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Next generation sequencing of breast implant associated anaplastic large cell lymphomas reveals a novel *STAT3-JAK2* fusion among other activating genetic alterations within the *JAK-STAT* pathway

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Abstract

Breast implant associated anaplastic large cell lymphoma (BIA-ALCL) is a distinct type of ALCL, and a new provisional entity by the 2016 revision of the World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues. In contrast to systemic and primary cutaneous ALCLs, BIA-ALCLs have been genetically characterized by the absence of fusions and frequent activation of the *JAK-STAT3* pathway through mutations in *JAK1* and *STAT3*. In this study, we report the results of the genetic profiling of 9 BIA-ALCL casessupportingthe role of the *JAK-STAT* pathway activation in this entity, including the identification of an activating*STAT3-JAK2* fusion similar to those recently reported in T-cell lymphoproliferative disorders of the gastrointestinal tract. To our knowledge, this is the first fusion reported in BIA-ALCL, providing further insight into the overall genetic landscape of this rare entity as well as uncovering potential options for targeted therapy in cases with advanced disease.

Introduction

Breast implant associated anaplastic large cell lymphoma (BIA-ALCL) has been recently incorporated as a provisional category of ALCL in the 2016 revision of the World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues.[1]This is a rare and distinct entity which arises in the capsule surrounding textured saline or silicone breast implants at a median intervalof 9 years and typically presents as a unilateral peri-implant effusion with no grossly identifiable masslesion. The morphologic and immunophenotypic features are indistinguishablefrom those of systemic or cutaneous ALK negative ALCL, with the presence of large cells with pleomorphic and anaplastic

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Data available on request from the authors.

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morphology andcharacteristic strong / uniform expression of CD30. The prognosisis generally excellent,provided that the disease is confined to the effusion and capsule,as it can be successfullytreated with complete capsulectomy alone.[2–5]The presence of an associated mass, invasion through the capsule and lymph node involvementare seen in approximately 20% of patients and are characteristics associated with worseoutcome.[6]

While BIA-ALCL appears to have distinct biologic features that sets itapart from other ALCL subtypes, the genomic landscape and genetic events specifically responsible for its development remain poorly understood, in part due to the rarity and likely underdiagnosis of the disease.Activating mutations in *STAT3* and *JAK1* have been recently described as common events,[7–10] suggesting that *JAK-STAT3* signaling pathway constitutive activation may be at least partially involved in its oncogenesis.[11,12]To date, however, structural gene rearrangements, including those commonly associated with other ALCLs, such as translocations involving *ALK*, *DUSP22* or *TP63*, have never been reported in BIA-ALCL.[12–15] In this report, we describethe genomic profile of 9 patients with BIA-ALCL using MSK-IMPACT heme, a 400 gene hybrid capture next generation sequencing assay and the identification of an activating*STAT3-JAK2* fusion not previously described in this entity.

Materials and Methods

Nine cases with BIA-ALCL diagnosed at our institution were studied. Both DNA and RNA were extracted from sections of formalin-fixed paraffin embedded (FFPE)tumor tissue blocks. To address adequacy issues, between10–40 sections (5 μm thick) were used for extraction. Samples were sequenced using the MSK IMPACT HEME assay,a hybridization capture-based next generation sequencing panel capturing all coding regions of 400 genes[16]and the MSK-PanHeme Fusion assay, an RNA-based genefusion detection assay which utilizes the ArcherDx Anchored Multiplex polymerase chain reaction (PCR) (AMP™) technology and targets199 genes. Matched normal control DNA from all 9 patients(4 FFPE non-neoplastic tissue, 3 nail clippings, 2 blood samples) was used for paired tumor: normal analysis to ensure only somatic variant calling. Copy number alterations were assessed using FACETS.[17]To determineT-cell receptor (TCR) betaandTCR gammagene rearrangement status,conserved regions within the variable (Vb), diversity (Db), and the joining (Jb) regions of TCR beta and gamma were amplified by PCR in the presence of fluorescently-labeled primers (InVivoScribe BIOMED-2), and the amplified products were detected by capillary electrophoresis on an ABI 3730 DNA analyzer (Thermo FisherScientific). Fluorescent in-situ hybridization (FISH) was further performed on correspondingFFPE tissue sections using a *JAK2*specific break-apart probe set (Cytocell/Oxford Gene Technology, Tarrytown, NY) following manufacturer's protocol. Immunohistochemical (IHC) staining for pSTAT3 was performed using M9C6antibody (Cell Signaling Technology, Danvers, MA) at 1:250 dilution on the Leica Bond III staining platform (Leica Biosystems Inc, Buffalo Grove, IL).

