Lack of expression of ALK and CD30 in breast carcinoma by immunohistochemistry irrespective of tumor characteristics

Samer Nassif, MD, Ziad M. El-Zaatari, MD, Michel Attieh, MD, Maya Hijazi, MD, Najla Fakhreddin, MD, Tarek Aridi, BSc, Fouad Boulos, MD

Abstract
CD30 is a member of the tumor necrosis factor family of cell surface receptors normally expressed in lymphocytes, as well as some lymphomas, but has been described in other malignancies. Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor that belongs to the insulin receptor superfamily, and is normally expressed in neural cells, but has been detected in several malignancies. There is conflicting data in the literature that describes the expression of these receptors in breast cancer, and the aim of this study is to test the expression of CD30 and ALK in a cohort of Middle Eastern patients with breast carcinoma.

Our cohort showed complete negativity to both CD30 and ALK, adding to the conflicting data available in the literature, and more studies are needed to reliably identify a trend of expression of CD30 and ALK in breast carcinoma, especially in the Middle East.

Keywords: ALK, breast cancer, carcinoma, CD30, middle east

1. Introduction
CD30, also known as Ki-1 or Ber-H2, is a member of the tumor necrosis factor family of cell surface receptors and is a known activation antigen in lymphocytes, being rarely expressed in non-lymphoid non-neoplastic cells.[1,2] Its expression has been well studied in multiple lymphoid neoplasms including Hodgkin lymphoma,[3,4] anaplastic large cell lymphoma,[5,6] and diffuse large B-cell lymphoma,[7] among others. However, CD30 expression has rarely been described in non-lymphoid tissues,[8,9] and few studies have examined its expression in non-lymphoid neoplasms, namely epithelial tumors,[10-13] sometimes with conflicting results.[11,12] Specifically, very little data exists about CD30 expression in breast carcinoma or about its association with tumor characteristics. One study demonstrated CD30 protein expression by immunohistochemistry (IHC) in around 5% of triple negative breast cancer cases,[11] and another recent study showed that CD30 protein expression by immunohistochemistry was seen with a higher sensitivity in breast cancer with a high CD30 gene RNA level.[14] Such information could potentially be of great clinical benefit, especially given that anti-CD30 targeted therapy has been shown to be effective against CD30-positive neoplasms.[15]

Anaplastic lymphoma kinase (ALK, CD246) is a tyrosine kinase receptor that belongs to the insulin receptor superfamily. ALK is normally detected in neural cells and plays an important part in the early development of the nervous system.[16] Although it is not found in normal non-neural adult tissues, its expression has been variably described in several malignancies including lymphoid tumors such as ALK-positive anaplastic large cell lymphoma,[17,18] epithelial tumors such as lung adenocarcinomas,[19] pancreatic ductal adenocarcinomas and neuroendocrine tumors,[20] renal cell carcinomas,[21] mesenchymal tumors such rhabdomyosarcoma,[22] inflammatory myofibroblastic tumors,[23] and neuroblastoma.[24] In breast cancer, however, ALK expression and function are poorly understood, with relatively few studies describing ALK positive inflammatory[25] and triple negative breast carcinomas. Similar to CD30 expression, knowledge of ALK expression in malignant breast tumors could be of value given that ALK-targeted therapy has been shown to be effective against certain neoplasms such as ALK expressing lung adenocarcinoma[27] and neuroblastoma.[28]
any, with specific tumor characteristics. This is especially relevant in our country where almost half of breast cancer cases are diagnosed before age 50, and around 20% before age 40.\textsuperscript{[29]} Moreover, multiple studies have shown that breast cancer in young women tends to be more aggressive, with higher proportions of aggressive molecular groups, especially the Basal-Like/Triple Negative (BL/TN) subtype.\textsuperscript{[30,31]} It is therefore important to study the expression of ALK and CD30 in breast cancer in our population, particularly with the putative relationship between ALK expression and the BL/TN phenotype.\textsuperscript{[32]}

2. Material and methods

This study was approved by the Institutional Review Board along with waiver of consent due to patient anonymity.

