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ALK-positive large B-cell lymphomas express a terminal B-cell differentiation program and activated STAT3 but lack *MYC* rearrangements

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ALK-positive large B-cell lymphoma is an aggressive lymphoid neoplasm characterized by a monomorphic proliferation of immunoblast-like cells expressing a plasmablastic phenotype and carrying *ALK* rearrangements. *MYC* rearrangements are frequent in plasmablastic lymphomas, advanced plasma cell myelomas and a subgroup of diffuse large B-cell lymphomas, but their presence in ALK-positive large B-cell lymphomas is unknown. MYC expression is downregulated by BLIMP1, a master modulator of plasma cell differentiation. BLIMP1 and MYC are upregulated by STAT3, a signal transducer activated by ALK. To determine the role of BLIMP1, MYC and STAT3 in the pathogenesis of ALK-positive large B-cell lymphomas, we investigated *MYC* rearrangement and the expression of MYC, phosphorylated STAT3, BLIMP1, PAX5 and XBP1 in 12 ALK-positive large B-cell lymphomas. All cases expressed ALK with a granular cytoplasmic pattern. Nine cases had a split signal consistent with an *ALK* rearrangement. Three additional cases showed a deletion of the 5′ or 3′ end of the *ALK* probe consistent with cryptic translocation. PAX5 was virtually negative in all cases tested, whereas BLIMP1 was expressed in all tumors and XBP1 in 11 of 12. Phosphorylated STAT3 was observed in all cases with a strong and diffuse nuclear pattern. *MYC* rearrangements were not identified in any tumor, but *MYC* gains and amplification were detected in six cases and one case, respectively. MYC protein was expressed in all tumors independently of *MYC* gene alterations. These results indicate that ALK-positive large B-cell lymphomas express a complete plasmablastic differentiation program but, contrary to plasmablastic lymphomas, do not have *MYC* rearrangements. STAT3 is constantly activated and may be an alternative mechanism to promote MYC expression in these tumors. The relevance of the ALK/STAT3 pathway in the pathogenesis of ALK-positive large B-cell lymphomas may offer an attractive target for new therapies.
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ALK-positive large B-cell lymphoma was initially described by Delsol *et al*[1] in 1997 as an uncommon and aggressive subtype of diffuse large B-cell lymphoma with frequent immunoblastic morphology, a plasma cell phenotype and expression of the ALK protein. This protein is detected in most cases with a granular cytoplasmic pattern, which is commonly associated with the rearrangement of *ALK* with the clathrin gene *CTCL*, t(2;17).[2] However, other genetic partners as well as other patterns of cell localization of the ALK protein have been described.[3–5] In addition to the *ALK* rearrangements, conventional cytogenetic studies have reported

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the presence of frequent complex karyotypes with multiple structural and numerical alterations in other chromosomes.[2,6–14]

The oncogenic mechanisms involved in the pathogenesis of human ALK-positive large B-cell lymphomas are not well known. ALK protein is a cytoplasmic receptor with tyrosine kinase activity that is not normally expressed in lymphoid cells. *ALK* rearrangements upregulate oncogenic fusion proteins in which the ALK fragment contains the catalytic domain and the fused partner provides a dimerization domain that activates the receptor without the need of the ligand.[15,16] *ALK* activation has the oncogenic potential in different types of cells including B and T lymphocytes, and in transgenic mice, it induces the development of plasma cell tumors.[17,18] The dimerization of ALK leads to the activation of different downstream signaling pathways, one of the most relevant being the STAT3 pathway.[19,20] STAT3 is an important signal transducer that triggers the program of plasma cell differentiation through BLIMP1 activation, XBP1 and IRF4 expression.[21] ALK-positive large B-cell lymphomas have a phenotype similar to that of plasma cells, being negative for CD20 and positive for CD138. However, whether these tumors express a complete terminal B-cell differentiation program with the upregulation of BLIMP1 and XBP1 is not well known.[22] STAT3 also regulates many proteins involved in tumor cell proliferation including MYC that may have an important role in the pathogenesis of these tumors.[23]

The aggressive behavior of some B-cell lymphomas has been correlated with the presence of *MYC* rearrangement. This genetic alteration has been observed frequently in lymphoid neoplasms with plasma cell differentiation, such as plasmablastic plasma cell myelomas and plasmablastic lymphomas.[24–26] On the other hand, MYC protein expression has been recently described in some aggressive B-cell lymphomas, not always related to the presence of *MYC* gene alterations.[27–30] The presence of *MYC* alteration and protein expression, as well as the activation of STAT3 in ALK-positive large B-cell lymphomas are not well known.