Results

The clinical features of the 9 patients examined in this cohort are provided in Table 1.DNA sequencing was successfully performed on all 9 cases with mean sequencing depthof

461X(range, 184X-678X).Thirty-foursomatic mutations were detected across26genesin 8 of 9 patients (89%), at a median variant allele frequency (VAF) of 10% (range, 2.3–76.9%). The most commonly mutated gene was *JAK1*, with point mutations involving codon G1097 (D,V or S) identified in 44% (4/9) of the cases. Overall, missense mutations were the most common type of alteration (26/34, 76%) followed by splice site(3/34, 9%), nonsense (3/34, 9%), and frameshift (2/34, 6%) mutations. In all, 78% (7/9) of the cases had a somatic alteration affecting genes in the *JAK/STAT* pathway including *JAK1*, *JAK2*, *STAT3*, *STAT5B* and *SOCS1*and 56% (5/9) had mutations in epigenetic modifiers (Table 2, Figure 1). An in-frame fusion involving exon 24 of *STAT3* and exons 19–25 of *JAK2* (Figure 2) was detected in 1 case. Analysis of copy-number aberrations (CNAs) showed several losses and gains as summarized in Table 2 and Figure 1.

Targeted RNA sequencing (RNASeq)confirmed the fusion transcript and precise breakpoints of the*STAT3-JAK2* rearrangement. No other fusions transcripts were detected by RNAseq on 3 additional cases while the remaining 5 cases could not be successfully sequenced due to low quantity of RNA recovered from the FFPE tissue samples.FISH studies using the *JAK2* break-apart probe confirmed the presence of a*JAK2*rearrangement with a split signal pattern (Figure 3A). No additional *JAK2* rearrangements were detected by FISH, however, 6of 9 (67%) cases showed extra signals consistent with copy number gains.

TCR gene rearrangement analysis by fragment analysis was successfully performed on all 9 patients and showed clonal rearrangement in 8 of the 9 (89%) cases. One case showed a prominent peak within a polyclonal background.

Immunohistochemical staining for pSTAT3 was performed in 7 of the 9 cases (including the patient with *STAT3-JAK2* fusion). Of the patients tested, 6/7(86%) showed strong and diffuse expression of pSTAT3.

Case description

The patient harboring the *STAT3-JAK2* fusion is a 69-year-old woman with history of invasive ductal carcinoma diagnosed in 2003 and treated with bilateral mastectomy and chemotherapy. Initial reconstructive surgery with placement of breast silicone implants was performed in 2004,followed by implant replacement in 2010. In 2018,she developed left breast swelling and a pericapsular effusion. Cytologic assessment of aspirated fluidrevealed atypical cells suspicious for lymphoma. Concurrent flow cytometric analysis confirmed the presence of abnormal cells with aberrant immunophenotype (18.9% of the total white cells),includingbright expression of CD30, CD45, and CD56, as well as increased forward and side scatter suggestive of larger size but without expression of CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD22, CD23, CD38, CD200, CD279, or FMC7. Subsequent complete capsulectomy confirmed involvement by BIA-ALCL (Figure 3B).[4,5] There was no mass formation, extracapsular extension or lymphadenopathy. Immunohistochemicalstains (Figure 3B) showed strong positivity for CD30andlack of ALK1 or pan-cytokeratin expression;pSTAT3 showed strong expression in the neoplastic cells.The patient is currently doing well on expectant monitoring with no adjuvant therapy given.

Discussion

In the present study, we report the sequencing results of 9 BIA-ALCL cases including, to our knowledge, the first fusion identified in this entity. To date, theoverall understanding of the genetic landscape of BIA-ALCL remains limited due to the relatively small number of cases studied. Based on review of literature, only 57 total cases have been previously studied using NGS, of which 24underwent whole exome sequencing[7–10,18,19] while others were studied using narrow targeted panels.