2.1. Case identification and block selection

Cases of invasive breast carcinoma were retrieved from the archives of the department of Pathology and Laboratory Medicine at the American University of Beirut Medical Center using the Laboratory Information system search engine, over a period of 9 years. The same blocks that were used for immunohistochemical staining for Estrogen and Progesterone Receptors and Her-2/neu expression were identified and selected for this study. Additionally, data was retrieved from the pathology reports including patient gender, age, tumor size, tumor type and grade, lymphovascular invasion, status of Estrogen Receptors, Progesterone Receptors, and Her-2/neu expression. All cases with missing information or unavailable paraffin blocks were excluded. All selected cases were internal (no referred blocks) and therefore fixed in 10% buffered formalin for an appropriate duration (6–48 hours).

2.2. Immunohistochemistry

Immunohistochemical staining for CD30 (JCM182), ALK (5A4) and ALK (D5F3) was performed using the Ventana immunos-
could be due to a copy number increase of either chromosome 2 as a whole,\textsuperscript{33} or the ALK gene specifically.\textsuperscript{16} Interestingly, according to Kim et al’s study, copy number gain was associated with inflammatory breast cancer, but no significant correlation with positive immunohistochemical staining was found.\textsuperscript{16} Another example of discrepancy regarding ALK in breast cancers refers to the specific ALK-EML4 gene fusion typically identified in lung adenocarcinoma; this mutation was not detected in breast cancer by Fukuyoshi et al.\textsuperscript{37} but detected in 2.4% of breast cancer cases by Lin et al.\textsuperscript{38}

In addition to correlating very well with FISH negative and positive results, dichotomous immunohistochemical reactivity (0 vs 3+) with the ALK antibody seems to represent the strongest predictor of response to ALK inhibitor therapy.\textsuperscript{39} In fact, FISH-negative IHC-positive non-small cell lung cancer was repeatedly shown to respond to ALK-inhibitor therapy, while FISH-positive IHC-negative tumors failed treatment in 100% of cases.\textsuperscript{40–43}

Therefore, we aimed in our study to assess ALK expression by immunohistochemistry in different types of breast cancer using 2 different ALK antibody clones. Despite the average size of our study sample, the selected cases adequately represent the broad spectrum of breast cancer presentations at our institution. Results were similar with both clones in that there was complete absence of ALK expression in all stained samples.

Our results contrast with previous studies where ALK expression in breast cancer was demonstrated immunohistochemically\textsuperscript{34} and even when using the same antibody clone.\textsuperscript{32} The reasons for this discrepancy are not evident, but could be related to population-dependent genetic differences. In view of the marked variation in results in ALK expression/mutation in breast cancer, questions arise whether ALK-targeted treatment strategies in breast carcinoma can be of benefit.

Our study also showed complete lack of expression of CD30 in the selected breast cancer cases. These results argue against testing for CD30 positivity in breast cancer, and cast significant doubt on the potential for anti-CD30 targeted therapy in advanced or refractory breast cancer cases.

Of note, one limitation to this study was the lack of testing for ALK and CD30 mutations by molecular techniques due to funding restrictions. This could be the subject of future projects.

In summary, we showed the lack of ALK and CD30 immunoreactivity in breast cancer in our cohort, irrespective of tumor characteristics. The conflicting results between our study and that of Siraj et al warrant further investigation, given that 36% of their cases were IHC positive for ALK, whereas our cohort showed complete negativity using 2 different antibody clones. This raises the suspicion of possible methodological contributors from either study to this significant difference between 2 somewhat comparable patient populations. We, therefore, believe that studies on the expression of both ALK and CD30 in breast carcinoma are still necessary, given the potential benefit of targeted therapy on breast cancers with aggressive biology and poor response to conventional chemotherapeutic regimens.

