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ORIGINAL ARTICLE

Systemic, primary cutaneous, and breast implant-associated ALK-negative anaplastic large-cell lymphomas present similar biologic features despite distinct clinical behavior

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Abstract

Despite distinct clinical presentation and outcome, systemic, primary cutaneous, and breast implant-associated anaplastic large cell lymphomas (S-, PC-, BI-ALCL) ALK-negative (ALK−) show similar histopathological features including the presence of the "hallmark" cells with horseshoe-shaped nuclei and CD30 protein expression. The purpose was to better characterize these three entities using immunohistochemistry and FISH (Fluorescent in situ hybridization) to identify biomarkers differently expressed and that might be involved in their pathogenesis. Twenty-two S-ALCL ALK−, 13 PC-ALCL, and 2 BI-ALCL were included. Cases were tested for P53, P63, MUM1, MYC, GATA3, p-STAT3, PD1, and PDL1 protein expression and *DUP22, TP53*, *TP63*, *MYC*, and *PDL1* chromosomal aberrations. As expected, S-ALCL ALK− patients had adverse outcome compare to PC and BI-ALCL. No difference was observed between the three groups concerning protein expression except for MUM1 that was significantly more frequently expressed in S-ALCL ALK− compared to PC-ALCL. In particular, constitutive activation of the STAT3 pathway and PDL1/PD1 immune-checkpoint expression was present in the three entities. *TP53* deletion and *PDL1* gene amplification were the commonest cytogenetic alterations and were present in the three entities. None of the studied biological parameters was associated with prognosis. Despite distinct clinical behavior, S-ALCL ALK−, PC-ALCL, and BI-ALCL share similar biological features. Larger series should be investigated with the current approach to determine more precisely the activity and the prognostic value of these biomarkers and pathways in each group.

Keywords Anaplastic lymphoma · Cutaneous · Breast implant · PDL1 · STAT3

Introduction

Anaplastic large cell lymphomas ALK-negative (ALCL ALK−) comprise a group of CD30-positive non-Hodgkin lymphomas of T cell-origin that share common morphologic and phenotypic characteristics including the presence of the "hallmark" cells with horseshoe-shaped nuclei and strong and diffuse expression of CD30. The World Health Organization (WHO) 2016 classification of hematopoietic and lymphoid tissue recognizes three entities: systemic anaplastic large-cell lymphomas ALK-negative (S-ALCL ALK−), primary cutaneous anaplastic large-cell lymphoma (PC-ALCL), and the recently recognized breast implant-associated anaplastic large-cell lymphoma (BI-ALCL). Despite overlapping histopathologic features, these three entities differ in their clinical presentation and outcome. S-ALCL ALK−represents a subset of peripheral T cell lymphomas (PTCLs) with unfavorable prognosis. S-ALCL ALK− is genetically and clinically heterogeneous, with a subset showing

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DUSP22 rearrangement and more favorable prognosis and a subset harboring *TP63* translocation and associated with an aggressive outcome. PC-ALCL is a CD30+ lymphoproliferative disorder (LPD) of the skin with a relatively indolent course. Classically, it presents as persisting solitary, grouped, or multifocal nodules that may spontaneously regress although up to half of cases can recur. BI-ALCL is a malignancy of T cells arising in the capsule of breast implants. It presents either as a recurrent effusion or a tumor mass. Both clinical presentations are associated with the presence of the "hallmark" cells, either confined to the capsule or infiltrating the capsule and/or adjacent tissues, and strong expression of CD30. Prognosis is excellent although the presentation as a tumor mass is more aggressive.

In the present work, we choose to investigate a series of anaplastic large-cell lymphoma (ALCL) including S-ALCL ALK−, PC-ALCL, and BI-ALCL with the aim to identify biomarkers and genes differentially and specifically involved in the pathogenesis of these three entities. We focused on molecular abnormalities described in at least one of these entities but poorly investigated in the others including P53, P63, MUM1, MYC and GATA3 protein expression, the STAT3 pathway, and *DUP22*, *TP53*, *TP63*, and *MYC* chromosomal aberrations. As the three entities are localized in particular topography, we also raised the question of the role of the micro-environment. Thus, we investigated the PD1/PDL1 immune pathway with immunohistochemistry and FISH (fluorescent in situ hybridization) analysis.

Materials and methods

Patients

We reviewed 37 ALCL ALK-negative diagnosed between 2004 and 2018 in the Department of Pathology of the Centre-Hospitalo-Universitaire (CHU) of Montpellier, France. Cases were distributed as followed: 22 S-ALCL ALK−, 13 PC-ALCL, and 2 BI-ALCL. The following clinical data were collected: age at diagnosis, sex, clinical presentation, initial therapy, relapse, progression, death, number of lines of treatment, and clinical status at last follow-up. In each case, except one, Ann Arbor staging could be determined.

