



# CLA, TRAF1, BCL2, NFKB2, CD56, EMA and Fascin Expression in CD30+ Lymphoproliferative Disorders of the Skin



<sup>1</sup>Pathology, Moffitt Cancer Center, Tampa, FL, United States; <sup>2</sup>Genoptix Medical Laboratory, Carlsbad, CA, United States; <sup>3</sup>Cutaneous Oncology, Moffitt Cancer Center, Tampa, FL, United States and <sup>4</sup>Dermatology, Roger Williams Hospital, Providence, RI, United States

**Background:** Lymphomatoid papulosis (LyP), primary cutaneous (pcALCL) and systemic anaplastic large cell lymphoma (sALCL) are CD30+ lymphoproliferative disorders (LPD) with overlapping morphology, but widely different behavior. This study examines the role of BCL2, CD56, cutaneous lymphocyte antigen (CLA, skin homing), EMA, Fascin, NFKB2, and tumor necrosis factor receptor (TNFR) associated factor 1 (TRAF1, intracellular trafficking) in differentiating CD30+ lymphoproliferative disorders in the skin.

**Design:** With IRB approval, formalin-fixed, paraffin-embedded biopsy specimens from 13 LyP, 9 pcALCL and 8 sALCL were selected from the MCC Archives from 2000-07. All cases were stained with the above antibodies using manufacturers protocols. Manual morphometry and virtual flow cytometry were performed to quantitate antibody expression. Statistical analysis was performed using non-parametric analysis of variance (Kruskal-Wallis method).

**Results:** There was no significant difference of TRAF1 between LyP and pcALCL ( $p > 0.05$ ), but significant differences between LyP and sALCL ( $p < 0.01$ ), and pcALCL and sALCL ( $p < 0.001$ ). CLA expression showed significant differences between LyP and pcALCL ( $p < 0.05$ ) and LyP and sALCL ( $p < 0.001$ ), with insignificant differences between pcALCL and sALCL ( $p > 0.05$ ). BCL2 showed significant differences between pcALCL and sALCL ( $p < 0.001$ ) but not between LyP and pcALCL ( $p > 0.05$ ) and LyP and sALCL ( $p > 0.05$ ). NFKB2 showed significant differences between LyP and sALCL ( $p < 0.01$ ) and pcALCL and sALCL ( $p < 0.05$ ). CD56 ( $p = 0.5283$ ), Fascin ( $p = 0.2590$ ), and EMA ( $p = 0.1624$ ) were not contributory.

**Conclusion:** There was significant differential expression of TRAF1 and CLA in LyP. TRAF1 was negative in sALCL. CLA differentiates LyP from pcALCL and sALCL. BCL2 may differentiate pcALCL from sALCL. NFKB2 may be useful in distinguishing pcALCL from sALCL. This study indicates potential utility of TRAF-1, CLA, BCL2 and possibly NFKB2 in separating systemic from primary cutaneous CD30+ LPD. CD56, Fascin and EMA were non-contributory.

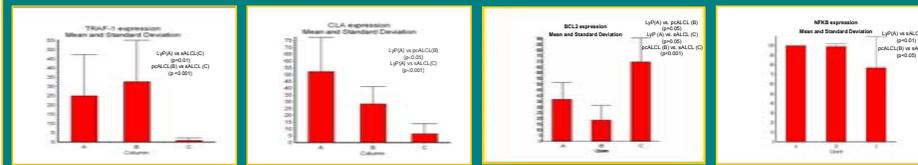
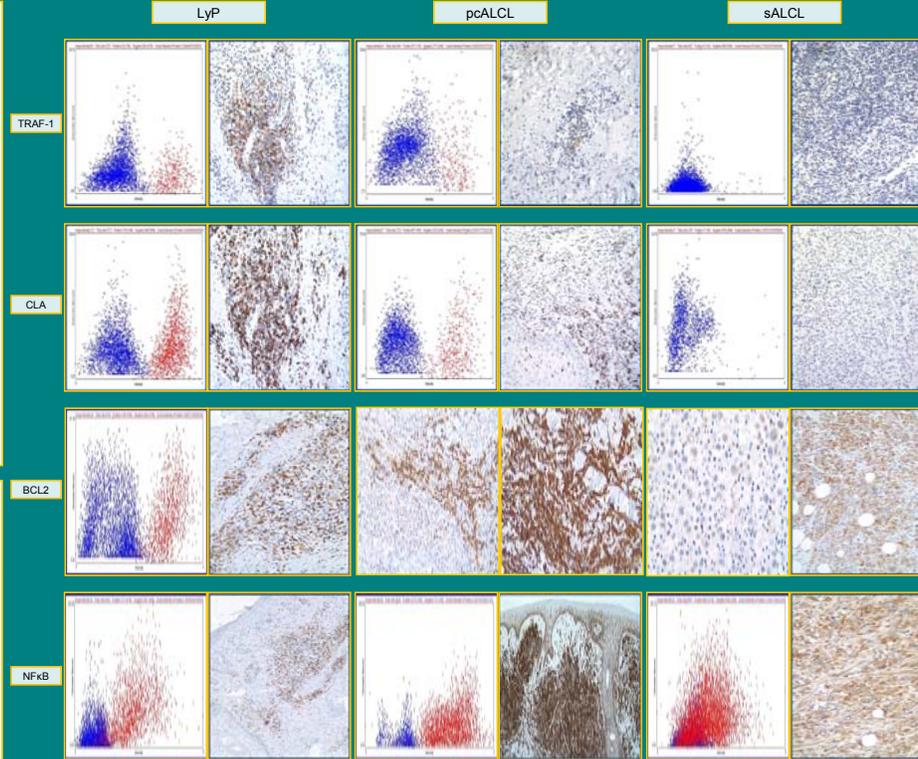
**INTRODUCTION:** The spectrum of diseases that constitute the CD30 positive lymphomas, with lymphomatoid papulosis (LyP) at one end, and anaplastic large cell lymphoma (ALCL) at the other end, show variable morphology and clinical behavior. The border between various diseases is sometimes difficult to establish. The central problem in the correct diagnosis and classification of this groups of diseases is that there are no reliable histologic criteria to differentiate between the different types of primary and secondary cutaneous CD30+ lymphoproliferations.

