Int J Clin Exp Pathol 2020;13(7):1682-1687
www.ijcep.com /ISSN:1936-2625/IJCEP0113476

Original Article

The expressions of MUM-1 and Bcl-6 in ALK-negative systemic anaplastic large cell lymphoma with skin involvement and primary cutaneous anaplastic large cell lymphoma

Yeo-Rye Cho[1], Jeong-Wan Seo[2], Sung Yong Oh[3], Min-Kyoung Pak[4], Ki-Ho Kim[1]

[1]Department of Dermatology, Dong-A University College of Medicine, Busan, South Korea; [2]Department of Dermatology, The Armed Forces Medical Gangneung Hospital, Gangwon-Do, South Korea; [3]Department of Internal Medicine, Dong-A University College of Medicine, Busan, South Korea; [4]Department of Pathology, Dong-A University College of Medicine, Busan, South Korea

Received April 28, 2020; Accepted June 3, 2020; Epub July 1, 2020; Published July 15, 2020

Abstract: Background: It is important to differentiate between primary cutaneous anaplastic large cell lymphoma (PC-ALCL) and ALK-negative systemic ALCL with skin involvement, as the prognoses and treatments for these two diseases are considerably different. Objective: This study aimed to compare the expressions of multiple myeloma oncogene 1 (MUM-1) and B-cell lymphoma 6 (Bcl-6) in PC-ALCL and ALK-negative systemic ALCL. Methods: This retrospective qualitative study investigated the clinical features of 7 patients with ALK-negative PC-ALCL, 5 patients with ALK-negative systemic ALCL with skin involvement, and 6 patients with ALK-positive systemic ALCL with skin involvement. The MUM-1 and Bcl-6 expressions were evaluated using immunohistochemistry. Results: The MUM-1 expression rates were 85.7% in the PC-ALCL cases and 100% in the ALK-negative systemic ALCL with skin involvement cases. The Bcl-6 expression rates were 28.5% in the PC-ALCL cases and 20% in the ALK-negative systemic ALCL cases with skin involvement. Conclusion: Although the cutaneous manifestations of ALK-negative systemic ALCL and PC-ALCL are similar, the prognoses and treatment approaches are considerably different. Our results indicate that MUM-1 expression is commonly expressed in both types of ALCL, but Bcl-6 is less commonly expressed in PC-ALCL cases and systemic ALCL with skin involvement cases.

Keywords: ALK, Bcl-6, MUM-1, primary cutaneous anaplastic large cell lymphoma, systemic anaplastic large cell lymphoma

Introduction

In 2016, the World Health Organization classified anaplastic large cell lymphoma (ALCL) as a mature T and NK neoplasm [1]. Systemic ALCL can be divided into the ALK-positive and ALK-negative types, and ALCL with skin manifestations is considered primary cutaneous ALCL (PC-ALCL). As a disease entity, ALK-positive ALCL typically affects children and young adults, has a morphological spectrum that includes small cell and lymphohistiocytic variants, and has a better prognosis than ALK-negative ALCL in many cases [2-5]. In contrast, PC-ALCL is a primary cutaneous CD30-positive T-cell lymphoproliferative disorder that does not typically involve ALK expression. It is important to differentiate between PC-ALCL and ALK-negative systemic ALCL with skin involvement, especially if the ALCL diagnosis is based on a skin biopsy, as the prognoses and treatments differ considerably between these two diseases [6]. For example, PC-ALCL has a good prognosis and a five-year survival rate of >90%, which is substantially higher than the five-year survival rate of 30-49% for ALK-negative systemic ALCL [7, 8]. However, these two diseases usually present with identical clinical morphologies and histological features during hematoxylin and eosin (H&E) staining, which highlights the importance of a systematic evaluation and the development of better histological screening tools.