The aims of this study were to determine whether ALK-positive large B-cell lymphomas express a complete terminal B-cell differentiation program with the upregulation of BLIMP1 and XBP1 and whether MYC genetic alterations and STAT3 activation may play a role in the pathogenesis of these tumors.

Materials and methods

Case Selection

Twelve ALK-positive large B-cell lymphoma cases were retrieved from the files of the Pathology Department of the Hospital Clinic of Barcelona; Netherlands Cancer Institute (Amsterdam, The Netherlands); Laboratorio de Hematopatología (Mendoza, Argentina) and Consultoria em Patologia (Botucatu, Brazil). The patients were predominantly males (92%) with a median age of 33 years (range 22–42 years). Ten patients (83%) presented with nodal disease and two had extranodal presentation: one in the right colon and the other one in the soft tissues of the thigh (Table 1).

For comparison purposes, we also studied 11 plasmablastic lymphomas and 16 diffuse large B-cell lymphomas that were selected because they were of the activated B-cell subtype by gene expression profiling (11 cases) or had been classified immunohistochemically as non-germinal center-like subtype using the Hans' algorithm (5 cases).[31]

Immunohistochemistry

Immunohistochemical studies were carried out with a panel of monoclonal and polyclonal antibodies

Table 1 Clinical and pathological features of ALK+ LBCL

Case	*Age (years)*	*Sex*	*Location*	*CD79a*	*PAX5*	*IGH IGL*	*IRF4*[a]	*BLIMP1*[a]	*XBP1*[a]	*ALK FISH*
1	34	M	Lymph node	−	ND	IgA+, lambda	−	+++	++	R
2	42	M	Lymph node	−	ND	IgA+, lambda	ND	++	−	DS+ALK extra copies
3	30	M	Lymph node	−	−	Lambda	++	+++	+++	R+ALK extra copies
4	38	M	Right colon mass	ND	−	IgA+, kappa	−	NV	++	R+ALK extra copies
5	29	M	Lymph node	ND	−	IgA+, lambda	+	+++	+	R
6	28	M	Soft tissue of the thigh	−	−	IgA+, lambda	+++	+++	+	D 3′ end+ALK extra copies
7	32	M	Lymph node	+	−	IgA+, kappa	+++	+++	+++	R
8	22	M	Lymph node	−	−	−	++	++	+++	DS
9	38	M	Lymph node	−	−	−	−	+	+	D 5′ end
10	34	M	Lymph node	ND	Weak +	IgA+, IgG+, lambda	+++	+++	+	R
11	Un.	F	Lymph node	−	−	IgA+, IgG+, lambda	+	+++	+++	D 5′ end+ALK extra copies
12	40	M	Lymph node	−	−	IgA+	++	+	++	R

R, rearrangement; DS, double split; D, deletion; ND, not determined; NV, not valuable; Un, unknown.
All cases showed positivity for EMA and CD138 and were negative for CD20.
[a]+: 25–50%; ++: 50–75%; and +++: >75%.

reactive in paraffin-embedded tissue sections using a peroxidase-labeled detection system, standard antigen retrieval protocols and an automated immunostainer (Dako Autostainer, Glostrup, Denmark and Bond-Max, Leica Microsystems, Wetzlar, Germany). The panel of antibodies used included CD20 (clone L26; Dako), CD79a (clone JCB117; Dako), PAX5 (clone 24; BD Biosciences), CD30 (clone Ber-H2; Dako) and EMA (clone E29; Dako). Polyclonal antibodies for IgA, IgG, kappa and lambda were applied (all from Dako). ALK expression was determined using the monoclonal ALK1 antibody (Dako).The terminal B-cell differentiation program was evaluated using antibodies anti-IRF4 (clone MUM1p; Dako) and CD138 (clone MI15; Dako), PRDM1/Blimp1 (clone Sc13206; Santa Cruz Biotechnology) and XBP1 (clone Sc7160; Santa Cruz Biotechnology). The conditions for all these antibodies were the same as described previously.[32] A cutoff of 25% was used to interpret the results as positive.

