blood

American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
editorial@hematology.org

Gene alterations in epigenetic modifiers and JAK-STAT signaling are frequent in breast implant-associated ALCL.

Tracking no: BLD-2019-001904R2

Camille Laurent (CHU Toulouse, IUCT Oncopole, Centre de Recherche en Cancerologie de Toulouse Inserm UMR 1037,, France) Alina Nicolae (CHU Hautepierre, France) Cécile Laurent (Institut Carnot CALYM, France) Fabien Le Bras (Hopital Henri Mondor, France) Corinne Haioun (CHU Henri Mondor,) Virginie Fataccioli (Hopital Henri Mondor, France) Nadia Amara (CHU Toulouse, IUCT Oncopole, France) José Adélaïde (CRCM, France) Arnaud Guille (Institut Paoli-Calmettes, Centre de Recherche en Cancérologie de Marseille, Inserm U1068, CNRS UMR7258, Aix-Marseille University, UM105, France) Jean-Marc Schiano (Institut Paoli Calmettes, France) Bruno Tesson (Institut Carnot CALYM, France) Alexandra Traverse-Glehen (Hospices Civils de Lyon, Centre Hospitalier Lyon Sud, France) Marie-Pierre Chenard (Département de Pathologie, Hôpitaux Universitaires de Strasbourg,) Lénaïg Mescam (Institut Paoli-Calmettes, France) Anne Moreau (CHU NANTES, France) Catherine Chassagne-Clement (Centre Leon Berard, France) Joan Somja (CHU de Liège, Belgium) Fréderic Escudié (CHU Toulouse, IUCT Oncopole, France) Marc André (CHU UCL Namur, Belgium) Nadine Martin (Hôpital Henri Mondor, France) Laetitia Lacroix (Hôpital Henri Mondor, France) François Lemonnier (INSERM U955, France) Anne-Sophie Hamy-Petit (Institut Curie, RT2Lab, INSERM, U932, PSL Research University, France) Fabien Reyal (Institut Curie, France) Marie Bannier (Institut Paoli-Calmettes, France) Lucie Oberic (IUCT - Oncopole, Toulouse, France) Nais Prade (CHU Toulouse, France) François-Xavier Frénois (IUCT Oncopole, France) Asma Beldi-Ferchiou (APHP, MONDOR, France) Marie-Helene Delfau-Larue (APHP, GH MONDOR, UPEC university, France) Reda Bouabdallah (Institut Paoli-Calmettes, France) Daniel Birnbaum (INSERM UMR1068, France) Pierre Brousset (IUCT-Oncopole, France) Luc Xerri (Institut Paoli-calmettes, AMU, CRCM, France) Philippe Gaulard (Hopital Henri Mondor, AP-HP, Creteil, France, France)

Abstract:
The oncogenic events involved in breast implant-associated anaplastic large cell lymphoma (BI-ALCL) remain elusive. To clarify this point, we have characterized the genomic landscape of 34 BI-ALCLs (15 tumor, 19 *in situ* subtypes) collected from 54 BI-ALCL patients diagnosed through the French *Lymphopath* network. Whole exome sequencing (n=22, with paired tumor/germline DNA) and/or targeted deep sequencing (n=24) showed recurrent mutations of epigenetic modifiers in 74% of cases, involving notably *KMT2C* (26%), *KMT2D* (9%), *CHD2* (15%) and *CREBBP* (15%). *KMT2D* and *KMT2C* mutations correlated with a loss of H3K4 mono- and tri-methylation by immunohistochemistry. Twenty cases (59%) showed mutations in at least one member of the *JAK/STAT* pathway including *STAT3* (38%), *JAK1* (18%), *STAT5B* (3%) and in negative regulators like *SOCS3* (6%), *SOCS1* (3%) and *PTPN1* (3%). These mutations were more frequent in tumor-type than *in situ* samples (p=0.038). All BI-ALCLs expressed pSTAT3, regardless of the mutational status of genes in *JAK/STAT* pathway. Mutations in *EOMES* gene (12%) involved in lymphocytes development, *PI3K-AKT/mTOR (6%)* and loss of function mutations in *TP53* (12%) were also identified. Copy number aberration (CNA) analysis identified recurrent alterations including gains on chromosomes 2, 9p, 12p and 21 and losses on 4q, 8p, 15, 16 and 20. Regions of CNA encompassed genes involved in *JAK/STAT* pathway and epigenetic regulators. Our results show that BI-ALCL genomic landscape is not only characterized by *JAK/STAT* activating mutations but also loss-of-function alterations of epigenetic modifiers.

Conflict of interest: No COI declared

COI notes:

Preprint server: No;

Author contributions and disclosures: Ca.L., A.N., V.F., N.A., P.B., L.X. and P.G. collected data; Ca.L., A.N., Ce.L., L.X. and P.G. analysed data and made the figures; Ca.L., A.N., Ce.L., P.B., L.X. and P.G. designed the research and wrote the paper; Ca.L., A.N., Ce.L., J.A., D.B., N.M., F.X.F, A.B.F, M.H.D, N.P., L.X. and P.G. performed experiments; Ce.L., F.E. and B.T. provided expert statistic analysis; C.H, M.A, F.L.B, L.O., J.M.S., F.R. and M.B. provided patient samples and clinical information; C.L., A.N., LX., P.B., P.G., A.T.G., M.P.C., L.M., A.S.H.P, A.M., F.L., C.C.C and J.S. provided samples for analysis.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: For original data, please contact laurent.c@chu-toulouse.fr

Clinical trial registration information (if any):

Gene alterations in epigenetic modifiers and JAK-STAT signaling are frequent in breast implant-associated ALCL.

Short title: BI-ALCL genomic landscape

Camille Laurent[1,2*] and Alina Nicolae[3,4*], Cécile Laurent[5], Fabien Le Bras[6], Corinne Haioun[4,6], Virginie Fataccioli[4,7], Nadia Amara[1], José Adélaïde[8,9], Arnaud Guille[8,9], Jean-Marc Schiano[10], Bruno Tesson[5], Alexandra Traverse-Glehen[11], Marie-Pierre Chenard[3], Lénaïg Mescam[12], Anne Moreau[13], Catherine Chassagne-Clement[14], Joan Somja[15], Frédéric Escudié[1], Marc André[16], Nadine Martin[4], Laetitia Lacroix[4], François Lemonnier[4,6], Anne-Sophie Hamy[17], Fabien Reyal[18], Marie Bannier[19], Lucie Oberic[20], Nais Prades[21], François-Xavier Frénois[1], Asma Beldi-Ferchiou[4,22], Marie-Helene Delfau-Larue[4,22], Reda Bouabdallah[10], Daniel Birnbaum[8,9], Pierre Brousset[1,2**] and Luc Xerri[12, 23**] and Philippe Gaulard[4,7**].

[1]Pathology and Cytology Department, Centre Hospitalo-Universitaire de Toulouse,Institut Universitaire du Cancer de Toulouse-Oncopole, Toulouse, France.

[2]Centre de Recherche en Cancerologie de Toulouse, Inserm, UMR1037 laboratoire d'excellence TOUCAN, Paul Sabatier University Toulouse III, Toulouse, France.

[3]Pathology and Cytology Department, CHU Hautepierre, Strasbourg, France.

[4]Institut Mondor de Recherche Biomédicale, INSERM U955 and Université Paris-Est, Créteil, France.

[5]CALYM - LYSARC, Institut Carnot, Pierre-Bénite, France.

[6]Lymphoid Malignancies Unit, AP-HP, Groupe Hospitalier Henri Mondor-Albert Chenevier, Créteil, France.

[7]Department of Pathology, Groupe Hospitalier Henri Mondor, AP-HP, Créteil, France.