Table 1. Clinical characteristics of the patients with ALK-negative anaplastic large cell lymphoma

	ALK-PC-ALCL	ALK-systemic ALCL	ALK+systemic ALCL
Number of patients	7	5	6
Sex (male:female)	4:3	4:1	3:3
Age of onset (years)	57 (31-82)	50.8 (27-79)	48 (15-73)
Therapy	Excision (7/7)	Surgery or excision (1/5)	Surgery or excision (0)
	Radiotherapy (7/7)	Chemotherapy (2/5)	Chemotherapy (5/6)
	Chemotherapy (1/7)	Radiotherapy (2/5)	Radiotherapy (1/6)
Follow-up (months)	78.5	60.8	51.2
Five-year survival rate	100%	40% (2/5)	33.3% (2/6)
Mortality rate (%)	0%	80% (4/5)	83.3% (5/6)
IHC staining			
CD30	100%	100%	100%
MUM-1	85.7% (6/7)	100% (5/5)	ND
Bcl-6	28.5% (2/7)	20% (1/5)	ND

ALK, anaplastic lymphoma kinase; PC, primary cutaneous; ALCL, anaplastic large cell lymphoma; IHC, immunohistochemistry; MUM-1, multiple myeloma oncogene 1; Bcl-6, B-cell lymphoma 6; ND, no data. Data are shown as the mean (range) or percentage (fraction of cases).

The MUM-1 and Bcl-6 proteins are transcription factors that are commonly used as B-cell markers and markers of B-cell neoplasms [9]. However, the expressions of both MUM-1 and Bcl-6 have also been observed in a small percentage of activated T-cells within germinal centers and peripheral T-cell lymphomas, and some studies have examined the expressions of both markers in ALCL [10, 11]. The present study examines the expressions of MUM-1 and Bcl-6 to determine whether they can be used in the diagnosis, differentiation, and prognostication of cases of PC-ALCL and ALK-negative ALCL with skin involvement.

Patients and methods

Patients

This retrospective qualitative study evaluated data from 18 patients who visited the Dermatology Department of Dong-A University Hospital between 2000 and 2014. Skin biopsies were performed to support the diagnoses of PC-ALCL (7 patients), ALK-negative ALCL with skin involvement (5 patients), and ALK-positive ALCL with skin involvement (6 patients). The present study involved reviewing the H&E-stained slides and additional immunohistochemical (IHC) staining as necessary. The diagnoses were confirmed based on the 2016 World Health Organization criteria. The study's retrospective protocol was approved by the Dong-A University institutional review board (DAUHIRB-19-155).

Immunohistochemistry

Paraffin blocks of the skin biopsy specimens were cut into 4-μm sections. The IHC staining was performed using antibodies that targeted CD30 (Ber-H2, 1:50, ThermoFisher, US), ALK (ALK1, 1:80, DAKO, US), MUM-1 (MUM1P, 1:50, DAKO, US), and Bcl-6 (PG-B6P, 1:10, DAKO, US). The sections were then counterstained using Mayer's hematoxylin. The staining for all the markers was matched and compared based on CD30 immunostaining during the evaluation of the cases with scattered tumor cells. Positive immunostaining was defined based on positive cell staining and intensity as >20% of lymphoma cells for MUM-1 and >10% for Bcl-6 [11-13]. The threshold value for Bcl-6 was chosen based on previous studies that used thresholds of 10-20% [11].

Results

We reviewed the medical records of the 18 patients (**Table 1**) with ALK-negative PC-ALCL (4 men, 3 women; mean age: 57.0 years [range: 31-82 years]), ALK-negative systemic ALCL with skin involvement (4 men, 1 woman; mean age: 50.7 years [range: 27-79 years]), and ALK-positive systemic ALCL with skin involvement (3 men, 3 women; mean age: 48.0 years [range: 15-73 years]). The PC-ALCL cases