MYC expression was assessed using the monoclonal Y69 antibody (Epitomics), at dilution 1/40, with incubation of the primary antibody for 1 h after antigen retrieval at pH 6 for 30 min. The activation of STAT3 was analyzed using the monoclonal phosphorylated-STAT3 (pSTAT3, phosphorylation on tyrosine 705) antibody (clone D3A7; Cell Signaling Technology). The samples were incubated with this antibody at a dilution of 1:100 for 2 h at 37 °C. MYC and pSTAT3 immunostaining were evaluated in a semiquantitative manner and a normal tonsil tissue was used as the control. A cutoff of 25% was also used to interpret the results as positive.

The presence of Epstein–Barr virus was examined by *in situ* hybridization to detect Epstein–Barr virus-encoded early nuclear RNAs. The latency-associated nuclear antigen 1 of human herpes virus type 8 was studied by immunohistochemistry using the clone LN53 (Advanced Biotechnologies), both as described earlier.[25]

Fluorescence *In Situ* Hybridization

Fluorescence *in situ* hybridization was performed on 3- to 4-μm-thick sections of formalin-fixed, paraffin-embedded tissues, using break-apart probes (Vysis, Abbott Molecular) specific for *ALK* (2p13) and *MYC* (8q24) as described previously.[25]

Tonsil sections were used as controls. For every tumor and tonsil sample, a minimum of 100 evaluable nuclei were scored. The cutoff values for the interphase FISH analyses were established following the criteria of Ventura *et al*.[33] To detect rearrangements, the cutoff value was 3%. Moreover, the mean number of numerical genetic alterations was evaluated in each case to assess the incidence of the genetic events in the tumors. Gains were considered when three to four copies of the gene studied were identified, whereas more than four copies were considered as amplification. A deletion was considered when one of the alleles lost one of their extremes in >40% of the neoplastic cells.

Results

Pathological Features of ALK-Positive Large B-Cell Lymphomas

The clinical and immunophenotypic features of the 12 ALK-positive large B-cell lymphomas are summarized in Table 1. All cases had a granular cytoplasmic distribution of ALK expression. CD20 was negative in all of them. CD79a, PAX5 and CD30 were detected in isolated cells in 2/9, 1/10 and 2/12 cases, respectively, but none of the tumors had positivity for more than one of these B-cell markers. EMA was expressed in all cases. IgA was positive in 9/12 cases and two of them also expressed IgG. No clear expression of immunoglobulin heavy chains could be demonstrated in three cases. Lambda restriction was detected in 7/12 and kappa restriction in 2/12, and in 3 the study was not contributory. Epstein–Barr virus and/or human herpes virus type 8 were negative in all the cases investigated.

CD138 was positive in all cases examined. IRF4 was positive in 8/11 (73%) cases; two had weak expression in 10–25% of the neoplastic cells and one was negative. BLIMP1 was positive in all cases, most of them with strong expression in >50% of the cells. Similarly, XBP1 was also expressed in 11 of 12, although the number of positive cells was slightly lower than for BLIMP1.

The *ALK* break-apart probe showed different patterns of rearrangement and alterations in all cases (Table 1 and Figure 1). Seven of 12 (58%) cases showed a split ALK signal consistent with a chromosomal rearrangement (cases 1, 3, 4, 5, 7, 10 and 12). Two of these cases also had extra normal copies of the normal allele (cases 3 and 4). A split signal of both alleles was observed in two other cases (cases 2 and 8) and one of them also showed extra copy signals of normal *ALK* allele (case 8). Two other cases exhibited isolated copies of 3′ end (telomeric region) (cases 9 and 11), one of them with additional copies of the normal *ALK* (case 11). One additional case showed isolated extra copies of the 5′ end of ALK (centromeric region) and of the normal allele (case 6). These three cases with deletions of the 3′ or 5′ ends had ALK protein expression with the same granular cytoplasmic pattern as the other cases.