Author contributions

Conceptualization: Samer Nassif, Ziad M. El-Zaatari, Maya Hijazi, Najla Fakhreddin, Fouad Boulos.

Data curation: Samer Nassif, Ziad M. El-Zaatari, Michel Attieh, Maya Hijazi, Tarek Aridi, Najla Fakhreddin, Fouad Boulos.

Formal analysis: Samer Nassif, Ziad M. El-Zaatari, Tarek Aridi, Fouad Boulos.

Funding acquisition: Samer Nassif, Fouad Boulos.

Investigation: Samer Nassif, Michel Attieh, Maya Hijazi, Najla Fakhreddin, Fouad Boulos.

Methodology: Samer Nassif, Ziad M. El-Zaatari, Maya Hijazi, Tarek Aridi, Najla Fakhreddin, Fouad Boulos.

Project administration: Samer Nassif, Fouad Boulos.

Resources: Samer Nassif, Fouad Boulos.

Software: Samer Nassif, Fouad Boulos.

Supervision: Samer Nassif, Najla Fakhreddin, Fouad Boulos.

Validation: Samer Nassif, Fouad Boulos.

Visualization: Samer Nassif, Michel Attieh, Maya Hijazi, Tarek Aridi, Fouad Boulos.

Writing – original draft: Samer Nassif, Ziad M. El-Zaatari, Michel Attieh, Maya Hijazi, Tarek Aridi, Najla Fakhreddin, Fouad Boulos.

Writing – review & editing: Samer Nassif, Ziad M. El-Zaatari, Michel Attieh, Fouad Boulos.

References

[1] Dürkop H, Foss HD, Eitelbach F, et al. Expression of the CD30 antigen in non-lymphoid tissues and cells. J Pathol 2000;190:613–8.

[2] Ollazoglu E, Grewal IS, Gerber H. Targeting CD30/CD30L in oncology and autoimmune and inflammatory diseases. Adv Exp Med Biol 2009;647:174–85.

[3] Schwab U, Stein H, Gerdes J, et al. Production of a monoclonal antibody specific for Hodgkin and Sternberg–Reed cells of Hodgkin’s disease and a subset of normal lymphoid cells. Nature 1982;299:65.

[4] von Wasielewski R, Menzel M, Fischer R, et al. Classical Hodgkin’s disease. Clinical impact of the immunophenotype. Am J Pathol 1997;151:1123.
[5] Schwarting R, Gerdes J, Durkop H, et al. BER-H2: a new anti-Ki-1 (CD30) monoclonal antibody directed at a formal-resistant epitope. Blood 1988;74:1678-89.

[6] Stein H, Foss H-D, Durkop H, et al. CD30+ anaplastic large cell lymphoma: a review of its histopathologic, genetic, and clinical features. Blood 2000;96:3681-95.

[7] Campuzano-Zuluaga G, Ciufetti-Lavina M, Lossos IS, et al. Frequency and extent of CD30 expression in diffuse large B-cell lymphoma and its relation to clinical and biologic factors: a retrospective study of 167 cases. Leuk Lymphoma 2013;54:2405-11.

[8] Taniolakis D, Menegaki M, Nikolaidou S, et al. Occurrence of CD30 antigen on tissues and cells other than lymphoid origin. A study of human fetal skin in 8th, 10th, and 12th gestational weeks. Romanian J Biophys 2004;14:59–67.

[9] Garcia-Prats M, Ballestin C, Sotelo T, et al. A comparative evaluation of immunohistochemical markers for the differential diagnosis of malignant pleural tumours. Histopathology 1998;32:462–72.

[10] Morris SW, Naeve C, Mathew P, et al. ALK, the chromosome 2 gene locus altered by the t (2; 5) in non-Hodgkin lymphoma: a review of its histopathologic, genetic, and clinical features. J Biomed Biotechnol 2007;2007:931648.

