Molecular profiling reveals a hypoxia signature in breast implant-associated anaplastic large cell lymphoma

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Supplemental Material

Supplemental Methods

Cell lines

Cell lines TLBR-1, TLBR-2, and TLBR-3 were established from BIA-ALCLs by A.L.E.\textsuperscript{1,2} and were maintained in RPMI-1640 (Gibco) supplemented with 10% FBS (Clontech), 1% penicillin/streptomycin (Gibco), and 100 U/mL IL2 (R&D 202-IL-050). TLBR-1, -2, and -3 cells were cultured at a concentration of $0.5 \times 10^6$/mL for 72 h and resuspended in PBS. For experiments involving hypoxia, cells were incubated for 12-24 h at 37$^\circ$C under humidified conditions in a Napco Series 8000 water-jacketed CO$_2$ incubator (Thermo Scientific) at 5% CO$_2$ and 1% O$_2$. For siRNA electroporation, cells in the same media without addition of penicillin/streptomycin were transfected with siRNA targeting CA9 (ON-TARGETplus SMARTpool, L-005244-00-0010; Dharmacon) or a non-targeting control pool (ON-TARGETplus, D-001810-10-05). Protein expression and cell proliferation were assessed after 72 h as described below.

Western blotting

For western blotting, proteins were isolated from cell culture lysates in radioimmunoprecipitation assay (RIPA) buffer containing HALT, PMSF and Roche cOmplete\textsuperscript{TM} Mini protease inhibitors.
Proteins were quantified using the Biorad DC™ assay and equal quantities were loaded and run on 10% Tris-HCl poly-acrylamide gels. Proteins were then transferred to nitrocellulose membranes and blocked with 1:1 TBS:Odyssey Blocking Buffer (LI-COR). Membranes were incubated overnight with the following primary antibodies: CA9 Rabbit mAb (D47G3; Cell Signaling), β-Actin (AC-15; Novus Biologicals LLC, Littleton, CO) and HIF-1α (EP1215Y; Abcam). Protein detection was performed on a LI-COR Odyssey Fc using IRDye® 680W and 800CW secondary antibodies (LI-COR).

**Cell proliferation assay**

The MTS assay (CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay, Promega) was used to assess cell viability per the manufacturer’s protocol. Briefly, 50,000 cells were plated per well after electroporation with control or CA9 siRNA. Plates were incubated under normoxic conditions for 48 h to allow cell recovery and then cultured under either normoxic or hypoxic conditions for 24 h. Colorimetric determination was performed measuring absorbance at 490 nm after incubation with MTS reagent for 2-3 h.

**Lentiviral CA9 overexpression**

Lentiviral vectors included a commercially available human CA9 lentiviral vector (pLenti-GIII-EF1a, ABM Inc., #LV102843) and empty pLenti-GIII-EF1a vector as a control (ABM Inc., #LV588). For viral packaging, 293T cells were maintained using DMEM complete media supplemented with 10% FBS. On the day of viral packaging, cell media was changed to RPMI1640 complete media supplemented with 10% FBS. Transfection solution was prepared in Opti-MEM™ I Reduced Serum Medium (Thermo Fisher Scientific, 31985070) using
Lipofectamine 3000 (Thermo Fisher Scientific, L3000015), lentiviral packaging plasmid psPAX2 (a gift from Didier Trono [Addgene plasmid #12260; http://n2t.net/addgene:12260; RRID:Addgene_12260]), and pMD2.G (a gift from Didier Trono [Addgene plasmid #12259; http://n2t.net/addgene:12259; RRID:Addgene_12259]), and added dropwise onto 293T cells. Packaged virus was harvested 24 h after transfection and purified by high speed centrifugation and filtration using 0.45 μm syringe filters (Fisher, 09-719D). TLBR-3 cells were maintained in RPMI1640 complete media supplemented with 10% FBS and IL2, transfected using titrated virus and polybrene (10 mg/mL), and selected in puromycin. Ten NSG mice per group were injected subcutaneously with 1.0 x 10^6 cells TLBR-3 cells transduced with CA9 or empty vector in 100 μL of PBS and tumors were measured as described.