MYC Gene Alterations and Protein Expression

The genetic alterations and protein expression of MYC in ALK-positive large B-cell lymphomas, plasmablastic lymphomas and diffuse large B-cell lymphomas are summarized in Table 2 and Figure 2. *MYC* analysis by FISH was successful in the 11

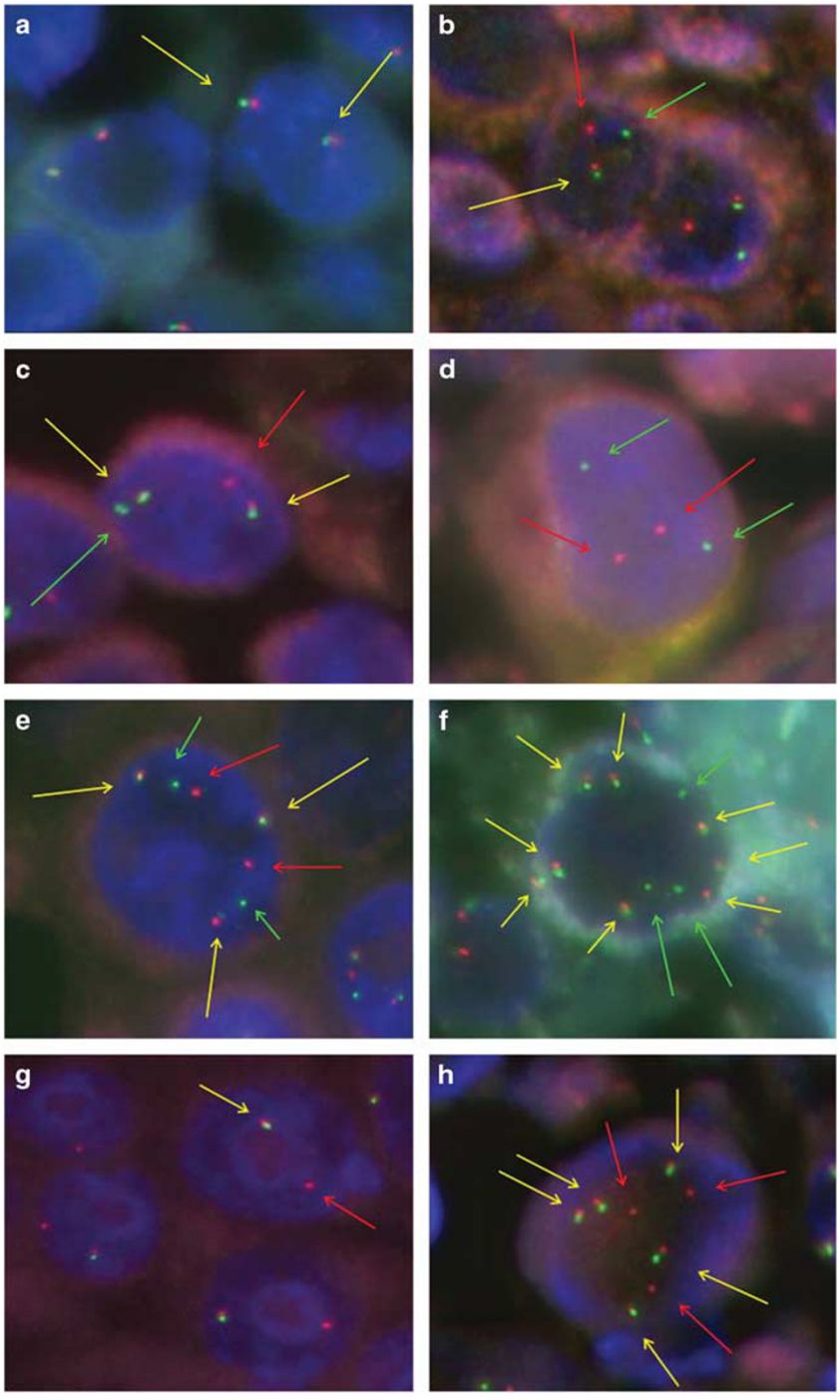

Figure 1 Different ALK patterns detected by fluorescence *in situ* hybridization (FISH). (**a**) Normal ALK pattern observed in a reactive lymph node; (**b**) case 1, simple rearrangement; (**c**) case 3, simple rearrangement and extra copies; (**d**) case 8, double rearrangement; (**e**) case 2, double rearrangement and extra copies; (**f**) case 6, three loci with deletion of the 3′ end and extra copies; (**g**) case 9, deletion of the 5′ end; (**h**) case 11, three loci with deletion of the 5′ end and extra copies. Yellow arrow: normal signal of *ALK*; red arrow: split signal of *ALK*, 3′ end; and green arrow: split signal of *ALK*, 5′ end.

