Genetic subtyping of breast implant-associated anaplastic large cell lymphomas

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# Supplemental Material

## Supplemental Methods

### Immunohistochemistry and fluorescence *in situ* hybridization (FISH)

Immunohistochemistry for ALK and p63 was performed as previously described.[1,2] Other phenotypic markers were evaluated when performed for diagnostic purposes or when sufficient material was available for additional immunohistochemistry, as previously reported.[1,3] Activation of STAT3 was assessed using immunohistochemistry for tyrosine 705-phosphorylated STAT3 ($pSTAT3^{Y705}$; clone D3A7, 1:400; Cell Signaling) in 25 available cases. Presence of *DUSP22* rearrangement was evaluated by FISH using a break-apart probe as published.[1] Presence of *TP63* rearrangement was evaluated by FISH using a *TP63* break-apart probe and a dual-fusion probe for *TBL1XR1-TP63* as previously published.[2] Both break-apart and dual-fusion probes were employed because the partner of *TP63* (3q28) is often the nearby gene *TBL1XR1* (3q26) and break-apart FISH alone can be difficult to interpret and may miss inv(3)(q26q28).[4] The distribution of genetic subtypes in BIA-ALCL was compared to that of other types of ALCL based on frequencies of ALK positivity in systemic ALCL (n=159),[5] *DUSP22* rearrangements in systemic ALK-negative (n=73)[1] and primary cutaneous (n=82)[6-8] ALCL, and *TP63* rearrangements in systemic ALK-negative (n=73)[1] and primary cutaneous (n=41)[4,9] ALCL.

### DNA sequencing and analysis

Genomic DNA was extracted from FFPE tissue sections macrodissected to enrich for tumor content using the Qiagen (Germantown, MD) AllPrep FFPE Kit. BIA-ALCL samples with adequate DNA (n=15) were interrogated for mutations of *JAK1*, *JAK3*, *STAT3*, *STAT5A*, and *STAT5B* using

targeted capture of the complete coding regions. Libraries were prepared from 200 ng of DNA per sample using baits designed with SureSelect (Agilent, Santa Clara, CA) software and the SureSelectXT Reagent Kit (Agilent). Libraries were pooled at equimolar concentrations and sequenced at 2x150 bp on an Illumina (San Diego, CA) MiSeq instrument using the 300-cycle MiSeq Reagent Kit V2 (Illumina). Raw sequence reads were extracted and de-multiplexed with Illumina CASAVA (Consensus Assessment of Sequence And VAriation) version 1.8.2 and FASTQ files were aligned to human genome build hg19 using CLC Genomics Workbench (Qiagen). Average coverage (read depth) for each gene was: *JAK1*, 740; *JAK3*, 536; *STAT3*, 724; *STAT5A*, 745; and *STAT5B*, 763. Orthogonal sequencing was performed on an Ion Torrent (Thermo Fisher, Waltham, MA), and variants identified on both platforms were visually evaluated using Alamut (Interactive Biosoftware, Rouen, France). Germline variants were excluded based on data in ExAC,[10] dbSNP,[11]and 1000 Genomes.[12] Functional annotation was derived from the literature or predicted using PolyPhen-2[13] and SIFT.[14] All reported variants had a variant base quality score >30, a minor allele frequency of >5%, and coverage of >95x.