[8]Department of Predictive Oncology, Institut Paoli-Calmettes,

[9]Centre de Recherche en Cancérologie de Marseille (CRCM), Inserm U1068, CNRS UMR7258, Aix-Marseille University, UM 105, Marseille, France.

[10]Department of Hematology, Institut Paoli-Calmettes, Marseille, France.

[11]Pathology Department, Centre Hospitalier Lyon-Sud, Pierre-Bénite, France.

[12]Department of Bio-Pathology Institut Paoli-Calmettes, Marseille, France.

[13]Pathology and Cytology Department, Centre Hospitalier Hôtel Dieu, Nantes, France.

[14]Department of Bio-Pathology Pathology and Cytology Department, Centre Léon Bérard, Lyon, France.

[15]Pathology and Cytology Department, Centre Hospitalo-Universitaire de Liège, Liège, Belgium.
[16]Department of Hematology, Centre Hospitalo-Universitaire UCL Namur, Yvoir, Belgium.
[17]Residual Tumour & Response to Treatment Laboratory, RT2Lab, INSERM, U932, PSL Research University, Translational Research Department, Institut Curie, Paris, France.
[18]Department of Surgery, Institut Curie, Paris, France.
[19]Department of Surgery, Institut Paoli-Calmettes, Marseille, France.
[20]Hematology Department, Centre Hospitalo-Universitaire de Toulouse, Institut Universitaire du Cancer de Toulouse-Oncopole, Toulouse, France.
[21]Laboratory of Hematology, Centre Hospitalo-Universitaire de Toulouse, Institut Universitaire de Cancérologie de Toulouse, Toulouse, France.
[22]Department of Immunobiology and Haematobiology, Groupe Hospitalier Henri Mondor, AP-HP, Créteil, France.
[23]Department Tumor Immunology, Institut Paoli-Calmettes, Aix-Marseille University, Centre de Recherche en Cancérologie de Marseille, Marseille, France.

*Co-first author.
**Co-Last author.

Corresponding authors :
Camille Laurent, Laboratoire d'Anatomie Pathologique, Institut Universitaire du Cancer de Toulouse-Oncopole, 1 avenue Irène Joliot-Curie 31059 Toulouse France; Université Paul-Sabatier, Toulouse, F-31400 France; e-mail :laurent.c@chu-toulouse.fr ; and Philippe Gaulard, Département de Pathologie, Groupe Hospitalier Henri Mondor, AP-HP, Créteil, France; INSERM U955, Université Paris-Est, Créteil, France ; e-mail: philippe.gaulard@aphp.fr

Text Word count: 3 977
Abstract Word count: 234
Figures: 3
Table: 1
References: 55

Scientific Categories: Lymphoid neoplasia and mutational landscape.

Key points

- Whole exome sequencing of a large series of BI-ALCL demonstrates recurrent mutations in epigenetic regulators.
- An accumulation of alterations in epigenetic modifiers and genes in the *JAK/STAT* pathway likely drives BI-ALCL oncogenesis.

Abstract

The oncogenic events involved in breast implant-associated anaplastic large cell lymphoma (BI-ALCL) remain elusive. To clarify this point, we have characterized the genomic landscape of 34 BI-ALCLs (15 tumor, 19 *in situ* subtypes) collected from 54 BI-ALCL patients diagnosed through the French *Lymphopath* network. Whole exome sequencing (*n*=22, with paired tumor/germline DNA) and/or targeted deep sequencing (*n*=24) showed recurrent mutations of epigenetic modifiers in 74% of cases, involving notably *KMT2C* (26%), *KMT2D* (9%), *CHD2* (15%) and *CREBBP* (15%). *KMT2D* and *KMT2C* mutations correlated with a loss of H3K4 mono- and tri-methylation by immunohistochemistry. Twenty cases (59%) showed mutations in at least one member of the *JAK/STAT* pathway including *STAT3* (38%), *JAK1* (18%), *STAT5B* (3%) and in negative regulators like *SOCS3* (6%), *SOCS1* (3%) and *PTPN1* (3%). These mutations were more frequent in tumor-type than *in situ* samples (*p*=0.038). All BI-ALCLs expressed pSTAT3, regardless of the mutational status of genes in *JAK/STAT* pathway. Mutations in *EOMES* gene (12%) involved in lymphocytes development, *PI3K-AKT/mTOR (6%)* and loss of function mutations in *TP53* (12%) were also identified. Copy number aberration (CNA) analysis identified recurrent alterations including gains on chromosomes 2, 9p, 12p and 21 and losses on 4q, 8p, 15, 16 and 20. Regions of CNA encompassed genes involved in *JAK/STAT* pathway and epigenetic regulators. Our results show that BI-ALCL genomic landscape is not only characterized by *JAK/STAT* activating mutations but also loss-of-function alterations of epigenetic modifiers.

Keywords

BI-ALCL, *JAK/STAT* pathway, epigenetic modifiers, *in-situ* and tumor subtypes.

Introduction

Breast implant-associated anaplastic large-cell lymphoma (BI-ALCL) is a rare form of T-cell lymphoma arising adjacent to a breast implant, recently recognized as a provisional entity in the 2017 revised World Health Organization (WHO) lymphoma classification.[1] The incidence of BI-ALCL varies from 1/30 000 to 1/1000 women with breast implants[2-5] with approximately 660 cases reported to the FDA.[4]

Most BI-ALCL patients (80%) present with an isolated effusion adjacent to the implant (seroma BI-ALCL) and have an excellent outcome.[5-7] In contrast, a minority of patients (20%) present with a breast tumor, which might disseminate outside the breast and have a worse prognosis.[5-7] We recently described two BI-ALCL histological subtypes that correlate with the clinical presentations: i) *in situ* BI-ALCL found in patients with seroma, characterized by lymphoma cells lining the capsule border and/or suspended in a serous/fibrinoid material; ii) tumor-type BI-ALCL mostly found in patients with tumor mass, characterized by capsule invasion.[6,8]

Although BI-ALCLs share a similar morphology and immunophenotype with systemic ALCLs, they lack rearrangements involving *ALK, DUSP22*, and *TP63* genes.[8,9] In a manner reminiscent of systemic ALCL, a few studies using next generation sequencing have pointed towards a putative dysregulation of *JAK/STAT* signalling in BI-ALCL oncogenesis. Recurrent somatic *STAT3* mutations have been indeed observed in 26% to 64% of analyzed cases.[6,9-15] However, since only two BI-ALCL cases have been thoroughly studied by whole exome sequencing (WES), the mutational landscape of this entity has not yet been reported.

To further explore potential molecular mechanisms involved in BI-ALCL pathogenesis, we have analyzed a large set of 34 BI-ALCL cases by WES and/or targeted deep sequencing (TDS). The mutational landscape of BI-ALCL showed both *JAK/STAT* signalling dysregulation and epigenetic alterations.

