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Original contribution

# MYC expression is associated with older age, common morphology, increased *MYC* copy number, and poorer prognosis in patients with ALK+ anaplastic large cell lymphoma☆,☆☆

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**Summary** The role of MYC dysregulation has been studied extensively in B-cell lymphomas, but little is known about its significance in T cell lymphomas. This study, for the first time in the literature, assessed the clinicopathologic and prognostic significance of MYC expression in ALK+ anaplastic large cell lymphoma (ALCL) cases. Using ≥50% as the cutoff value for positive MYC expression by immunohistochemistry, 17 of 46 (37%) cases were MYC+. Patients with MYC+ tumors were older (median age, 39 *versus* 29 years, p = 0.04) and more often showed a common morphologic pattern (100% *versus* 69%, $p = 0.02$), when compared with those with MYC-negative tumors. By fluorescence in situ hybridization analysis, 9 of 31 (29%) cases showed increased *MYC* copy number, and 1 of 31 (3%) case had an *MYC* rearrangement, and the remaining 21 (68%) cases showed no *MYC* aberrations. Among the cases with increased *MYC* copy number, 5 of 8 (62%) cases showed *MYC* copy gain and/or amplification and 3 of 8 (38%) had polysomy 8. MYC expression was associated with increased *MYC* copy number ($p = 0.01$). MYC expression, but not increased *MYC* copy number, correlated with shorter overall survival (OS) ($p = 0.03$). In conclusion, MYC expression identified a distinct group of ALK + ALCL patients with more aggressive behavior and shorter OS. Our data suggest that MYC

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expression is an adverse prognostic factor and may be useful in stratifying or predicting the prognosis of patients with ALK+ ALCL.


## 1. Introduction

Anaplastic large cell lymphoma (ALCL) is a T cell neoplasm usually characterized by large tumor cells with horseshoe-shaped nuclei and abundant cytoplasm (so-called *hallmark* cells), uniform and strong CD30 expression, and aberrant loss of one or more T cell antigens. These neoplasms can be further classified into ALK+ and ALK- negative types. ALK+ ALCL is characterized by ALK expression resulting from translocations involving *ALK* at 2p23, most commonly t(2; 5)(p23; q35)/*NPM1-ALK* [1]. Although the long-term overall survival (OS) rates are 70−90% in patients with ALK+ ALCL, some patients have refractory/relapsed disease with 5-year OS rate of <50% [2,3]. Therefore, identifying patients with a poorer prognosis who might benefit from more aggressive therapy is needed.

*MYC*, located on chromosome 8q24, encodes a transcription factor that regulates many genes involved in cell growth, proliferation, apoptosis, metabolism, and differentiation [4−7]. *MYC* is altered in many malignancies including lymphomas and some solid tumors [5]. The role of *MYC* dysregulation in lymphomas has been studied extensively in B-cell lymphomas. Translocations of *MYC* partnered with one of the immunoglobulin genes are the genetic hallmark of Burkitt lymphoma and can be detected in 90% of cases. These translocations lead to MYC overexpression and are thought to have an important role in pathogenesis of Burkitt lymphoma [8]. *MYC* translocations also occur in 10−15% of diffuse large B-cell lymphoma (DLBCL), predominantly in the germinal center B-cell type [8,9]. In addition to *MYC* rearrangements, numerical alterations of *MYC* are also detected in 7−21% of DLBCL. *MYC* translocations and increased copy number may be later events in the pathogenesis of DLBCL and have been associated with a poorer prognosis [10−19]. MYC expression assessed by immunohistochemistry also has been correlated with a poorer prognosis in DLBCL.

Studies of *MYC* dysregulation in T cell lymphomas including ALCL are much more limited than in B-cell lymphomas. An early study reported MYC overexpression in all 15 cases of pediatric ALK+ ALCL assessed, although MYC cutoff value was not specified [20]. A more recent study, using a cutoff level of ≥30%, reported that 31 of 38 (82%) primary ALK+ ALCL samples were MYC+ by immunohistochemistry [21]. However, the clinicopathologic features and outcome of patients with MYC+ ALCL and the underlying mechanisms of MYC expression in those patients have not yet been reported. In this study, we assessed MYC expression in ALK+ ALCL cases using immunohistochemistry, and the results were correlated with clinicopathologic features and patient outcome. We also investigated *MYC* aberrations by fluorescence in situ hybridization (FISH) analysis and its relationship with MYC expression.