In keeping with prior reports, our study supports that the *JAK-STAT* pathway is frequently dysregulated in BIA-ALCL. Both activating and putative loss-of function mutations were identified within this pathway in 78% of cases. *JAK2* copy number gains were identified by FISH analysis in the remaining caseswhich could potentially elicit or enhance aberrant activation of the *JAK-STAT3* pathway.[20]Immunohistochemistryfor expression of pSTAT3 was also positive in all cases tested except for one.In contrast to prior studies, mutationsin the JH1 kinase domain of *JAK1,* rather than STAT3, were the most common variants in our cohort (44% of cases). Alterations involved codon G1097 exclusively (G1097D, G1097S and G1097V), all of which are well recognized gain-of-function mutations which trigger aberrant phosphorylation of *STAT3* downstream in hematologic malignancies. Other alterationsinvolving thispathwayincluded mutations in *STAT3, STAT5* and *SOCS1.*The *STAT3*mutation, S614R,has been previously described as the most common activating mutation in BIA-ALCL in all previous studies, while the *STAT5B* mutation is a variant not previously reported. A*SOCS1* truncating mutationwith putative loss-of-function effectwas also detected,predicting deregulation through to loss of inhibition on *JAK-STAT* signaling. Similar mutations in this gene have been identified in various types of lymphomas, including BIA-ALCL.[21–24]

Of particular interest, the*STAT-JAK2* fusion detected in this study was recentlyreported as a recurrent genetic alterationinindolent T-cell lymphoproliferative disorder of the gastrointestinal tract (GI TLPDs).[25]To date, only 4 cases have been reported in the literature, exclusively associated with GI TLPDs.The finding of a similar fusion in a similarly indolent process may support the potential role of these rearrangements in a distinct subset of less aggressive lymphoproliferative disorders. Similar to the previously reported fusions, the *STAT3-JAK2* rearrangement in BIA-ALCL retains the entire catalytic domain (JH1) of *JAK2*and shares the same *STAT3* RNA breakpoint. Recent functional studies performed by Hu et al, confirm this fusion to be oncogenic both in vitro and in-vivo using various models.[26]Coincidentally, to specifically assess function in human T-cell lymphoma model, this groupevaluated the effect of the fusion on the growth of TLBR-3 cells derived from a BIA-ALCL, which are dependent on IL2. Fusion expression rescued the cells from IL2 withdrawal causing marked independent growth in vitro as well as large subcutaneous tumor formation in-vivo on NSG mice. Studies also demonstrated that fusion expression activatesSTAT5 and constitutively enhances transcriptional activity within the *JAK-STAT* pathway. More importantly, preliminary work by the same group using various JAK inhibitors in-vitro and in an animal model, characterize this fusion as a potential targetable event with differential impact on the activity of the fusion protein.[26]

JAK2 fusions or rearrangements involving other partners have been identified in a variety of myeloid and precursor lymphoid neoplasms[27,28] but are rare in mature T-cell lymphomas.[29–32] In a previous study by Ehrentraut et al.,for example, the authors used a FISH break apart probe to assess for *JAK2* rearrangements in 200 patients with various T-cell lymphomas, including 53 patients with ALCL and ALCL cell lines, finding no evidence of *JAK2* fusions in any of the samples studied.[31]Although our combined assessment for fusions using DNA and RNA in this study was partially limited by the quality and quantity of RNA that could be effectively isolated from some of the samples, targeted FISH analysis did not identify any other *JAK2*rearrangement outside of our index case.

In addition to alterations involving the *JAK-STAT* pathway, somatic events involving epigenetic modifiers were the second most common alterations identified. Mutations or gene losses involving *TET2*, *TET3*, *ARID4B*, KDM5C, *KDM6A*, *KMT2C/D*, and *SMARCB1* were detected in 56% of cases. Alterations in epigenetic modifiers are common in T-cell lymphomas and have also been recently reported as common events in the largest series of BIA-ALCL studied by NGS.[19]

Taken together, while our current understanding of the genetics of BIA-ALCL still remains limited, thus far, a unifying mechanism of lymphomagenesis in this rare entity seems to be the activation of the *JAK-STAT* pathway which also offers possibilities for targeted treatment in patients with advanced disease. A more recent finding is the accumulation of alterations in epigenetic modifiers which are also common in other T-cell lymphomas. These findings also have important diagnostic and therapeutic implications. Diagnostically, the presence of mutations, while not necessarily diagnostic of the entity itself, could greatly facilitate the diagnosis of BIA-ALCL in conjunction with the morphologic and immunophenotypic findings and could also provide a molecular marker for staging and monitoring.