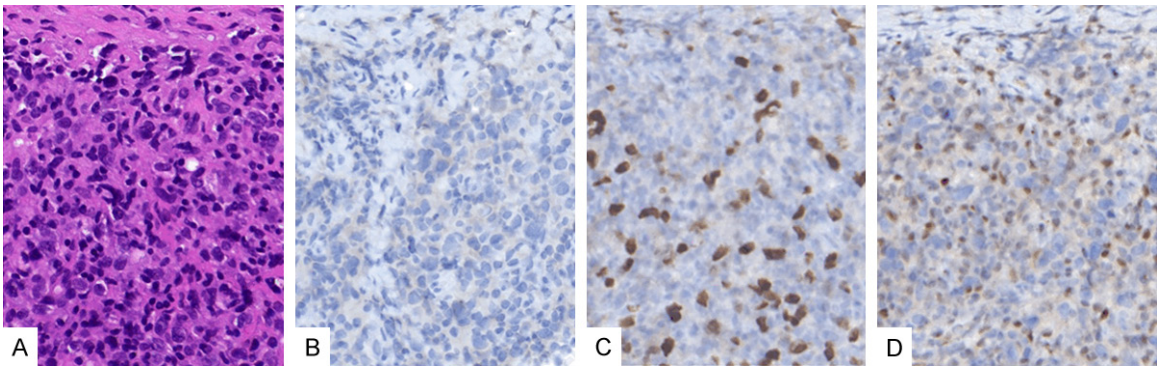

Figure 1. Findings for the ALK-negative PC-ALCL. The hallmark cells are visible during the hematoxylin and eosin staining (A), with a negative expression of ALK (B), a positive expression of MUM-1 (C), and a weak, positive expression of Bcl-6 (D). ALK, anaplastic lymphoma kinase; PC-ALCL, primary cutaneous anaplastic large cell lymphoma; MUM-1, multiple myeloma oncogene 1; Bcl-6, B-cell lymphoma 6.

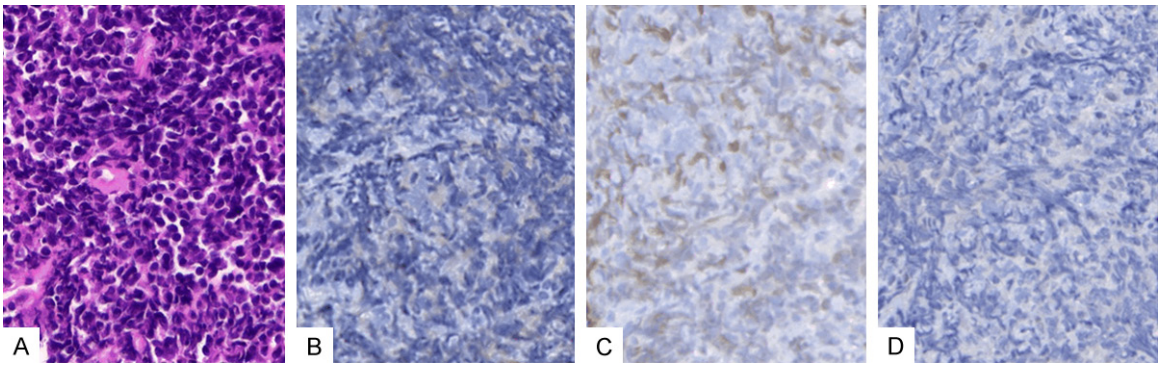

Figure 2. Findings for ALK-negative systemic ALCL with skin involvement. The hallmark cells are visible during the hematoxylin & eosin staining (A), with a negative expression of ALK (B), a positive expression of MUM-1 (C), and a negative expression of Bcl-6 (D). ALK, anaplastic lymphoma kinase; PC-ALCL, primary cutaneous anaplastic large cell lymphoma; MUM-1, multiple myeloma oncogene 1; Bcl-6, B-cell lymphoma 6.

generally involved lesions at the extremities (legs for 3 patients and arms for 4 patients), and 6 of the 7 patients (87.1%) presented with a solitary lesion. In contrast, diffuse or multifocal skin lesions were observed in 5 patients (45%) with ALK-negative or ALK-positive systemic ALCL with skin involvement. The 5-year survival rates were 100% for PC-ALCL and only 40% for ALK-negative systemic ALCL with skin involvement, despite those patients undergoing surgical removal, various chemotherapy regimens, and radiotherapy. The 5-year survival rate was 33.3% for ALK-positive systemic ALCL with skin involvement.

Negative expressions of ALK and positive expressions of CD30 were observed in all the patients with PC-ALCL and in the 5 patients with ALK-negative systemic ALCL and skin involvement. The MUM-1 expression rates were 85.7% (6/7 patients) in the PC-ALCL cases and 100% (all 5 patients) in the ALK-negative systemic ALCL with skin involvement cases. Diffuse staining for MUM-1 was predominantly observed in the nucleus, with moderate-to-strong staining intensity. The Bcl-6 expression rates were 28.5% (2/7 patients) in the PC-ALCL cases but only 20.0% (1/5 patients) in the ALK-negative systemic ALCL with skin involvement cases. The Bcl-6 staining in the positive cases generally involved weak foci in the nuclei. Based on these characteristics, we failed to detect any noticeable differences in the MUM1 and BCL-6 expressions in the PC-ALCL and ALK-negative systemic ALCL cases (**Figures 1** and **2**).