## 2. Materials and methods

### 2.1. Case selection

We searched the database of the Department of Hematopathology at The University of Texas MD Anderson Cancer Center from January 1, 2007 through December 31, 2018 for cases of ALK+ ALCL that had materials available for assessment of MYC by immunohistochemistry. The diagnosis and subclassification of ALCL were based on criteria specified in the 2016 WHO classification [1]. The diagnosis of ALK+ ALCL was confirmed by ALK expression by immunohistochemistry with or without t(2; 5)(p23; q35) by conventional cytogenetics or *ALK* rearrangement by FISH analysis. Clinical information was obtained by review of medical records. This study was approved by the institutional review board.

### 2.2. Immunophenotypic analysis

Immunohistochemical studies were performed using formalin-fixed paraffin-embedded (FFPE) tissue sections, either at the time of diagnosis or retrospectively for this study as described previously [22]. Immunohistochemical analysis was performed on an automated immunostainer (Leica Bond-Max IHC Stainer, San Diego, CA). Tissue sections, 4-μm-thick, were deparaffinized and underwent heat-induced antigen retrieval using the Bond Max Epitope Retrieval 1 solution for 15 min. The antibodies used were specific for CD2, CD7, EMA (Leica Biosystems, Newcastle, United Kingdom); CD3, CD20, CD43, CD45 (Dako, Carpinteria, CA, USA); CD4 (Cell Marque, Rocklin, CA, USA); CD5 (SP4; Labvision/Neomarkers, Fremont, CA, USA); CD8, granzyme B (Thermo Fisher, Waltham, MA, USA); ALK (Cell Signaling, Danvers, MA, USA); c-MYC (clone Y69, Epitomics, Burlingame, CA, USA) and PAX5 (Transduction Labs, San Diego, CA, USA). The Bond Refine Polymer detection system was used for visualization.

**Table 1** Clinical features of patients with MYC+ and MYC-negative ALK+ anaplastic large cell lymphoma.

| Clinical features | MYC+ (n = 17) | MYC-negative (n = 29) | *P* value |
|---|---|---|---|
| Male:female | 1.4:1 (10/7) | 1.2:1 (16/13) | 1.00 |
| Median age, yrs (range) | 39 (17–64) | 29 (5–58) | 0.04 |
| B symptoms | 64% (7/11) | 62% (13/21) | 1.00 |
| Nodal presentation | 93% (13/14) | 81% (22/27) | 0.64 |
| Extranodal involvement | 7% (1/14) | 19% (5/27) | 1.00 |
| Bone marrow involvement | 23% (3/13) | 19% (4/21) | 1.00 |
| Stage III or IV | 75% (9/12) | 77% (17/22) | 1.00 |
| Elevated WBC | 50% (5/10) | 38% (5/13) | 0.69 |
| Absolute lymphocytosis | 10% (1/10) | 0% (0/11) | 0.48 |
| Elevated serum LDH | 70% (7/10) | 31% (4/13) | 0.08 |
| IPI ≥3 | 22% (2/9) | 13% (2/15) | 0.61 |
| Initial treatment | | | |
| CHOP or modified CHOP | 82% (9/11) | 70% (16/23) | 0.68 |
| Other* | 18% (2/11) | 30% (7/23) | |
| Initial CR | 64% (7/11) | 87% (20/23) | 0.18 |
| With SCT | 18% (2/11) | 50% (11/22) | 0.13 |

ALCL, anaplastic large cell lymphoma; WBC, white blood cells; LDH, lactate dehydrogenase; IPI, International Prognostic Index; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; *, including Hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, and dexamethasone), EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin), ICE (ifosfamide, carboplatin, etoposide), BV (Brentuximab vedotin), and so on R, rituximab; CR, complete response; SCT, stem cell transplant.