Although the majority of patients diagnosed with BIA-ALCL can be cured with complete capsulectomy alone, those that present with a mass lesion or disease beyond the capsule are considered high risk for recurrence and progression.[6]Within our small cohort, for instance, patients 4 and 8 presented with advanced disease, requiring systemic therapy and radiotherapy and one ultimately required stem cell transplant as outlined in table 1. Patients with advanced disease often constitute a therapeutic challenge for current treatment modalities, underscoring the need to explore alternate therapeutic options. At present, significant pre-clinical and early clinical data support the potential efficacy of therapies targeting the *JAK-STAT* pathway and epigenetic regulators, with several clinical trials currently underway,underscoring the importance of comprehensively evaluating patients for potential therapeutic targets.

An important and critical consideration for future studies, is the adequate preservation of material for broad molecular testing. Two recent publications by Jaffe, et al and Lyapichev, et aloutline key strategies in processing of theseroma fluid/effusion surrounding the implant and the handling of capsulectomy specimens following removal ofimplant(s).[33,34]Of note, based on our own experience, we find that while these guidelines effectively address the basic needs for morphologic and immunophenotypic assessment in clinical

practice, comprehensive molecular testing remains a challenge.Due to the nature of this disease,involvement of the capsule is often very focal, making it difficult to obtain sufficient tumor from FFPE capsuletissue. Alternatively, the effusion fluid may contain many more tumor cells than the capsule itself and could provide more material for downstream studies but the recommendations to prioritize the creation of a cell block over fresh material for flow cytometry and other studies, limit the amount and quality of material that can be recovered for molecular assays, particularly for RNA studies. In our small series, despite extensive efforts to maximize tumor and nucleic acid yield by using up to 40 sections of FFPE tissue, most samples could not be tested by RNA seq due to low quantity and quality of RNA. In light of the scant nature of the material, protocols designed for maximal preservation of fresh pericapsular fluid for molecular studies would be highly valuable to obtain high quality sequencing data.The concentration of the cellular content by centrifugation from larger volumesof pericapsular fluid would also greatly facilitate future studies.

Acknowledgments

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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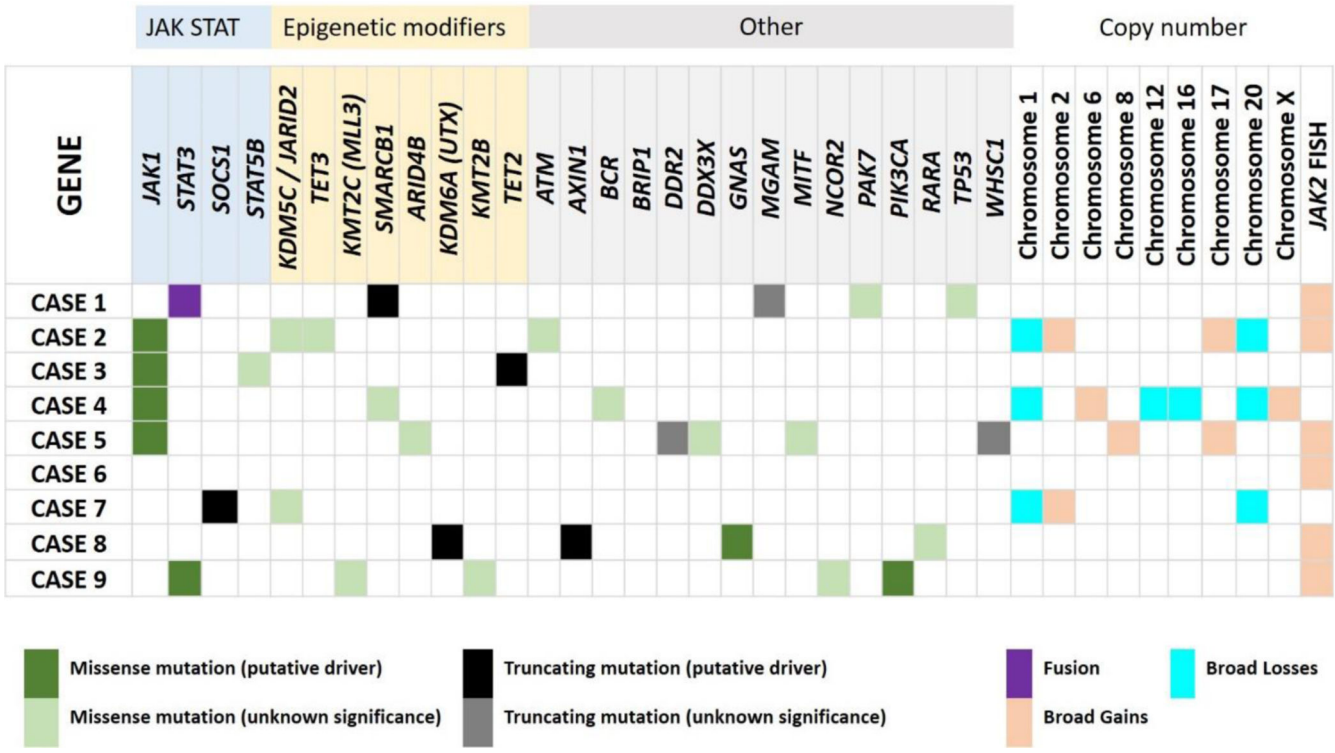

