

# Immunohistochemical expression of Mum-1, Oct-2 and Bcl-6 in systemic anaplastic large cell lymphomas

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## ABSTRACT

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**Aims and background.** Several transcription factors predominantly used for B-cell lineage identification are also expressed in a small percentage of T cells within germinal centers and interfollicular areas. The aim of the study was to evaluate the expression of Mum-1, Oct-2 and Bcl-6 in systemic anaplastic large cell lymphoma.

**Methods.** Thirty cases of anaplastic large cell lymphoma were retrieved from our archives and tissue microarray constructed. Immunohistochemistry was carried out using an avidin-biotin peroxidase complex method.

**Results.** A predominance of nuclear staining was observed for all transcription factors. Mum-1 was positive in all but one case (96.7%). Half of the cases displayed Oct-2 expression (15/30 cases). A considerable number of cases also had Bcl-6 expression (9/30). Bcl-6 staining was noted to be more common in ALK positive cases.

**Conclusion.** Our findings emphasize that these markers are not restricted to B-cell lineage and that extensive expression can be observed in anaplastic large cell lymphoma of T/null cell phenotype.

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## Introduction

Antibodies to transcription factors are promising antibodies that are becoming available as useful tools in diagnostic pathology. Their number is increasing steadily. Being expressed in early embryogenesis, they are regarded to be highly specific for certain differentiation pathways and hence very helpful in determining cell lineage. Furthermore being nuclear stains, these antibodies are very easy to interpret, increasing their diagnostic appeal.

Recently, there has been an expansion in the array of transcription factors available in diagnostic hematopathology<sup>1</sup>. Although initially described for specific lymphoid lineages, knowledge and experience with these markers is increasing, with expansion of their expression spectrum. With regard to the transcription factors discovered so far, studies show that their expression within the lymphoid system is not as cell type specific as hoped.

MUM-1, Oct-2 and Bcl-6 are three of these transcription markers commonly identified as B-cell markers and predominantly used in B-cell neoplasms. However, their expression has also been shown in a small percentage of activated T cells within germinal centers and interfollicular areas<sup>2-4</sup>. Studies have also reported expression in anaplastic large cell lymphoma (ALCL) and peripheral T-cell lymphomas<sup>3,5-10</sup>.

The expression of these transcription factors, mainly used for diagnosis of B-cell lymphoma, has been evaluated in a wide variety of tumors. However, only a few ALCL were incorporated in most of the study groups. The present study was aimed at evaluating the expression of these transcription factors more systematically in ALCL and confirming previous findings using a relatively large series of 30 cases.

**Key words:** anaplastic large cell lymphoma, Bcl-6, Mum-1, Oct-2.

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## Materials and methods

### Case selection

The archives of the Pathology Department of the Hacettepe University Faculty of Medicine were retrospectively reviewed, and cases of ALCL were identified. From these, cases with sufficient diagnostic material suitable for tissue microarray (TMA) construction were chosen. This resulted in a total of 30 cases belonging to 26 patients. Cases of primary cutaneous ALCL were excluded. The hematoxylin-eosin (H&E) sections and available immunohistochemical slides of all cases were reviewed, and the diagnosis was confirmed based on the criteria of the WHO 2008 classification. Additional immunohistochemistry was performed when necessary to aid the diagnosis.

### Tissue microarray

Construction of TMA was carried out using a manual precision instrument (ATA100, Chemicon International, Temecula CA, USA). An H&E slide of each donor block was used to define representative tumor regions. In those cases with scattered rather than diffuse infiltration, regions with a large number of anaplastic cells were selected. Tissue cylinders of 1-mm diameter were cut out and incorporated within the recipient block. Two replicates of each case were used to increase the number of assessable cases.

### Immunohistochemistry

Immunohistochemical investigation was carried out on 5- $\mu$ m-thick sections mounted on poly-L-lysine-coated slides. Antibodies against the following antigens were used: CD30, Mum-1, Oct-2 and Bcl-6. Pretreatment conditions, dilutions and product source are listed in Table 1. Primary antibodies were visualized with an avidin-biotin peroxidase complex method. Diaminobenzidine was used as substrate. Sections were counterstained by hematoxylin. Normal tonsils were used as positive controls.

### Morphological analysis

Staining with all markers was matched/compared with CD30 immunostaining during evaluation. This was most helpful in analyzing cases with scattered tumor cells. Nuclear staining in more than 20% of the neoplas-

tic cells was considered positive. This threshold value was chosen with respect to previous studies where a threshold ranging from 10 to 20% was used<sup>3,5,9,11,12</sup>. Staining intensity and distribution of expression was also noted. Staining intensity was graded as weak, moderate or strong.

### Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Sciences 11.0 for Windows. The chi-square test was performed to test the association between ALK status and Mum-1, Oct-2 and Bcl-6 expression.  $P < 0.05$  was considered to be statistically significant.

## Results

The study group consisted of 26 patients and 30 specimens. Patient ages ranged between 16 and 75, with a mean of 37 (36.9). There were 8 female and 18 male patients. Most of the cases were lymph node specimens (27/30), there was one case with pleural involvement (this case also had a lymph node sample), one case with gastric involvement, and one case with skin involvement (representing cutaneous involvement of systemic ALCL). Twenty-five of the cases were of the T-cell phenotype, 5 of null cell type. There were 15 ALK-positive and 15 ALK-negative cases.

CD30 positivity was present in all cases, in accord with a diagnosis of ALCL. A predominance of nuclear staining with occasional accompanying weak cytoplasmic staining was observed with all evaluated markers, in keeping with the transcriptional role of these molecules (Figures 1 and 2). Diffuse nuclear staining with moderate to strong intensity was observed in all positive cases with Mum-1 and Oct-2. Mum-1 was positive

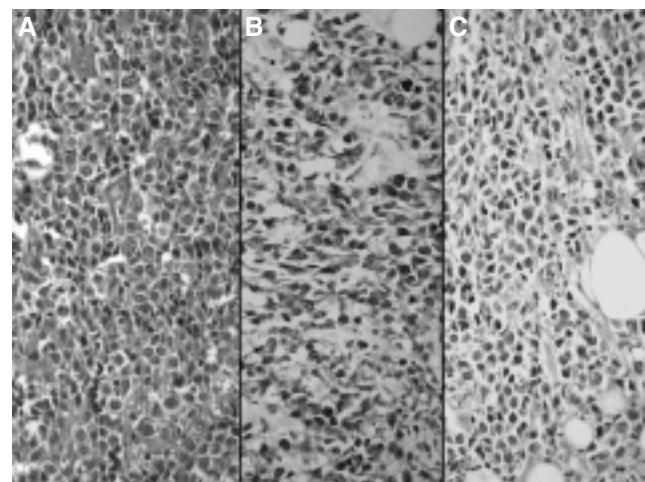


Figure 1 - A case of ALCL (A, H&E staining) demonstrating diffuse and strong nuclear staining for MUM-1 (B) and Oct-2 (C).

**Table 1 - Source, dilution and retrieval technique for antibodies used**

Antibody	Dilution	Retrieval	Source
CD30	1/40	Pressure cooker, 3 min, EDTA	Neomarkers
MUM-1	1/25	Pressure cooker, 3 min, citrate	DAKO
Oct-2	1/40	Pressure cooker, 3 min, citrate	Neomarkers
Bcl-6	1/75	Pressure cooker, 3 min, EDTA	Neomarkers

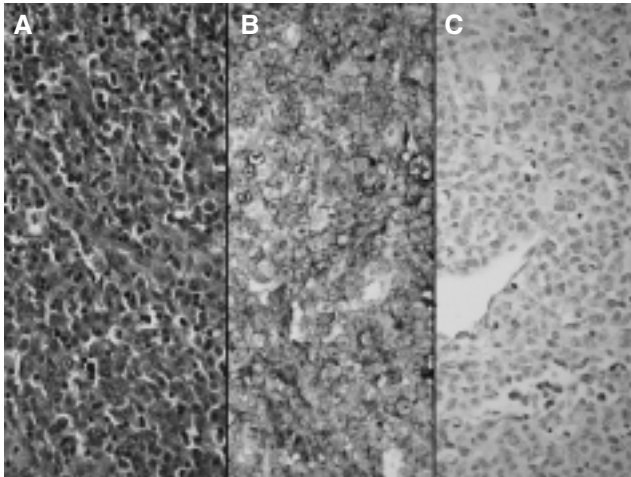


Figure 2 - A case of ALCL with focal and weak nuclear staining with Bcl-6. A) H&E, B) CD30, and C) Bcl-6 staining.

in all but one case (96.7%). Half of the cases displayed Oct-2 expression (15/30 cases). A considerable number of cases (30%, 9/30) also had Bcl-6 expression. However, Bcl-6 mainly demonstrated focal and weak nuclear staining (staining intensity compared to that of germinal center cells within the control tonsil specimen).