Histological and immunohistochemical analysis

All cases were reviewed by four pathologists (VS, AG, LD, and VCM) without the knowledge of the ALCL entity. Institutional ethical approval was obtained in compliance with the Helsinki agreement. The diagnosis of S-ALCL ALK−, PC-ALCL, and BI-ALCL was made on hematoxylin and eosin (H&E) and was based on the WHO 2016 classification of hematopoietic and lymphoid tissue. For immunohistochemical examination, 3-μm thick tissue sections from the formalin-fixed paraffin-embedded (FFPE) blocks were subjected to antigen retrieval and immunostained on a Ventana Benchmark XT autostainer (Ventana Tucson, AZ, USA). The following antibodies were used after appropriate antigen retrieval according to the manufacturer's instructions: CD3 (clone 2GV6,Ventana, PREP Kit Ventana), CD2 (clone MRQ-11 Ventana, PREP Kit Ventana), CD5 (clone 4C7, DAKO, Denmark A/S 1:100), CD7 (clone CBC-37, DAKO, 1:25), CD4 (clone SP35, Ventana, PREP Kit Ventana), CD8 (clone SP57, Ventana, PREP Kit Ventana), and TCRβF1 (clone 8A3, Thermo scientific, Courtaboeuf, France,1:50). CD30 (clone Ber-H2, Ventana, PREP Kit Ventana), ALK1 (clone ALK1, DAKO, 1:50), CD15 (clone MMA, Ventana, PREP Kit Ventana), Granzyme B (clone GR B-7, DAKO, 1:25), TIA1 (clone 2G9, Immunotech, Marseille, France, 1:100), Perforin (clone 5B10, Menarini, California USA 1:20, CD20 (clone L26, Dako, Denmark A/S, 1:300), PAX5 (clone DAK-PAX5, DAKO, 1:25), MUM1 (clone MUM1p, Dako, 1:50), P63 (clone 4A4, Ventana, PREP Kit Ventana), P53 (clone DO7, Ventana, PREP Kit Ventana), MYC (clone EP 121, Epitomics, Burlingame, CA, USA 1:100), GATA3 (clone L50–823 Biocare medical, California USA 1:50), CD68 (clone KP1, DAKO, 1:400), PD1 (clone NAT105, Abcam, Paris, France 1:100), PDL1 (clone E1L3M, cell signaling, Leiden The Netherlands, 1:200), and phospho-STAT3 (p-STAT3, clone M9C6, cell signaling, 1:50). Association with Epstein-Barr virus (EBV) was examined by in-situ hybridization (ISH) using EBV-encoded early nuclear RNA (EBER). For MUM1, MYC, GATA3, P63, and P53, we evaluated the percentage of positive neoplastic cells as previously described. MUM1, MYC, GATA3, P63, and P53 protein expression was considered as positive if nuclear staining was observed respectively in at least 30%, 40% [1], 10% [2], 30% [3], and 10% [4] of the neoplastic cells. For evaluation of PD1 and PDL1, slides were scanned at high magnification with a × 20 objective and digitized on the iScan Coreo scanner (Ventana, Roche, France) to generate an image of the whole slide. Images were obtained for CD30, CD3, CD68, PD1, and PDL1. Images were then compared on the same screen to evaluate PDL1 expression specifically in CD30-positive tumor cells and CD68-positive macrophages and PD1 expression specifically in CD3-positive tumor infiltrating lymphocytes (TILs). For PD-L1, both a membranous immunostaining signal on the cell surface and cytoplasmic staining within cells were recorded [5]. PD1 positive staining was evaluated in the membrane and cytoplasm of TILs [6]. The percentage of positive cells was evaluated for both antibodies, and samples showing staining of any intensity in 1% or more of the respective cells were considered positive [5].

Interphase fluorescence in situ hybridization (FISH)

Interphase FISH was performed on 3-μm thick tissue sections using split signal FISH DNA probes for *DUSP22-IRF4*

(Zytolight SPEC IRF4, DUSP22 dual color break apart probe, 6p25.3, Zytovision), *MYC*/8q24 (probe Y5410; DAKO A/S), *PDL1* (PDL1, CD274 break apart probe, 9p24.1, Empire Genomics), *TP63* (*TP63* break apart probe, 3q28, Empire Genomics), and deletion DNA probes for *TP53* (*TP53* deletion probe, 17p13.1, Cytocell) according to the manufacturer's instructions. Digital images were captured with a Metafer Slide Scanning Platform using a Leica Axioplan fluorescence microscope (Zeiss Axio Imager M1) equipped with a charge-coupled device (CCD) camera coupled to and driven by ISIS software (MetaSystem, FISH Imaging System, Germany). At least, 100 nuclei were evaluated independently by three scorers (AG, AT, VS). Cases were considered positive when more than 15% of the cells displayed abnormalities on the FFPE tissue sections.

DNA extraction and PCR analysis

Rearrangements of T cell receptor (TCR) were studied on DNA extracted from FFPE tissue sections using Qiamp DNA Blood mini kit® (QIAGEN, France) according to the manufacturer's instructions. The clonal status of each sample was determined as previously descried by the BIOMED-2 group [7].

Statistical analysis

Survival was determined from time of diagnosis until time of death or last follow-up. Survival curves were constructed by the Kaplan–Meier method. Survival distributions were compared with the log-rank test. Statistical significance was set at a p value of 0.05. Analyses were performed using SAS (statistical analysis system) 9 software.

Results

Clinical features and follow-up

The clinical characteristics of the 37 patients are depicted in Table 1. Median of follow-up was 15 months (12–108). As expected, patients with S-ALCL ALK− presented more aggressive disease with poor outcome compared to PC-ALCL and BI-ALCL. Most patients in this subgroup had high-stage disease at initial diagnosis (Ann Arbor stage III or IV) whereas all patients with PC-ALCL and BI-ALCL were stage IE. Compared to PC-ALCL and BI-ALCL, S-ALCL ALK− were more frequently treated with systemic chemotherapy (77.8% versus 30.8%, $p = 0.013$), underwent mostly at least two lines of treatment (44.4% versus 0%, $p = 0.01$), less frequently harbored complete remission at last follow-up (36.4% versus 92.9%, $p = 0.0013$), and usually died of disease (50% versus 0%, $p = 0.002$). Concerning the two patients with BI-ALCL, the two previously described distinct clinical presentations were observed [8]. One patient presented with seroma without tumor mass. She underwent capsulectomy with implant removal. The second patient had effusion with palpable breast tumor mass. She had implant removal with additional treatment including polychimiotherapy. Both patients had no relapse and were in complete remission at last follow-up.

