical outcomes in 46 DDLPS cases with prominent myxoid features.

Outcome	*DDIT3*-positive (n = 11)	*DDIT3*-negative (n = 35)	p value
Local recurrence*	3/8 (37.5%)	11/25 (44.0%)	0.337
RR (95% CI)	—	—	0.85 (0.29–2.51)
Distant metastasis†	1/6 (16.7%)	5/23 (21.7%)	0.825
RR (95% CI)	—	—	0.77 (0.11–5.50)
Death (all-cause)	5/11 (45.5%)	11/35 (31.4%)	0.805
RR (95% CI)	—	—	1.45 (0.62–3.37)

*Recurrence status available in 33 cases (*DDIT3*-positive n = 8; *DDIT3*-negative n = 25). †Metastasis status available in 29 cases (*DDIT3*-positive n = 6; *DDIT3*-negative n = 23). RR, relative risk; CI, confidence interval. p values from Fisher's exact test.

Figure Legends

ARTICLE IN PRESS

Fig. 1. Heterogeneous histological appearance with MLPS-like myxoid areas (left), dedifferentiated component (centre), and geographic necrosis (right) (haematoxylin and eosin, ×40).

Fig. 2. Fibromyxoid areas of hypocellular DDLPS (haematoxylin and eosin, ×100).

Fig. 3. Myxoid stroma-rich areas with branching thin-walled capillaries and cellular and hypocellular zones (haematoxylin and eosin, ×100).

Fig. 4. Geographic necrosis adjacent to myxoid stroma-rich hypocellular areas (haematoxylin and eosin, ×40).

Fig. 5. Strong diffuse nuclear *MDM2* immunoreactivity in DDLPS (immunohistochemistry, ×200).

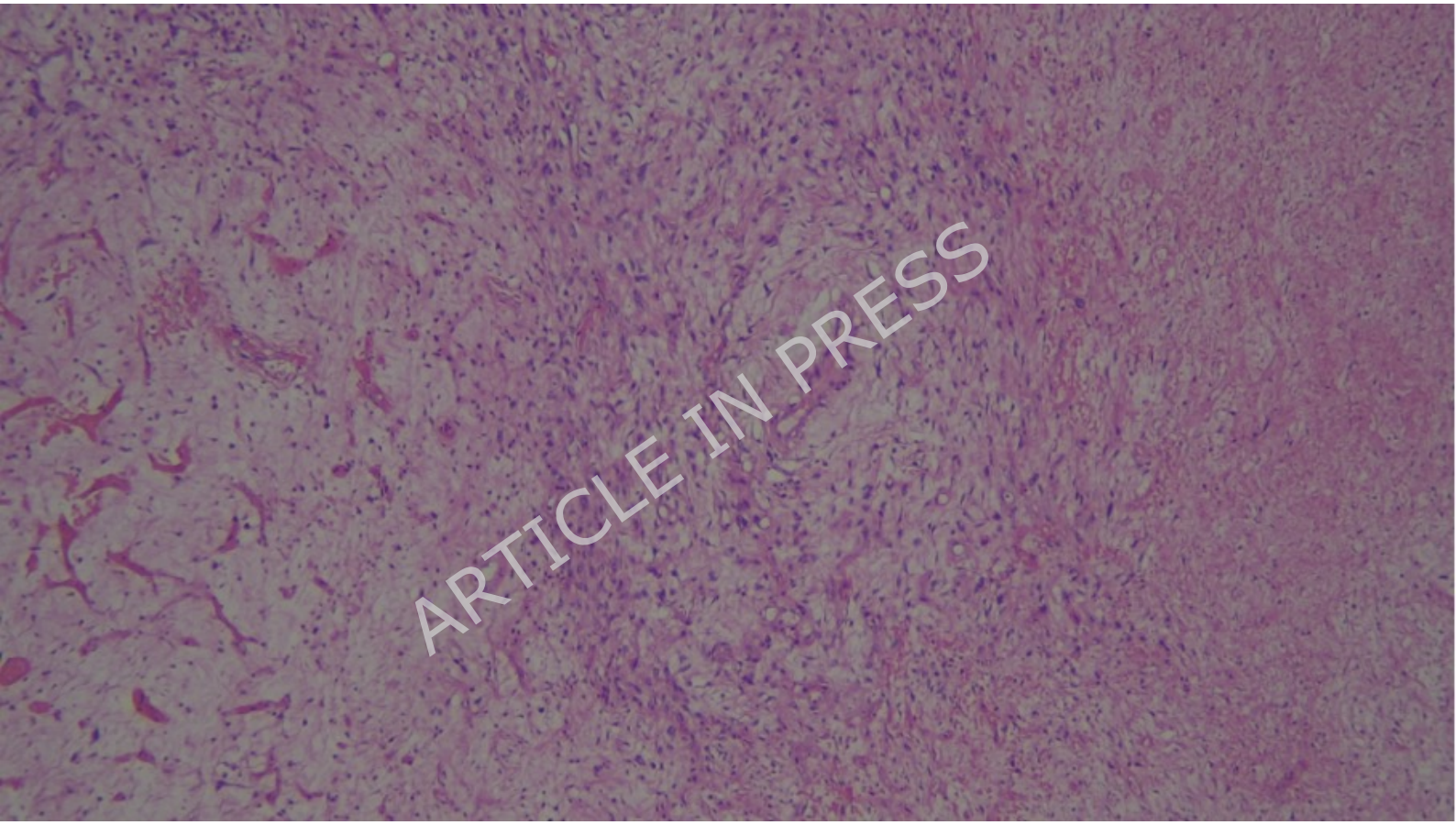
Fig. 6. Weak (1+) focal nuclear *DDIT3* immunoreactivity in DDLPS with myxoid features (immunohistochemistry, ×200).