Material and methods

Cases selection and tumor samples

Fifty-four BI-ALCL cases (including two BI-ALCL cases from Belgium pathology departments at CHU de Liège and CHU UCL Namur) were collected between 2010 and May 2018 via the French Lymphopath network aiming at providing a systematic review of every newly diagnosed or suspected lymphoma.[16] Among them, 34 cases were selected based on matched germline samples availability and/or tumor DNA quality for downstream genomic

analysis. All these cases were further reviewed by four of the coauthors (CL, AN, PG and LX). They were histologically subclassified as *in situ* or tumor-type BI-ALCL and staged according to the MD Anderson TNM classification[7,17,18] based on the pathological material submitted to the *Lymphopath* network. Clinical and pathological features are detailed in **Table 1**. Based on H&E sections and appropriate immunostainings a semi-quantitative estimation of tumor cell content was given as ≤20%, 20-50% and >50% tumor cells. In addition, 20 cases with available remaining DNA were analyzed for their T-cell receptor (TCR) repertoire profile using a CapTCR-seq NGS technique (see details in Supplemental methods) in order to evaluate the proportion of neoplastic T-cells in comparison to the total T-cell population (number of reads corresponding to the dominant clonal TCRβ gene rearrangement among the total number of reads with TCRβ gene rearrangements) (**Supplemental Table S1).**

These patients were included in the framework of a T-cell lymphoma consortium (Tenomic), which was approved by the local ethical committee (Comité de Protection des Personnes Ile de France IX 08-009). Informed consent was obtained from patients for the analysis of tumor and germline specimens. Tissue samples were collected and processed following standard ethical procedures (Helsinki Declaration of 1975). Clinical and pathological features of 17 cases have been previously reported.[8]

Immunohistochemistry (IHC)

Immunohistochemical studies were performed on 3 µm-thick FFPE sections using routine protocols on fully-automated platforms (Ventana, Benchmark XT, Tucson, AZ, USA). For diagnostic purpose, the cases were tested for a wide panel of antibodies detailed in **Supplemental Table S2.** To functionally validate the mutations affecting *STAT3* and *STAT5B* genes, antibodies against phospho-STAT3 (clone D3A7; 1:50; Cell signalling; Beverly; USA) and phospho-STAT5 (clone E208; 1:100; Abcam, Cambridge, United Kingdom) were used. The histone H3K4 methylation status was evaluated using antibodies against histone-3 lysine-4 (H3K4)me3 (Tri-methyl Lys4) (polyclonal GTX128954; 1/100; Gene Tex, Irvine, USA) and H3K4me1 (ab8895 clone; 1/500; Abcam, Cambridge, United Kingdom). Computer assisted software was used to score the histone H3K4 methylation status (See details in Supplementary methods).

DNA extraction

Genomic tumor DNA was extracted from 10 µm-thick formalin-fixed paraffin embedded (FFPE) tissue sections using Qiagen QIAamp DNA FFPE Tissue Kit (Qiagen Inc., Valencia,

CA) or Maxwell® 16 FFPE Plus LEV DNA Purification Kit (Promega) according to the manufacturers' recommendations. To enrich in tumor cells content, a macrodissection was performed for 8 cases. For the cases analyzed by whole exome sequencing (WES), the paired germline DNA was extracted, either from peripheral blood (n=14) or from macrodissected non-tumoral FFPE capsular tissue (n=8).

Whole exome sequencing

WES was performed on 22 samples including 12 *in situ* and 10 tumor-type BI-ALCL tissues and their matched germline DNA. DNA libraries were prepared using NEBU ultra V2 and Agilent SureSelect Clinical Research Exome V2. Sequencing was performed on an Illumina HiSeq4000 with an expected mean depth of 200X for the tumor and 70X for the germline. Sequences analysis was performed using Bcbio-Nextgen somatic variant calling pipeline (version 1.0.7a). Sequences were aligned to the human genome (version GRCh37) with the Burrows-Wheeler Aligner software (version 0.7.17). Single-nucleotide variants (SNVs) and indels were detected with the combination of 4 algorithms [Mutect2 version 3.8[19], Vardict version 1.5[20], Strelka2[21] and Freebayes version 1.1.046[22]]. Variants were annotated using VEP (version 94) and were filtered as follows: coverage in tumor and corresponding normal samples ≥15, alternative bases coverage ≥3, and variant allele frequency (VAF) > 2% (ratio between alternative allele reads and total reads). Variants described in 1000 Genomes Project, gnomAD database and ExAC database over 1% of the population were discarded. The calls with reads showing strong bias or present in the majority of the samples were filtered out. All reported variants passed visual inspection using the Integrative Genomics Viewer.

Copy number aberrations (CNA) analysis

Analysis of CNA for paired tumor-normal whole exome sequenced samples was performed using FACETS algorithm.[23] Critical value for segmentation was set to 150. Other parameters remain the same as default values. To estimate the status calling, we extracted the estimated total copy number (under column tcn.em) and the ploidy from the segmentation files produced by FACETS. We also estimated the genotype to identify regions of copy-neutral loss of heterozygosity (cnLOH). Amplification was defined as more than 4 DNA copies.

Targeted deep sequencing (TDS)

To validate WES results, 24 BI-ALCL (12 *in situ* and 12 tumor-type cases) were analysed using a TDS approach, with 500X average depth (497X [265-698X]). Twelve of them were initially analysed by WES and 12 additional cases were used. The 406 genes FundationOne Heme panel

used includes genes previously reported to be mutated in T-cell lymphomas, members of cytokine signaling and of T-cell receptor signaling pathways (https://assets.ctfassets.net/vhribv12lmne/zBxaQC12cScqgsEk8seMO/a70860fea48927c7ff8b70e90c292182/F1H_TechnicalInformation.pdf).

Reads were aligned on human genome (GRCh37) with BWA (version 0.7.17). Variant calling was done with Vardict (version 1.5) and GATK Haplotype Caller (GATK 3.8) algorithms. They were filtered on their allele frequency (VAF > 2%), on strand bias, on their depth (>50), and on the depth of the alternative allele (>3). Variants were annotated with VEP (version 94). The variants presented in a pool of normal based on whole exome sequencing analysis were discarded. Variants known as polymorphic were eliminated (variants known in 1000 Genomes or ExAC or GnomAD with a frequency greater than 1%). Only the variants associated with a frameshift, in-frame indel, start/stop codon change, missense change were kept. The sequence alignments for all JAK/STAT variants were visually inspected and artefacts excluded.

Results

Clinical data and pathological features

Patients' age at diagnosis ranged from 39 to 82 years. Thirty-two breast implants were textured and the remaining two cases were of unknown status. Seventeen patients had experienced 2 or more implant surgeries before BI-ALCL diagnosis with a median interval time between last implant surgery and BI-ALCL diagnosis of 7.3 years (range 6 to 273 months). Twenty-three patients (70%) were Ann Arbor stage I/II and 30% were stage IV. Regarding the histological subtype, 19 patients had an *in situ* BI-ALCL (T1 stage according to MD Anderson TNM staging[7,17,18] and 15 a tumor-type BI-ALCL (4 corresponding to T2, 4 to T3, 6 to T4 stage and 1 non–evaluable). The main clinical and pathological characteristics of the 34 BI-ALCL patients are given in **Table 1**. By immunohistochemistry, all cases were strongly and uniformly positive for CD30 and showed incomplete T-cell phenotype, with a common activated cytotoxic profile. Neoplastic cells were often positive for EMA (90%) and ALK1 was consistently negative. Most patients had a favorable outcome except 3 patients who died of lymphoma progression (cases #13, #18 and #37).[24]

Genomic landscape of BI-ALCL

WES was performed on 22 paired tumor/normal samples. The mean sequencing depth was 131X (84-153X) for the tumor and 75X (47X-145X) for the germline DNA. All but three BI-ALCLs showed somatic mutations in genes considered as relevant to oncogenesis, according to our bioinformatics filtering. In total, we identified 1248 non synonymous somatic mutations involving 1118 genes after variant filtering. SNV represented 86% of the mutations (1069 SNV for 979 genes). Among them, missense mutations were the most frequent alterations (88%) followed by nonsense (7%) and splice-site mutations (4%) (**Supplemental Figure S1A)**. Indels represented 14% of somatic mutations (179 mutations involving 153 genes), including 74% frameshift and 26% inframe insertions or deletions. Among single-nucleotide mutations, G>T and C>A transversions were the most common (56%), followed by G>A and C>T transitions (30%), consistent with similar observations in other cancers and lymphomas.[25] The mean count of non-synonymous somatic alterations was 57 with a minimum of 9 and a maximum of 193 (See **Supplemental Table S3** for the complete list of mutations). The mutational landscape of BI-ALCL was remarkably homogeneous with enriched clusters among *JAK-STAT*, epigenetic modifier genes, and cell cycle or apoptosis pathways (**Supplemental Figure S1B**).