Histopathologic features

Tumor cells in all cases were large and pleomorphic with irregular nuclei (Fig. 1a). Furthermore, in addition, the "hallmark" cells with eccentric horseshoe-shaped nuclei were observed in all cases in variable number. Phenotypic analyses are summarized in Table 1. All cases were strongly positive for CD30 (Fig. 1b) and consistently negative for ALK and EBV. All cases had incomplete T cell phenotype with extensive T cell antigen loss including CD2, CD3, CD5, and CD7. Ten cases (10/37, 27%) showed these four antigens loss including 4 S-ALCL ALK−, 4 PC-ALCL, and the 2 BI-ALCL. Most cases expressed at least one cytotoxic enzyme including TIA1, Granzyme B, and Perforin whereas 12 cases were negative for the three, including 9 S-ALCL and 3 PC-ALCL. The majority of cases were positive for CD4. Interestingly, one case (S-ALCL ALK−) co-expressed CD4 and CD8 whereas nine cases (5 S-ALCL ALK− and 4 PC-ALCL) were negative for both CD4 and CD8. When looking at the protein of interest (MUM1, P63, P53, MYC, GATA3, and p-STAT3), MUM1 was significantly more frequently expressed in S-ALCL ALK− compared to PC-ALCL (90.9% versus 46.2%, $p = 0.006$). Only two cases that were S-ALCL ALK− expressed P63 (Fig. 1c) and both patients died of disease. No significant difference was observed between the three groups concerning P53, MYC, and GATA3 protein expression and respectively 43.2% (16/37), 27% (10/37), and 8.6% (3/35) of cases in the global cohort expressed these protein. For p-STAT3 evaluation, only nuclear expression in tumor cells was taken into account (Fig. 1d). Although the difference was not significant ($p = 0.073$), p-STAT3 positive expression tended to be associated with PC-ALCL (84.6%) and BI-ALCL (100%) compared to S-ALCL ALK− (54.5%).

FISH and TCR gene rearrangements results

Cytogenetic features are presented Table 1. FISH testing with the *MYC* probe was negative in all contributive cases tested. The presence of a *DUSP22/IRF4* translocation was observed both in S-ALCL ALK− (20%, 4/20) and PC-ALCL (8.3%, 1/12) (Fig. 2a). The 4 S-ALCL ALK− had strong expression of MUM1 protein whereas the PC-ALCL was negative. Moreover, 3/5 cases had no expression of cytotoxic enzymes (TIA1, Granzyme B, and Perforin) whereas the two others (one S-ALCL ALK− and the PC-ALCL) expressed at least

Table 1 Clinicopathologic features

	ALCL ALK− $N = 37$	S- ALCL ALK− $N = 22$	PC-ALCL $N = 13$	BI-ALCL $N = 2$
Clinic				
Patients				
Mean age, years (range)	58 (7–91)	59 (9–91)	56 (7–80)	65 (55–75)
Male, *n* (%)	24/37 (64.9)	14/22 (63.6)	10/13 (76.9)	0/2 (0)
Female, *n* (%)	13/37 (35.1)	8/22 (36.4)	3/13 (23.1)	2/2 (100)
Ann Arbor staging				
I, *n* (%)	1/35 (2.9)	1/20 (5)	0/13 (0)	0/2 (0)
II, *n* (%)	2/35 (5.7)	2/20 (10)	0/13 (0)	0/2 (0)
III, *n* (%)	4/35 (11.4)	4/20 (20)	0/13 (0)	0/2 (0)
IV, *n* (%)	12/35 (34.3)	12/20 (60)	0/13 (0)	0/2 (0)
IE, *n* (%)	16/35 (45.7)	1/20 (5)	13/13 (100)	2/2 (100)
Initial management				
Surgical excision, *n* (%)	12/29 (41.4)	4/18 (22.2)	8/11 (72.7)	NA
Local radiation, *n* (%)	3/29 (10.3)	0/18 (0)	3/11 (27.3)	NA
Chemotherapy, *n* (%)	17/29 (58.6)	14/18 (77.8)	3/11 (27.3)	NA
Implant removal, *n* (%)*	1/2 (50)	NA	NA	1/2 (50)
Implant removal + chemotherapy, *n* (%)*	1/2 (50)	NA	NA	1/2 (50)
Lines of treatment**				
0, *n* (%)	13/31 (41.9)	4/18 (22.2)	8/11 (72,7)	1/2 (50)
1, *n* (%)	10/31 (32.3)	6/18 (33,3)	3/11 (27.3)	1/2 (50)
≥ 2, *n* (%)	8/31 (25.8)	8/18 (44.4)	0/11 (0)	0/2 (0)
Outcome				
CR at last follow-up, *n* (%)	21/26 (58.3)	8/22 (36.4)	11/12 (91.7)	2/2 (100)
AWD at last follow-up, *n* (%)	4/36 (11.1)	3/22 (13.6)	1/12 (8.3)	0/2 (0)
Relapse, *n* (%)	9/36 (25)	6/22 (27.3)	3/12 (25)	0/2 (0)
DOD, *n* (%)	11/36 (30.6)	11/22 (50)	0/12 (0)	0/2 (0)
Phenotype				
CD2 positive, *n* (%)	23/37 (61.2)	15/22 (68.2)	8/13 (61.5)	0/2 (0)
CD3 positive, *n* (%)	13/37 (35.1)	8/22 (36.4)	5/13 (38.5)	0/2 (0)
CD5 positive, *n* (%)	9/37 (24.3)	4/22 (18.2)	5/13 (38.5)	0/2 (0)
CD7 positive, *n* (%)	2/35 (5.7)	1/20 (5)	1/13 (7.7)	0/2 (0)
CD30 positive, *n* (%)	37/37 (100)	22/22 (100)	13/13 (100)	2/2 (100)
TCRβF1 positive, *n* (%)	4/37 (10.8)	2/22 (9.1)	2/13 (15.4)	0/2 (0)
CD4 positive, *n* (%)	28/37 (75.7)	17/22 (77.3)	9/13 (69.2)	2/2 (100)
CD8 positive, *n* (%)	4/37 (10.8)	3/22 (13.6)	1/13 (7.7)	0/2 (0)
CD15 positive, *n* (%)	6/37 (16.2)	4/22 (18.2)	1/13 (7.7)	1/2 (50)
TIA1 positive, *n* (%)	19/36 (52.8)	10/21 47.6)	9/13 (69.2)	1/2 (50)
Granzyme B positive, *n* (%)	22/36 (61.1)	11/21 (52.4)	10/13 (76.9)	1/2 (50)
Perforine positive, *n* (%)	18/37 (48.7)	11/22 (50)	6/13 (46.2)	1/2 (50)
CD20 positive, *n* (%)	1/37 (2.7)	0/22 (0)	1/13 (7.7)	0/2 (0)
PAX5 positive, *n* (%)	1/37 (2.7)	1/22 (4.5)	0/13 (0)	0/2 (0)
ALK1 positive, *n* (%)	0/37 (0)	0/22 (0)	0/13 (0)	0/2 (0)
MUM1 positive, *n* (%)	28/37 (75.7)	20/22 (90.9)	6/13 (46.2)	2/2 (100)
P63 positive, *n* (%)	2/37 (5.4)	2/22 (9.1)	0/13 (0)	0/2 (0)
P53 positive, *n* (%)	16/37 (43.2)	11/22 (50)	5/13 (38.5)	0/2 (0)
MYC positive, *n* (%)	10/37 (27)	6/22 (27.3)	3/13 (23.1)	1/2 (50)
GATA3 positive, *n* (%)	3/35 (8.6)	2/21 (9.5)	0/12 (0)	1/2 (50)
p-STAT3 positive, *n* (%)	25/37 (67.6)	12/22 (54.5)	11/13 (84.6)	2/2 (100)
EBER positive, *n* (%)	0/37 (0)	0/22 (0)	0/13 (0)	0/2 (0)
Cytogenetic				
MYC translocation positive, *n* (%)	0/31 (0)	0/20 (0)	0/9 (0)	0/2 (0)
DUSP22/IRF4 translocation positive, *n* (%)	5/33 (15.2)	4/20 (20)	1/12 (8.3)	0/1 (0)
TP53 deletion positive, *n* (%)	8/27 (29.6)	5/15 (33.3)	2/10 (20)	1/2 (50)
P63 translocation positive, *n* (%)	1/32 (3.1)	1/19 (5.3)	0/11 (0)	0/2 (0)

