Whole exome sequencing reveals activating *JAK1* and *STAT3* mutations in breast implant-associated anaplastic large cell lymphoma anaplastic large cell lymphoma

Systemic anaplastic large cell lymphoma (sALCL) is an aggressive T-cell non Hodgkin lymphoma which is divided into two categories based on the expression of the anaplastic lymphoma kinase (ALK) protein (i.e. ALK-positive ALCL and ALK-negative ALCL). ALK-negative sALCL typically presents with advanced-stage nodal or extranodal disease with B-symptoms and has a poor clinical outcome with conventional therapies.[1] The association between ALK-negative ALCL arising in the fluid and capsule around breast prostheses has led to the recognition of a distinct clinicopathological entity termed breast implant-associated ALCL (BIA-ALCL).[2,3] This relatively new disease entity has numerous unique features, including the limitation of malignant infiltration to a peri-prosthetic effusion in the majority of patients and a favorable clinical outcome.[4,5]

One of the central pathogenic drivers of sALCL is aberrant activation of STAT3. In addition, STAT3 activation has been demonstrated in BIA-ALCL patient samples by immunohistochemistry.[6] Whilst STAT3 activation is the direct result of the chimeric ALK protein in ALK-positive ALCL, multiple genetic lesions appear to be responsible for STAT3 activation in ALK-negative sALCL, including activating mutations in *JAK1*, *STAT3* and fusion proteins involving the tyrosine kinases TYK2 and ROS1.[7-9] Despite this recent molecular characterization of sALCL, the genetic lesions present in BIA-ALCL as an individual entity are unknown. We performed whole exome sequencing (WES) on two patients with effusion-limited BIA-ALCL with the aim of characterizing this unique entity and to provide insights into the genetic lesions present in this rare disease.

Informed consent was obtained from patients for the analysis of tumor/germline specimens. DNA libraries were prepared using Agilent SureSelect XT Human All Exon V5 and sequenced on an Illumina HiSeq 2500. WES data was processed and analyzed using an in-house bioinformatics pipeline (see *Online Supplementary Methods*). Somatic variants associated with a frameshift, in-frame indel, start/stop codon change, missense change or canonical splice site were kept, and variants from known highly polymorphic genes were excluded. The sequence alignments for all remaining variants were visually inspected and artefacts excluded.

Case 1. A 42 year old woman presented with right breast swelling approximately three-and-a-half years after the insertion of custom-made, high-profile, saline-filled salt-loss textured silicone implants (Nagor, Glasgow, UK). Breast MRI showed a moderate-sized peri-prosthetic effusion. Fluid aspirated from this effusion showed numerous large atypical appearing cells which were: $CD2^-$, $CD3^+$, $CD4^+$, $CD5^-$, $CD7^-$, $CD8^+$, $CD30^+$, and ALK-, by immunohistochemistry. She underwent bilateral removal of the implants and was found to have involvement of the right effusion fluid by lymphoma without infiltration of the pseudocapsule. Staging bone marrow biopsy and FDG-PET scans showed no evidence of systemic disease (Stage IA (T1N0M0)[5]). The patient was treated with radiotherapy to the right breast and remains in remission with no evidence of recurrence at six years follow-up. Further clinicopathological features of this case have been previously described.[10]

WES was performed on DNA extracted from the effusion cytology fluid and germline DNA (uninvolved bone marrow) yielding a mean target base coverage of 113x (tumor) and 135x (germline). After filtering, 51 non-synonymous somatic variants were detected (*Online Supplementary Table S1*). Copy number analysis revealed multiple somatic alterations. Selected variants and copy number changes are detailed in Table 1, and copy number data are presented in Figure 1.

All variants detected were considered variants of uncertain significance (VOUS) except for a variant detected in *STAT3* (NM_139276.2:c.1840A>C, p.S614R). The *STAT3* S614R results in enhanced transcriptional activity of STAT3 and has been previously observed in a range of T-cell and NK lymphoproliferative disorders.[11-13] Of note, primary tumor cells from this patient had previously been used to establish the first model cell line of BIA-ALCL (TLBR-1), which shows significantly increased STAT3 activation/phosphorylation as well as cell death when exposed to STAT3-specific inhibitors.[14]

Case 2. A 56 year old woman presented with a three month history of breast swelling approximately seven years after insertion of anatomic, salt-loss textured silicone-filled breast implants (Allergan, NJ, USA). Fluid aspirated from the left breast showed large atypical cells

Table 1. Selected variants and focal copy number changes from exome sequencing of two cases of effusion-limited breast implant-associated anaplastic large cell lymphoma.

	Origin	Comment
Case 1		
STAT3 S614R (NM_139276.2; c.1840A>C)	Somatic	Pathogenic mutation leading to enhanced STAT3 activation
1p copy number loss*	Somatic	Focal deleted region containing tumor suppressor gene *RPL5*
10p copy number loss*	Somatic	Focal deleted region containing tumor suppressor gene *GATA3*
19p copy number gain*	Somatic	Focal gained region containing JAK family kinase *TYK2*
Case 2		
JAK1 G1097V (NM_002227.2:c.3290_3291 delinsTT)	Somatic	Frequently mutated codon in ALK-negative systemic ALCL. Other amino acid changes at this site result in constitutive JAK1 activation and enhanced STAT3 signaling
JAK3 V722I (NM_000215.3:c.2164G>A)	Germline	Population frequency 0.86% in ExAC**. Somatic mutations associated with enhanced STAT3 activation.