# Supplemental Tables

## Supplemental Table 1. Phenotype of breast-implant-associated anaplastic large cell lymphoma (BIA-ALCL)

| | Marker | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CD2 | CD3 | CD4 | CD5 | CD7 | CD8 | CD30 | CD43 | CD45 | CD56 | EMA | TIA1 | GrzB | Perf. | TCRβF1 | TCRγδ | p63 | EBER* |
| Total%† | 55 | 31 | 75 | 34 | 6 | 9 | 100 | 86 | 44 | 28 | 48 | 58 | 63 | 50 | 19 | 0 | 12 | 0 |
| N tested | 22 | 36 | 36 | 29 | 18 | 33 | 36 | 29 | 32 | 18 | 25 | 24 | 24 | 2 | 21 | 20 | 33 | 21 |
| Patient | | | | | | | | | | | | | | | | | | |
| 1 | . | - | + | + | . | . | + | + | - | . | . | . | . | . | . | . | - | . |
| 2 | - | - | + | . | . | - | + | . | . | . | - | - | + | . | . | . | - | . |
| 3 | . | + | + | - | . | - | + | + | + | . | - | + | + | . | . | . | - | . |
| 4‡ | - | - | + | + | - | - | + | - | + | - | - | + | - | . | - | - | - | - |
| 5 | + | + | + | + | . | - | + | + | + | . | . | . | . | . | . | . | - | - |
| 6 | + | - | + | - | - | - | + | +/- | . | . | +/- | - | - | . | . | . | + | . |
| 7 | + | + | + | + | -/+ | - | + | + | - | - | - | -/+ | +/- | . | . | . | - | . |
| 8 | + | + | + | - | . | - | + | . | + | . | - | . | . | . | . | . | - | . |
| 9 | - | - | + | - | . | - | + | + | -/+ | . | -/+ | -/+ | -/+ | . | - | - | - | - |
| 10 | + | + | - | - | - | - | + | . | - | . | -/+ | - | - | . | . | . | - | . |
| 11 | . | - | - | - | - | - | + | - | - | . | -/+ | + | - | . | +/- | - | - | - |
| 12 | - | - | + | - | - | . | + | + | - | . | . | . | . | . | - | . | . | - |
| 13 | + | + | + | - | . | - | + | . | + | . | . | + | + | . | . | . | - | . |
| 14 | - | - | + | - | . | - | + | . | - | . | + | . | . | . | . | . | - | . |
| 15 | . | - | - | - | . | . | + | . | . | . | . | . | . | . | . | . | + | . |
| 16 | - | - | - | . | . | - | + | + | - | . | . | . | . | . | . | . | - | - |
| 17 | . | + | + | + | +/- | - | + | . | + | . | . | . | . | . | . | . | . | . |
| 18 | - | - | + | - | - | - | + | - | - | . | - | . | . | . | - | - | - | . |
| 19 | +/- | -/+ | + | - | -/+ | - | + | +/- | - | . | . | . | . | . | - | - | - | . |
| 20 | . | - | + | + | . | - | + | + | - | + | + | + | + | . | - | - | - | - |
| 21 | . | - | + | . | . | - | + | + | - | - | + | - | + | . | + | - | - | - |
| 22 | + | + | + | + | . | - | + | + | + | - | + | + | + | . | - | - | - | - |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 23 | + | - | - | + | - | + | + | + | - | + | - | . | + | . | + | - | - | - |
| 24 | - | - | + | - | - | - | + | + | + | - | + | + | + | . | - | - | - | - |
| 25 | . | + | + | . | . | + | + | + | + | - | + | + | + | . | - | - | - | - |
| 26 | + | - | + | - | - | - | + | + | - | + | - | + | + | . | - | - | - | - |
| 27 | . | - | - | - | - | + | + | + | - | + | - | - | + | . | - | - | - | - |
| 28 | . | - | - | . | - | - | + | + | . | - | . | . | + | . | - | - | + | - |
| 29 | . | + | + | + | . | - | + | + | - | - | + | + | + | . | - | - | - | - |
| 30 | . | - | + | + | - | - | + | + | + | - | - | - | - | . | - | - | - | - |
| 31 | + | + | + | . | . | - | + | + | - | + | . | + | . | . | . | . | - | . |
| 32 | . | - | - | . | . | - | + | + | - | - | + | - | + | . | - | - | - | - |
| 33 | + | - | + | - | - | - | + | + | + | - | . | - | - | - | + | - | - | - |
| 34 | . | - | + | - | . | - | + | + | + | - | + | + | - | . | - | - | +/- | - |
| 35 | - | - | - | - | - | - | + | - | + | - | + | + | - | + | - | - | - | - |
| 36 | - | - | + | - | - | - | + | + | +/- | . | + | + | . | . | . | . | . | . |

Abbreviations: EBER, Epstein-Barr virus-encoded small RNA; EMA, epithelial membrane antigen; GrzB, granzyme B; Perf., perforin; TCR, T-cell receptor; TIA1, T-cell intracellular antigen-1.

*EBER analyzed by *in situ* hybridization; all other results based on immunohistochemistry.

†Total% represents percentage of cases tested with at least partial staining based on the following scoring system: +, positive (≥30% tumor cells staining); +/-, partial (10-29%); -/+, focal (1-9%); -, negative (0%); ., not done.

