

## Targeted next generation sequencing of breast implant-associated anaplastic large cell lymphoma reveals mutations in JAK/STAT signalling pathway genes, *TP53* and *DNMT3A*

Breast implant-associated anaplastic large cell lymphoma (BI-ALCL) is an uncommon neoplasm occurring in women with either cosmetic or reconstructive breast implants (Clemens *et al*, 2016). Until now, most studies have focused on defining the clinico-pathological features of BI-ALCL, leading to its inclusion as a new provisional entity, a subtype of anaplastic lymphoma kinase (ALK)-negative ALCL, in the revised World Health Organization classification of lymphoid malignancies (Swerdlow *et al*, 2016). BI-ALCL is characterized by the presence of CD30<sup>+</sup> large atypical lymphocytes frequently confined to the peri-implant seroma fluid. Nevertheless, solid infiltrating masses and cases pursuing an aggressive clinical course have been reported. The surgical and pathological staging system designed by Clemens *et al* (2016) suggests that BI-ALCL has a pattern of progression similar to that of solid tumours rather than non-Hodgkin lymphomas, and that the effusion- and solid-types might represent different stages of the same disease rather than two distinct variants.

The molecular pathogenesis and mechanisms of progression of BI-ALCL, however, remain largely unknown, thus limiting the identification of biomarkers that enable disease prognostication and optimal treatment. Hence, we performed targeted next generation sequencing of seven BI-ALCL, identified in the archives of three institutions over 7 years, to investigate the presence of underlying somatic mutations. Informed consent was obtained from patients and the study was performed in accordance with the Declaration of Helsinki. DNA extracted from micro-dissected tumour cells of formalin-fixed paraffin-embedded BI-ALCL samples (QIAamp DNA Mini kit; Qiagen, Germantown, MD, USA) was used to prepare DNA libraries (Sureselect kit; Agilent Technologies, Santa Clara, CA, USA). Sequencing was performed on a HiSeq2500 (Illumina, San Diego, CA, USA) using a panel of 465 cancer-associated genes (Table S1). The sequence data were aligned to the human reference genome (hg19) and variants were identified using NextGENE (SoftGenetics, State College, PA, USA). The average read depth of the samples was 400× (Table SII). Somatic mutations were identified by comparison of variants detected in lymphoma with those from matched constitutional DNA. Common variants (>1% frequency) present in the 1000 genomes database, and the database of Columbia University were removed. Somatic mutations were classified using the

prior literature, and two different prediction algorithms (SIFT <http://sift.bii.a-star.edu.sg> and Polyphen-2 [PP2] <http://genetics.bwh.harvard.edu/pph2/>). The exonic somatic variants were confirmed by bidirectional Sanger sequencing using Big-Dye terminators v3.1 (Applied Biosystems, Carlsbad, CA, USA).

The clinical and pathological features of the patients are summarized in Table I. Informative results were obtained in five of seven cases (Table SII); analysis failed in two cases due to the poor quality of DNA. Five somatic variants affecting four genes were identified in two cases: one intronic and four within coding regions (Fig 1 and Table SIII).

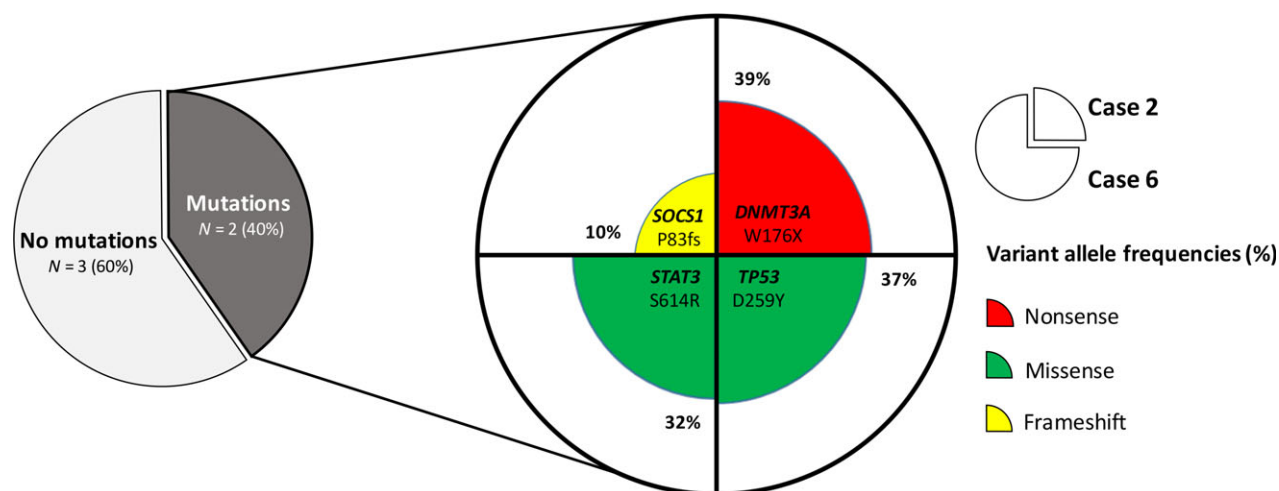
A *STAT3* missense variant (p.S614R) affecting the SH2 domain, which mediates *STAT3* dimerization, was detected in one of these two BI-ALCLs. JAK/STAT signalling is implicated in cell proliferation, differentiation and apoptosis, and aberrant activation of *STAT3* has been reported in several human cancers associated with persistent immune stimulation and/or inflammation. Notably, the gain-of-function mutation (S614R) was recently described in one BI-ALCL (Blombery *et al*, 2016), and has been reported in angioimmunoblastic T cell lymphomas, chronic lymphoproliferative disorders of natural killer cells, and T-cell large granular lymphocyte leukaemias (Odejide *et al*, 2014). Moreover, gain-of-function mutations in *STAT3* have been reported in 18% of systemic ALK-negative ALCLs and 5% of cutaneous ALCLs (Crescenzo *et al*, 2015). An *in vitro* study using BI-ALCL-derived cell lines also showed activation of the JAK/STAT pathway through autocrine production of interleukin 6, suggesting a possible pathogenic mechanism (Lechner *et al*, 2012).