**Table 2** Pathological features of patients with MYC+ and MYC-negative ALK+ anaplastic large cell lymphoma.

| Pathologic features | MYC+ (n = 17) | MYC-negative (n = 29) | *P* value |
|---|---|---|---|
| Morphologic type | | | |
| Common pattern | 100% (17/17) | 69% (20/29) | **0.02** |
| Noncommon pattern | 0% (0/17) | 31% (9/29) | |
| Immunophenotype | | | |
| CD2+ | 36% (4/11) | 67% (14/21) | 0.14 |
| CD3+ | 18% (3/17) | 37% (10/27) | 0.20 |
| CD4+ | 60% (6/10) | 68% (15/22) | 0.70 |
| CD5+ | 50% (5/10) | 36% (8/22) | 0.70 |
| CD7+ | 0% (0/6) | 50% (7/14) | 0.05 |
| CD8+ | 29% (2/7) | 17% (3/18) | 0.60 |
| CD25+ | 67% (2/3) | 83% (10/12) | 0.52 |
| CD43+ | 56% (5/9) | 82% (9/11) | 0.34 |
| CD45+ | 67% (10/15) | 88% (15/17) | 0.21 |
| TCR A/B+ | 100% (3/3) | 38% (3/8) | 0.18 |
| TCR G/D+ | 0% (0/2) | 0% (0/8) | 1.00 |
| Granzyme B+ | 60% (3/5) | 100% (8/8) | 0.13 |
| TIA1+ | 100% (3/3) | 100% (4/4) | 1.00 |
| EMA+ | 90% (9/10) | 100% (11/11) | 0.48 |

Immunophenotypic analysis by flow cytometry was performed on cell suspensions of tissue biopsy specimens or bone marrow aspirates using either a FACScanto II or FACSCalibur cytometer (Becton-Dickinson Biosciences, San Jose, CA, USA) as has been described [22]. Lymphocytes were gated for analysis using side scatter *versus* forward scatter and CD45 expression *versus* side scatter. The panel of monoclonal antibodies included reagents specific for CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD25, CD30, CD45, TCR alpha/beta, TCR gamma/delta (Becton-Dickinson Biosciences, San Jose, CA, USA).

### 2.3. Fluorescence in situ hybridization

FISH analysis was performed on FFPE tissue sections using *ALK* dual color break-apart probe, and *MYC* dual color break-apart probe (Abbott Molecular, Des Plaines, IL, USA) according to the manufacturer's instructions, 200 nuclei were analyzed [10,22]. The cutoffs established in our cytogenetic laboratory were 18.1% for increased *MYC* copy number and 8.1% for *MYC* rearrangement, respectively. *MYC* amplification was designated as ≥ 6 copies or clusters of *MYC* signals. For cases with increased *MYC* copy number without amplification (i.e. 3-5 copies), FISH analysis using *MYC/CEP8* dual color probes (Abbott Molecular, Des Plaines, IL, USA) was performed to differentiate true *MYC* copy gain from polysomy 8: the former showing copy number increase in *MYC* only (not in *CEP8*), whereas the latter showing copy number increase in both *MYC* and *CEP8*.

### 2.4. Statistical analysis

Statistical analyses were performed using the Graph-Pad Prism 8. Fisher's exact test was utilized to compare the clinicopathologic features between MYC+ *versus* MYC-negative groups in patients with ALK+ ALCL. OS was calculated from the date of initial diagnosis to the date of death or last follow-up. Survival was analyzed using the Kaplan-Meier method and was compared using the log rank test. A p value of less than 0.05 was considered statistically significant.

## 3. Results

### 3.1. Clinical findings

We assessed MYC expression in 46 cases of ALK+ ALCL. Using ≥50% as the cutoff value for MYC positivity, 17 (37%) cases were MYC+ and 29 (63%) cases were MYC-negative. The clinical features of these patients are summarized in Table 1. The MYC+ group included 10 men