This study attempts to use a panel of 6 immunohistochemical stains in differentiating CD30-positive lymphoproliferations involving the skin. The expression profiles of these markers in CD30-positive lymphoproliferations were analyzed and correlated with the pathologic diagnosis for these entities.

In this study we show that tumor necrosis factor receptor (TNFR)-associated factor 1 (TRAF-1) is differentially expressed in LyP and primary cutaneous ALCL compared to systemic ALCL. As a member of the TRAF family, TRAF-1 is involved in the intracellular signal transduction of TNFR family members without death domain. TRAF-1 function is complex and not completely understood. However, it is possible that TRAF1 is involved in the sensitivity of cells to apoptosis. TRAF-1 is expressed mainly in activated lymphocytes and strongly induced by NFK-B2. Therefore, cells with high NFK-B2 activity generally express high levels of TRAF-1. A member of the TNFR family, like CD30, could also mediate intracellular signaling through NFK-B2 activation leading to subsequent TRAF-1 induction.

Cutaneous lymphocyte antigen (CLA) is an E-selectin ligand which facilitates adhesion of T lymphocytes to cutaneous vascular endothelium, initiating egress of T lymphocytes from the skin to systemic circulation. We found that CLA is highly expressed in CD30+ large atypical cells in LyP but expressed at lower level in primary cutaneous ALCL with minimal to absent level in systemic ALCL. The mechanism of decreased CLA expression in extra-cutaneous spread of lymphoma is presently unknown.

BCL2 is a proto-oncogene that is involved in preventing normal cellular caspases from allowing cells to enter apoptosis. BCL2 has been shown to be expressed in ALK negative sALCL and pcALCL. CD56 is an adhesion marker that is expressed by both NK cells and a subset of CD4 and CD8 positive T cells. It may be involved in homophilic binding in lymphocytes, though it's function in lymphocytes is relatively unknown. CD56 expression has been reported in pcALCL. Fascin is an actin bundling protein that has been identified in both Hodgkin lymphoma and anaplastic large cell lymphoma. EMA is a transmembrane glycoprotein that has been previously identified in systemic anaplastic large cell lymphoma.



**DESIGN:** After obtaining SRC and IRB approval, formalin-fixed and paraffin-embedded biopsy specimens from well-characterized diagnostically confirmed CD30 positive lymphomas, both systemic and involving the skin, examined at the Moffitt Cancer center during years 2000 to 2007 were selected from the pathology department of Moffitt Cancer and Research Institute database. All cases were stained with TRAF-1 (Imgenex), CLA (HEC4), NF-kB2 p100/p52 (Cell Signaling), BCL2 (Dako), EMA (Dako), CD56 (Sigma) and Fascin (Fisher Scientific). Manual morphometry of 500 lymphocytes counted under high power view (40x HPF) and virtual flow cytometry were performed to quantitate the degree of antibody expression. The antibody expression was scored as negative (less than 5%), weak positive (5 to 50%), intermediately positive (50-90%) and strongly positive (90-100%). Immunohistochemistry was performed on paraffin fixed samples at the University of South Florida Core Laboratory Department of Pathology & Cell Biology according to the manufacturer's protocols. The study initially included 40 sections from cases with CD30 positive lymphoproliferative disorders. All skin and lymph node samples were examined using hematoxylin-eosin staining and immunohistochemical staining. After pathology review, 10 case were excluded from the study due to scant tissue for adequate diagnosis. The remainder included 13 LyP, 9 pcALCL, and 8 sALCL. All diagnosis were confirmed by expert hematopathologists.