**Serum and tumor CA9 determination in BIA-ALCL xenograft models**

Eight NSG mice per group were injected subcutaneously with 10.0 x 10^6 cells TLBR-1, -2, or -3 cells in 100 μL of PBS. When each tumor reached 1,000 mm^3 the corresponding mouse was euthanized and blood was obtained by cardiac puncture. Blood was similarly obtained from a fourth group of mice injected with PBS only. Blood samples were immediately stored in serum separator tubes (BD Microtainer), allowed to clot for 1 h, and centrifuged to collect serum. Subcutaneous tumors were harvested from tumor-bearing mice immediately after euthanasia. Tumor tissue was pulverized and lysed for 15 min on ice using RNase-free disposable pellet pestles (Fischer Scientific) in 120 μL of RIPA buffer containing HALT, PMSF, and Roche cOmplete™ Mini protease inhibitors. Samples were centrifuged at 13,000 rpm for 15 min at 4°C. Supernatant protein concentrations were determined using a DC protein assay and adjusted to a concentration of 1 μg/μL for CA9 measurement by ELISA.
CA9 ELISA

Human serum (n=1), plasma (n=3), and seroma (n=13) samples were collected at MD Anderson Cancer Center between May 2014 and March 2019 as previously published. Ten seromas were involved by BIA-ALCL based on standard pathologic criteria. Control seromas were collected from 3 patients, 2 with benign seromas and 1 from an uninvolved seroma contralateral to BIA-ALCL. CA9 concentrations in seroma, serum, plasma, cell culture supernatant, and tumor lysate samples were measured using a commercially available solid-phase ELISA kit (Quantikine, R&D Systems, Minneapolis, MN) following the manufacturer’s instructions, diluting samples as needed to allow interpretation within the dynamic range of the reference standard curve. Briefly, 100 μL of appropriately diluted sample was added to each well and the plate was shaken for 2 h at room temperature. Wells were washed, incubated with 200 μL of biotinylated anti-CA9 conjugate for 2 h, washed again, and then incubated with 200 μL of streptavidin-horseradish peroxidase and stabilized chromogen solution for 0.5 h. Finally, 50 μL of stop solution (2N H₂SO₄) was added and absorbance was measured at 450 nm on a photometric plate reader. Concentrations were interpolated from triplicate measurements using a second-order polynomial quadratic model.

Statistical analysis

Statistical analyses were performed using JMP Pro 14 (SAS Institute), GraphPad Prism 7, or in the R statistical environment. Statistical tests are as indicated. P-values <0.05 were considered statistically significant.
### Supplemental Tables

#### Supplemental Table 1. Top gene sets positively associated with BIA-ALCLs

| NAME                                                      | NES  | Nom P-val | FDR q-val |
|-----------------------------------------------------------|------|-----------|-----------|
| HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION                | 2.963| 0.000     | 0.000     |
| HALLMARK_HYPOXIA                                         | 2.727| 0.000     | 0.000     |
| HALLMARK_TNFA_SIGNALING_VIA_NFKB                         | 2.530| 0.000     | 0.000     |
| REACTOME_COLLAGENFORMATION                                | 2.358| 0.000     | 0.000     |
| KEGG_ECM_RECEPTOR_INTERACTION                            | 2.140| 0.000     | 0.001     |
| REACTOME_EXTRACELLULARMATRIXORGANIZATION                 | 2.139| 0.000     | 0.001     |
| REACTOME_A_TETRASACCHARIDE_LINKERSEQUENCE_ISRQURED_FORGAG_SYNTHESIS | 2.072| 0.000     | 0.002     |
| BIOCARTA_CDMACPATHWAY                                    | 2.052| 0.000     | 0.002     |
| HALLMARK_ANGIOGENESIS                                    | 2.022| 0.000     | 0.004     |
| REACTOME_ACTIVATION_OF_THE_AP1_FAMILY_OF_TRANSCRIPTIONFACTORS | 1.959| 0.000     | 0.010     |
Supplemental Table 2. Top gene sets associated with hypoxia vs. normoxia in TLBR-2 cells