**See Figure 1. **Exome Aggregation Consortium (http://exac.broadinstitute.org/).*[26]

which were: CD2$^-$, CD3$^-$, CD4$^+$, CD5$^+$, CD7$^-$, CD8$^-$, CD30$^+$, and ALK$^-$, by immunohistochemistry. Histological examination showed disease confined to the effusion with no evidence of infiltration of the pseudocapsule. (Stage IA (T1N0M0)[5])(Figure 2A). She underwent removal of the implant with no other local or systemic therapy, and remains clinically well with no evidence of recurrence at six months follow-up.

WES was performed on DNA extracted from effusion cytology fluid yielding a mean target base coverage of 145x (tumor) and 103x (germline). After filtering, 24 non-synonymous somatic variants were detected (*Online Supplementary Table S2*). No copy number changes were detected.

All somatic variants detected were considered VOUS except for a variant detected in *JAK1* (NM_002227.2:c.3290_3291delinsTT, p.G1097V). The *JAK1* G1097V occurs in the kinase domain of JAK1. Multiple amino acid substitutions have been observed at this site in ALK-negative sALCL which have been shown to be associated with increased levels of pSTAT3 *in vitro*,[7] suggesting that the G1097V is likely to be a pathway activating mutation. pSTAT3 immunohistochemistry was performed in this case and showed strong staining of tumor cell nuclei (Figure 2B).

A germline *JAK3* variant (NM_000215.3:c.2164G>A, p.V722I) was also detected in this patient. The *JAK3* V722I variant occurs in the pseudokinase domain and has been hypothesized to disrupt its interaction with the kinase domain, resulting in constitutive activation of the JAK3 protein.[15,16] Expression of the *JAK3* V722I leads to transformation, cytokine independence and sensitivity to JAK3 inhibitors *in vitro*.[16] Although the *JAK3* V722I has been observed as an acquired variant in extranodal NK/T-cell lymphoma and other hematological malignancies,[15-17] it also occurs at approximately 0.5-1% in population SNP databases (without a clear associated clinical phenotype).[26] Of note, concomitant acquired activating *JAK1* and *JAK3* mutations have been shown to have synergistic effects on STAT3 activation in cell lines.[18]

Aberrant JAK/STAT3 signaling has an established role in inflammation-associated cancers.[19] One model of BIA-ALCL pathogenesis hypothesizes the stimulation of malignant lymphocyte clones by chronic inflammation induced by either the implant contents or its surface characteristics/biofilm.[20-23] The observation of variants leading to aberrant STAT3 activation in our cases is consistent with this model. Of note, the presence of JAK/STAT3 activating variants in our cases were compatible with prolonged remission when treated with local therapy alone (surgery +/- radiotherapy without systemic chemotherapy), which suggests that additional modifying disease factors (genetic or otherwise) are present in "typical" ALK-negative sALCL in order to give rise to the markedly inferior outcomes observed.

In the context of a chronic inflammatory stimulus, underlying host genetic factors play a role in influencing the likelihood of malignant lymphoid transformation.[24,25] The presence of a rare activating germline *JAK3* variant (V722I) in our case may have provided such a genetic predisposition. Moreover, the observation of combined *JAK1/JAK3* mutations is highly reminiscent of the finding of co-occurrence of acquired activating mutations in *JAK1* and *STAT3* in the same tumor in a significant proportion of patients with ALK-negative sALCL, which is hypothesized to be related to a selective advantage afforded by the synergistic effect of combined mutations.[7]

In summary, we have identified acquired activating mutations in *JAK1* and *STAT3* in two cases of effusion-limited BIA-ALCL and identified a possible contribution to disease development from a germline *JAK3* variant. Further investigation in a larger cohort is required in order to determine the exact incidence of *JAK1/STAT3* mutations in BIA-ALCL as well as any predisposing genetic factors. The aberrancy in the JAK/STAT3 pathway implicated in our cases supports the current inflam-