	LyP	pcALCL	sALCL
BCL2	37.0 ± 14.8	18.7 ± 12.7	69.5 ± 21.1
CLA	52.4 ± 25.1	28.6 ± 12.7	6.5 ± 7.6
TRAF1	25.1 ± 22.4	32.8 ± 22.3	0.9 ± 1.4
NFKB2	100 ± 0	95.9 ± 3.3	76.8 ± 32.5
CD56	0.2 ± 0.4	0.1 ± 0.3	0
EMA	2.7 ± 5.6	13.3 ± 28.3	18.8 ± 26.9
Fascin	16.4 ± 12.7	47.6 ± 31.7	43.1 ± 44.7

**RESULTS:** The result shows differential utility for TRAF-1 and CLA expression. TRAF-1 is expressed more than 5% in 12 out of 13 LyP cases and in all nine pcALCL. TRAF-1 expression is virtually absent in sALCL with or without skin manifestations (Kruskal Wallis statistic). There was no significant difference of TRAF-1 staining between LyP and pcALCL analyzed for TRAF1 expression ( $p > 0.05$ ), but significant between LyP vs sALCL ( $p < 0.01$ ), and between pcALCL vs sALCL ( $p < 0.001$ ). The tumor cells of all cases of sALCL case were negative for TRAF-1 ( $p = 0.0003$ ). CLA expression shows extremely significant differences between LyP vs pcALCL ( $p < 0.05$ ) as well as LyP vs sALCL ( $p < 0.001$ ) and insignificant differences between pcALCL vs sALCL ( $p > 0.05$ ). Mean expression of TRAF-1 is 25% in LyP vs 32% in pcALCL and 0.87% in sALCL. CLA had a mean of 52% for LyP, 28.5% for pcALCL and 6.5% for sALCL (see graphs). These results indicate statistically significant differences in TRAF-1 expression in cases with LyP / pcALCL vs sALCL, but no significant difference between LyP and pcALCL. CLA on the other hand shows significant differences between LyP vs pcALCL and sALCL. Cutaneous Lymphocyte Antigen (CLA), showed strong expression in LyP with variable expression in primary cutaneous ALCL and low expression in systemic ALCL (see table). BCL2 showed significant differences between pcALCL and sALCL ( $p < 0.001$ ) but not between LyP and pcALCL ( $p > 0.05$ ) and LyP and sALCL ( $p > 0.05$ ). NFKB2 showed significant differences between LyP and sALCL ( $p < 0.01$ ) and pcALCL and sALCL ( $p < 0.05$ ). NFKB2 staining in LyP was cytoplasmic, while in pcALCL and sALCL it was cytoplasmic and variable nuclear staining. CD56 ( $p = 0.5283$ ), Fascin ( $p = 0.2590$ ), and EMA ( $p = 0.1624$ ) were not contributory.

**CONCLUSION:** There is significant differential expression of TRAF-1 and CLA in lymphomatoid papulosis, primary cutaneous anaplastic large T cell lymphomas and systemic anaplastic large T cell lymphoma. CLA can differentiate LyP from pcALCL. Strong TRAF-1 expression is almost restricted to CD30-positive cells in LyP and intermediate to high expression in pcALCL. sALCL is uniformly negative for TRAF-1. This study, though of limited sample size, shows significant differential expression of TRAF1 and CLA in LyP. TRAF1 was negative in sALCL. CLA differentiates LyP from pcALCL and sALCL. BCL2 may differentiate pcALCL from sALCL. NFKB2 may be useful in distinguishing pcALCL from sALCL. This study indicates potential utility of TRAF-1, CLA, BCL2 and possibly NFKB2 in separating systemic from primary cutaneous CD30+ LPD. CD56, Fascin and EMA were non-contributory.

**References:**

- Assaf C, Hirsch B, Wagner F, Lucka L, Grünbaum M, Gelrich S, Lukowsky A, Sterry W, Stein H and Dürkop H. Differential expression of TRAF1 aids in the distinction of cutaneous CD30-positive lymphoproliferations. *Journal of Investigative Dermatology*. 2007; 127: 1898-1904.
- Droc C, Cuañing HD, Kadiri ME. Need for an improved molecular/genetic classification for CD30+ lymphomas involving the skin. *Cancer Control*. 2007 Apr;14(2):124-32.
- Cuañing HD, Zhong E, Mosticinski L. "Virtual Flow Cytometry" of immunostained lymphocytes on microscopic tissue slides: iHCFLOW™ Tissue Cytometry. *Cytometry*. 2007;72B:83-76.
- Vitale C, Bougrier F, Delol and Brousset P. Bcl-2 expression in anaplastic large cell lymphoma. *American Journal of Pathology*. 2001; 158:1889-90.

Special Thanks to the Histopathology Core, USF Department of Pathology & Cell Biology for technical assistance.

Please direct inquiries to **Hernani Cuañing** at [Hernani.Cuañing@moffitt.org](mailto:Hernani.Cuañing@moffitt.org)