JAK/STAT pathway activation in BI-ALCL

Most common alterations observed in BI-ALCL samples involved members of the *JAK/STAT* pathway, found in 64% (*n*=14/22) of WES analysed cases. *STAT3* and *JAK1* were the most frequently mutated genes, accounting for 41% (*n*=9/22) and 18% (*n*=4/22) of cases, respectively. These were followed by *SOCS3* (9%), *SOCS1* (5%), *PTPN1* (5%) and *STAT5B* (5%). Interestingly, these mutations were not mutually exclusive and co-occurrence of mutations in two genes of the *JAK/STAT* pathway was found in 4 cases (**Supplemental Figure S1B**). All *STAT3* mutations were missense mutations and occurred at p.S614R, p.G618R, p.D661Y and p.I659L hotspots. Except one nonsense mutation (p.R174*), *JAK1* mutations were missense and related to the p.G1097D/V/C/S hotspots. All these aberrations have been already shown to be gain-of function mutations resulting in *JAK/STAT* pathway activation in various types of T cell lymphoma.[26,27]

Highly recurrent alterations in epigenetic modifiers in BI-ALCL

We detected frequent mutations of epigenetic regulators and histone-modifiers. Genes encoding H3K4 methyltransferases such as *KMT2C* (p.S3213L and p.K2797Rfs*26) (*n*=4, 18%) or *KMT2D* (p.P2781T) (*n*=1, 4.5%) were the most frequently mutated, followed by mutations of genes coding for chromodomain helicase DNA-binding proteins (*n*=5, 23%) such as *CHD2* (p.G870R, p.P1796S, p.E1242K) (**Supplemental Figure S1B**). These missense and frameshift

indel mutations were predicted to be deleterious, conducting to impairment of protein function. Besides, we found nonsense or missense mutations in other epigenetic genes previously described as frequently mutated in T- and B-cell lymphomas such as *TET2* (p.E149*), *DNMT3A* (p.Q678*) and *CREBBP* (p.L1825I).[28-32]

Other pathways involved in BI-ALCL

Genomic alterations in the *PI3K* signalling pathway were observed in 14% of cases and included point mutations of *PTPN11* (*n*=2) and *PIK3CG* (*n*=1) genes. In 18% of cases, *EOMES*, known to be involved in lymphocyte development, showed deleterious inframe insertion-deletion. One patient harboured missense mutation in *CARD11* (p.S621F), involved in TCR signalling. As to genes involved in cell cycle control and apoptosis, 2 patients carried mutations in *TP53 (*p.R282W and p.C238Y) and 3 showed inframe deletion in *TSC22D1* (p.Q509del) acting as a tumor suppressor through apoptosis induction. None of the samples showed germline mutation of *TP53* (**Supplemental Figure S1B**).

Copy number aberrations in BI-ALCL

All 22 samples were analysed for CNA profiling, but CNA estimation from WES data was successful for 8 samples (2 *in situ* and 6 tumor-type BI-ALCL cases) whereas it was not evaluable in the remaining 12 samples probably due to degradation of DNA from FFPE samples and/or low proportion of neoplastic cells. The mean WES coverage was satisfying in CNA informative cases as well as in those with non-informative CNA (139 X [113-192]; and 129 X [83.5-153]; respectively). Four out of the 8 interpretable cases were polyploid (3 triploids, one tetraploid). All analyzed BI-ALCL genomes showed multiple aberrations and most genomes were complex. CNA analysis showed more losses than gains (61% vs 39%). Recurrent CNAs were gains on chromosomes 2, 9p, 12p and 21 and losses on 4q, 8p, 15, 16 and 20 (**Supplemental Figures S2A and S2B**). The main gain region on chromosome 2 (:173,929,213-176,957,814) encompassed *CDCA7* which is known to contribute to *MYC*-mediated tumorigenesis.[33] Genes of the *JAK/STAT* pathway including *JAK1*, *STAT3* and *STAT5B* were affected by CNAs, with concomitant gene mutations in some cases (detailed in **Supplemental Figure S2C**). Several regions of loss and copy-neutral loss of heterozygosity (cnLOH) encompassed *JAK/STAT* inhibitors and epigenetic regulators (**Supplemental Figure S2C**). Loss of the 17p region harbouring *TP53* was detected in 3 cases, with concomitant *TP53* mutation in 2 cases.

Targeted deep sequencing

In order to validate and further expand our WES data, we performed TDS in 24 BI-ALCL cases, including 12 additional cases. The 12 double-screened cases showed a perfect concordance (**Figure 1A**). This extended BI-ALCL cohort confirmed the high rate of mutations targeting the *JAK/STAT* pathway, since at least one member of this pathway was mutated in 50% of the 12 additional cases. These recurrent mutations were observed in *STAT3* (*n*=3/12), *JAK1* (*n*=1/12), *STAT5B* (*n*=1/12), whereas one case harboured concurrent *STAT3* and *JAK1* mutations. Furthermore, 66% (*n*=8/12) of the analysed cases carried one or several deleterious mutations in epigenetic modifiers, like *KMT2C* (25%), *CHD2* (17%) and *CREBBP* (17%).

Altogether, 74% of the entire cohort of 34 BI-ALCLs sequenced by WES and/or TDS, contained alterations of epigenetic regulators and histone-modifiers**,** whereas mutations involving the *JAK/STAT* pathway were found in 59% of the cases (**Figure 1B)**. Somatic mutations in regulators of the cell cycle and apoptosis were less common (26%) including *TP53* (12%) and *PI3K/AKT* (6%). Other altered pathways included lymphocyte development or TCR signalling (26%). A linear depiction of the protein domains of frequently mutated genes is shown in **Figure 2 and Supplemental Figure S3.**

Immunohistochemistry

Using IHC, we have investigated the consequences at the protein level of mutations targeting *STAT3*, *STAT5B*, *KMT2C* and *KMT2D* **(Figure 3)**. In this respect, 20 BI-ALCL cases with available material, including 15 *STAT3* wild-type and 5 *STAT3* mutated cases were analyzed using an anti-pSTAT3 antibody. All tested cases exhibited a strong nuclear positive staining indicating the presence of the phosphorylated form of STAT3 in lymphoma cells regardless of the mutational status (**Figure 3A and 3B**). Six cases including the 2 *STAT5B* mutated BI-ALCL (case #6 and #18) were also tested for the phospho-STAT5 (p-STAT5) expression. The two *STAT5B* mutated cases showed a strong nuclear expression of p-STAT5 in tumor cells, whereas the wild type *STAT5B* cases were negative (**Supplemental Figure S4**). In addition, as *KMT2C* and *KMT2D* take part in H3K4 trimethylation, staining using anti-H3K4me3 and H3K4me1 antibodies was performed in available *KMT2C/D* wild-type and mutated cases. As illustrated in **Figure 3C-F**, KMT2C/D wild-type BI-ALCLs disclosed H3K4me3 or H3K4me1 nuclear positivity in most of neoplastic cells (**Figure 3C and 3D)**, whereas tumor cells of *KMT2C/2D* mutated cases were negative for H3K4me1 and me3 antibodies or showed a weak staining as

compared to reactive cells (**Figure 3E and 3F)**. Automated quantification of the H3K4me1 and H3K4me3 staining further confirmed a significant difference between *KMT2D/2C* mutated and *KMT2D/2C* wild-type cases with a higher percentage of tumor cells negative and/or with a lower expression of H3K4me3 and H3K4me1 staining in mutated cases (n=4 and n=6, respectively) than in unmutated ones (n=8 and n=4, respectively) ($p< 0.0398$ and $p<0.0086$, respectively) (**Supplementary Figure S5**).