A frameshift deletion causing a premature stop codon in *SOCS1* (p.P83Rfs\*20) was detected in the BI-ALCL harbouring the *STAT3* mutation. *SOCS1* is a negative feedback regulator of the JAK/STAT pathway. The p.P83Rfs\*20 mutation deletes the C-terminal SOCS box domain and partially deletes the SH2 domain, which downregulates the kinase activity of JAK. Loss-of-function mutations of *SOCS1*, leading to constitutive activation of JAK/STAT signalling, have been described in B-cell lymphomas and in classical Hodgkin lymphomas (Mottok *et al*, 2009). Moreover, *SOCS1* was found to be silenced by miR-155 in ALK-negative ALCL (Merkel *et al*, 2015). Mutations in *STAT3* and *SOCS1* suggest that deregulated activation of the JAK/STAT pathway may contribute to the development of BI-ALCL.

Table 1. Clinical and pathological features of breast implant-associated anaplastic large cell lymphoma.

Case ID	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Age at diagnosis (years)	47	54	72	49	35	47	68
Years between implant and BL-ALCL	6	5	14	8	4	n/a	16
Side	Left	Right	Right	Left	Left	Left	Right
Reason for implant	Reconstruction for left-sided infiltrating ductal breast carcinoma	Reconstruction for right-sided infiltrating ductal breast carcinoma	Cosmetic	Reconstruction for left-sided infiltrating lobular breast carcinoma	Cosmetic	Reconstruction for left-sided breast cancer	Reconstruction for right-sided infiltrating ductal breast carcinoma
Type of implant	BIOCELL <sup>®</sup> textured silicone gel-filled (Inamed); contralateral breast augmentation with BIOCELL <sup>®</sup> textured silicone gel-filled (Inamed)	BIOCELL <sup>®</sup> textured silicone gel-filled (McGhan)	1999 = BIOCELL <sup>®</sup> textured silicone gel-filled (Inamed) 2007 = polyurethane-coated silicone (Polytech Silimed) 2013 = polyurethane-coated silicone (Polytech Silimed)	BIOCELL <sup>®</sup> textured silicone gel-filled (Inamed)	Polyurethane-coated silicone (Polytech Silimed)	n/a	BIOCELL <sup>®</sup> textured silicone gel-filled (McGhan)
Clinical presentation	Left-side effusion and contralateral axillary lymphadenopathy	Effusion	Right-side effusion	Effusion	Left-side effusion	Left-side effusion and contralateral enlarged axillary lymph node	Right-side effusion and contralateral axillary lymphadenopathy
Immunophenotype	CD30 <sup>+</sup> , ALK <sup>-</sup> , CD8 <sup>-</sup> , GrB <sup>+/-</sup> , CD4 <sup>+</sup> , CD3 <sup>-</sup> , CD15 <sup>+/-</sup> , MUM1 <sup>+</sup> , CD25 <sup>+</sup>	CD30 <sup>+</sup> , ALK <sup>-</sup> , CD8 <sup>-</sup> , GrB <sup>+/-</sup> , CD4 <sup>+</sup> , CD3 <sup>-</sup> , CD15 <sup>+/-</sup> , MUM1 <sup>+</sup> , CD25 <sup>+</sup>	CD30 <sup>+</sup> , ALK <sup>-</sup> , CD8 <sup>+</sup> , GrB <sup>+</sup> , CD4, CD3 <sup>-</sup> , CD15 <sup>-</sup> , MUM1 <sup>+/-</sup> , CD25 <sup>+</sup>	CD30 <sup>+</sup> , ALK <sup>-</sup> , CD8 <sup>-</sup> , GrB <sup>-</sup> , CD4 <sup>+</sup> , CD3 <sup>+/-</sup> , CD15 <sup>-</sup> , CD25 <sup>+/-</sup>	CD30 <sup>+</sup> , ALK <sup>-</sup> , CD8 <sup>-</sup> , GrB <sup>+/-</sup> , CD4 <sup>-</sup> , CD3 <sup>+/-</sup> , CD15 <sup>-</sup>	CD30 <sup>+</sup> , ALK <sup>-</sup> , CD3 <sup>-</sup> , PAX5 <sup>-</sup>	CD30 <sup>+</sup> , CD15 <sup>+</sup> , EMA <sup>+</sup> , MUM1 <sup>+</sup> , CD2 <sup>+</sup> and CD4 <sup>+</sup> , CD45 <sup>+/-</sup> , CD3 <sup>-</sup> , CD5 <sup>-</sup> , CD7 <sup>-</sup> , CD8 <sup>-</sup> , ALK <sup>-</sup> , CD56 <sup>-</sup> , CD20 <sup>-</sup> , PAX5 <sup>-</sup> , CD79a <sup>-</sup> , OCT2 <sup>-</sup> , CD138 <sup>-</sup> , p53 <sup>-</sup> , TIA-1 <sup>-</sup> , Granzyme-B <sup>-</sup> , Perforin <sup>-</sup>
TRG gene rearrangement	Polyclonal	Monoclonal	Monoclonal	Monoclonal	Monoclonal	Monoclonal	Monoclonal
BL-ALCL stage*	T1N1M0	T1N0M0	T1N0M0	T2N0M0	T2N0M0	T1N1M0	T3N2M0