Discussion

The recognition of ALCL is difficult, especially because it is challenging to differentiate

between PC-ALCL, lymphomatoid papulosis, mycosis fungoides, and systemic ALCL with skin involvement. Moreover, ALK dysregulation is not unique to ALCL, and a nested reverse transcription-polymerase chain reaction has been used to determine its expressions in inflammatory myofibroblastic tumors, carcinoma, tumors of neural origin, and peripheral blood cells from healthy people [14]. Thus, questions remain regarding the biology of the ALK-positive and ALK-negative subgroups, as well as their relationships.

Similar clinical features are observed in PC-ALCL and systemic ALCL with skin involvement, although these two diseases have substantially different treatments and prognoses. For example, approximately 70% of patients with systemic ALCL present at an advanced stage (III or IV) and with B symptoms, which are associated with a rapidly progressive clinical course, non-contiguous lymphadenopathy, and a five-year survival rate of 30-49% in ALK-negative ALCL cases [5, 15, 16]. In contrast, patients with PC-ALCL generally have a favorable prognosis and high five-year survival rates (>90%) [17]. In the present study, the five-year survival rates were 100% for PC-ALCL but only 40% for ALK-negative systemic ALCL with skin involvement, despite those patients undergoing surgical removal, radiotherapy, and various chemotherapies, including the CHOP regimen (cyclophosphamide, hydroxydaunorubicin, oncovin, and prednisolone), the VIPD regimen (cisplatin, etoposide, ifosfamide, and dexamethasone), and rituximab. Therefore, the findings from the present study confirm the importance in differentiating between these two diseases, given the remarkable survival difference. This differentiation should be based on a clinical examination, pathological findings, and a staging work-up that includes computed tomography and a whole-body bone scan. Accurate diagnoses may also require the use of IHC staining and a fluorescence in situ hybridization analysis.

The MUM-1 transcription factor is encoded by an oncogene and was first identified in multiple myeloma cells [18]. In the normal lymphoid system, MUM-1 is expressed in plasma cells, a small percentage of B-cells, light zones of the germinal center, and activated T-cells [12]. One study found that MUM-1, Oct-2, and Bcl-6 are extensively expressed in ALCL of the T/null cell phenotype, rather than being restricted to the B-cell lineage [19]. Wasco et al. also compared the MUM-1 expressions in 7 cases of cutaneous ALCL and 5 cases of systemic ALCLs, which revealed positive MUM-1 expressions in all but one case of systemic ALCL [10]. The present study also confirmed that positive MUM-1 expressions were observed in a high proportion of patients with ALK-negative PC-ALCL and in patients with systemic ALCL and skin involvement, which are difficult to differentiate in the clinical setting. In this context, Wada et al. indicated that IRF4 non-translocation abnormalities (involving a gene encoding MUM-1) are widely distributed in cases of systemic ALCL, PC-ALCL, and other T-cell lymphoproliferative disorders [20]. However, IRF4 translocation is rarely observed in other T-cell lymphoproliferative disorders and in only 20% of PC-ALCL cases, which indicates that IRF4 translocation is highly specific for PC-ALCL [20]. Our results also confirmed positive MUM-1 expression in various T-cell lymphoproliferative disorders, and they indicate that MUM-1 expression is not significantly associated with IRF4 translocation, which is weakly detected in PC-ALCL.

The Bcl-6 protein was originally identified as a chromosomal translocation product in diffuse large B-cell lymphomas, where it functions as a transcriptional repressor [21]. This expression of Bcl-6 is mainly observed in germinal center B lymphocytes and in germinal center B-cell lymphomas. While Bcl-6 is known to be expressed in germinal centers and perifollicular CD4+ T lymphocytes (mainly co-expressing CD30), few studies have examined Bcl-6 expression in T-cell lymphomas [21, 22]. Carbone et al. reported that Bcl-6 is rarely expressed in other peripheral and precursor T-cell neoplasms, but is expressed in approximately 45% of ALCL cases [11]. Lamant et al. also reported that Bcl-6 was expressed in only 28% of patients with ALK-negative ALCL [23]. The findings from the present study also confirm that there are low rates of Bcl-6 expression in cases of ALK-negative PC-ALCL and cases of ALK-negative systemic ALCL with skin involvement.