Correlation between genetic alterations, T-cell repertoire, tumor cell content and pathological features

Fisher exact test was used to compare the mutational profiles of the two BI-ALCL subtypes. Across all the samples, there was no significant difference of the total number of detected mutations and in the number of mutations involving the epigenetic modifiers between these two subtypes. However, tumor-type cases showed a higher rate of mutations in members of *JAK/STAT* pathway as compared to *in situ* samples across all the samples (80% vs 42% of cases; p=0.038). Particularly, *STAT3* was significantly more mutated in tumor-type than *in situ* cases (n=9/15 vs n=4/19; p=0.034). In addition, tumor-type cases were more often mutated in genes controlling the cell cycle than *in situ* cases (n=7/15 vs n=1/19, respectively; p=0.025). A tendency for higher genomic complexity (i.e. total number of breakpoints) in tumor-type than *in situ* samples (mean count 118 [82-194] and 76 [73-78], respectively) (p=0.06) was seen. When samples were categorized according to the percentage of tumor cells (≤20%, 20-50% and >50% of tumor cells per sample) determined by optical evaluation, there was no significant difference in terms of number of overall mutations as well as in mutations in the *JAK/STAT* pathway between the 3 categories. In an attempt to determine more precisely whether the number of alterations was related or not to the tumor cell content, we compared genetic alterations (VAF of the dominant pathogenic mutation) with the tumor cell content analyzed by the TCR repertoire using CapTCR-seq NGS. We overall found that the proportion of the dominant T-cell clone estimated by TCR-seq was well-correlated with the percentage of tumor cells evaluated by pathological examination (Spearman correlation s=0.6) (**Supplemental Table S1**). Considering the VAFs of the dominant pathogenic variant, the correlation with the dominant clonal TCRß rearrangement was moderate (Spearman correlation s=0.51). Indeed, whereas several cases – mostly tumor-type (7/9) - displayed high values of both the dominant pathogenic mutation and the dominant T-cell clone (VAF above half of TCR value), others - mainly *in-situ* BI-ALCLs (6/11) - disclosed low VAF of the dominant mutation compared to the dominant T-cell clone (VAF below half of TCR value), a finding which suggests the

possibility that the pathogenic mutation might belong to a sub-clone in these later cases (**Supplemental Table S1 and Supplemental Figure S6**).

Discussion

Chronic inflammation and subversion of cytokine receptors signalling was previously suspected to play a role in BI-ALCL pathogenesis. Our results strongly support this hypothesis, since about 60% of our cases harboured somatic mutations in at least one member of the *JAK/STAT* cascade, among which *STAT3* and *JAK1* were the most frequently mutated genes. This is in keeping with the known STAT3 activation in BI-ALCL cell lines, with concomitant high levels of cytokines such as IL6 and IL10.[34] We also observed constant nuclear expression of pSTAT3 in all BI-ALCL cases regardless of their mutational status. Since *STAT3* mutations have been shown to increase the phosphorylation of STAT3 in response to IL-6,[35] overexpression of cytokines and/or somatic mutations might be responsible for constitutive STAT3 activation in BI-ALCL.

This pathogenic mechanism appears not BI-ALCL specific since STAT3 activation is known to occur in systemic ALCLs. *JAK/STAT* activation in BI-ALCL may be exacerbated since the tumor occurs in an enclosed space in which cytokines can reach high levels due to chronic inflammation. Furthermore, 15% of our cases exhibited co-occurring mutations in two genes of the *JAK/STAT* pathway. Such double hits could potentialize *JAK/STAT* signalling in BI-ALCL. It was indeed previously demonstrated *in vitro* that double *JAK1/STAT3* mutants could act synergistically to amplify STAT3 activation and sustain cell transformation.[25] Likewise, the observed mutations in *JAK/STAT* negative regulators and deletions in chromosomal regions containing these genes might further boost BI-ALCL growth.

In our series, 74% of cases showed deletions or inactivating mutations targeting *KMT2C*, *CHD2*, *CREBBP* and *KMT2D*. The relevance of epigenetic alterations has been already emphasised in other T-cell lymphomas such as those of TFH origin, hepatosplenic T-cell lymphoma, ALCLs, intestinal T cell lymphoma, peripheral T-cell lymphoma, NOS (PTCL, NOS) and extranodal NK/T-cell lymphoma.[15,30,31,36-40] These latter malignancies, however, harbour mutations of the *TET2*, *IDH2*, *DNMT3* and *SETD2* genes, which were either absent or uncommon in our series (see **Supplemental Figure S7 and Supplemental Table S4**).

KMT2D and *KMT2C* are members of the MLL (mixed lineage leukemia) methyltransferase family involved in the methylation of H3K4, an important process regulating gene

transcription.[41,42] So far, hundreds of *KMT2C* and *KMT2D* mutations have been identified, making them among the most frequently mutated genes in human cancers.[41,42] Our results are in keeping with recent reports depicting alterations of these two genes in T-cell lymphomas like Sézary syndrome, extranodal NK/T-cell lymphoma and PTCL, NOS.[40,43-45] Loss of MLL proteins function is known to affect cell differentiation and proliferation programs.[46] Accordingly, we observed an absence or decrease of H3K4 methylation in *KMT2C* or *KMT2D* mutated BI-ALCL cases compared to wild-type cases. By contrast to the high frequency of missense mutation affecting the *STAT3* gene, *KMT2* genes were more often targeted by non-sense or frameshift mutations. This could be explained by the fact that *STAT3* mutations (especially in its kinase domain) are known to induce oncogenic activation[25,47] whereas *KMT2* mutations in the C-terminal portion (including the SETdomain-containing lysine methyltransferase) are potentially inactivating, with subsequent loss of KMT2 protein reducing the methyltransferase activity.[48,49] We could also detect frequent mutations in other genetic modifiers such as *CHD2, CREBBP* and *HDAC* family members.

Altogether, we report here that the genomic profiling of BI-ALCL identifies common alterations in genes of the JAK-STAT pathway and in epigenetic modifiers. Most of the mutations observed in BI-ALCL have been reported in other PTCL subtypes,[25,36,37,40,50-55] though at variable frequencies (**Supplemental Figure S7).** However, the limited number of systemic or cutaneous ALCL cases with available sequencing data reported so far[25,52] precludes definitive conclusion as to putative differences between the mutational landscape of BI-ALCL and other ALK-negative, systemic or cutaneous ALCL. Further larger studies are warranted to compare the genetic landscape of these different ALCL subsets.

TP53 germline mutations in BI-ALCL have been recently reported.[14] We could not identify such mutations in our cases, although *TP53* somatic mutations were present in 12% of cases and were associated with loss of the corresponding 17p region in 2 cases, whereas 17p loss was detected in another non-mutated case. These data suggest a significant impact of *TP53* defect on BI-ALCL growth.

Five BI-ALCL cases studied by both WES and/or TDS did not show pathogenic mutations. For 2 samples with low tumor cell content, which also lacked *TCR* rearrangements, we cannot exclude a false negative result. In the 3 remaining samples, we could ensure enrichment in tumour cell content by macrodissection and detect a monoclonal *TCR* rearrangement. The lack of mutations in these cases suggests that other mechanisms could contribute to STAT3

activation. This is consistent with our observation that all tested BI-ALCLs expressed pSTAT3, regardless to their mutational status. However, the possibility of minor subclones below the detection threshold of our sequencing approach cannot be totally ruled out.