Figure 1.

Alterations detected in 9 patients with breast implant associated anaplastic large cell lymphoma. Detailed list of alteration in each case included in Table 2.

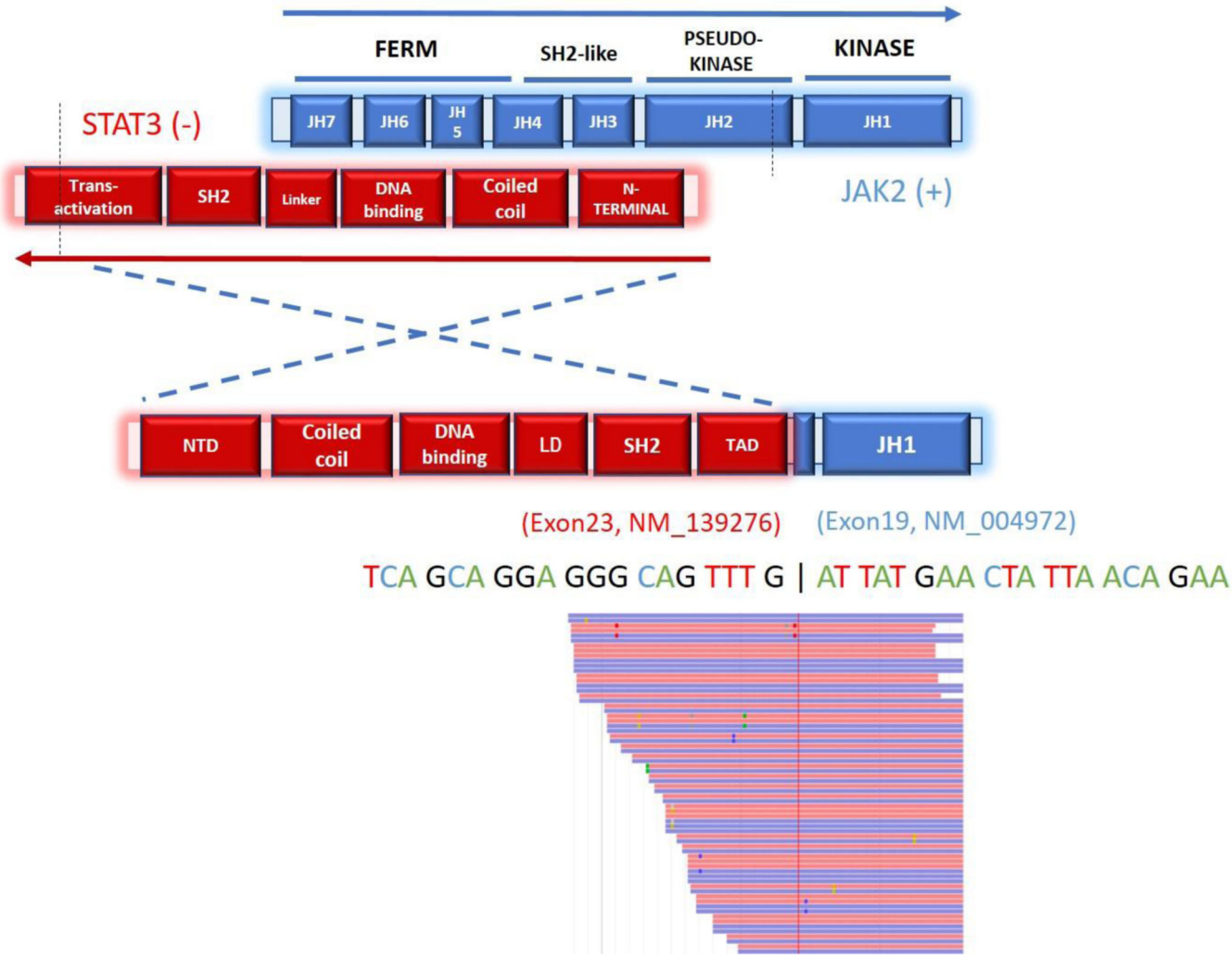

Figure 2.

Schematic illustration of the protein structure and transcript sequence of the *STAT3-JAK2* in-frame fusion product. Exons 1–23 of *STAT3* are fused to exons 19–25 of *JAK2* which include the kinase domain. Red and blue rectangles represent bi-directional RNA sequencing reads supporting the fusion breakpoint.