| NAME                                             | NES   | Nom P-val | FDR q-val |
|--------------------------------------------------|-------|-----------|-----------|
| HALLMARK_HYPOXIA                                 | 2.514 | 0.000     | 0.000     |
| HALLMARK_GLYCOLYSIS                              | 2.145 | 0.000     | 0.000     |
| REACTOME_3 UTR_MEDIATED_TRANSLATIONAL_REGULATION  | 2.090 | 0.000     | 0.000     |
| REACTOME_NONSENSE_MEDIATED_DECAY_ENHANCED_BY_THE_EXON_JUNCTION_COMPLEX | 2.023 | 0.000     | 0.000     |
| KEGG_NITROGEN_METABOLISM                         | 1.950 | 0.000     | 0.002     |
| REACTOME_METABOLISM_OF_CARBOHYDRATES             | 1.918 | 0.000     | 0.005     |
| REACTOME_GLUCOSE_METABOLISM                      | 1.920 | 0.000     | 0.005     |
| REACTOME_SRP_DEPENDENT_COTRANSLATIONAL_PROTEIN_TARGETING_TO_MEMBRANE | 1.894 | 0.000     | 0.010     |
| HALLMARK_IL2_STAT5_SIGNALING                     | 1.870 | 0.000     | 0.016     |
| HALLMARK_KRAS_SIGNALING_UP                       | 1.856 | 0.000     | 0.019     |
| KEGG_JAK_STAT_SIGNALING_PATHWAY                  | 1.846 | 0.000     | 0.022     |
| KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION      | 1.842 | 0.000     | 0.022     |
| Gene Set                                         | FDR   | P-value | q-value |
|------------------------------------------------|-------|---------|---------|
| REACTOME_GLYCOLYSIS                            | 1.823 | 0.002   | 0.029   |
| KEGG_PENTOSE_PHOSPHATE_PATHWAY                 | 1.819 | 0.002   | 0.030   |
| REACTOME_GLUCONEOGENESIS                       | 1.823 | 0.000   | 0.031   |
| KEGG_STARCH_AND_SUCROSE_METABOLISM             | 1.799 | 0.002   | 0.042   |

**Down-regulated Gene Sets**

| Gene Set                                         | FDR   | P-value | q-value |
|------------------------------------------------|-------|---------|---------|
| HALLMARK_MYC_TARGETS_V2                         | -1.937| 0.000   | 0.001   |
### Supplemental Table 3. Top gene sets associated with CA9 siRNA vs. control siRNA in TLBR-2 cells