Such an alternative mechanism is also supported by the finding of *STAT3* being less frequently mutated in *in situ* than in tumor-type cases. This suggests a continuum of *JAK/STAT* pathway activation ranging from cytokine hypersecretion to *JAK/STAT* mutations. High cytokine levels could be solely responsible for some *in situ* lesions, whereas the aggressive tendency of tumor-type cases may be underline by mutant *JAK/STAT* clones. In accordance with this hypothesis, TCR repertoire profile analysis indicated that in most tumor-type samples, the dominant pathogenic mutation was present in the majority of neoplastic T-cells.

To conclude, this genomic characterization of the largest BI-ALCL series reported to date not only confirms the key role of the *JAK/STAT* pathway, but also highlights the importance of epigenetics in BI-ALCL pathogenesis. Compounds targeting these molecular alterations, which are already being explored in systemic ALCL and other T-cell lymphomas, might be thus considered in the future to treat the small proportion of BI-ALCL patients with aggressive refractory disease.

Acknowledgments

This work was supported in part by institutional grants from the Institut National du Cancer (INCA, 2017-007), the Fondation pour la Recherche Médicale (FRM, Equipe Labellisée DEQ20160334875), the Leukemia Lymphoma Society (LLS SCOR 7013-17).), the Laboratoire d'Excellence Toulouse Cancer (TOUCAN) (contract ANR11-LABX), the Programme Hospitalo-Universitaire en Cancérologie CAPTOR (contract ANR11-PHUC0001), the Lymphome Study Association (LYSA) and the Institut Carnot Lymphome (CALYM). We thank Nathalie Van Acker (H3K4me1 staining), Eric Delabesse (TCR analysis), Giorgio Inghirami (expertise in ALCLs sequenced data), Andrea Cerqueira Do Vale (technical support for TCR repertoire analysis) and François Lozach (probe design for TCR repertoire analysis). We acknowledge the experts from the Lymphopath network and from the French registry on BI-ALCL and the participants of the Tenomic consortium a complete membership list appears in "Appendix."

Authorship Contribution

Ca.L., A.N., V.F., N.A., P.B., L.X. and P.G. collected data; Ca.L., A.N., Ce.L., L.X. and P.G. analysed data and made the figures; Ca.L., A.N., Ce.L., P.B., L.X. and P.G. designed the research and wrote the paper; Ca.L., A.N., Ce.L., J.A., D.B., N.M., F.X.F, L.L, A.B.F, M.H.D, N.P., L.X. and P.G. performed experiments; Ce.L., F.E. and B.T. provided expert statistic analysis; C.H, M.A, F.L.B, L.O., J.M.S., F.R., RB and M.B. provided patient samples and clinical information; C.L., A.N., LX., P.B., P.G., A.T.G., M.P.C., L.M., A.S.H.P, A.M., F.L., C.C.C and J.S. provided samples for analysis.

Conflicts of interest disclosure

The authors declare that we have no conflict of interest.

Data Sharing Statement

For original data, please contact laurent.c@chu-toulouse.fr

References

1. Swerdlow S, Campo E, Harris NL, et al. World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues (revised 4th edition. Vol. 2.). *IARC, Lyon, France* 2017.
2. Doren EL, Miranda RN, Selber JC, et al. U.S. Epidemiology of Breast Implant-Associated Anaplastic Large Cell Lymphoma. *Plast Reconstr Surg*. 2017;139(5):1042-1050.
3. Brody GS, Deapen D, Taylor CR, et al. Anaplastic large cell lymphoma occurring in women with breast implants: analysis of 173 cases. *Plast Reconstr Surg*. 2015;135(3):695-705.
4. https://www.tga.gov.au /alert/breast-implants-and-anaplastic-large-cell -lymphoma.
5. Leberfinger AN, Behar BJ, Williams NC, et al. Breast Implant-Associated Anaplastic Large Cell Lymphoma: A Systematic Review. *JAMA Surg*. 2017;152(12):1161-1168.
6. Laurent C, Haioun C, Brousset P, Gaulard P. New insights into breast implant-associated anaplastic large cell lymphoma. *Curr Opin Oncol*. 2018;30(5):292-300.
7. Miranda RN, Aladily TN, Prince HM, et al. Breast implant-associated anaplastic large-cell lymphoma: long-term follow-up of 60 patients. *J Clin Oncol*. 2014;32(2):114-120.
8. Laurent C, Delas A, Gaulard P, et al. Breast implant-associated anaplastic large cell lymphoma: two distinct clinicopathological variants with different outcomes. *Ann Oncol*. 2016;27(2):306-314.
9. Oishi N, Brody GS, Ketterling RP, et al. Genetic subtyping of breast implant-associated anaplastic large cell lymphoma. *Blood*. 2018;132(5):544-547.
10. Blombery P, Thompson E, Ryland GL, et al. Frequent activating STAT3 mutations and novel recurrent genomic abnormalities detected in breast implant-associated anaplastic large cell lymphoma. *Oncotarget*. 2018;9(90):36126-36136.
11. Blombery P, Thompson ER, Jones K, et al. Whole exome sequencing reveals activating JAK1 and STAT3 mutations in breast implant-associated anaplastic large cell lymphoma anaplastic large cell lymphoma. *Haematologica*. 2016;101(9):e387-390.
12. Di Napoli A, Jain P, Duranti E, et al. Targeted next generation sequencing of breast implant-associated anaplastic large cell lymphoma reveals mutations in JAK/STAT signalling pathway genes, TP53 and DNMT3A. *Br J Haematol*. 2018;180(5):741-744.
13. Letourneau A, Maerevoet M, Milowich D, et al. Dual JAK1 and STAT3 mutations in a breast implant-associated anaplastic large cell lymphoma. *Virchows Arch*. 2018;473(4):505-511.
14. Blombery P, Thompson ER, Prince HM. Molecular Drivers of Breast Implant-Associated Anaplastic Large Cell Lymphoma. *Plast Reconstr Surg*. 2019;143(3S A Review of Breast Implant-Associated Anaplastic Large Cell Lymphoma):59S-64S.
15. Oishi N, Miranda RN, Feldman AL. Genetics of Breast Implant-Associated Anaplastic Large Cell Lymphoma (BIA-ALCL). *Aesthet Surg J*. 2019;39(Supplement_1):S14-S20.
16. Laurent C, Baron M, Amara N, et al. Impact of Expert Pathologic Review of Lymphoma Diagnosis: Study of Patients From the French Lymphopath Network. *J Clin Oncol*. 2017;35(18):2008-2017.
17. Clemens MW, Medeiros LJ, Butler CE, et al. Complete Surgical Excision Is Essential for the Management of Patients With Breast Implant-Associated Anaplastic Large-Cell Lymphoma. *J Clin Oncol*. 2016;34(2):160-168.
18. Clemens MW, Jacobsen ED, Horwitz SM. 2019 NCCN Consensus Guidelines on the Diagnosis and Treatment of Breast Implant-Associated Anaplastic Large Cell Lymphoma (BIA-ALCL). *Aesthet Surg J*. 2019;39(Supplement_1):S3-S13.
19. Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol*. 2013;31(3):213-219.
20. Lai D, Meyer IM. A comprehensive comparison of general RNA-RNA interaction prediction methods. *Nucleic Acids Res*. 2016;44(7):e61.
21. Kim JM, Park JE, Yoo I, et al. Integrated transcriptomes throughout swine oestrous cycle reveal dynamic changes in reproductive tissues interacting networks. *Sci Rep*. 2018;8(1):5436.
22. Garrison E, Marth G. Haplotype-based variant detection from short-read sequencing. *arXiv preprint arXiv:12073907*. 2012.