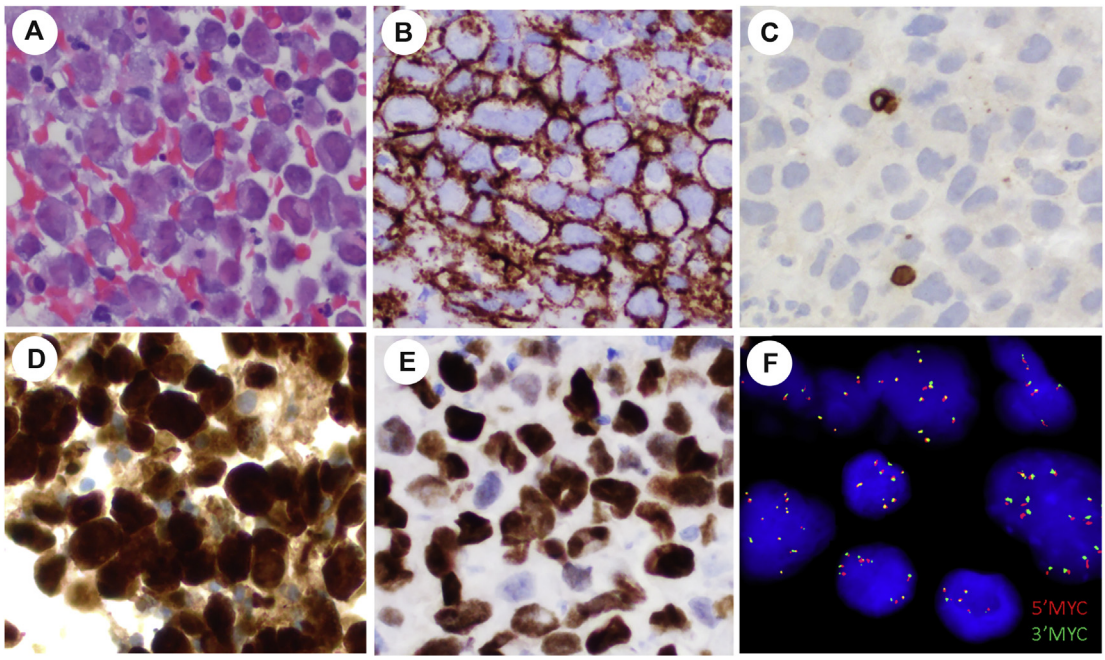


**Fig. 1** A MYC+ ALK+ ALCL case with *MYC* amplification. (A) This case showed common morphologic pattern. (B−C) The lymphoma cells were uniformly and strongly positive for CD30 (B, membranous staining) but were negative for CD3 (C). Rare small lymphocytes with strong positivity for CD3 (C) were the background reactive T cells. (D-E) The lymphoma cells were positive for ALK (D, nuclear and cytoplasmic staining) and MYC (E, nuclear staining). (F) FISH analysis showed the lymphoma cells had *MYC* amplification (≥6 copies) in majority of the cells. A. Hematoxylin-eosin stain, x400. B-E. Immunohistochemistry, x400. F. FISH analysis using *MYC* dual color (3′*MYC* labeled as green, 5′*MYC* labeled as red) break-apart probe, x600. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.) FISH, fluorescence in situ hybridization; ALCL, anaplastic large cell lymphoma.

and 7 women with a median age of 39 years (range, 17−64 years) at the time of diagnosis. Seven of 11 (64%) patients had B symptoms. Lymphadenopathy was identified in 13 of 14 (93%) patients and 1 of 14 (7%) had extranodal involvement. Bone marrow was involved in 3 of 13 (23%) patients. Nine of 12 (75%) fully staged patients had stage III or IV disease. Five of 10 (50%) patients had leukocytosis and 1 of 10 (10%) had absolute lymphocytosis. Seven of 10 (70%) patients tested showed an elevated serum LDH level. Two of 9 (22%) patients had an International Prognostic Index score of >3 (Table 1).

The MYC-negative group included 16 men and 13 women with a median age of 29 years (range, 5−58 years) at time of diagnosis. The median age of MYC+ group was older than MYC-negative group (39 years vs. 29 years; $p = 0.04$). Patients had a similar frequency of B symptoms, lymphadenopathy, and advanced stage disease. Other than age, there were no significant differences in the clinical features of patients with MYC+ *versus* MYC-negative ALK+ ALCL (Table 1).