The present study revealed that MUM-1 is not a suitable marker for differentiating between PC-ALCL and ALK-negative systemic ALCL with

skin involvement. This result is consistent with the high expressions of MUM-1 in both T-cell lymphomas from a previous study [24]. However, these results also indicate that B-cell lymphoma and ALCL should be considered in the differential diagnosis when MUM-1 is found to be expressed in cutaneous lymphoma. The present study also revealed relatively low Bcl-6 expression rates in both diseases, and we are only aware of a few studies that have analyzed Bcl-6 expression in PC-ALCL [25]. Therefore, further studies are needed to better understand the nature and significance of the Bcl-6 expressions in PC-ALCL and ALK-negative systemic ALCL.

Our study has several limitations that should be considered. First, the sample size was small, so a larger prospective study is needed to more comprehensively examine the clinical relevance of the MUM-1 and/or Bcl-6 expressions in systemic ALCL and PC-ALCL. Second, no data were available regarding the expressions of MUM-1 and Bcl-6 in the ALK-positive systemic ALCL with skin involvement cases, which would have yielded potentially useful results.

The diagnosis and staging of ALCL currently involves a clinical work-up, radiographic imaging, and laboratory testing. We hypothesized that immunohistochemical testing (e.g., to determine the expressions of MUM-1 and Bcl-6) may help improve this diagnostic process. Unfortunately, neither protein appears to be a useful marker for differentiating between systemic ALCL and PC-ALCL. Further studies are needed to identify any immunohistochemical markers that could be used to differentiate between ALK-negative PC-ALCL and ALK-negative systemic ALCL with skin involvement.

Acknowledgements

This study was supported by the Dong-A University Research fund.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ki-Ho Kim, Department of Dermatology, Dong-A University College of Medicine, 26 Dongdaesingongwon-Ro, Seo-Gu, Busan 49201, South Korea. Tel: +82-51-240-5435; Fax: +82-51-253-0787; E-mail: khkim@dau.ac.kr; Dr. Sung Yong Oh, Department of Internal Medicine, Dong-A University College of Medicine, 26 Dongdaesingongwon-Ro, Seo-Gu, Busan 49201, South Korea. Tel: +82-51-240-5044; Fax: +82-51-242-5852; E-mail: drosy@dau.ac.kr

References

[1] Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles GA, Zelenetz AD and Jaffe ES. The 2016 revision of the world health organization classification of lymphoid neoplasms. Blood 127: 2375-2390.

[2] Irshaid L and Xu ML. ALCL by any other name: the many facets of anaplastic large cell lymphoma. Pathology 2020; 52: 100-110.

[3] Leventaki V, Bhattacharyya S and Lim MS. Pathology and genetics of anaplastic large cell lymphoma. Semin Diagn Pathol 2020; 37: 57-71.

[4] Wang X, Wu J and Zhang M. Advances in the treatment and prognosis of anaplastic lymphoma kinase negative anaplastic large cell lymphoma. Hematology 2019; 24: 440-445.

[5] Shustov A and Soma L. Anaplastic large cell lymphoma: contemporary concepts and optimal management. Cancer Treat Res 2019; 176: 127-144.

[6] Prieto-Torres L, Rodriguez-Pinilla SM, Onaindia A, Ara M, Requena L and Piris MA. CD30-positive primary cutaneous lymphoproliferative disorders: molecular alterations and targeted therapies. Haematologica 2019; 104: 226-235.

[7] Hapgood G and Savage KJ. The biology and management of systemic anaplastic large cell lymphoma. Blood 2015; 126: 17-25.

[8] Ferreri AJ, Govi S, Pileri SA and Savage KJ Anaplastic large cell lymphoma, ALK-negative. Crit Rev Oncol Hematol 2013; 85: 206-215.

[9] Abdulla M, Hollander P, Pandzic T, Mansouri L, Ednersson SB, Andersson PO, Hultdin M, Fors M, Martin Erlanson M, Degerman S, Petersen HM, Asmar F, Grønbaek K, Enblad G, Cavelier L, Rosenquist R and Amini RM. Cell-of-origin determined by both gene expression profiling and immunohistochemistry is the strongest predictor of survival in patients with diffuse large B-cell lymphoma. Am J Hematol 2020; 95: 57-67.