Fig. 7. Moderate (2+) focal nuclear *DDIT3* immunoreactivity in DDLPS with myxoid features (immunohistochemistry, ×200).

Fig. 8. *MDM2* amplification demonstrated by FISH. Multiple red signals (*MDM2* locus) relative to green signals (chromosome 12 centromere) indicate high-level *MDM2* amplification (×1000).

ARTICLE IN PRESS

ARTICLE IN PRESS



ARTICLE IN PRESS



ARTICLE IN PRESS



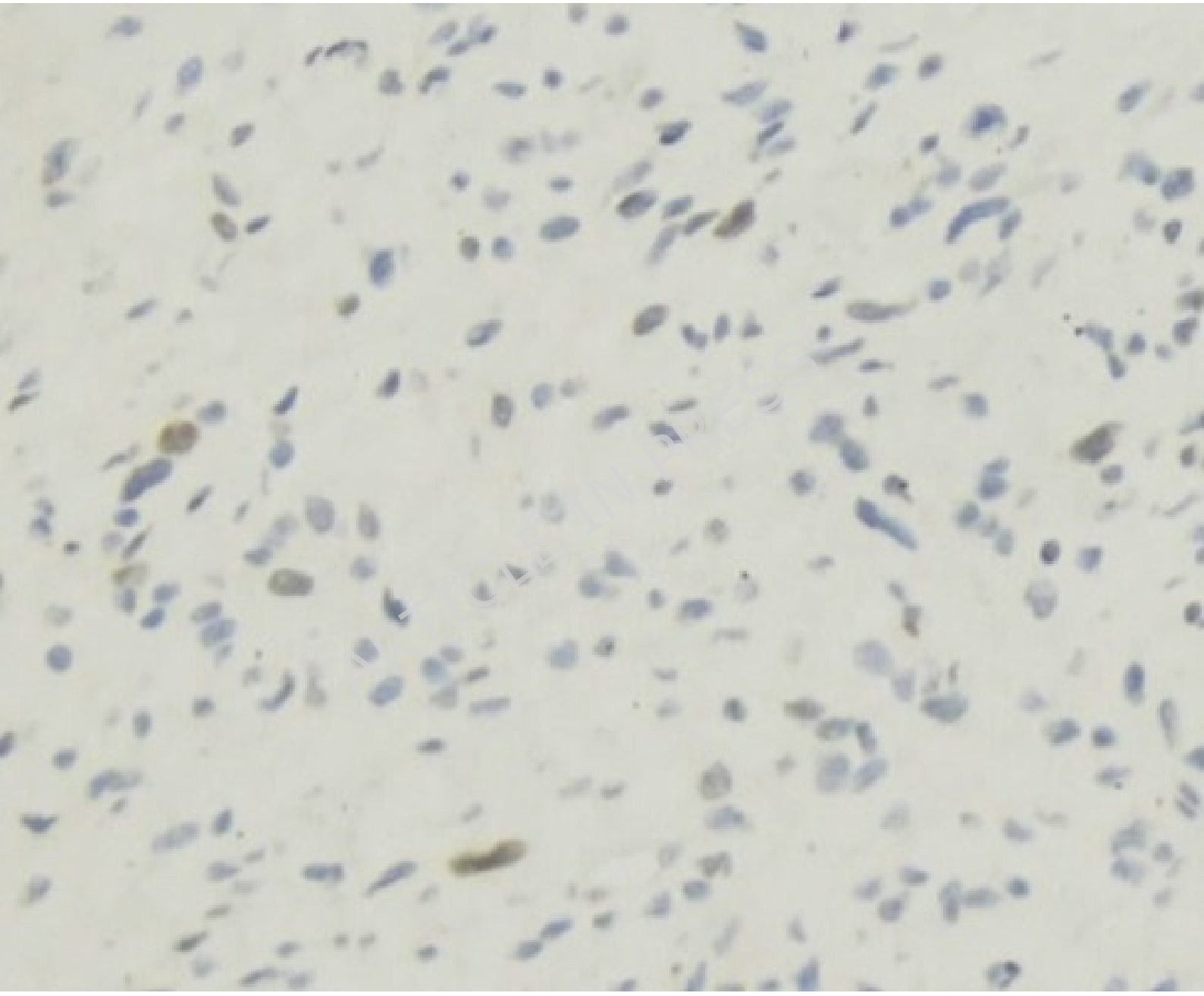
ARTICLE IN PRESS



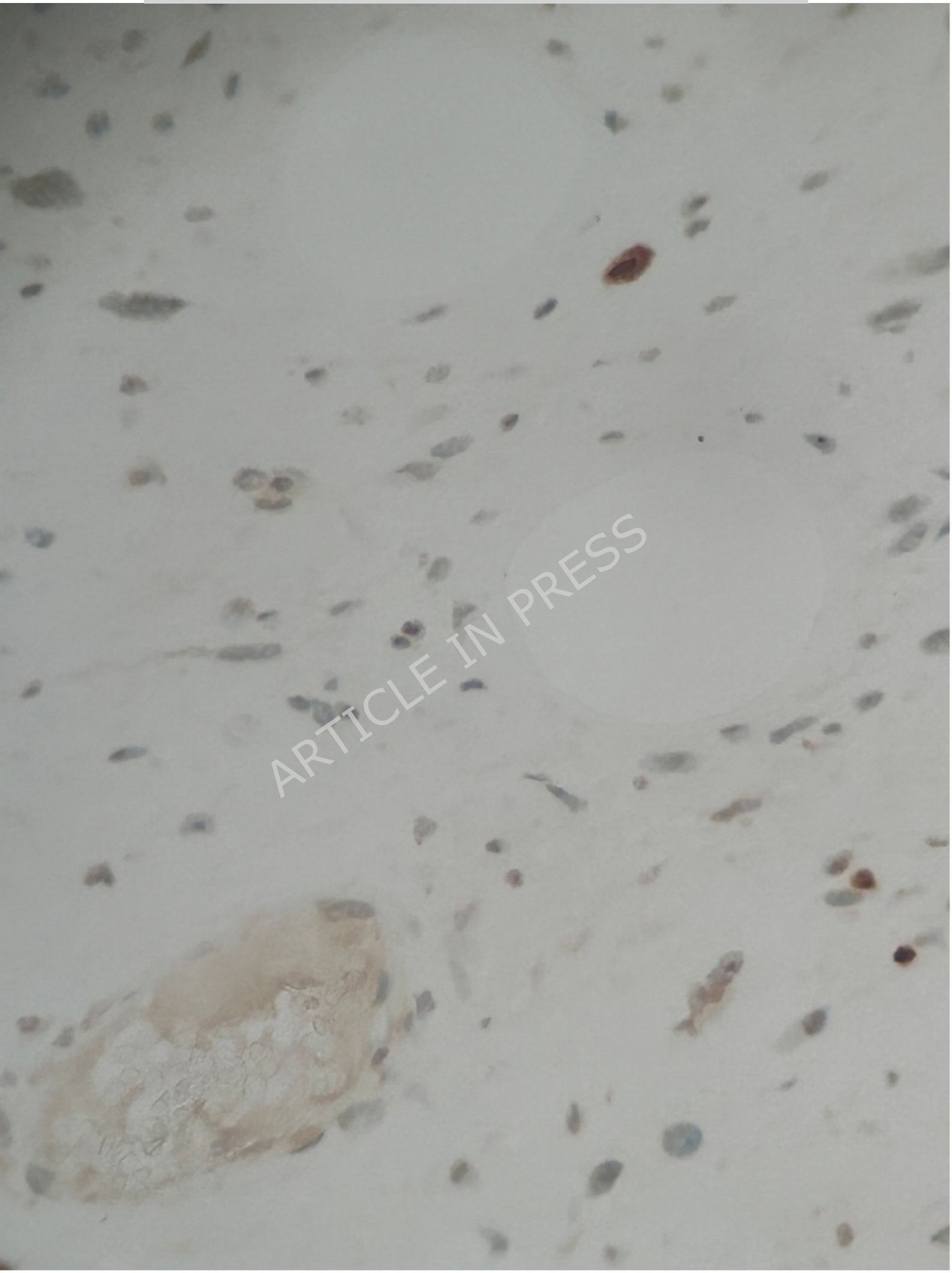
ARTICLE IN PRESS



ARTICLE IN PRESS