### 3.2. Pathologic findings

The pathological features of these patients are summarized in Table 2. All 17 cases of ALK+ ALCL that were MYC+ showed common (classic) morphology (Table 2; Fig. 1). In the 29 MYC-negative neoplasms, 20 (69%) cases showed common morphology and 9 (31%) cases had noncommon morphologic patterns: 5 (17%) small cell and 4 (14%) lymphohistiocytic (Fig. 2). The MYC + subset of ALK + ALCL more frequently had common morphology (100% vs. 69%, $p = 0.02$). MYC+ cases also had variable expression of T cell antigens (Table 2). Most of the MYC+ cases were positive for CD4 (6/10; 60%), CD5 (5/10; 50%), CD25 (2/3; 67%), CD43 (5/9; 56%), and CD45 (10/15; 67%). In addition, most MYC+ cases were positive for EMA (9/10; 90%) and 3 of 5 (60%) were positive for granzyme B. Small subsets of MYC+ cases were positive for CD2 (4/11; 36%), CD3 (3/17; 18%), and CD8 (2/7; 29%). None of the MYC+ cases were positive for CD7 (n = 6). There was no significant difference in the frequency of expression of T cell antigens and cytotoxic markers between MYC+ *versus* MYC-negative ALK+ ALCL cases (all $p > 0.05$; Table 2).

Thirty-one cases were assessed for *MYC* aberrations by FISH analysis. Nine (29%) cases showed increased *MYC* copy number (Table 3). One (3%) case had a *MYC* rearrangement [23]. The remaining 21 (68%) cases showed no *MYC* aberrations (Fig. 2). Among the 9 cases with increased *MYC* copy number, 3 had MYC amplification