ALCL ALK−, anaplastic large cell lymphomas ALK-negative; *S-ALCL ALK−*, systemic anaplastic large-cell lymphomas ALK-negative; *PC-ALCL*, primary cutaneous anaplastic large-cell lymphoma; *BI-ALCL*, breast implant-associated anaplastic large-cell lymphoma; *NA*, not applicable; *Concerning only BI-ALCL, **Among patient having systemic treatment by chemotherapy at initial tretament; *CR*, complete remission; *PR*, partial remission; *AWD*, alive with disease; *DOD*, dead of disease

TIA1 and Granzyme B. One case had a *TP63* translocation (1/32) (Fig. 2c). It was one of the S-ALCL ALK− showing strong P63 protein expression. Unfortunately, FISH analysis with TP63 FISH probe was not evaluable in the other case of S-ALCL ALK− with P63 protein expression. Of interest, *TP53* deletion was the most frequent cytogenetic aberration

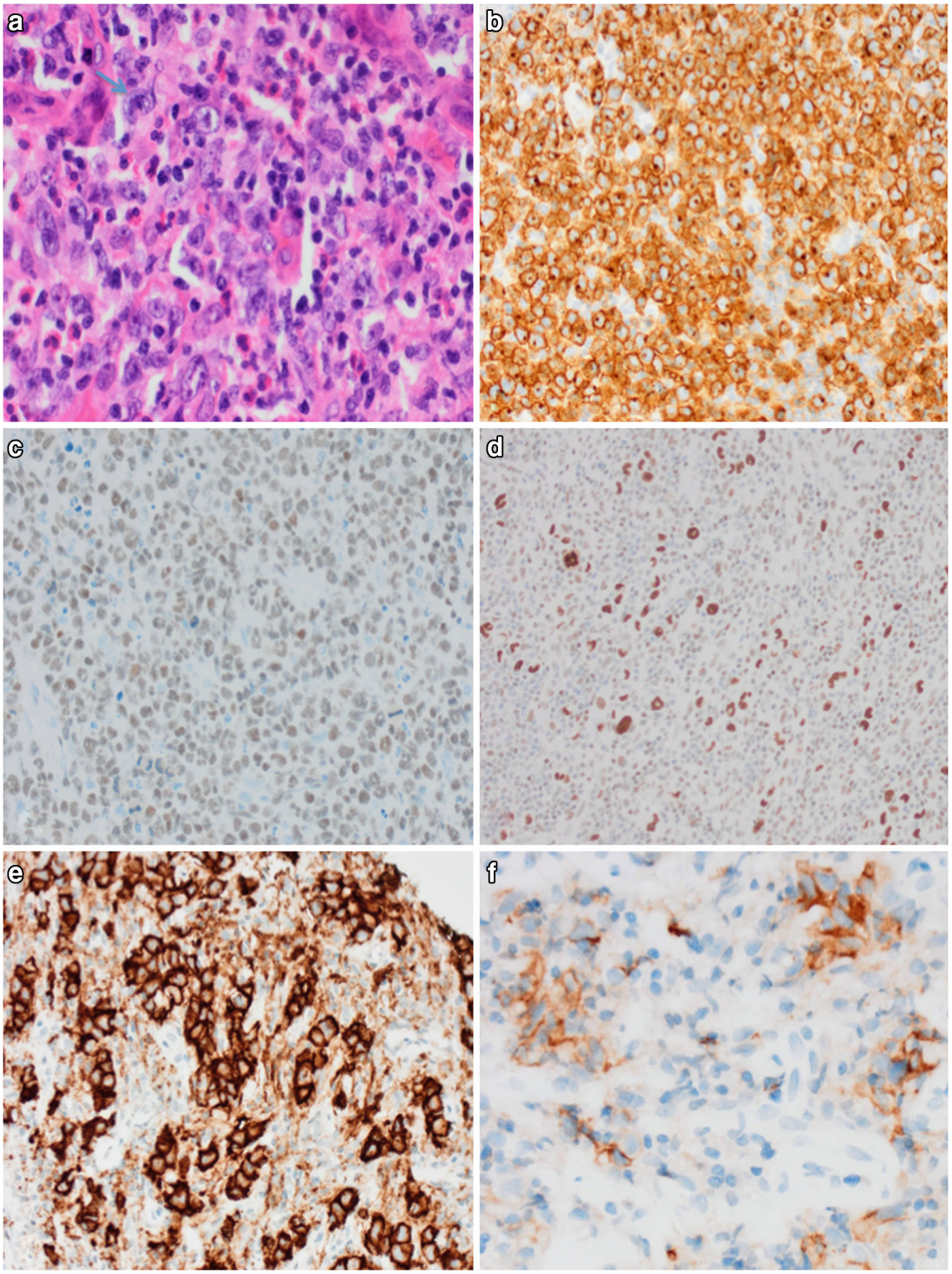

Fig. 1 Histologic and phenotypic features of ALCL ALK. **a**, **e**, **f** A case of PC-ALCL. **a** Hematoxylin and eosin (H&E)-stained tissue sections (× 40), blue arrow showing the "hallmark" cell with eccentric horseshoe-shaped nuclei. **e** PDL1 expression in tumor cells (× 20). **f** PD1 expression in TILs (× 20). **b**, **c**, **d** A case of S-ALCL ALK−. **b** CD30 expression (× 20). **c** P63 expression (× 20). **d** p-STAT3 (× 10)