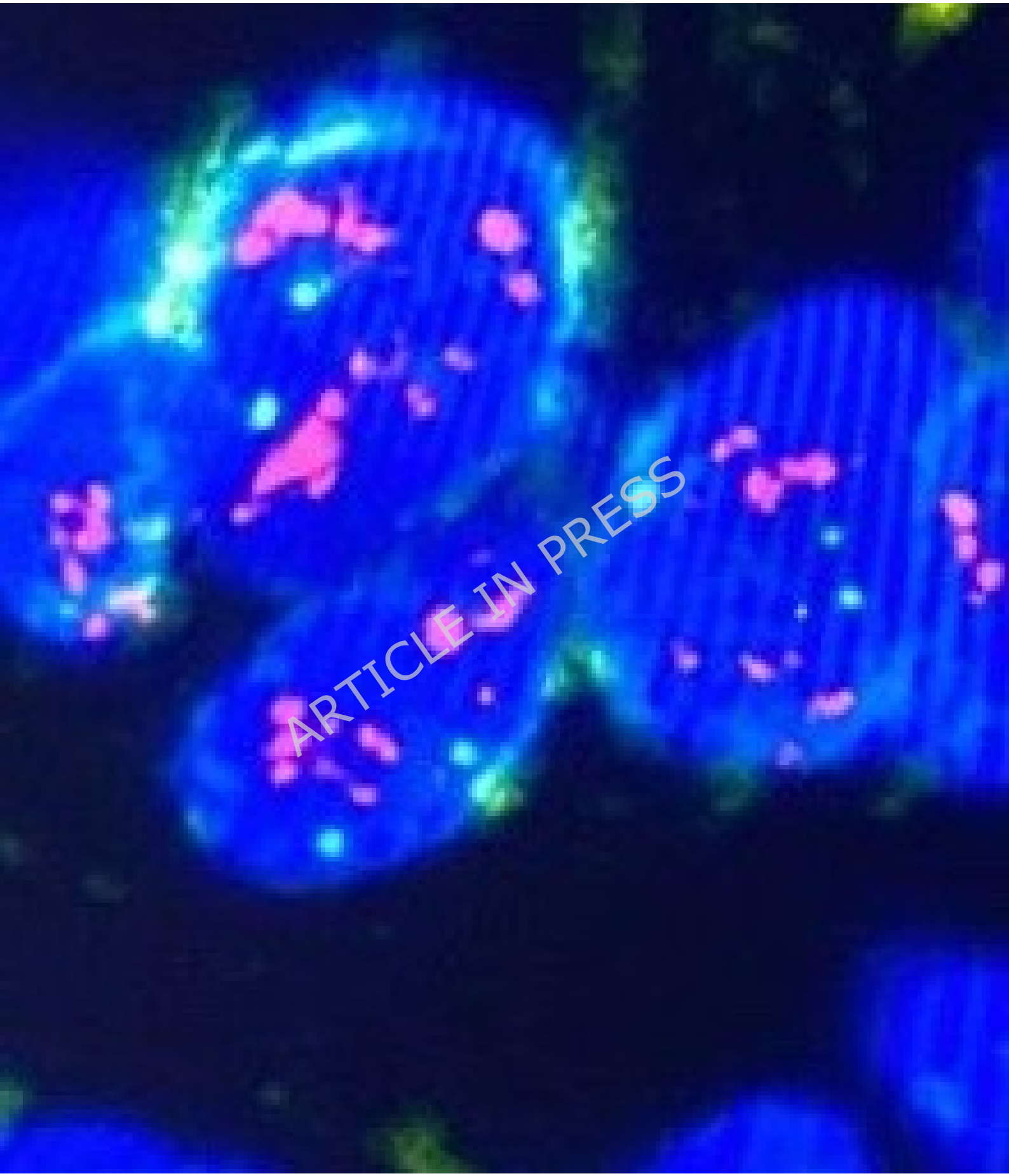


ARTICLE IN PRESS



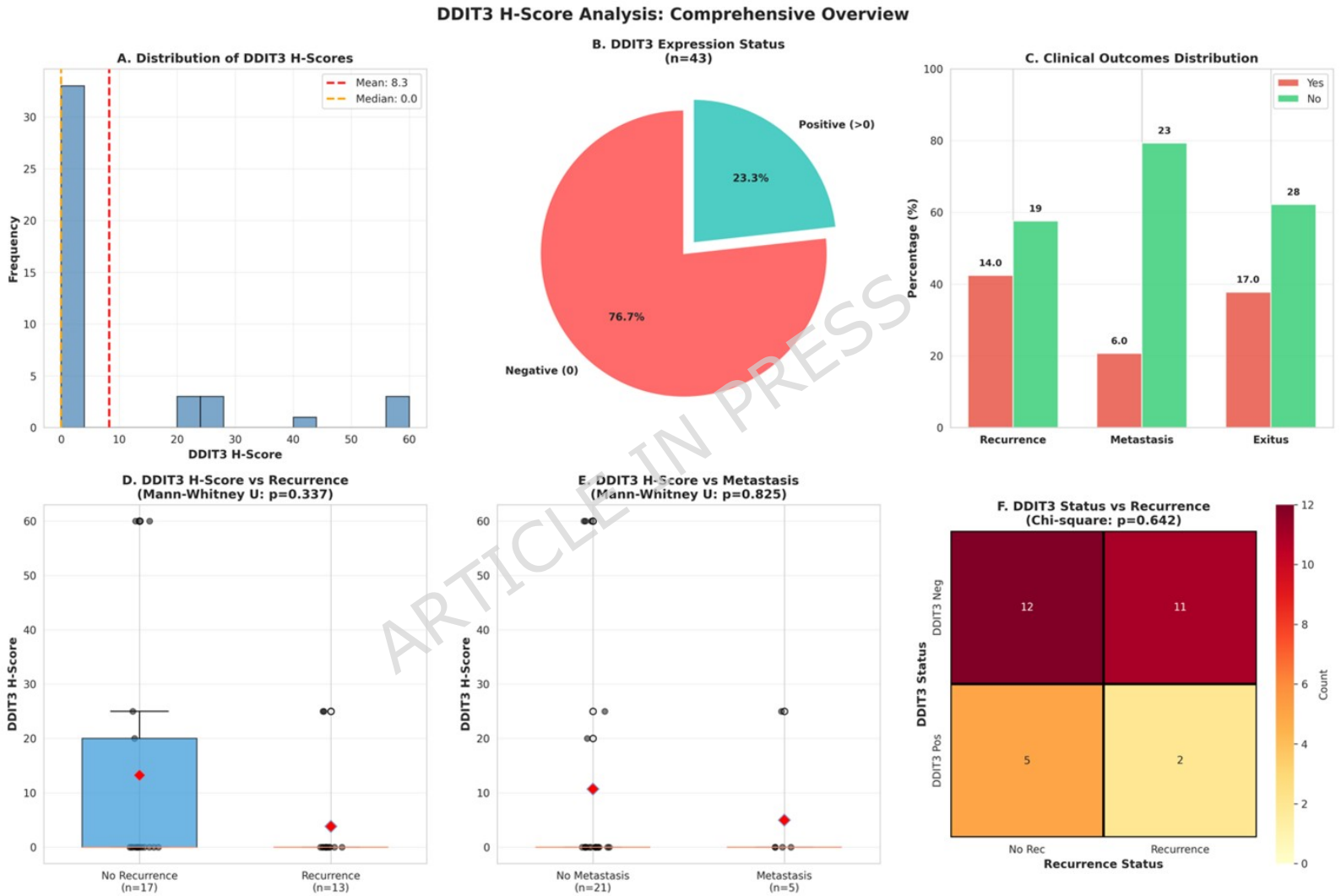
ARTICLE IN PRESS

ARTICLE IN PRESS



ARTICLE IN PRESS

ARTICLE IN PRESS



ARTICLE IN PRESS