ALK-positive large B-cell lymphomas cases studied. Six cases showed an increased copy number of the gene, 5 gains (cases 4, 5, 10, 11 and 12) and 1 amplification (case 3), whereas 5 cases did not show any genetic alteration. The mean copy number gains per cell was 3.26 (range 2.76–3.87) and the amplified copies 5.84. No evidence of *MYC* rearrangement was detected in any case.

MYC protein was expressed in the 12 cases. Six tumors had MYC positivity in 25–50% of the cells and three of them had *MYC* gains (cases 10, 11 and 12). Three cases had MYC expression in 50–75% of the

Table 2 MYC: Genetic alterations by FISH and protein expression by IHC

Case no.	*Diagnosis*	*MYC IHC (%)*	*MYC FISH*	*pSTAT3 (%)*
1	ALK+LBCL	>90	N	>75
2	ALK+LBCL	>75	N	100
3	ALK+LBCL	>75	Amplifications	100
4	ALK+LBCL	50–75	Gains	100
5	ALK+LBCL	50–75	Gains	100
6	ALK+LBCL	50–75	N	100
7	ALK+LBCL	25–50	N	100
8	ALK+LBCL	25–50	N	100
9	ALK+LBCL	25–50	N	100
10	ALK+LBCL	25–50	Gains	100
11	ALK+LBCL	25–50	Gains	100
12	ALK+LBCL	25–50	Gains	50–75
13	PBL	50–75	Rearrangement	50–75
14	PBL	50–75	Rearrangement	ND
15	PBL	25–50	Rearrangement	50–75
16	PBL	25–50	Gains	NV
17	PBL	—	Gains	ND
18	PBL	50–75	Rearrangement	ND
19	PBL	25–50	Gains	50–75
20	PBL	—	Gains	—
21	PBL	—	Gains	100
22	PBL	25–50	N	—
23	PBL	—	N	ND
24	DLBCL	>75	N	50–75
25	DLBCL	25–50	Rearranged	ND
26	DLBCL	25–50	N	ND
27	DLBCL	25–50	N	—
28	DLBCL	—	N	50–75
29	DLBCL	—	N	—
30	DLBCL	—	N	—
31	DLBCL	—	N	—
32	DLBCL	—	N	ND
33	DLBCL	—	N	ND
34	DLBCL	—	Gains	—
35	DLBCL	>75	N	—
36	DLBCL	50–75	N	—
37	DLBCL	50–75%	N	50–75
38	DLBCL	—	N	50–75
39	DLBCL	—	N	ND

ALK+ LBCL, ALK-positive large B-cell lymphoma; PBL, plasmablastic lymphoma; DLBCL, diffuse large B-cell lymphoma, all cases were ABC subtype by gene expression profile or non-GCB by immunohistochemistry; N, normal FISH pattern; NV, not valuable; ND, not determined; Gains, 3–4 copies; amplification, >4 copies.

neoplastic cells, and two of them had *MYC* gains (cases 4 and 5). The other three tumors showed MYC expression in >75% of the tumor cells and one of them demonstrated amplification of the gene (case 3). No gene alterations were observed in six cases.

In plasmablastic lymphomas, *MYC* genetic alterations were observed in 9/11 cases, 4 rearrangements and 5 gains. MYC expression was detected in 7/11 cases. Four cases were positive in 25–50% of the neoplastic cells, two of them showed *MYC* gains (cases 16 and 19), one had a gene rearrangement and no gene alterations were observed in one. Three tumors were MYC positive in 50–75% and all of them had *MYC* rearrangement (cases 13, 14 and 18). No MYC expression was observed in three plasmablastic lymphomas with *MYC* gains (cases 17, 20 and 21) (Table 2).

In diffuse large B-cell lymphomas, *MYC* genetic alterations were observed in 2/16 cases, one rearrangement and one gain. MYC expression was detected in 7/16 cases. Two cases showed MYC expression in >75% of the neoplastic cells, both without any *MYC* gene alteration (cases 24 and 35). In two cases, MYC expression was observed in 50–75% of the tumor cells, but none of them showed *MYC* gene alterations by fluorescence *in situ* hybridization (cases 36 and 37). Three additional cases showed MYC expression in 25–50% of the neoplastic cells (cases 25, 26 and 27) and one of them presented rearrangement of the gene (case 25).

pSTAT3 Expression

pSTAT3 expression in ALK-positive large B-cell lymphomas, plasmablastic and diffuse large B-cell lymphoma-activated B-cell subtype is detailed in Table 2 and Figure 2. All ALK-positive large B-cell lymphomas were strongly positive for pSTAT3. This overexpression was observed in virtually all neoplastic cells, except in one case (case 12).