| NAME | NES   | Nom P-val | FDR q-val |
|------|-------|-----------|-----------|
| **Up-regulated Gene Sets** | | | |
| None passing FDR≤0.05 | | | |
| **Down-regulated Gene Sets** | | | |
| HALLMARK_MYC_TARGETS_V1 | -2.460 | 0.000 | 0.000 |
| REACTOME_S_PHASE | -2.236 | 0.000 | 0.000 |
| HALLMARK_MYC_TARGETS_V2 | -2.228 | 0.000 | 0.000 |
| REACTOME_ASSEMBLY_OF_THE_PRE_REPLICATIVE_COMPLEX | -2.143 | 0.000 | 0.000 |
| REACTOME_ORC1_REMOVAL_FROM_CHROMATIN | -2.174 | 0.000 | 0.000 |
| REACTOME_DNA_REPLICATION | -2.182 | 0.000 | 0.000 |
| HALLMARK_UNFOLDED_PROTEIN_RESPONSE | -2.191 | 0.000 | 0.000 |
| REACTOME_M_G1_TRANSITION | -2.135 | 0.000 | 0.000 |
| REACTOME_SYNTHESIS_OF_DNA | -2.198 | 0.000 | 0.000 |
| REACTOME_G1_S_TRANSITION | -2.129 | 0.000 | 0.000 |
| Pathway                                                                 | t-value | p-value 1 | p-value 2 |
|------------------------------------------------------------------------|---------|-----------|-----------|
| REACTOME_MITOTIC_M_M_G1_PHASES                                          | -2.126  | 0.000     | 0.000     |
| REACTOME_HOST_INTERACTIONS_OF_HIV_FACTORS                              | -2.098  | 0.000     | 0.001     |
| REACTOME_REGULATION_OF_MITOTIC_CELL_CYCLE                               | -2.105  | 0.000     | 0.001     |
| KEGG_PROTEASOME                                                        | -2.095  | 0.000     | 0.001     |
| HALLMARK_E2F_TARGETS                                                   | -2.071  | 0.000     | 0.001     |
| REACTOME_VIF_MEDIATED_DEGRADATION_OF_APOBEC3G                           | -2.069  | 0.000     | 0.001     |
| REACTOME_REGULATION_OF_ORNITHINE_DECARBOXYLASE_ODC                      | -2.062  | 0.000     | 0.001     |
| REACTOME_MRNA_PROCESSING                                                | -2.072  | 0.000     | 0.001     |
| REACTOME_MITOTIC_G1_G1_S_PHASES                                        | -2.062  | 0.000     | 0.001     |
| REACTOME_SCF_BETA_TRCP_MEDIATED_DEGRADATION_OF_EMI1                     | -2.063  | 0.000     | 0.001     |
| REACTOME_CDT1_ASSOCIATION_WITH_THE_CDC6_ORC_ORIGIN_COMPLEX              | -2.063  | 0.000     | 0.001     |
| REACTOME_PROCESSING_OF_CAPPED_INTRON_CONTAINING_PRE_MRNA               | -2.074  | 0.000     | 0.001     |
| REACTOME_CYCLIN_E_ASSOCIATED_EVENTS DURING_G1_S_TRANSITION_            | -2.075  | 0.000     | 0.001     |
| REACTOME_APC_C_CDH1_MEDIATED_DEGRADATION_OF_CDC20_AND_OTHER_APC_C_CDH1_ | -2.075  | 0.000     | 0.001     |
| TARGETED_PROTEINS_IN_LATE_MITOSIS_EARLY_G1                              |         |           |           |
| HALLMARK_MTORC1_SIGNALING                                              | -2.075  | 0.000     | 0.001     |
| REACTOME_SCFSKP2_MEDIATED_DEGRADATION_OF_P27_P21                        | -2.054  | 0.000     | 0.001     |
| REACTOME_CROSS_PRESENTATION_OF_SOLUBLE_EXOGENOUS_ANTIGENS_ENDOSONES     | -2.052  | 0.000     | 0.001     |
| Pathway                                        | Z-score | P-value | FDR       |
|-----------------------------------------------|---------|---------|-----------|
| REACTOME APC C CDC20 MEDIATED DEGRADATION OF MITOTIC PROTEINS | -2.077  | 0.000   | 0.001     |
| REACTOME ER PHAGOSOME PATHWAY                 | -2.079  | 0.000   | 0.001     |
| KEGG ASThma                                   | -2.045  | 0.000   | 0.001     |
| HALLMARK INFLAMMATORY RESPONSE                | -2.035  | 0.000   | 0.002     |
| REACTOME METABOLISM OF RNA                    | -2.037  | 0.000   | 0.002     |
| REACTOME ACTIVATION OF NF KAPPA IN B CELLS    | -2.029  | 0.000   | 0.002     |
| REACTOME CDK MEDIATED PHOSPHORYLATION AND REMOVAL OF CDC6 | -2.039  | 0.000   | 0.002     |
| REACTOME DESTABILIZATION OF mRNA BY AUF1 HNRNP D0 | -2.030  | 0.000   | 0.002     |
| REACTOME AUTODEGRADATION OF CDH1 BY CDH1 APC C | -2.041  | 0.000   | 0.002     |
| REACTOME TRNA AMINOACYLATION                  | -2.003  | 0.000   | 0.002     |
| REACTOME CELL CYCLE CHECKPOINTS              | -2.000  | 0.000   | 0.002     |
| REACTOME MRNA SPlicing                       | -2.001  | 0.000   | 0.002     |
| REACTOME CELL CYCLE MITOTIC                  | -1.978  | 0.000   | 0.004     |
| REACTOME REGULATION OF APOPTOSIS              | -1.970  | 0.000   | 0.005     |
| BIOCARTA CD40 PATHWAY                        | -1.972  | 0.000   | 0.005     |
| REACTOME MITOCHONDRIAL PROTEIN IMPORT         | -1.941  | 0.000   | 0.009     |
| REACTOME SIGNALING BY WNT                     | -1.941  | 0.000   | 0.009     |
| KEGG SPliceosome                             | -1.941  | 0.000   | 0.009     |
| Pathway                                                                 | Z-score | P-value 1 | P-value 2 |
|------------------------------------------------------------------------|---------|-----------|-----------|
| REACTOME_REGULATION_OF_MRNA_STABILITY_BY_PROTEINS_THAT_BIND_AU_RICHLEMENTS | -1.942  | 0.002     | 0.009     |
| BIOCARTA_PROTEASOME_PATHWAY                                            | -1.937  | 0.002     | 0.009     |
| HALLMARK_G2M_CHECKPOINT                                                | -1.935  | 0.000     | 0.009     |
| REACTOME_ANTIGEN_PROCESSING_CROSS_PRESENTATION                        | -1.931  | 0.000     | 0.010     |
| HALLMARK_CHOLESTEROL_HOMEOSTASIS                                       | -1.917  | 0.002     | 0.012     |
| REACTOME_PROTEIN_FOLDING                                               | -1.914  | 0.000     | 0.012     |
| REACTOME_PREFOLDIN_MEDIATED_TRANSFER_OF_SUBSTRATE_TO_CCT_TRIC          | -1.909  | 0.002     | 0.013     |
| REACTOME_CYTOSOLIC_TRNA_AMINOACYLATION                                 | -1.905  | 0.004     | 0.013     |
| HALLMARK_UV_RESPONSE_UP                                                | -1.902  | 0.000     | 0.014     |
| REACTOME_P53_DEPENDENT_G1 DNA DAMAGE_RESPONSE                           | -1.896  | 0.002     | 0.015     |
| REACTOME_METABOLISM_OF_MRNA                                            | -1.897  | 0.000     | 0.015     |
| KEGG_AMINOACYL_TRNA_BIOSYNTHESIS                                       | -1.891  | 0.002     | 0.016     |
| REACTOME_P53_INDEPENDENT_G1 S DNA DAMAGE_CHECKPOINT                    | -1.887  | 0.004     | 0.016     |
| REACTOME_HIV_LIFE_CYCLE                                                | -1.881  | 0.002     | 0.017     |
| REACTOME_LATE_PHASE_OF_HIV_LIFE_CYCLE                                  | -1.874  | 0.000     | 0.018     |
| REACTOME_DOWNSTREAM_SIGNALING_EVENTS_OF_B_CELL_RECEPTOR_BCR           | -1.876  | 0.000     | 0.018     |
| REACTOME_TRANSPORT_OF_MATURE_TRANSCRIPT_TO_CYTOPLASM                   | -1.875  | 0.004     | 0.018     |
| Pathway                                         | Z-score | P-value  | FDR      |
|------------------------------------------------|---------|----------|----------|
| HALLMARK_ALLOGRAFT_REJECTION                   | -1.876  | 0.000    | 0.018    |
| REACTOME_CHOLESTEROL_BIOSYNTHESIS              | -1.866  | 0.006    | 0.020    |
| REACTOME_METABOLISM_OF_NON_CODING_RNA          | -1.862  | 0.002    | 0.020    |
| REACTOME_AUTODEGRADATION_OF_THE_E3_UBIQUITIN_LIGASE_COP1 | -1.864  | 0.002    | 0.020    |
| KEGG_ALLOGRAFT_REJECTION                       | -1.858  | 0.010    | 0.021    |
| BIOCARTA_TNFR2_PATHWAY                         | -1.851  | 0.000    | 0.022    |
| REACTOME_MRNA_SPLICING_MINOR_PATHWAY          | -1.848  | 0.004    | 0.023    |
| REACTOME_INTERACTIONS_OF_VPR_WITH_HOST_CELLULAR_PROTEINS | -1.844  | 0.002    | 0.024    |
| KEGG_CYTOSOLIC DNA_SENSING_PATHWAY             | -1.838  | 0.013    | 0.025    |
| REACTOME_PERK_REGULATED_GENE_EXPRESSION        | -1.836  | 0.004    | 0.025    |
| KEGG_JAK_STAT_SIGNALING_PATHWAY                | -1.817  | 0.002    | 0.030    |
| KEGG_PYRIMIDINE_METABOLISM                     | -1.815  | 0.002    | 0.031    |
| REACTOME_TRANSCRIPTION                         | -1.807  | 0.000    | 0.031    |
| KEGG_DNA_REPLICATION                           | -1.808  | 0.008    | 0.031    |
| REACTOME_RNA_POL_II_TRANSCRIPTION              | -1.808  | 0.000    | 0.032    |
| REACTOMEFORMATION_OF_TUBULIN_FOLDING_INTERMEDIATES_BY_CCT_TRIC | -1.809  | 0.008    | 0.032    |
| REACTOME_EXTENSION_OF_TELOMERES                | -1.800  | 0.012    | 0.032    |
| HALLMARK_TNFA_SIGNALING_VIA_NFKB               | -1.801  | 0.000    | 0.032    |
| Pathway                                                        | Score  | P-value | FDR   |
|---------------------------------------------------------------|--------|---------|-------|
| BIOCARTA_RANMS_PATHWAY                                       | -1.795 | 0.000   | 0.033 |
| REACTOME_DNA_STRAND_ELONGATION                               | -1.795 | 0.010   | 0.033 |
| REACTOME_CLEAVAGE_OF_GROWING_TRANSCRIPT_IN_THE_TERMINATION_REGION_ | -1.788 | 0.014   | 0.035 |
| KEGG_VIRAL_MYOCARDITIS                                       | -1.779 | 0.006   | 0.037 |
| REACTOME_APOPTOSIS                                           | -1.775 | 0.002   | 0.038 |
| BIOCARTA_RACCYCD_PATHWAY                                     | -1.776 | 0.004   | 0.038 |
| REACTOME_INFLUENZA_LIFE_CYCLE                                | -1.769 | 0.000   | 0.039 |
| BIOCARTA_TH1TH2_PATHWAY                                      | -1.759 | 0.006   | 0.042 |
| KEGG_AUTOIMMUNE_THYROID_DISEASE                              | -1.760 | 0.015   | 0.042 |
| BIOCARTA_41BB_PATHWAY                                        | -1.760 | 0.000   | 0.042 |
| REACTOME_ACTIVATION_OF_THE_PRE_REPLICATIVE_COMPLEX           | -1.747 | 0.016   | 0.045 |
| KEGG_STEROID_BIOSYNTHESIS                                    | -1.747 | 0.013   | 0.045 |
| REACTOME_METABOLISM_OF_AMINO_ACIDS_AND_DERIVATIVES           | -1.739 | 0.002   | 0.048 |
Supplemental Figures