\*TNM (size or direct extent of the primary tumour; degree of spread to regional lymph nodes; presence of distant metastasis) Staging for breast implant-associated anaplastic large cell lymphoma (BL-ALCL) proposed by Clemens *et al.* (2016). Inamed and McGhan implants are manufactured by Inamed Corp. (Goleta, CA, USA); Polytech Silimed implants are manufactured by Silimed (Rio de Janeiro, Brazil).



**Fig 1.** Somatic mutation frequency and spectrum in five breast implant-associated ALK-anaplastic large cell lymphomas evaluated by targeted next-generation sequencing. Exonic variants affecting four genes were identified in two out of five cases (Cases 2 and 6, Table I). In Case 2 a nonsense mutation of *DNMT3A* was detected, while Case 6 harboured missense mutations in *STAT3* and *TP53* as well as a frameshift mutation in *SOCS1* (variant allele frequencies are shown in each quadrant).

A missense mutation of *TP53* (p.D259Y) affecting the DNA binding domain was also observed in the aforementioned case. Although *TP53* mutations are uncommon in peripheral T-cell lymphomas (PTCLs) (Odejide *et al*, 2014), p.D259Y has been documented in several solid tumours (Petitjean *et al*, 2007).

The second BI-ALCL case with a somatic variant harboured a nonsense mutation in *DNMT3A* (p.W176X). *DNMT3A* is a DNA methyltransferase required for genome-wide *de novo* methylation. Mutations in *DNMT3A*, mostly impairing *DNMT3A* catalytic activity, have been reported in 8–22% of myeloid neoplasms and 33% of PTCLs (Odejide *et al*, 2014; Yang *et al*, 2015). Recent studies have identified *DNMT3A* mutations in pre-leukaemic haematopoietic stem cells (HSCs) within the founding clones of acute myeloid leukaemias (Yang *et al*, 2015), in CD34<sup>+</sup> progenitors of T-cell lymphomas (Odejide *et al*, 2014) and in 16% of early T-cell precursor acute lymphoblastic leukaemias (ALLs), a subgroup of T-lineage ALLs characterized by specific stem cell and myeloid features (Yang *et al*, 2015). It has been speculated that *DNMT3A*-mutant HSCs are predisposed to undergo neoplastic transformation upon acquiring mutations in other genes, which cooperate with *DNMT3A* mutations, fostering the development of distinct haematological malignancies. Our finding suggests that *DNMT3A* mutations in BI-ALCL might contribute to malignancy by deregulating the DNA methylation landscape of neoplastic T-cells or their precursors.

No somatic mutations were identified in three out of five evaluable cases. Nevertheless, it remains plausible that, similar to observations in systemic and cutaneous ALCL (Crescenzo *et al*, 2015), deregulation of the JAK/STAT pathway via the generation of fusion transcripts might occur in subsets of non-mutated BI-ALCLs.

Altogether our results confirm that mutations in JAK-STAT pathway genes occur in BI-ALCL, with the identification of *SOCS1* mutation for the first time in this disease and *TP53* and *DNMT3A* mutations as additional somatic events. Our findings add to the nascent literature regarding the genetic alterations underlying BI-ALCL and suggest further exploration of the involved molecular pathways in this malignancy.

### Authorship contributions

A.D.N. and G.B. designed experiments, analysed the data and wrote the paper; M.M., P.J. and E.M. analysed the data and wrote the paper; E.D. performed experiments; W.A. performed bioinformatic analysis, A.D.N., G.B., B.A., F.F., E.M. and F.S.D.P. collected the samples and reviewed histology.

### Conflict-of-interest disclosure

All the authors declare that they have no conflict of interest.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table SI.** Genes included in targeted next-generation sequencing panel.

**Table SII.** Coverage statistics for targeted next-generation sequencing assay.

**Table SIII.** Somatic variants in BI-ALCL.

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