In plasmablastic lymphomas, pSTAT3 expression was observed in 4/6 cases. Three of them (cases 13, 15 and 19) showed pSTAT3 expression in 50–75% of the neoplastic cells. MYC expression correlated with pSTAT3 expression in these cases. The fourth case (case 21) showed pSTAT3 expression in virtually 100% of the neoplastic cells, but MYC expression was negative. Two cases were negative for pSTAT3, one expressed MYC in 25–50% and the other was negative.

pSTAT3 expression was detected in 4/11 diffuse large B-cell lymphoma cases in 50–75% of the neoplastic cells. Two of these cases (cases 24 and 37) also showed MYC expression in a similar percentage, whereas the other two cases were negative for MYC (cases 28 and 38). None of them had *MYC* genetic alterations. Three cases without pSTAT3 expression (cases 27, 35 and 36) expressed MYC, but none of them showed genetic alterations of the gene.

Discussion

ALK-positive large B-cell lymphoma is an uncommon subtype of diffuse large B-cell lymphoma carrying *ALK* rearrangement and protein expression. The most common gene partner of *ALK* in these tumors is *CLTC* at 17q23[2,8,14,34–41] followed by *NPM1* at 5q35[6,10] and other genes identified in occasional cases such as *SQSTM1*[42] and *SEC31A*.[13] Genetic studies have recognized other translocations between *ALK* and chromosomal regions at 4q22, 12q24 or Xq21, but the corresponding gene partner has not been identified.[11,12] All these translocations lead to the overexpression and oncogenic activation of the ALK protein. The *CLTC-ALK* fusion generates a protein recognizable by immunohistochemistry with a cytoplasmic

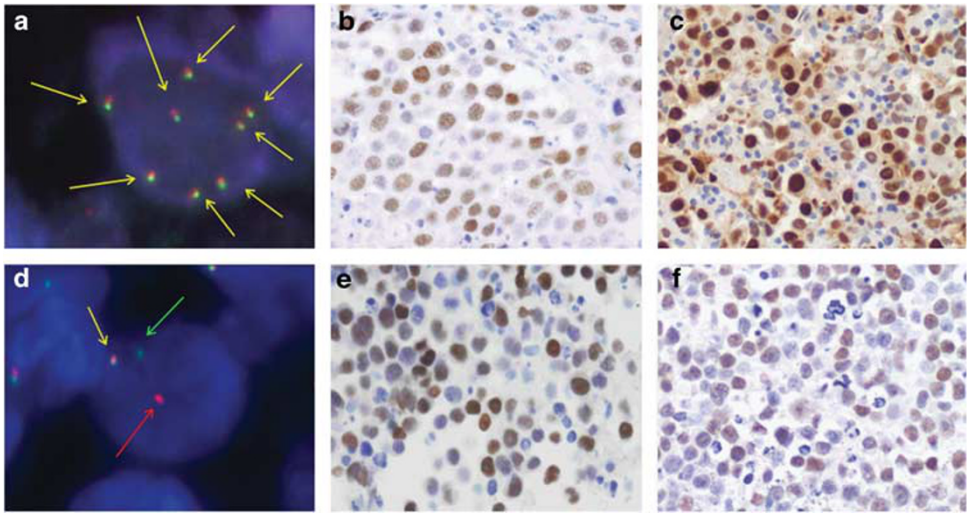

Figure 2 Phosphorylated signal transducers and activators of transcription-3 (pSTAT3) and MYC in ALK+ large B-cell lymphoma (LBCL), plasmablastic lymphoma (PBL) and activated B-cell-like (ABC) diffuse large B-cell lymphoma (DLBCL). (**a**) Case 3, *MYC* amplified in an ALK+ LBCL; (**b**) case 3, MYC protein expression in >75% of the cells; (**c**) case 3, pSTAT3 expression in all tumor cells; (**d**) case 13, *MYC* rearranged in a PBL case; (**e**) case 13, MYC protein expression in 50–75% of the neoplastic cells; (**f**) case 13, pSTAT3 protein expression in 50–75% of the tumor cells. Immunohistochemistry (IHC) images (**b**, **c**, **e** and **f**) photographed at ×400. Yellow arrow: normal signal of *MYC*; red arrow: split signal of *MYC*, 5′ end; green signal: split signal of *MYC*, 3′ end.