When the cases were analyzed with respect to ALK status, the expression of Mum-1 and Oct-2 was seen to be similar among ALK-positive and negative cases (Table 2). However, Bcl-6 expression was seen to be more common in ALK-positive cases. Whereas 46.7% (7/15 cases) of ALK-positive cases had Bcl-6 expression, only 15.4% (2/15 cases) of ALK-negative cases were positive for Bcl-6 (Table 2). The difference was close to the level of statistical significance ( $P = 0.054$ , chi-square test).

## Discussion

The number of transcription factors involved in diagnostic hematopathology is steadily increasing, as is experience with their expression patterns<sup>1</sup>. Most of these markers are directed mainly at B-cell differentiation and activation pathways. Several transcription factors predominantly used for B-cell lineage identification and classification such as Mum-1, Oct-2, Oct-1, Bob-1 and

Bcl-6 are also expressed in a small percentage of T cells within germinal centers and interfollicular areas<sup>1</sup>. Studies show that these positive T cells are activated T cells coexpressing CD30. In this study, we analyzed the expression of Mum-1, Oct-2 and Bcl-6 in 30 systemic ALCL using TMA, a technique which allows simultaneous examination of a large number of cases processed in identical conditions and the use of small amounts of archival tissues<sup>13</sup>. Prior studies with these markers only included a small number of ALCL lymphoma cases within the study group.

MUM-1 (multiple myeloma oncogene 1)/IRF-4 (interferon regulatory factor 4) is a transcription factor initially characterized as the product of an oncogene in multiple myeloma cells. Later studies showed that its expression was restricted to melanocytes and lymphoid cells<sup>14,15</sup>. Within the lymphoid system it is normally identified in plasma cells, a small percentage of B cells mainly located in the light zone of germinal centers, and activated T cells, the highest expression being noted in plasma cells and activated T lymphocytes<sup>2,3</sup>. Its exact function is yet unknown. Mice deficient in MUM-1 demonstrate deficient B- and T-cell function, with lack of germinal center formation, absence of plasma cells, severe reduction in immunoglobulin production, and appropriate B and cytotoxic T-cell activation<sup>16</sup>. Therefore, it is reasoned that one of its main roles is to facilitate plasma cell differentiation. In line with its normal expression pattern, positivity with the marker has been noted in a variety of B-cell lymphoproliferative disorders, mainly those with plasmacytic differentiation<sup>3,5</sup>. Within B-cell neoplasms, its presence has been associated with a more aggressive clinical course in diffuse large B-cell lymphoma<sup>17</sup>. Recently, a limited number of studies with a limited number of cases have demonstrated its expression in T-cell lymphomas<sup>2,3,5,12</sup>.

With respect to ALCL, four prior studies reported MUM-1 expression in 80-100% of cases. In total, of the 29 cases of ALCL studied, 27 were seen to express MUM-1 (Table 3). We also observed widespread MUM-1 expression, with all but one case demonstrating diffuse nuclear positivity (96.7%). MUM-1 expression paralleled CD30 expression. Wasco *et al.*<sup>12</sup>, who analyzed systemic and cutaneous ALCL (5 of each), also noted

Table 2 - Expression of MUM-1, Oct-2 and Bcl-6 with respect to ALK status

	MUM-1 (no. cases)		Oct-2 (no. cases)		Bcl-6 (no. cases)	
	+	-	+	-	+	-
ALK +	14	1	7	8	7	8
-	15	0	8	7	2	13

Table 3 - MUM-1 expression in ALCL, summary of prior studies

Study	Diagnosis	No. positive cases (no./total)	Percentage of positive cases
Natkunam <i>et al.</i> , 2001 <sup>3</sup>	ALCL	4/4	100%
Falini <i>et al.</i> , 2000 <sup>2</sup>	ALCL	10/10	100%
Wasco <i>et al.</i> , 2008 <sup>12</sup>	ALCL (cutaneous & systemic, 5 of each)	9/10	90%
Tsuboi <i>et al.</i> , 2000 <sup>5</sup>	ALCL	4/5	80%

MUM-1 expression to parallel that of CD30, being present in large activated T cells. A similar observation was true of the 10 ALCL cases analyzed by Falini *et al.*<sup>2</sup>

Oct-2 is another transcription factor predominantly expressed in B cells. It is involved in the regulation of genes engaged in immunoglobulin expression, CD20, CRISP-3 and CD36. It is also engaged in the transcriptional regulation of T-cell activation, Kang *et al.*<sup>4</sup> have suggested that Oct-2 is a downstream component of the regulatory cascade that follows T-cell activation. Weak expression has been noted in interfollicular T cells<sup>6</sup>. Saez *et al.*<sup>6</sup> investigated Oct-2 expression in various lymphomas and found that within T-cell lymphomas Oct-2 was predominantly expressed in the angioimmunoblastic type (all four cases showing moderate to weak expression). Weak expression was also noted in one of the two anaplastic large cell lymphomas within their series. The high level of expression was restricted to B cells. However, the study only had 2 ALCL cases within the T-cell lymphoma group. We also found Oct-2 expression in half of the cases in our series (15 of 30). The intensity of expression was strong to moderate.