(29.6%) in the global cohort and was observed in the three entities (Fig. 2d). We did not identify any correlation between the presence of a *TP53* deletion and positive expression of the protein in tumor cells ($p = 0.209$). The 5 S-ALCL ALK− patients with *TP53* deletion had stage III–IV disease and three of them (3/5, 60%) died of disease whereas the patients with PC-

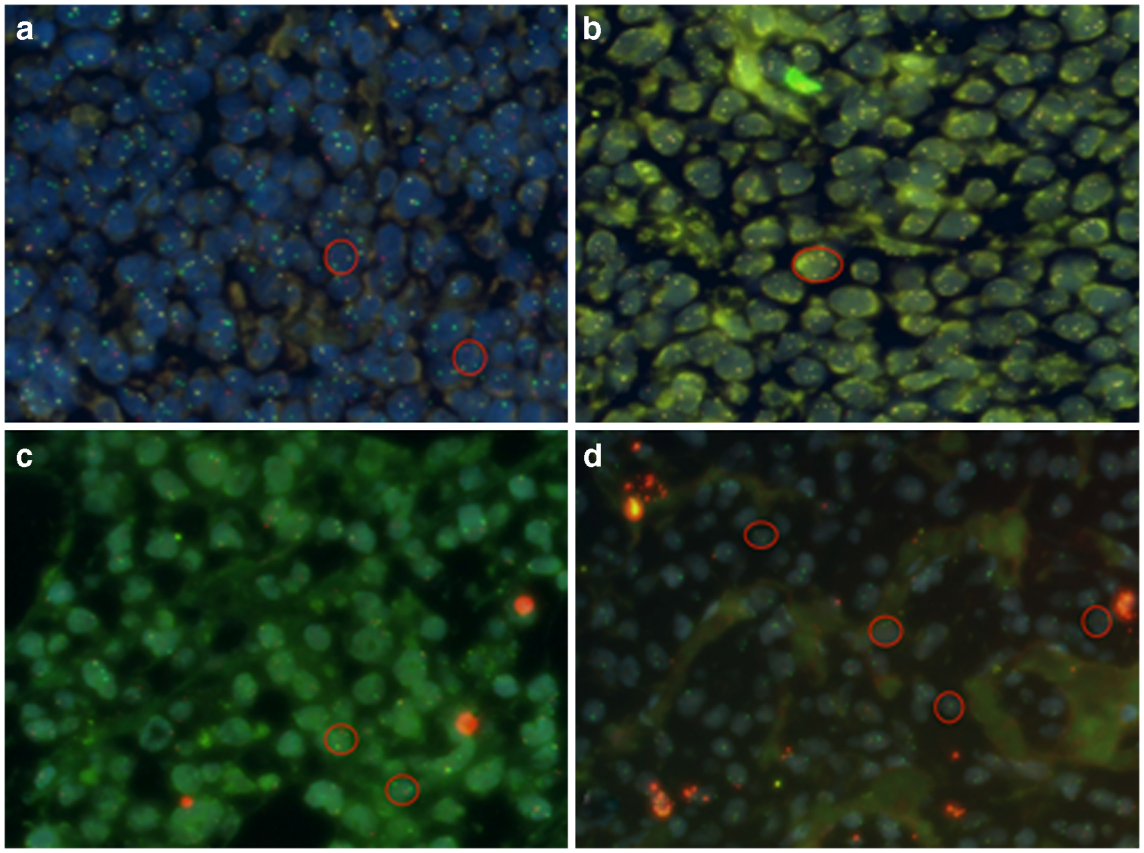

Fig. 2 Cytogenetic features of ALCL ALK. **a**, **b** A case of S-ALCL ALK −. **a** Presence of *IRF4/DUSP22* gene break with isolated green and red signals in most cells (red circle) (× 20). **b** PDL1 gene amplification with 5–6 signals in most cells (red circle) (× 20). **c** A case of S-ALCL ALK demonstrating a *TP63* gene break with isolated green and red signals in most cells (red circle) (× 20). **d** A case of PC-ALCL demonstrating a *TP53* deletion unique red signals (TP53) whereas two green signals (control) are observed in most cells (red circles) (× 20)

ALCL and BI-ALCL associated with a*TP53* deletion were stage IE and achieved complete remission at last follow-up.