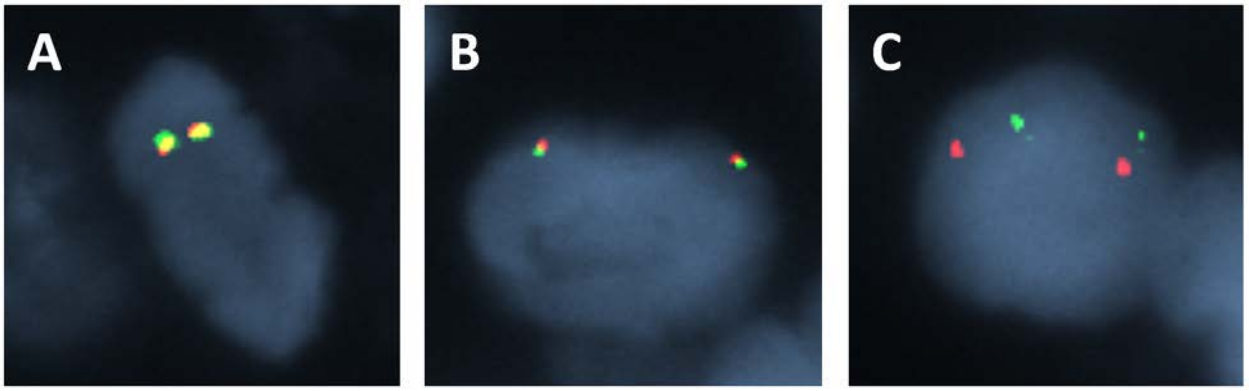
‡Clinical data on Patient 4 and Patients 20-35 have been previously published by Miranda et al.[15]

**Supplemental Table 2. Staging, pSTAT3, and genetic data by case**

| Patient | Stage* | pSTAT3† | Gene | Variant | VAF (%) |
|---|---|---|---|---|---|
| 1 | n/a | 90 | . | . | . |
| 2 | IA | n/a | . | . | . |
| 3 | IA | 90 | *STAT3* | c.1842C>G_p.Ser614Arg | 42 |
| 4 | IA | 50 | . | . | . |
| 5 | IA | 60 | . | . | . |
| 6 | IA | 30 | . | . | . |
| 7 | n/a | 90 | . | . | . |
| 8 | n/a | 90 | *STAT3* | c.1919A>T_p.Tyr640Phe | 6 |
| 9 | n/a | 70 | . | . | . |
| 10 | n/a | 50 | . | . | . |
| 11 | n/a | 70 | . | . | . |
| 12 | IIA | n/a | . | . | . |
| 13 | IA | 70 | . | . | . |
| 14 | IA | 100 | . | . | . |
| 15 | III | 100 | *STAT3* | c.1919A>T_p.Tyr640Phe | 27 |
| 16 | IA | 80 | . | . | . |
| 17 | n/a | n/a | . | . | . |
| 18 | n/a | 80 | . | . | . |
| 19 | IA | n/a | . | . | . |
| 20 | IC | 90 | . | . | . |
| 21 | IIA | 60 | . | . | . |
| 22 | IIA | 70 | . | . | . |
| 23 | IA | 30 | *JAK1* | c.3290G>A_p.Gly1097Asp | 55 |
| 24 | IC | 90 | . | . | . |
| 25 | IB | 100 | . | . | . |
| 26 | IB | 100 | . | . | . |
| 27 | IB | 100 | . | . | . |
| 28 | IA | 80 | . | . | . |
| 29 | IIA | 70 | . | . | . |
| 30 | IIA | n/a | . | . | . |
| 31 | IB | 70 | . | . | . |
| 32 | IC | 60 | . | . | . |
| 33 | IIA | n/a | . | . | . |
| 34 | IB | n/a | . | . | . |
| 35 | IIA | n/a | . | . | . |
| 36 | III | n/a | . | . | . |

Abbreviations: n/a, not available; VAF, variant allele frequency.
*Based on the TNM staging system of Clemens et al.[16]
†Percentage of tumor cells with nuclear staining for $pSTAT3^{Y705}$, scored to the nearest decile.

## Supplemental Figure




**Supplemental Figure 1. Representative fluorescence *in situ* hybridization (FISH) in BIA-ALCL.** (A) FISH using a breakapart probe to the *DUSP22-IRF4* gene region on 6p25.3 shows 2 intact red-green fusion signals, indicating the absence of a rearrangement. (B) FISH using a breakapart probe to the *TP63* gene region on 3q28 shows 2 intact red-green fusion signals, indicating the absence of a rearrangement. (C) FISH using a dual-fusion probe to the *TBL1XR1* gene region on 3q26 and the *TP63* gene region on 3q28 shows 2 copies of both gene regions without evidence of fusion. (All: original magnification, ×600).

## Supplemental References

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