granular pattern. However, this pattern is also detected in other translocations, with the exception of the nuclear and cytoplasmic pattern of the *NPM1-ALK* fusion. All our cases had ALK expression with a cytoplasmic granular pattern, although the partner involved could not be determined since we used a break-apart *ALK* probe. A split signal consistent with an ALK translocation was detected in 7 of the 12 cases. Intriguingly, two cases showed only a deletion of the 5′ end of the probe. This FISH pattern has been observed occasionally in ALK-positive large B-cell lymphomas.[4,11–13] The combination of FISH and molecular studies have confirmed that this pattern is due to cryptic translocations of the 3′ end of *ALK* fused with different partners and associated with the deletion of the *ALK* 5′ end. Similar to the reported cases, our two tumors with the 5′ end deletion expressed ALK with the same granular cytoplasmic pattern as the other cases with the typical split signal. In addition to the rearranged gene, our FISH study detected double splits in two cases and extra copies of the normal *ALK* gene in five cases. These findings are consistent with the multiple chromosomal alterations and karyotypes close to the tetraploidy detected in around 50% of these tumors by conventional cytogenetics.[2,6–14] However, as we have not used centromer probe in this study, we cannot distinguish between true polysomies or partial chromosomal gains. We also found a case with deletion of 3′ end, which has not been reported before in the literature. This case also expressed ALK protein with the same granular and cytoplasmic pattern of expression, suggesting that *ALK* may have a cryptic rearrangement with a similar functional effect upregulating ALK protein.

The oncogenic pathways involved in the pathogenesis of ALK-positive large B-cell lymphomas are not well defined. *ALK* rearrangements produce fusion proteins with constitutive tyrosine kinase activity, which activates several downstream pathways. One of them is the activation of STAT3 signaling that seems to be required for ALK-mediated lymphomagenesis in different cell models including murine and human B and T cells.[19,20,43] In this study, we have demonstrated that activated pSTAT3 is strongly expressed in all ALK-positive large B-cell lymphomas. These observations expand the previous identification of pSTAT3 in two ALK-positive large B-cell lymphomas[44] and suggest that this pathway may be important in the pathogenesis of these lymphomas. The relevance of STAT3 in ALK-positive large B-cell lymphomas is highlighted by its downregulation in cell lines derived from these tumors treated with an ALK inhibitor.[7,13]

The strong and constant expression of pSTAT3 in ALK-positive large B-cell lymphomas contrasted with the lower expression observed in non-germinal center B-cell-like diffuse large B-cell lymphomas (36%) and slightly lower in plasmablastic lymphomas (67%). STAT3 has not been previously investigated in plasmablastic lymphoma and our findings suggest that this pathway may be also relevant in these tumors. The expression of pSTAT3 observed in our subset of non-germinal center B-cell-like diffuse large B-cell lymphomas is similar to the frequency observed in previous studies.[45,46] Although the prognostic significance of this finding in diffuse large B-cell lymphoma is controversial,[47,48] studies in cell lines and animal models suggest that STAT3 may be a relevant therapeutic target for this subgroup of diffuse large B-cell lymphomas.[45,46,49]

The high expression of pSTAT3 in ALK-positive large B-cell lymphomas may be relevant for the plasmablastic phenotype of these tumors since STAT3 promotes the upregulation of BLIMP1, a master regulator of the plasma cell differentiation program.[21] Previous studies have characterized the phenotype of ALK-positive large B-cell lymphomas with CD138 positivity and lack of mature B-cell markers such as CD20 and CD79a, but the expression of BLIMP1 and other markers of the plasma cell differentiation program was not known. Consistent with the role of pSTAT3, we have demonstrated that ALK-positive large B-cell lymphomas constantly express BLIMP1 and it seems to be functional because the tumors lack *PAX5* and express *XBP1*, two genes, respectively, repressed and upregulated by BLIMP1. PAX5 is essential to maintain the mature B-cell identity and its repression is required for the development of the plasma cell differentiation program, whereas XBP1 is an important transcription factor that modulates the terminal B-cell differentiation program.[50,51] This complete plasmablastic phenotype observed in ALK-positive large B-cell lymphomas is less common in plasmablastic lymphomas. These tumors constantly express BLIMP1 in virtually all cases, but XBP1 is only expressed in approximately 50% of them.[52,53]