TCRgamma genes were clonally rearranged in 21/30 of cases tested.

PD1/PDL1 pathways

Results of PD1 expression in TILs, PDL1 expression both in macrophages and tumor cells, and of FISH analysis for *PDL1* gene are presented Table 2. PDL1 expression in tumor cells (Fig. 1e) and macrophages and presence of PD1-positive TILS in the microenvironment (Fig. 1f) were observed in the three groups without significant difference. FISH testing with the *PDL1* break-apart probe did not show any translocation in all contributive cases. Nevertheless, six cases had *PDL1* gene amplification (6/33, 18.2%) including S-ALCL ALK−, PC-ALCL, and BI-ALCL (Fig. 2b). Of interest, three cases (2 S-ALCL ALK− and one BI-ALCL) had *TP53* deletion in association with *PDL1* amplification. The case of BI-ALCL harboring these two cytogenetic aberrations correspond to the patient presenting effusion with palpable breast tumor mass.

Interestingly, the presence of PD1-positive TILs was significantly associated with PDL1 positive expression in tumor cells ($p = 0.02$) (Table 3). Although the difference was not

Table 2 PD1/PDL1 pathway. Results of immunohistochemistry analysis for PDL1 and PD1 and of FISH analysis for PDL1 gene

	ALCL ALK− *N* = 37	S- ALCL ALK− *N* = 22	PC-ALCL *N* = 13	BI-ALCL *N* = 2
Immunohistochemistry				
PDL1 tumor cells positive, *n* (%)	22/37 (59.5)	14/22 (63.6)	6/13 (46.2)	2/2 (100)
PDL1 macrophages positive, *n* (%)	25/37 (67.6)	16/22 (72.7)	7/13 (53.8)	2/2 (100)
PD1 TILs positives, *n* (%)	19/37 (51.4)	13/22 (59.1)	4/13 (30.8)	2/2 (100)
Cytogenetic				
PDL1 amplification, *n* (%)	6/33 (18.2)	4/19 (21.1)	1/12 (8.3)	1/2 (50)

significant, PDL1 protein expression in tumor cells tended to be associated with the presence of a *PDL1* gene amplification ($p = 0.065$) (Table 4). Finally, PDL1 positive expression and p-STAT3 positive expression in tumor cells were correlated ($p = 0.0049$) (Table 5).

Relationship between biologic parameters and outcome

We evaluated the correlation between variables of interest with event free survival (EFS) in the 37 ALCL ALK− patients. Events included death, relapse, and progression. Although the difference was not significant, cox analysis demonstrated that p-STAT3 positive expression tended to be associated with poorer outcome (Supplementary Table 1A). No significant association between other biologic parameters, including protein expression and cytogenetic aberrations, and EFS was observed (Supplementary Table 1A-B).

Discussion

In the present study, we evaluated the potential role of relevant biomarkers that were analyzed and compared in different entities of ALCL ALK− including: 22 S-ALCL ALK−, 13 PC-ALCL, and 2 BI-ALCL.

Our study confirms the main clinical characteristics of ALCL ALK−. In agreement with previous reports, most patients with S-ALCL ALK− were diagnosed in stages III–IV of disease and showed an unfavorable prognosis [9] with relapse and death of disease despite systemic treatment with polychemotherapy. On the contrary, all patients with PC-ALCL or BI-ALCL had localized disease at diagnosis and were stage IE. Although, some patients with PC-ALCL underwent chemotherapy or had relapse, as previously reported [10, 11], all were in complete remission at last follow-up. Of interest, we identified the two distinct clinical forms of BI-ALCL [8, 12, 13]. One patient presented as an effusion around the implant whereas the second presented a palpable tumor confined to the breast. Both patients had indolent course without relapse and were in complete remission at the last follow-up. Nevertheless the patient with palpable breast tumor mass required more intensive therapeutic approaches as recommended [8, 12–14].

As expected, we did not identify any difference concerning classical histologic and phenotypic features of the three entities. All cases were composed of large and pleomorphic neoplastic cells, with the presence of the "hallmark" cells in variable number, and showed characteristically strong and homogeneous expression of CD30. T cell antigens loss was frequent in the three entities. Most of the cases expressed cytotoxic-associated antigens such as TIA1, Granzyme B, and Perforine, as described [8, 15]. Nevertheless, some cases, including S-ALCK ALK− and PC-ALCL were negative for these three enzymes. Of interest, unlike many ALCL ALK−, those with *DUSP22/IRF4* translocation typically lack cytotoxic marker expression [16, 17]. In the present series, we identified five cases with such cytogenetic aberration and three had no cytotoxic marker expression. *IRF4/MUM1* (interferon regulatory factor 4/multiple myeloma oncogene-1) gene belongs to the IRF (interferon regulatory factor) family of transcription factors. MUM1 protein expression has been shown to be associated with poor survival outcomes in PTCL [18]. Of interest, we identified that MUM1 was significantly over-expressed in S-ALCL ALK− compared to PC-ALCL. MUM1 is also a target of immunomodulatory drugs such as lenalidomide that has demonstrated clinical activity in PTCL [19, 20]. Thus, MUM1 could be a therapeutic target in S-ALCL ALK−. *DUSP22/IRF4* translocations have been suggested as the driver of MUM1 protein expression in PTCL [17] (Fig. 3). Nevertheless, in this series, fewer cases harbored this cytogenetic aberration than the number of total cases with MUM1 protein expression. Furthermore, the sole PC-ALCL harboring a *DUSP22/IRF4* translocation did not show any MUM1