[11] Gopalan A, Dhall D, Olgac S, et al. Testicular mixed germ cell tumors: a morphological and immunohistochemical study using stem cell markers, OCT3/4, SOX2 and GDF3, with emphasis on morphologically difficult-to-classify areas. Mod Pathol 2009;22:1066.

[12] Latza U, Foss H-D, Dürkop H, et al. CD30 antigen in embryonal rhabdomyosarcomas. Mod Pathol 1995;14:463.

[13] Stein H, Foss H-D, Dürkop H, et al. CD30+ anaplastic large cell lymphoma. Histopathology 2006;48:555–61.

[14] Li B, Eschrich SA, Olgac S, et al. Testicular mixed germ cell tumors: a morphological and immunohistochemical study using stem cell markers, OCT3/4, SOX2 and GDF3, with emphasis on morphologically difficult-to-classify areas. Mod Pathol 2009;22:1066.

[15] Kneile J, Tan G, Suster S, et al. Expression of CD30 (Ber-H2) in nasopharyngeal carcinoma, undifferentiated type and lymphoepithelioma-like carcinoma. A comparison study with anaplastic large cell lymphoma. Histopathology 2006;48:555–61.

[16] Latza U, Foss H-D, Durkop H, et al. CD30 antigen in embryonal carcinoma and embryogenesis and release of the soluble molecule. Am J Pathol 1995;146:463.

[17] Sharanjan JP, Goldschmidt JH, Burke JM, et al. CD30 expression in nonlymphomatous malignancies. J Clin Oncol 2012;30(suppl 30):3069.

[18] Li B, Eschrich SA, Berghlund A, et al. Use of the total cancer care system to enrich screening for CD30-positive solid tumors for patient enrollment into a brentuximab vedotin clinical trial: a pilot study to evaluate feasibility. JMIR Res Protoc 2017;6:e45.

[19] Campuzano-Zuluaga G, Ciofi-Lavina M, Lossos IS, et al. Frequency and extent of CD30 expression in diffuse large B-cell lymphoma and its relation to clinical and biologic factors: a retrospective study of 167 cases. Leuk Lymphoma 2013;54:2405-11.

[20] Garcia-Prats M, Ballestin C, Sotelo T, et al. A comparative evaluation of immunohistochemical markers for the differential diagnosis of malignant pleural tumours. Histopathology 1998;32:462–72.

[21] Morris DH, Rahardja D, King E, et al. Tumour biomarker expression relative to age and molecular subtypes of invasive breast cancer. Br J Cancer 2012;107:382.

[22] Siraj AK, Beg S, Jehan Z, et al. ALK alteration is a frequent event in breast cancer cases. Breast Cancer Res Treat 2013;131:1061-6.

[23] Lamant L, Pulford K, Bischof D, et al. Expression of the ALK tyrosine kinase gene in neuroblastoma. Am J Pathol 2000;156:1711-21.

[24] Younes A. CD30-targeted antibody therapy. Curr Opin Oncol 2011;23:140-8.

[25] Kneile J, Tan G, Suster S, et al. Expression of CD30 (Ber-H2) in nasopharyngeal carcinoma, undifferentiated type and lymphoepithelioma-like carcinoma. A comparison study with anaplastic large cell lymphoma. Histopathology 2006;48:555–61.

[26] Li B, Eschrich SA, Olgac S, et al. Testicular mixed germ cell tumors: a morphological and immunohistochemical study using stem cell markers, OCT3/4, SOX2 and GDF3, with emphasis on morphologically difficult-to-classify areas. Mod Pathol 2009;22:1066.

[27] Lamant L, Pulford K, Bischof D, et al. Expression of the ALK tyrosine kinase gene in neuroblastoma. Am J Pathol 2000;156:1711-21.

[28] Casaluce F, Scambiato A, Maione P, et al. ALK inhibitors: a new targeted therapy in the treatment of advanced NSCLC. Targeted Oncol 2013;8:55–67.