23. Shen R, Seshan VE. FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. *Nucleic Acids Res*. 2016;44(16):e131.
24. Le Bras F, Laurent C, Bosc R, et al. Breast Implant Associated-Anaplastic Large Cell Lymphoma (BIA-ALCL): The French Lymphoma Study Association (LYSA) Registry Data. *ASH*. 2018,:abstract 111659.
25. Crescenzo R, Abate F, Lasorsa E, et al. Convergent mutations and kinase fusions lead to oncogenic STAT3 activation in anaplastic large cell lymphoma. *Cancer Cell*. 2015;27(4):516-532.
26. Waldmann TA, Chen J. Disorders of the JAK/STAT Pathway in T Cell Lymphoma Pathogenesis: Implications for Immunotherapy. *Annu Rev Immunol*. 2017;35:533-550.
27. Pizzi M, Margolskee E, Inghirami G. Pathogenesis of Peripheral T Cell Lymphoma. *Annu Rev Pathol*. 2018;13:293-320.
28. Couronne L, Bastard C, Bernard OA. TET2 and DNMT3A mutations in human T-cell lymphoma. *N Engl J Med*. 2012;366(1):95-96.
29. Lee G, Ryu HJ, Choi JW, et al. Characteristic gene alterations in primary gastrointestinal T- and NK-cell lymphomas. *Leukemia*. 2019.
30. da Silva Almeida AC, Abate F, Khiabanian H, et al. The mutational landscape of cutaneous T cell lymphoma and Sezary syndrome. *Nat Genet*. 2015;47(12):1465-1470.
31. Lemonnier F, Couronne L, Parrens M, et al. Recurrent TET2 mutations in peripheral T-cell lymphomas correlate with TFH-like features and adverse clinical parameters. *Blood*. 2012;120(7):1466-1469.
32. Vallois D, Dobay MP, Morin RD, et al. Activating mutations in genes related to TCR signaling in angioimmunoblastic and other follicular helper T-cell-derived lymphomas. *Blood*. 2016;128(11):1490-1502.
33. Gill RM, Gabor TV, Couzens AL, Scheid MP. The MYC-associated protein CDCA7 is phosphorylated by AKT to regulate MYC-dependent apoptosis and transformation. *Mol Cell Biol*. 2013;33(3):498-513.
34. Kadin ME, Deva A, Xu H, et al. Biomarkers Provide Clues to Early Events in the Pathogenesis of Breast Implant-Associated Anaplastic Large Cell Lymphoma. *Aesthet Surg J*. 2016;36(7):773-781.
35. Chen J, Zhang Y, Petrus MN, et al. Cytokine receptor signaling is required for the survival of ALK- anaplastic large cell lymphoma, even in the presence of JAK1/STAT3 mutations. *Proc Natl Acad Sci U S A*. 2017;114(15):3975-3980.
36. McKinney M, Moffitt AB, Gaulard P, et al. The Genetic Basis of Hepatosplenic T-cell Lymphoma. *Cancer Discov*. 2017;7(4):369-379.
37. Roberti A, Dobay MP, Bisig B, et al. Type II enteropathy-associated T-cell lymphoma features a unique genomic profile with highly recurrent SETD2 alterations. *Nat Commun*. 2016;7:12602.
38. Jiang L, Gu ZH, Yan ZX, et al. Exome sequencing identifies somatic mutations of DDX3X in natural killer/T-cell lymphoma. *Nat Genet*. 2015;47(9):1061-1066.
39. Joosten M, Seitz V, Zimmermann K, et al. Histone acetylation and DNA demethylation of T cells result in an anaplastic large cell lymphoma-like phenotype. *Haematologica*. 2013;98(2):247-254.
40. Fernandez-Pol S, Ma L, Joshi RP, Arber DA. A Survey of Somatic Mutations in 41 Genes in a Cohort of T-Cell Lymphomas Identifies Frequent Mutations in Genes Involved in Epigenetic Modification. *Appl Immunohistochem Mol Morphol*. 2019;27(6):416-422.
41. Ford DJ, Dingwall AK. The cancer COMPASS: navigating the functions of MLL complexes in cancer. *Cancer Genet*. 2015;208(5):178-191.
42. Rao RC, Dou Y. Hijacked in cancer: the KMT2 (MLL) family of methyltransferases. *Nat Rev Cancer*. 2015;15(6):334-346.
43. Kiel MJ, Sahasrabuddhe AA, Rolland DC, et al. Genomic analyses reveal recurrent mutations in epigenetic modifiers and the JAK-STAT pathway in Sezary syndrome. *Nat Commun*. 2015;6:8470.
44. Schatz JH, Horwitz SM, Teruya-Feldstein J, et al. Targeted mutational profiling of peripheral T-cell lymphoma not otherwise specified highlights new mechanisms in a heterogeneous pathogenesis. *Leukemia*. 2015;29(1):237-241.
45. Lee S, Park HY, Kang SY, et al. Genetic alterations of JAK/STAT cascade and histone modification in extranodal NK/T-cell lymphoma nasal type. *Oncotarget*. 2015;6(19):17764-17776.

46. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat Rev Genet*. 2012;13(5):343-357.
47. Koskela HL, Eldfors S, Ellonen P, et al. Somatic STAT3 mutations in large granular lymphocytic leukemia. *N Engl J Med*. 2012;366(20):1905-1913.
48. Zhang J, Dominguez-Sola D, Hussein S, et al. Disruption of KMT2D perturbs germinal center B cell development and promotes lymphomagenesis. *Nat Med*. 2015;21(10):1190-1198.
49. Ortega-Molina A, Boss IW, Canela A, et al. The histone lysine methyltransferase KMT2D sustains a gene expression program that represses B cell lymphoma development. *Nat Med*. 2015;21(10):1199-1208.
50. Moffitt AB, Ondrejka SL, McKinney M, et al. Enteropathy-associated T cell lymphoma subtypes are characterized by loss of function of SETD2. *J Exp Med*. 2017;214(5):1371-1386.
51. Watatani Y, Sato Y, Miyoshi H, et al. Molecular heterogeneity in peripheral T-cell lymphoma, not otherwise specified revealed by comprehensive genetic profiling. *Leukemia*. 2019.
52. Luchtel RA, Zimmermann MT, Hu G, et al. Recurrent MSC (E116K) mutations in ALK-negative anaplastic large cell lymphoma. *Blood*. 2019;133(26):2776-2789.
53. Palomero T, Couronne L, Khiabanian H, et al. Recurrent mutations in epigenetic regulators, RHOA and FYN kinase in peripheral T cell lymphomas. *Nat Genet*. 2014;46(2):166-170.
54. Choi J, Goh G, Walradt T, et al. Genomic landscape of cutaneous T cell lymphoma. *Nat Genet*. 2015;47(9):1011-1019.
55. Yoo HY, Sung MK, Lee SH, et al. A recurrent inactivating mutation in RHOA GTPase in angioimmunoblastic T cell lymphoma. *Nat Genet*. 2014;46(4):371-375.

Abbreviations:

BI-ALCL, Breast implant-associated anaplastic large cell lymphoma; WES, whole exome sequencing; TDS, targeted deep sequencing; CNA, copy number aberrations; IHC, immunohistochemistry.

Table legend:

Table 1. Main clinical characteristics and histological features of 34 BI-ALCL patients.

Figure legends:

Figure 1. Mutational landscape of BI-ALCL. (**A**) Whole-exome sequencing and/or targeted deep sequencing in 34 BI-ALCL. The heatmap of most significantly mutated genes is ordered according to biological pathways. (**B**) Functional pathways involved in BI-ALCL genomic abnormalities. The percentages refer to the frequency of alterations in the respective pathways.

Figure 2. Mapping of protein variants produced by frequently mutated genes. Protein domains of STAT3, JAK1, KMT2C and KMT2D were annotated according to PFAM database.