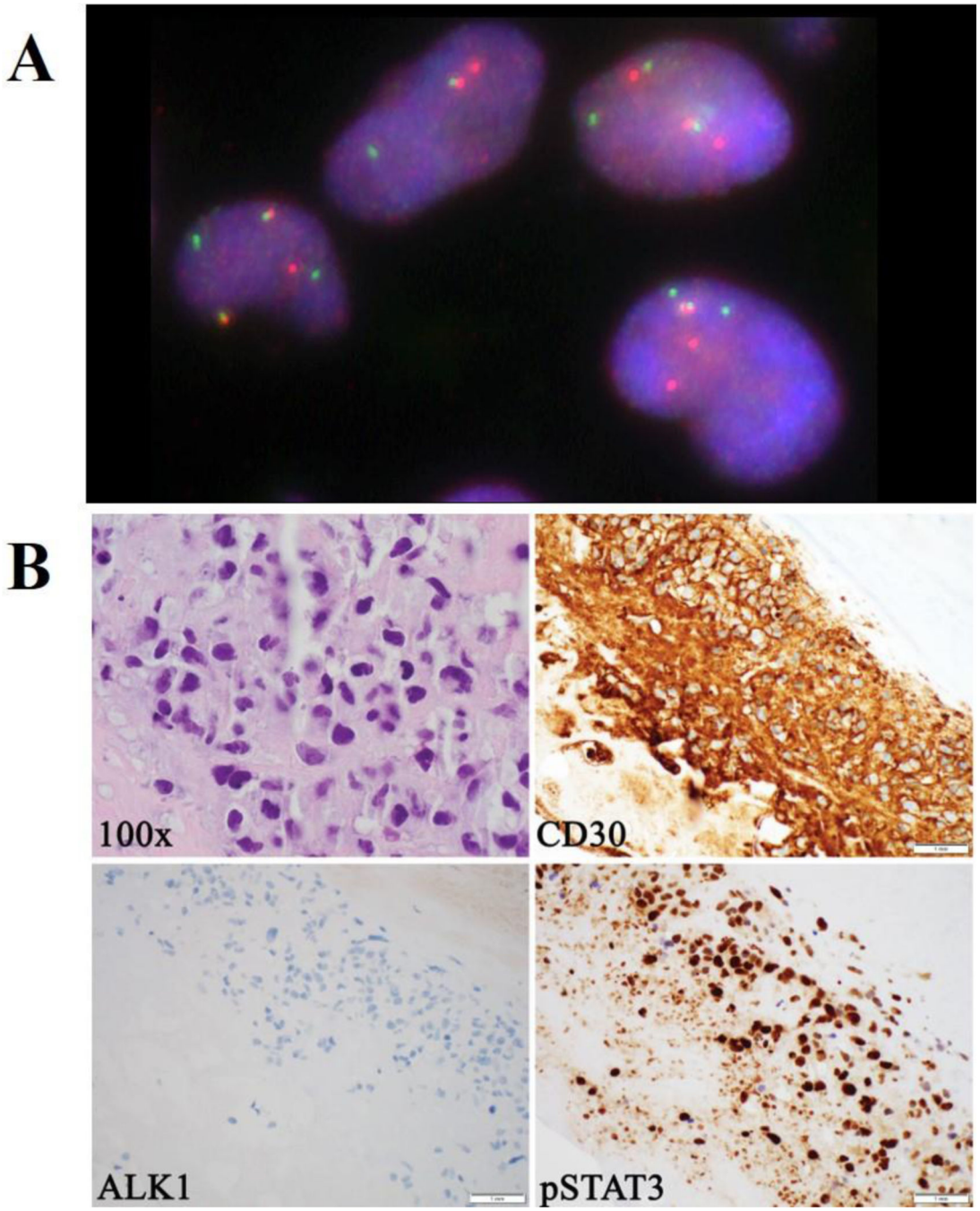

Figure 3.

A, Fluorescence in situ hybridization performed on FFPE using a *JAK2* specific break-apart probe set showing a split signal pattern, indicative of a *JAK2* rearrangement. B, Large anaplastic cells with irregular and multilobated nuclei with occasional “hallmark” cells (H&E, 1000x, oil immersion); the anaplastic cells strongly and uniformly express CD30 (400x); the cells lack expression of ALK1 (400x); the anaplastic cells show strong expression of pSTAT3 (400x).

Table 1.

Clinicopathologic features of 9 patients with breast implant associated anaplastic large cell lymphoma.

Patient	Age	Reason for Implant	Type of Implant	Number of Years after Implant	Presentation	Stage	Treatment	pSTAT IHC
1	68	IDC	Textured	8	Left breast swelling	T2	Bilateral complete capsulectomies	Positive
2	77	IDC	Textured	12	Right breast mass	T4, stage IIA	Excision and complete capsulectomy	Positive
3	61	IDC and ILC	Textured	8	Right breast swelling, effusion	T1 *	Bilateral complete capsulectomies	N/A
4	58	DCIS	Textured	6	Firmness above her left breast implant and change in shape of implant	T4N2M0	Capsulectomy and BV-CHP x 6 cycles + consolidative radiotherapy (3060 cGY in 17 fractions)	Positive
5	65	IDC	Textured	10	Right breast effusion	T1 *	Complete capsulectomy	Positive
6	35	Cosmetic	Textured	8	Right breast swelling, effusion	T1	Bilateral complete capsulectomies	N/A
7	51	IDC	Textured	6	Left breast swelling, effusion	T1, stage IA	Bilateral complete capsulectomies	Positive
8	66	IDC	Textured	11	Cutaneous lesion	T4	XRT (15 fractions, total 3000cGY)-, 6 cycles BV-CHOP, SCT	Negative
9	50	ILC	Textured	6	Left breast swelling, fevers, night sweats	T3, stage 1C	Bilateral complete capsulectomies	Positive

IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; DCIS, ductal carcinoma in situ; SCT, stem cell transplant; IHC, Immunohistochemistry;

* lymphoma confined to fluid only

Table 2.

Alterations detected by 400 gene hybrid capture next generation sequencing panel in 9 patients with breast implant associated anaplastic large cell lymphoma.