[10] Wasco MJ, Fullen D, Su L and Ma L. The expression of MUM1 in cutaneous T-cell lymphoproliferative disorders. Hum Pathol 2008; 39:557-563.

[11] Carbone A, Gloghini A, Gaidano G, Dalla-Favera R and Falini B. BCL-6 protein expression in hu-

man peripheral T-cell neoplasms is restricted to CD30+ anaplastic large-cell lymphomas. Blood 1997; 90: 2445-2450.
[12] Natkunam Y, Warnke RA, Montgomery K, Falini B and van De Rijn M. Analysis of MUM1/IRF4 protein expression using tissue microarrays and immunohistochemistry. Mod Pathol 2001; 14: 686-694.
[13] Tsuboi K, Iida S, Inagaki H, Kato M, Hayami Y, Hanamura I, Miura K, Harada S, Kikuchi M, Komatsu H, Banno S, Wakita A, Nakamura S, Eimoto T and Ueda R. MUM1/IRF4 expression as a frequent event in mature lymphoid malignancies. Leukemia 2000; 14: 449-456.
[14] Trumper L, Pfreundschuh M, Bonin FV and Daus H. Detection of the t(2;5)-associated NPM/ALK fusion cDNA in peripheral blood cells of healthy individuals. Br J Haematol 1998; 103: 1138-1144.
[15] Tsuyama N, Sakamoto K, Sakata S, Dobashi A and Takeuchi K. Anaplastic large cell lymphoma: pathology, genetics, and clinical aspects. J Clin Exp Hematop 2017; 57: 120-142.
[16] Zing NPC, Fischer T, Zain J, Federico M and Rosen ST. Peripheral T-cell lymphomas: incorporating new developments in diagnostics, prognostication, and treatment into clinical practice-PART 2: ENKTL, EATL, indolent T-cell LDP of the GI tract, ATLL, and hepatosplenic T-cell lymphoma. Oncology 2018; 32: e83-e89.
[17] Brown RA, Fernandez-Pol S and Kim J. Primary cutaneous anaplastic large cell lymphoma. J Cutan Pathol 2017; 44: 570-577.
[18] Gualco G, Weiss LM and Bacchi CE. MUM1/IRF4: a review. Appl Immunohistochem Mol Morphol 2010; 18: 301-310.
[19] Saglam A and Uner AH. Immunohistochemical expression of Mum-1, Oct-2 and Bcl-6 in systemic anaplastic large cell lymphomas. Tumori 2011; 97: 634-638.
[20] Wada DA, Law ME, Hsi ED, Dicaudo DJ, Ma L, Lim MS, de Souza A, Comfere NI, Weenig RH, Macon WR, Erickson LA, Ozsan N, Ansell SM, Dogan A and Feldman AL. Specificity of IRF4 translocations for primary cutaneous anaplastic large cell lymphoma: a multicenter study of 204 skin biopsies. Mod Pathol 2011; 24: 596-605.
[21] Mlynarczyk C, Fontan L and Melnick A. Germinal center-derived lymphomas: the darkest side of humoral immunity. Immunol Rev 2019; 288: 214-239.
[22] Rai MP, Bedi PS, Marinas EB and Khan NNS. Angioimmunoblastic T-cell lymphoma: a rare subtype of peripheral T-cell lymphoma. Clin Case Rep 2018; 6: 750-752.
[23] Lamant L, de Reynies A, Duplantier MM, Rickman DS, Sabourdy F, Giuriato S, Brugières L, Gaulard P, Espinos E and Delsol G. Gene-expression profiling of systemic anaplastic large-cell lymphoma reveals differences based on ALK status and two distinct morphologic ALK+ subtypes. Blood 2007; 109: 2156-2164.
[24] Benner MF, Jansen PM, Meijer CJ and Willemze R. Diagnostic and prognostic evaluation of phenotypic markers TRAF1, MUM1, BCL2 and CD15 in cutaneous CD30-positive lymphoproliferative disorders. Br J Dermatol 2009; 161: 121-127.
[25] Loseto G, Ciavarella S, Scattone A, Calabrese A, Quinto AM and Guarini A. Long-term complete remission of refractory primary cutaneous anaplastic large T cell lymphoma treated with brentuximab vedotin: a case report. J Cancer Sci Ther 2017; 09: 503-504.