Each variant is represented by a colored circle with missense mutation in blue, nonsense mutation in red, frameshiftIndel mutation in pink and inframeIndel in light pink colors.

Figure 3. pSTAT3 and H3K4me3 immunostaining in BI-ALCL. Representative cases of *STAT3* wild-type (**A**) and *STAT3* mutated (**B**) BI-ALCL showing nuclear expression of pSTAT3 in tumor cells regardless of the mutational status (**A** x200; B x100). Representative cases of *KMT2C/2D* wild-type BI-ALCLs showing strong nuclear expression of H3K4me3 (**C**, x400) and H3K4me1 (**D**, x400) with similar intensities in lymphoma cells and reactive surrounding cells. By contrast, the nuclear H3K4me3 staining in a case of *KMT2C* mutated BI-ALCL was weaker in tumoral cells than in reactive cells such as neutrophils (**E**, x200). Similarly, the nuclear H3K4me1 staining was lost in most neoplastic cells of a *KMT2D* mutated BI-ALCL case (**F,** x200).

Table 1. Main clinical characteristics and histological features of 34 BI-ALCL patients.

N°	Age (years)	BI-ALCL histological subtype (T component of MD Anderson TNM staging)	Implant characteristics			Ann Arbor staging	Therapy (implant removal/CT/RT)	Overall Survival in months (Outcome NED/DOD/DOC)	T cell clone (% tumor cells per sample)	Exome sequencing by WES/TDS
			Number of implant surgeries prior to BI-ALCL diagnosis (reason for implant)	At least one macro-textured of implant (yes or no)	Delay (years) from last implant to BI-ALCL diagnosis (delay from first implant)					
2	42	*in situ* (T1)	2 (cosmetic)	yes	10.5y (20y)	I	implant removal + RT	56.48 (NED)	yes (≤20%)	**WES**
5	54	*in situ* (T1)	NA (cosmetic)	NA	9 y (NA)	IE	NA	NA	NA (20%-50%)	**WES**
6	62	*in situ* (T1)	2 (breast carcinoma)	yes	0.26y (17y)	IV	implant removal	55.59 (NED)	yes (>50%)	**WES**
7	76	*in situ* (T1)	1 (breast carcinoma)	yes	14.23y	I	implant removal	44.91 (NED)	yes (≤20%)	**WES + TDS**
8	43	*in situ* (T1)	1 (breast carcinoma)	yes	6.7y	I	implant removal	42.81 (NED)	yes (20%-50%)	**WES + TDS**
10	61	tumor-type (T4)	4 (cosmetic)	yes	7.2y (17y)	IV	implant removal + CT[1]	77.24 (NED)	yes (>50%)	**TDS**
11	54	tumor-type (T4)	NA (cosmetic)	yes	3y (NA)	II	NA	NA	yes (>50%)	**TDS**
13	82	tumor-type (T4)	1 (breast carcinoma)	yes	6.96y	IV	implant removal + CT[1]	10.45 (DOD)	yes (>50%)	**WES**
14	80	tumor-type (T3)	NA (cosmetic)	yes	8y	IE	implant removal + CT[1]	3 (NED)	NA (>50%)	**TDS**
15	50	*in situ* (T1)	NA (cosmetic)	yes	8y	IE	implant removal	NA	yes (≤20%)	**TDS**
16	77	*in situ* (T1)	3 (breast carcinoma)	yes	1.23y (NA)	I	implant removal + CT[2]	60.29 (NED)	NA (≤20%)	**WES**
17	74	tumor-type (T4)	2 (breast carcinoma)	yes	8.12y (NA)	IV	implant removal + CT[1]	44.88 (NED)	yes (≤20%)	**WES**
18	75	*in situ* (T1)	5 (breast carcinoma)	yes	1.27y (NA)	IV	implant removal + CT[1]	16.39 (DOD)	NA (≤20%)	**TDS**
19	57	tumor-type (T4)	2 (breast carcinoma)	yes	7.57y (NA)	IV	NA	3.71 (DOC)	yes (20%-50%)	**WES + TDS**
21	62	*in situ* (T1)	1 (breast carcinoma)	yes	9.37y	I	implant removal	27.73 (NED)	yes (≤20%)	**WES + TDS**
22	39	*in situ* (T1)	1 (cosmetic)	yes	6.48y	I	implant removal	23.95 (NED)	NA (20-50%)	**WES**
24	62	*in situ* (T1)	2 (breast carcinoma)	NA	4.57y (NA)	I	implant removal + CT	30.39 (NED)	NA (≤20%)	**WES + TDS**
26	54	*in situ* (T1)	1 (cosmetic)	yes	9.88y	I	implant removal	27.17 (NED)	NA (≤20%)	**TDS**
28	66	tumor-type (T2)	2 (breast carcinoma)	yes	5.36y (NA)	II	implant removal + CT	22.01 (NED)	no (≤20%)	**WES + TDS**
29	44	tumor-type (T2)	1 (cosmetic)	yes	8.07y	I	implant removal	22,64 (NED)	no (20%-50%)	**WES + TDS**
30	54	tumor-type (T2)	2 (breast carcinoma)	yes	3.6y (NA)	I	implant removal + CT	25.26 (NED)	no (>50%)	**WES + TDS**
31	54	*in situ* (T1)	1 (cosmetic)	yes	5.11y	IV	implant removal	24.67 (NED)	yes (≤20%)	**TDS**
32	65	*in situ* (T1)	3 (cosmetic)	yes	4.25y (NA)	I	implant removal	21.32 (NED)	yes (≤20%)	**WES**
33	40	tumor-type (T3)	1 (cosmetic)	yes	10.12y	IV	implant removal + CT	19.22 (NED)	yes (>50%)	**WES + TDS**
34	50	tumor-type (T3)	1 (cosmetic)	yes	9.85y	I	implant removal	15.74 (NED)	yes (>50%)	**WES + TDS**
35	43	tumor-type (Tx)	1 (cosmetic)	NA	12.31y	IV	implant removal + CT	23.49 (NED)	yes (>50%)	**TDS**
36	72	in situ (T1)	1 (cosmetic)	yes	22.77y	I	implant removal	11.76 (NED)	yes (>50%)	**TDS**
37	63	tumor-type (T3)	1 (breast carcinoma)	yes	8.74y	I	CT	7.75 (DOD)	NA (>50%)	**WES**
38	55	*in situ* (T1)	3 (breast carcinoma)	yes	5.31y (NA)	I	implant removal	13.11 (NED)	yes (>50%)	**TDS**
42	48	*in situ* (T1)	2 (cosmetic)	yes	4.88y (NA)	I	implant removal	13.54 (NED)	yes (>50%)	**WES + TDS**
48	65	tumor-type (T4)	3(cosmetic)	yes	0.5y (NA)	I	implant removal	7.66 (NED)	yes (≤20%)	**TDS**
50	58	*in situ* (T1)	2 (cosmetic)	yes	1.89y (11y)	IV	implant removal	NA	no (≤20%)	**TDS**
Bel1	59	*in situ* (T1)	2 (NA)	yes	8y (NA)	I	implant removal	18 (NED)	NA (>50%)	**WES**
Bel2	47	tumor-type (T2)	2 (NA)	yes	6y (13y)	NA	implant removal	22 (NED)	yes (20%-50%)	**WES + TDS**

Abbreviations note: CT, chemotherapy not specified, CT^1, cyclophosphamide, adriamycin, vincristine and prednisone (6 cures) [CHOP] or [CHOP like]; CT^2, dexamethasone, high dose aracytine, oxaliplatin [DHAOX]; DOD, dead of disease; DOC, dead from other cause (breast carcinoma); NA, not available; NED, no evidence of disease; RT, radiotherapy; TDS, Targeted deep sequencing; WES, Whole exome sequencing.