[29] Garcia-Prats M, Ballestin C, Sotelo T, et al. A comparative evaluation of immunohistochemical markers for the differential diagnosis of malignant pleural tumours. Histopathology 1998;32:462–72.

[30] Latza U, Foss H-D, Durkop H, et al. CD30 antigen in embryonal carcinoma and embryogenesis and release of the soluble molecule. Am J Pathol 1995;146:463.

[31] Sharanjan JP, Goldschmidt JH, Burke JM, et al. CD30 expression in nonlymphomatous malignancies. J Clin Oncol 2012;30(suppl 30):3069.

[32] Li B, Eschrich SA, Berghlund A, et al. Use of the total cancer care system to enrich screening for CD30-positive solid tumors for patient enrollment into a brentuximab vedotin clinical trial: a pilot study to evaluate feasibility. JMIR Res Protoc 2017;6:e45.

[33] Younes A. CD30-targeted antibody therapy. Curr Opin Oncol 2011;23:587–93.

[34] Iwahara T, Fujimoto J, Wen D, et al. Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. Oncogene 1997;14:439.

[35] Tamiolakis D, Menegaki M, Nikolaidou S, et al. Occurrence of CD30 antigen on tissues and cells other than lymphoid origin. A study of human fetal skin in 8th, 10th, and 12th gestational weeks. Romanian J Biophys 2004;14:59–67.

[36] Schwarting R, Gerdes J, Durkop H, et al. BER-H2: a new anti-Ki-1 (CD30) monoclonal antibody directed at a formal-resistant epitope. Blood 1988;74:1678-89.

[37] Rago RA, Oliveira AM, Zheng L, et al. Expression of the ALK tyrosine kinase gene in neuroblastoma. Am J Pathol 2000;156:1711-21.

[38] El NS, Shamseddine A, Gera F, et al. Age distribution of breast cancer in Lebanon: increased percentages and age adjusted incidence rates of younger-aged groups at presentation. Le Journal medical libanais. J Med Liban 2002;50:3–9.

[39] Collins LG, Marotti JD, Gelber S, et al. Pathologic features and molecular phenotype by patient age in a large cohort of young women with breast cancer. Breast Cancer Res Treat 2012;131:1061-6.

[40] Kim MH, Lee S, Koo JS, et al. Anaplastic lymphoma kinase gene copy number gain in inflammatory breast cancer (IBC): prevalence, clinicopathologic features and prognostic implication. PLoS One 2015;10:e0120320.

[41] Lefebvre C, Bachelot T, Filleron T, et al. Mutational profile of metastatic breast cancers: a retrospective analysis. PLoS Med 2016;13:e1002201.

[42] Perez-Pimera P, Chang Y, Astudillo A, et al. Anaplastic lymphoma kinase is expressed in different subtypes of human breast cancer. Biochem Biophys Res Commun 2007;358:399–403.

[43] Kim MH, Lee S, Koo JS, et al. Anaplastic lymphoma kinase gene copy number gain in inflammatory breast cancer (IBC): prevalence, clinicopathologic features and prognostic implication. PLoS One 2015;10:e0120320.

[44] Lin E, Li L, Guan Y, et al. Exon array profiling detects EML4-ALK fusion in breast, colorectal, and non-small cell lung cancers. Mol Cancer Res 2009;7:1466–76.

[45] Sun J-M, Choi Y-L, Won J-K, et al. A Dramatic response to crizotinib in a non-small-cell lung cancer patient with IHC-positive and FISH-negative ALK. J Thorac Oncol 2012;7:e36–8.

[46] Van der Wekken A, Pelgrim R, Werner N, et al. Dichotomous ALK-IHC is a better predictor for ALK inhibition outcome than traditional ALK-FISH in advanced non-small cell lung cancer. Clin Cancer Res 2017;23:4251–8.