MYC rearrangements have been observed in 50% of plasmablastic lymphomas and plasmablastic plasma cell myelomas. BLIMP1, constantly expressed in these tumors, represses genes related to cell proliferation and growth including MYC.[54] The high frequency of *MYC* rearrangements in these tumors may be an oncogenic mechanism to overcome the repressor effect of BLIMP1 on MYC expression.[25] In this study, we have observed that, contrary to plasmablastic lymphomas, ALK-positive large B-cell lymphomas do not have *MYC* rearrangements, although one case had *MYC* amplifications. In spite of the low incidence of genetic alterations, MYC protein was expressed in all tumors, suggesting that it may be important for the pathogenesis of the tumor. Previous studies have shown that MYC is downstream of ALK[55] and may also be a target of activated STAT3.[56,57] Therefore, MYC expression in ALK-positive large B-cell lymphomas may be driven by these alternative mechanisms that, as postulated for plasmablastic lymphomas, may lead to overcome the repressor effect of BLIMP1.

In conclusion, our study shows that ALK-positive large B-cell lymphomas express markers associated with a complete plasma cell differentiation program and active STAT3, but contrary to other aggressive neoplasms with plasmablastic phenotype lack *MYC* rearrangements. These findings are consistent with a model in which *ALK* rearrangements activate STAT3 that in turn promotes the plasma cell differentiation program through BLIMP1, and upregulates the expression of MYC (Figure 3). The relevance of the ALK/STAT3 pathway in the pathogenesis of these lymphomas may offer an attractive target for new therapies.

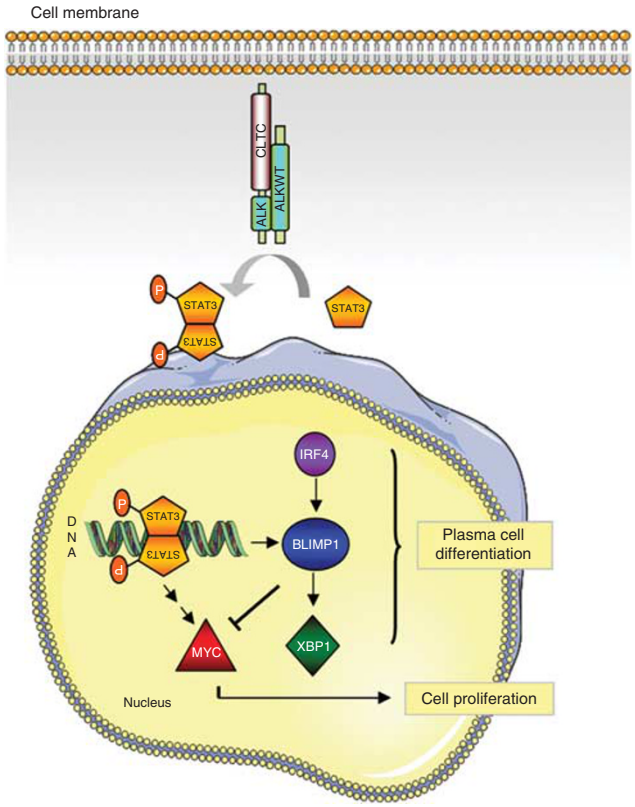

Figure 3 Model of signal transducers and activators of transcription-3 (STAT3) activation and BLIMP1 and MYC expression in ALK+ large B-cell lymphoma (LBCL). ALK rearranged receptor bound to a non-rearranged ALK receptor (ALK wild type, ALK WT) triggers a STAT3 homodimer formation without the presence of an external ligand. STAT3 dimerization results in the phosphorylation of its tyrosine residues. This activation allows the entrance of the STAT3 dimer into the nucleus of the cell, where it promotes the activation of BLIMP1, which jointly with interferon regulatory factor-4 (IRF4) and X-box binding protein 1 (XBP1) trigger the plasma cell differentiation, and the activation of MYC in the cell proliferation.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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