Supplemental Figure 1. BIA-ALCLs show significantly higher expression of hypoxia-related genes such as *VEGFA*, *CA9*, and *SLC2A3* encoding GLUT3 than non-BIA-ALCLs. No differential expression of *SLC2A1* encoding GLUT1 is observed. See also Figure 1, main manuscript. ***P<0.001; n.s., not significant.
**Supplemental Figure 2. Effect of CA9 overexpression in vitro.** (A) Western blot showing CA9 expression in TLBR-3 cells stably transduced with CA9 lentivirus or control vector under hypoxic conditions. (B) Relative growth of TLBR-3 cells stably transduced with CA9 lentivirus or control vector (MTS assay).
Supplemental Figure 3. Serum and plasma measurements of CA9 concentration in a limited number of samples from patients with BIA-ALCL.
Supplemental Figure 4. Subcutaneous TLBR-1, -2, and -3 tumors were grown in NSG mice to a tumor volume of 1000 mm³. Animals were euthanized and tumor tissue CA9 concentrations were measured by ELISA. See also Figure 6C. **, P<0.01; ***, P<0.001 (Mann-Whitney test).
Supplemental Figure 5. Gene expression data were derived from RNA sequencing of TLBR-1, -2, and -3 cell lines at normoxic baseline. Genes from the HALLMARK HYPOXIA gene set with log2 fold-change (FC) values ≥1 in either TLBR-2 or TLBR-3 compared to TLBR-1 are shown.
Supplemental Figure 6. Genes reported as significantly down- or up-regulated comparing BIA-ALCL to systemic ALCL in Di Napoli et al.\textsuperscript{5} were evaluated for fold-change differences in expression in the present study comparing BIA-ALCL to ALCLs of triple-negative genetic subtype. Means and standard deviations are shown.
Supplemental References