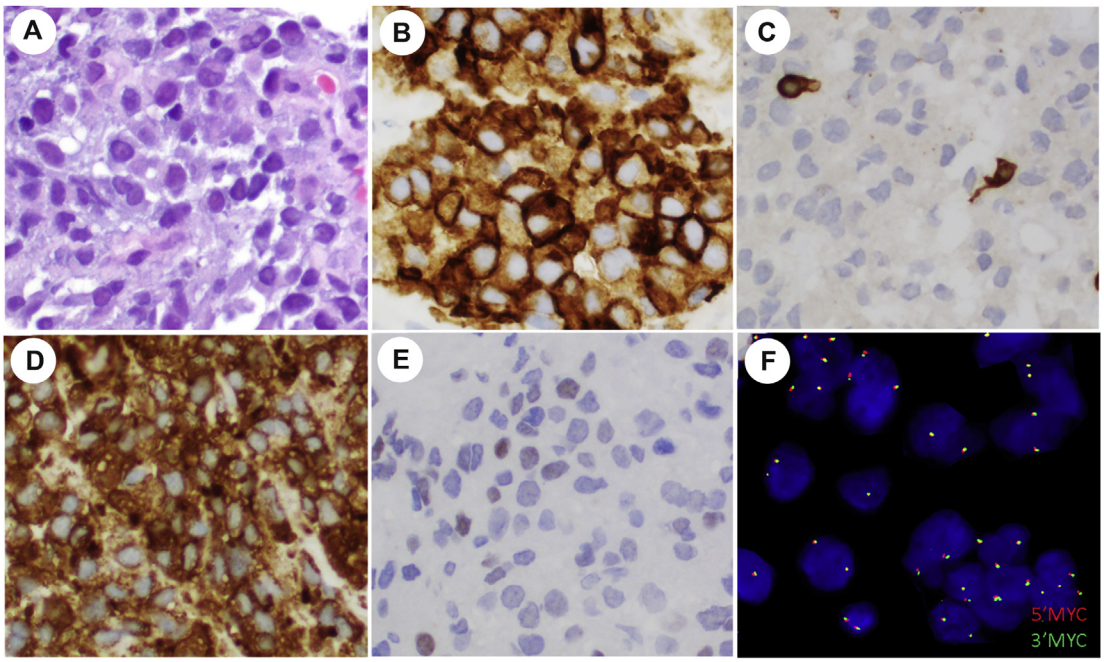


**Fig. 2** A MYC-negative ALK+ ALCL case with normal *MYC* copy number. (A) This case showed small cell morphologic pattern. B−C. The lymphoma cells were uniformly and strongly positive for CD30 (B) but were negative for CD3 (C). (D) The lymphoma cells were positive for ALK (cytoplasmic staining). (E) Only a small subset (<50%) of lymphoma cells showed MYC staining (weak). (F) FISH analysis showed normal signal pattern, with 2 fusion signals in majority of the cells. A. Hematoxylin-eosin stain, x400. B-E. Immunohistochemistry, x400. F. FISH analysis using *MYC* dual color (3′*MYC* labeled as green, 5′*MYC* labeled as red) break-apart probe, x600. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.) FISH, fluorescence in situ hybridization; ALCL, anaplastic large cell lymphoma.

(Figs. 1F, 3D) 1 had *MYC* copy gain (Fig. 4A), 1 had MYC copy gain and amplification, 3 had polysomy 8 (Fig. 4B), and 1 did not have tissue for FISH analysis for *MYC/CEP8* (Table 3). In the subgroup of cases with *MYC* aberrations, a mean of 63% lymphoma cells were positive for MYC expression, significantly higher than the mean of 41% lymphoma cells observed in cases without *MYC* aberrations (Fig. 5, $p = 0.02$). MYC was positive in 8 of 9 (89%) cases with increased *MYC* copy number *versus* 7 of 21 (33%) cases without increased *MYC* copy number ($p = 0.01$; Table 4).

**Table 3** Increased *MYC* copy number and MYC expression in ALK+ anaplastic large cell lymphoma.

| Case number | *MYC* copy number by FISH | MYC+ lymphoma cells by IHC (%) |
|---|---|---|
| 1 | ≥6 (*MYC* amplification) | 90 |
| 2 | 5′ *MYC* amplification | 60 |
| 3 | 5′ *MYC* amplification | 60 |
| 4 | 3-5 (*MYC* copy gain) | 60 |
| 5 | 3-7 (*MYC* copy gain/ amplification) | 50 |
| 6 | 3-5 (polysomy 8) | 90 |
| 7 | 3-5 (polysomy 8) | 50 |
| 8 | 3-5 (polysomy 8) | 10 |
| 9 | 3-4 (*MYC* copy gain or polysomy 8?)[a] | 70 |

ALCL, anaplastic large cell lymphoma; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.
[a] no tissue for FISH analysis for *MYC/CEP8*.

### 3.3. Treatment and response

Treatment and follow-up data were available for 34 patients with ALK+ ALCL: 11 MYC+ and 23 MYC-negative. All patients were treated with various chemotherapy regimens over the time interval of this study, with or without stem cell transplant (SCT). In patients with MYC+ neoplasms, 9 of 11 (91%) were treated with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or modified CHOP (Table 1). After initial induction chemotherapy, 7 of 11 (64%) patients achieved complete remission. Two of 11 (18%) patients received SCT: 1 autologous and 1 allogeneic. In patients with MYC-negative neoplasms, 16 of 23 (70%) were treated with CHOP or a modified CHOP regimen and 20 (87%) achieved complete remission. Eleven of 22 were treated with SCT. There were no differences in initial treatment or complete remission rate between the MYC+ *versus* MYC-negative groups (all $p > 0.05$; Table 1).