Table 4 PD1/PDL1 pathway. Correlations between PDL1 expression in tumor cells and PDL1 gene amplification ($n = 33$)

	PDL1-positive expression	No PDL1 expression	*p* value
PDL1 gene amplification			
Positive, *n* (%)	6 (100)	0 (0)	
Negative, *n* (%)	15 (55.6)	12 (44.4)	0.065

Table 3 PD1/PDL1 pathway. Correlations between PD1 expression in TILs and PDL1 expression in tumor cells ($n = 37$)

	PDL1 positive expression	No PDL1 expression	*p* value
PD1 expression			
Positive, *n* (%)	15 (78.9)	4 (21.1)	
Negative, *n* (%)	7 (38.9)	11 (61.1)	0.02

Table 5 PD1/PDL1 pathway. Correlations between PDL1 and p-STAT3-positive expression in tumor cells ($n = 37$)

	p-STAT3-positive expression	No p-STAT3 expression	*p* value
PDL1 expression			
Positive, *n* (%)	19 (86.4)	3 (13.6)	
Negative, *n* (%)	6 (40)	9 (60)	0.0049

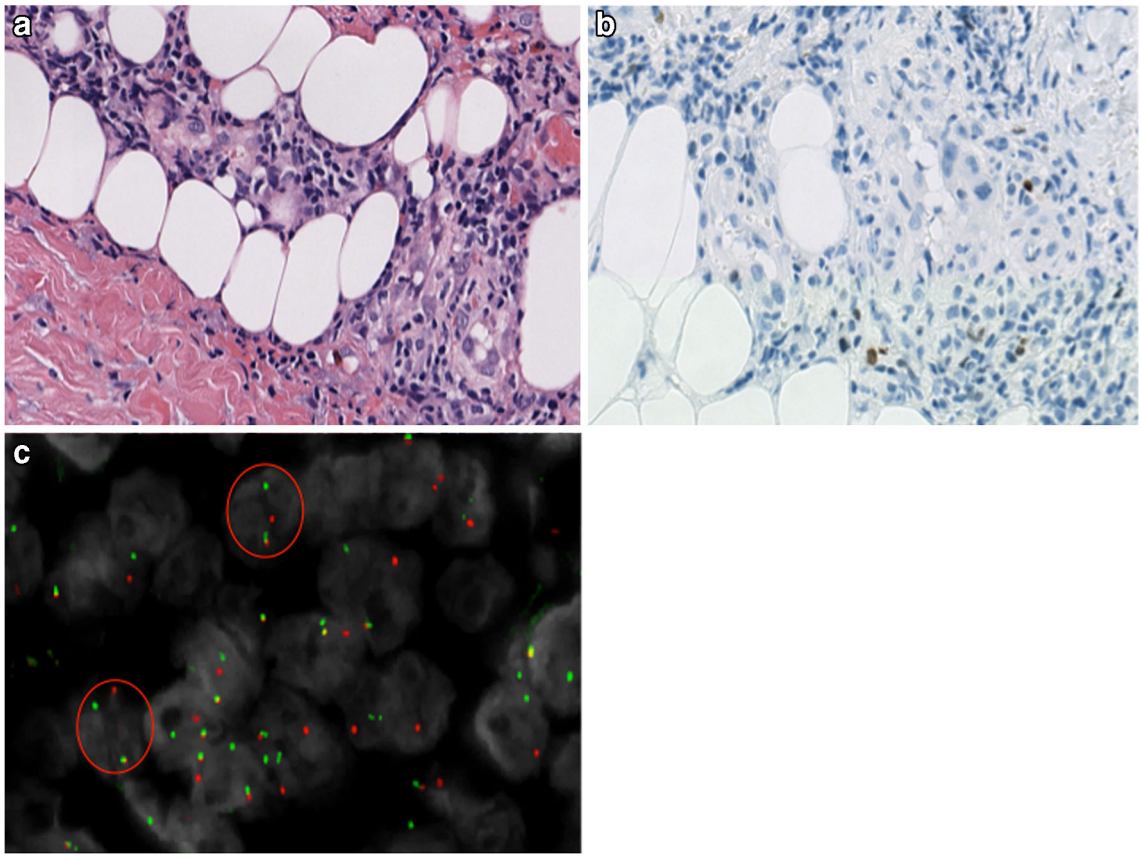

Fig. 3 Histologic phenotypic and cytogenetic features of PC-ALCL with *IRF4/DUSP22* gene break but without MUM1 protein expression. **a** Hematoxylin and eosin (H&E)-stained tissue (× 40). **b** MUM1 immunohistochemistry (× 40). Neoplastic cells are negative. **c** Presence of *IRF4/DUSP22* gene break with isolated green and red signals in most cells (red circle) (× 100)

protein expression. *TP63* gene (tumor protein p63) encodes a member of the p53 family of transcription factors. *TP63* rearrangement has been reported in 8% of S-ALCL ALK− and is associated with poor prognosis [16, 21]. P63 protein expression in ALCL is associated with *TP63* structural chromosomal abnormalities including translocation and extra copies [21, 22]. In the present series, only two cases of the total cohort expressed P63. They were two patients with S-ALCL ALK− who died of disease. One of these cases could be investigated with *TP63* FISH probe and showed translocation of this gene. As *TP63* gene abnormalities have not been reported in PC and BI-ALCL and as we only identified P63 aberration in S-ALCL ALK−, it could be speculated that P63 is only involved in S-ALCL ALK− pathogenesis and is associated with adverse outcome.