1. Lechner MG, Lade S, Liebertz DJ, et al. Breast implant-associated, ALK-negative, T-cell, anaplastic, large-cell lymphoma: establishment and characterization of a model cell line (TLBR-1) for this newly emerging clinical entity. Cancer. 2011;117(7):1478-1489.

2. Lechner MG, Megiel C, Church CH, et al. Survival signals and targets for therapy in breast implant-associated ALK--anaplastic large cell lymphoma. Clin Cancer Res. 2012;18(17):4549-4559.

3. Hanson SE, Hassid VJ, Branch-Brooks C, et al. Validation of a CD30 Enzyme-Linked Immunosorbant Assay for the Rapid Detection of Breast Implant-Associated Anaplastic Large Cell Lymphoma. Aesthet Surg J. 2019;Feb 21. pii: sjy327. doi: 10.1093/asj/sjy327. [Epub ahead of print]

4. Feldman AL, Harris NL, Stein H, et al. Breast implant-associated anaplastic large cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4th ed. Lyon: International Agency for Research on Cancer, 2017:421-422.

5. Di Napoli A, De Cecco L, Piccaluga PP, et al. Transcriptional analysis distinguishes breast implant-associated anaplastic large cell lymphoma from other peripheral T-cell lymphomas. Mod Pathol. 2019;32(2):216-230.