Patient	Gene Altered	Reference Sequence	Protein Change	cDNA Change	Location	Type of Alteration	VAF (%)	JAK2 FISH Results	FACETS Results
1	*TP53* *MGAM* *PAK7* *SMARCB1*	NM_000546 NM_004668 NM_177990 NM_003073	p.A307P p.X1449_splice p.P358R p.Q368*	c.919G>C c.4345+1G>A c.1073C>G c.1102C>T	exon 8 exon 36 exon 5 exon 8	Missense Splice Missense Nonsense	6.9 9.9 3.9 8.4	*JAK2* translocation positive; 70%, large cells, complex patterns	No calls
	STAT3-JAK2	NM_139276 NM_004972	t(17;9)(q21.1)			Fusion			
2	*ATM* *JAK1* *JARID2* *TET3* *TET3*	NM_000051 NM_002227 NM_004973 NM_144993 NM_144993	p.M900I p.G1097S p.V1019L p.V595L p.V787I	c.2700G>C c.3289G>A c.3055G>C c.1783G>T c.2359G>A	exon 18 exon 24 exon 14 exon 1 exon 4	Missense Missense Missense Missense Missense	5.1 27 8.6 15.8 17.1	Gain of extra copies; 3–5 signals, 50–60%	No calls
3	*TET2* *JAK1* *STAT5B*	NM_001127208 NM_002227 NM_012448	p.Q960* p.G1097D p.I174S	c.2878C>T c.3290G>A c.521T>G	exon 3 exon 24 exon 5	Nonsense Missense Missense	2.4 10.1 5.6	Normal	No calls
4	*BCR* *JAK1* *SMARCB1*	NM_004327 NM_002227 NM_003073	p.R162C p.G1097D p.D101N	c.484C>T c.3290G>A c.301G>A	exon 1 exon 24 exon 3	Missense Missense Missense	34.4 41.4 25.5	Normal	Gain:1p32–36, 2p (including *ALK*, *REL*), 2q11–35, 6p, 17q11–21, Xq25, Xq26.1, Xq26.2 (including *STAG2*, *BCORL1*, and *PHF6*) CNLOH: SP140 Loss: on 1p (*JAK1*, *BCL10*), 12q13 (including *ARID2*, *HDAC7* and *KMT2D/MLL2*), 16q23 (including *PLCG2*, *IRF8* and *FANCA*), 20p and 20q12–13, Xq12 (AR)
5	*ARID4B* *BRIP1* *DDR2* *DDX3X* *JAK1*	NM_016374 NM_032043 NM_006182 NM_001356 NM_002227 NM_198159 NM_001042424	p.P134A p.G763A p.X189_splice p.G533E p.G1097V p.E303V p.X961_splice	c.400C>G c.2288G>C c.566–2A>G c.1598G>A c.3290G>T c.908A>T c.2882–28_2895del	exon 7 exon 16 exon 7 exon 14 exon 24 exon 7 exon 16	Missense Missense Splice Missense Missense Missense Splice	11.6 33 9.7 13.4 36 7 11.3	Gain of extra copies; 3–4 signals, 30–40%	Gain: 8q, 17q

Patient	Gene Altered	Reference Sequence	Protein Change	cDNA Change	Location	Type of Alteration	VAF (%)	JAK2 FISH Results	FACETS Results
	MITF *WHSC1*								
6	None	N/A	N/A	N/A	N/A	N/A	N/A	Gain of extra copies; 3–5 signals, 60–80%	No calls
7	*JARID2* *SOCS1*	NM_004973 NM_003745	p.A700V p.S32Ffs*44	c.2099C>T c.95_142delins TCCTTCCTCCTCCTCGCCCG	exon 8 exon 2	Missense Frameshift, deletion	37.9 76.9	Gain of extra copies; 3 signals, 10–20%	Gain: 2p, 2q11–35, 17q11–21, ASXL1 (20q11.21) CNLOH: HDAC4, chr22q Loss: 1p22.3-p12, 20p, 20q12–13
8	*AXIN1* *GNAS* *KDM6A* *RARA*	NM_003502 NM_000516 NM_021140 NM_000964	p.Q476* p.R201C p.V1113Sfs*8 p.R272W	c.1426C>T c.601C>T c.3334_3335dupGT c.814C>T	exon 6 exon 8 exon 23 exon 7	Nonsense Missense Frameshift, insertion Missense	2.3 2.4 14.2 41.1	Gain of extra copies; 3–4 signals, 40–50%	No calls
9	*PIK3CA* *STAT3* *KMT2B* *KMT2C* *KMT2C* *NCOR2*	NM_006218 NM_139276 NM_014727 NM_170606 NM_170606 NM_006312	p.H1047R p.S614R S1739F L596V S593L S1518L	c.3140A>G c.1842C>G c.5216C>T c.1786C>G c.1778C>T c.4553C>T	exon 21 exon 20 exon 25 exon 13 exon 13 exon 34	Missense Missense Missense Missense Missense Missense	3.2 7.7 14.3 5.9 6.1 5.2	Gain of extra copies; 3–4 signals, 50–60%	No calls

VAF, variant allele frequency; Chr, chromosome; CNLOH, copy neutral loss of heterozygosity; FISH, fluorescence in situ hybridization.