Constitutive activation of the STAT3 pathway has been reported in ALCL, in particular, in ALCL ALK+ [23–25]. Of interest, we identified p-STAT3 positive expression in 67.6% (25/37) of cases in the total cohort. Also the difference was not significant, p-STAT3 positive expression was more frequent in PC-ALCL and BI-ALCL compared to S-ALCL ALK−, and p-STAT3 expression tends to correlate with EFS ($p = 0.08$). Differently, others have reported that p-STAT3 positivity correlated with inferior OS in T cell lymphoma [23]. Nevertheless, this association was only observed in angio-immunoblastic T cell lymphoma (AITL) using a 80% cut-off of positivity for p-STAT3 but not in ALCL.

GATA3 expression seems to correlate with adverse outcome in PTCL [2, 26]. P53 is usually overexpressed in ALCL [27] and its overexpression is associated with poor outcome [28, 29]. MYC signaling promotes ALCL survival [30]. Thus, we focused on these protein expressions in our cohort of ALCL ALK−. We did not identify any significant difference of expression between the three entities. Furthermore, none of these protein expressions correlated with poor outcome.

MYC translocations have been reported in rare cases of ALCL, in particular, in pediatric ALCL, and is associated with aggressive clinical behavior [31, 32]. However we did not find such cytogenetic alteration in the present series.

IRF4/DUSP22 translocations have been reported in both S-ALCL ALK− and PC-ALCL with an overall frequency of 30% [16, 17, 33, 34] but not in BI-ALCL [8]. Similarly, we identified this cytogenetic alteration in these two entities but not in BI-ALCL. Of interest, *TP53* deletion was the commonest cytogenetic abnormality in our series of ALCL

and was present in the three entities at quite similar frequency. *TP53* mutations have been reported in T cell lymphomas [35] but not *TP53* deletions in particular in the ALCL. Some have reported that P53 protein overexpression was positively correlated with *TP53* mutations [35] or deletions [36]. In this work, we did not find any correlation between P53 protein expression and the presence of *TP53* deletion (data not shown, $p = 0.20$). *TP53* gene alterations including mutations and/or deletions are associated with adverse prognosis in hematologic malignancies [37, 38]. Although the presence of *TP53* deletion did not correlate with EFS in our series, three of the five patients with S-ALCL ALK− harboring this alteration died of the disease, and the patient with BI-ALCL associated with the del *TP53* had breast tumor mass.

The homeostasis of immune system is mediated by the coordinated expression of stimulatory and inhibitory signals. PD1/PDL1 pathway is a relevant immune checkpoint in cancer. PD1 expressed on T cells interacts with PDL1 expressed on tumor cells or adjacent cells in micro-environment inducing T cell exhaustion and allowing tumor cells to evade immune surveillance. PD1/PDL1 expression in lymphomas has been investigated by a variety of studies. It can be demonstrated in B cell lymphomas in particular in those occurring in immunoprivileged sites including the skin, mediastin or central nervous system [39–42]. We identified PD1/PDL1 expression in most of the cases of ALCL ALK− and without difference between those occurring in breast-implant, skin, or lymph nodes.

The genetic mechanisms inducing PDL1 overexpression on lymphoma cells include alterations in chromosome 9p24.1 (*PDL1* gene translocations or amplifications) [43, 44], the presence of disruption of the 3′-untranslated region (UTR) of the *PDL1* gene [45], or activation of the STAT3 pathway [46–48]. Of interest, *PDL1* gene amplification was the second commonest cytogenetic aberration in this series of ALCL ALK− and was observed in the three entities. We were able to demonstrate a trend to a correlation between PDL1 expression and *PDL1* gene amplification and a significant association between PDL1 and pSTAT3 expression in tumor cells. A direct applicability of PD1/PDL1 expression to predict prognosis remains to be fully elucidated in lymphomas. In diffuse large B cell lymphomas (DLBCL), some have reported a negative association between PDL1 expression and outcome [49] whereas other reported a positive correlation [50]. Our data did not show an association between PD1/PDL1 expression or *PDL1* gene amplification and EFS.

In conclusion, despite distinct clinical behavior, systemic, primary cutaneous, and breast implant-associated ALK-negative large cell lymphomas share similar biological features including constitutive activation of the STAT3 pathway, PDL1/PD1 immune-checkpoint expression, *PDL1* gene amplification, and *TP53* deletion. None of the studied biological parameters was associated with prognosis. Nevertheless, although our study was limited by the number of patients especially in the subgroup with BI-ALCL, p-STAT3 protein expression and P63 aberrations (including protein overexpression and cytogenetic break) seem to correlate with poorer outcome. Larger series should be investigated with the current approach to determine more precisely the activity and the prognostic value of these biomarkers and pathways in each group.

Author contributions V S, M A, V C-M, and V C designed the research project. V S, A G, L D, and V C-M evaluated the histological and immunohistochemical findings. V S, M A, A T, L D O, A T, and V C evaluated the cytogenetic findings. V S and A G obtained data and wrote the main part of the manuscript. M A, V C-M, O D, S G, L D, A T, and V C reviewed the draft with critical comments.

Compliance with ethical standards

This study was carried out in agreements with the Declaration of Helsinki and was approved by the Centre des Ressources Biologiques (CRB) of the Centre-Hospitalo-Universitaire (CHU) of Montpellier, France.

Informed consent Written informed consent for the study was obtained from the patient.

Conflict of interest The authors declare that they have no conflicts of interest.

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