Bcl-6, a gene originally identified due to chromosomal translocations in diffuse large B-cell lymphomas, encodes for a transcription factor which acts as a transcriptional repressor<sup>18</sup>. According to recent studies, it functions as an anti-apoptotic molecule<sup>19,20</sup>. It is predominantly expressed in germinal center B lymphocytes and germinal center-derived B-cell lymphomas and is absent in B-cell precursors and plasma cells and neoplasms derived from these cells<sup>21</sup>. Expression has also been reported in a subpopulation of germinal center and perfollicular CD4-positive T lymphocytes, mainly coexpressing CD30<sup>7,9</sup>, hence associated with an activated state<sup>9,22</sup>. Cortical thymocytes have also been shown to express Bcl-6<sup>7,11</sup>. Only a couple of studies have investigated its expression in T-cell lymphomas<sup>8-11</sup>.

Carbone *et al.*<sup>9</sup> investigated a series of T-cell lymphomas and noted positivity only in ALCL (12 of 27 ALCL cases, 45%), suggesting that its expression is restricted to CD30-positive ALCL within the T-cell lymphomas and that it could be used to differentiate ALCL from other T-cell lymphomas. Later, Ree *et al.*<sup>8</sup> reported Bcl-6 positivity in some of the neoplastic cells in their 6 cases of angioimmunoblastic lymphoma. Hyjek *et al.*<sup>11</sup> observed expression in 4 of 8 cases of T lymphoblastic lymphoma, contrasting with the observations of Carbone *et al.*<sup>9</sup>, who had not observed positivity in any of their 8 lymphoblastic lymphoma cases. Likewise, Smock *et al.*<sup>23</sup> also investigated Bcl-6 immunostaining in T-lymphoblastic lymphomas and showed expression in only 3 of 22 cases (14%).

In a more recent study with a relatively large number of cases, Lamant *et al.*<sup>10</sup> showed Bcl-6 expression in ALCL to be correlated with ALK status. Using gene expression profiling, they found Bcl-6 overexpression in ALK-positive ALCL. They confirmed the observation with im-

munohistochemistry. Overall, 75% of their ALCL cases showed Bcl-6 expression; 81.6% (40/49 patients) of ALK-positive cases showed Bcl-6 expression, and Bcl-6 expression was present in only 28% (2/7 cases) of ALK-negative cases. However, the authors noted that ALK-positive cases were over-represented in their series.

In our study, we noted Bcl-6 positivity in 9 cases (30%). Bcl-6 positivity was weak and focal compared with the other two markers studied and with the intensity of staining within germinal centers in the reactive lymph nodes used for positive control. When we analyzed Bcl-6 expression with respect to ALK status, we also noted more frequent expression in ALK-positive cases (46.7% positivity in ALK-positive cases compared to 15.4% positivity in ALK-negative cases), although not as predominant as that noted by Lamant *et al.*<sup>10</sup> ALK status of our cases was equally distributed, with 15 positive and 15 negative cases. The difference in Bcl-6 expression with respect to ALK status was close to the level of statistical significance.

In summary, using a series of 30 ALCL cases we have shown staining of MUM-1 in 96.7% (29/30), Oct-2 in 50% (15/30) and Bcl-6 in 30% (9/30) of cases. Our findings confirm and extend data reported in prior studies. These markers are predominantly used to show B-cell lineage. Our findings once again emphasize that extensive expression of these markers can be observed in ALCL of T/null-cell phenotype. Furthermore, we noted Bcl-6 expression to be more frequent in ALK-positive cases.

The most probable explanation for the presence of these markers in ALCL is the fact that these markers have been shown to be expressed in a small number of T cells within and between germinal centers of normal lymphoid tissue, although at lower levels compared to B cells. Data suggest that these are cells coexpressing CD30 and in most cases CD4 and that they represent activated T cells. In turn, at least a proportion of ALCL are thought to be derived from these activated CD30-positive T cells, which could explain the considerable amount of expression present in our cases and those in the previous literature<sup>9,24</sup>.

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