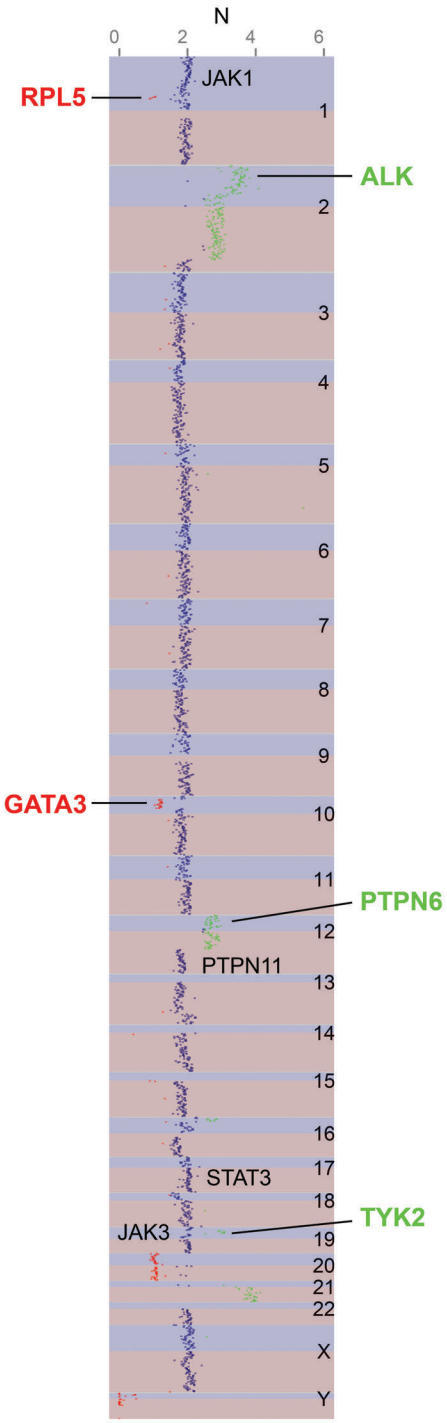

Figure 1. Whole genome copy number changes in a case of BIA-ALCL. Number of genome copies (N) shown by chromosome with diploid regions shown in blue and copy number gains and losses indicated in green and red, respectively. Selected genes of relevance to JAK/STAT signaling and tumor suppressors in sites of focal copy number loss are also indicated.

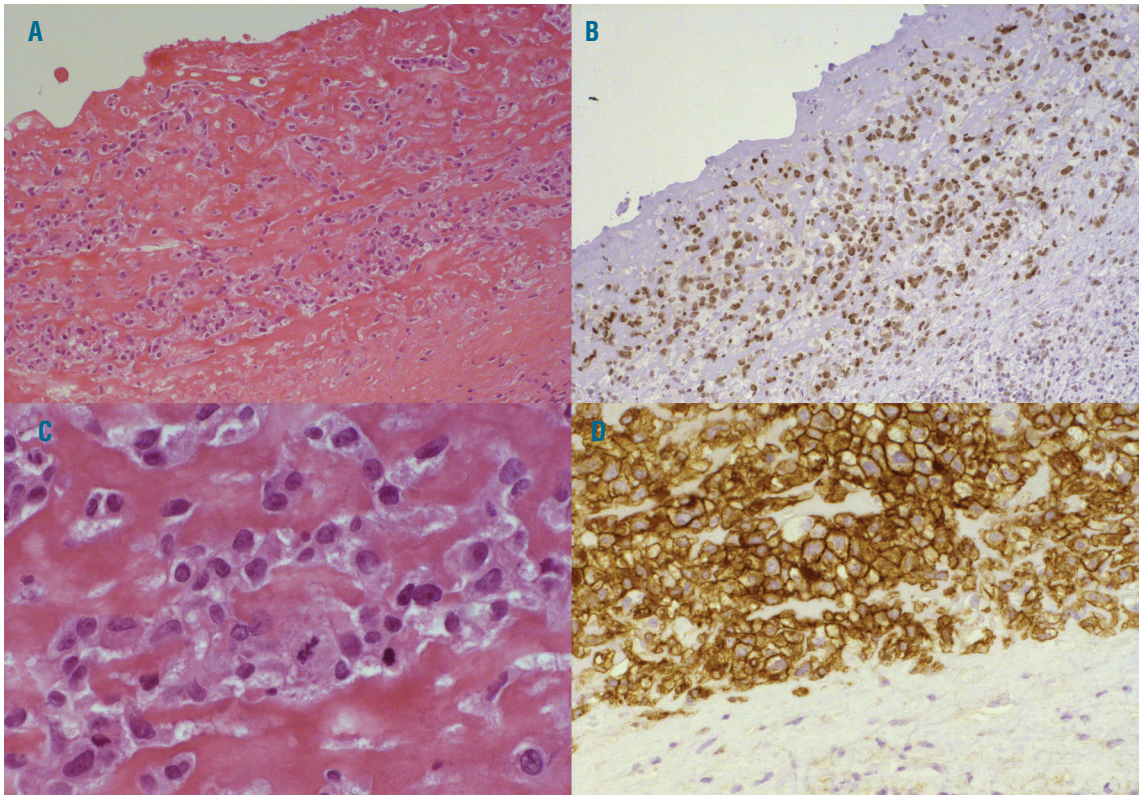

Figure 2. (A) Histology from Case 2 demonstrating large anaplastic tumor cells within the effusion fluid (Hematoxylin and eosin stain (H&E), x100) (B) pSTAT3 immunohistochemical staining showing positive staining in anaplastic tumor cell nuclei (x100) (C) Anaplastic tumor cells (H&E, x400) (D) CD30 immunohistochemical staining showing membrane staining in anaplastic tumor cells.

matory model of pathogenesis and suggests that, despite the unique clinicopathological features of BIA-ALCL compared to systemic ALK-negative ALCL, the fundamental driving genetic lesions between these two entities are similar.

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