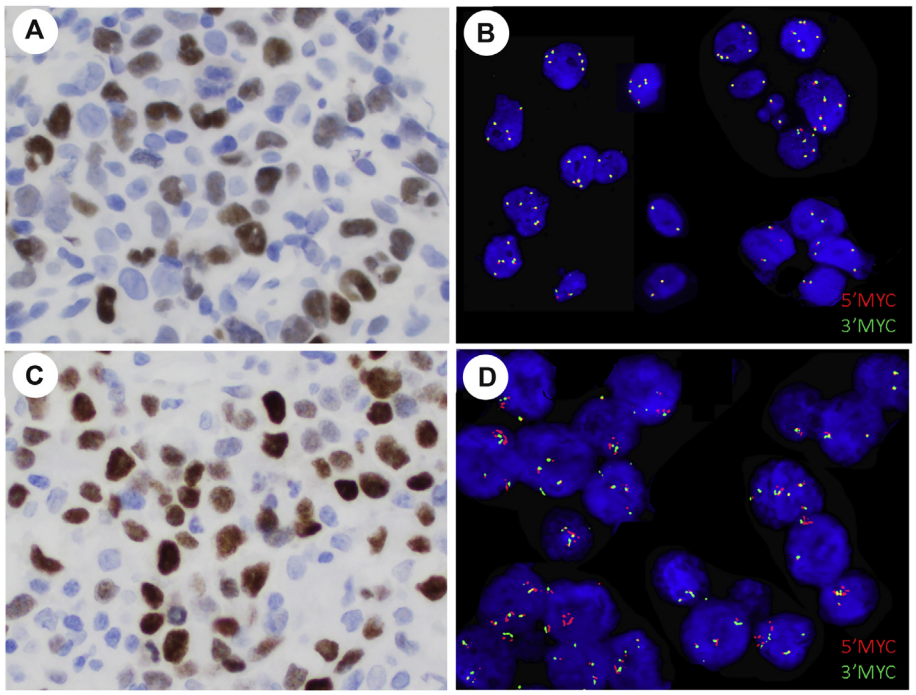


**Fig. 3** Two MYC+ ALK+ ALCL cases: one with 3−5 MYC copy number and one with 5′ *MYC* amplification. A and B. In this case, the lymphoma cells were positive for MYC expression (A) and FISH analysis showed 3∼5 MYC copy number in majority of the cells (B). C and D. In this case, the lymphoma cells were positive for MYC expression (C) and FISH analysis showed 5′*MYC* amplification with clusters of red signals in many cells (D). A and C. Immunohistochemistry, x400. B and D. FISH analysis using *MYC* dual color (3′*MYC* labeled as green, 5′*MYC* labeled as red) break-apart probe, x600. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.) FISH, fluorescence in situ hybridization; ALCL, anaplastic large cell lymphoma.

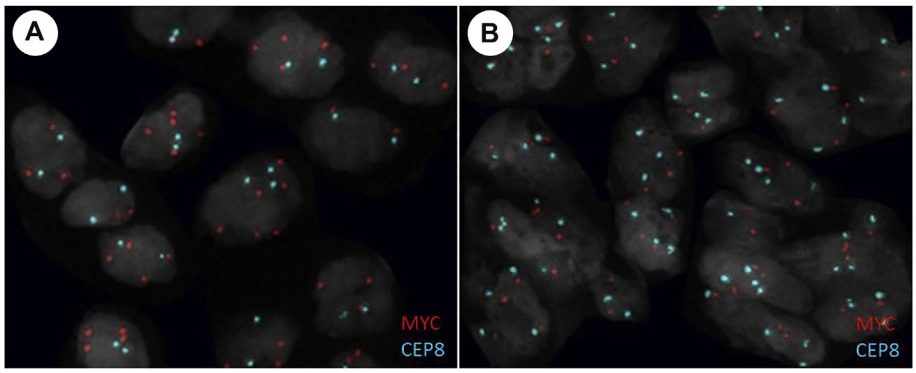


**Fig. 4** Two ALK+ ALCL cases with increased *MYC* copy number: one with *MYC* copy gain and one with polysomy 8. (A) FISH analysis showed increased *MYC* copy number (3−5 copies) but normal *CEP8* copy number in majority of the cells, i.e. *MYC* copy gain. B. FISH analysis showed increased copy number (3−5 copies) in both *MYC* and *CEP8,* i.e. polysomy 8. A and B. FISH analysis using *MYC/CEP8* dual color (*MYC* labeled as red, *CEP8* labeled as blue) probes, x600. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.) FISH, fluorescence in situ hybridization; ALCL, anaplastic large cell lymphoma.

### 3.4. Outcome

After a median clinical follow-up of 23 months (range, 0−224 months), 12 of 37 (32%) patients died. Death occurred in 6 of 13 (46%) patients with MYC+ neoplasms and in 6 of 24 (25%) patients with MYC-negative neoplasms. Survival analysis was performed in patients based on MYC expression status. Positive MYC expression, using a cutoff value of 50% (rather than 40% or less), was associated with significantly shorter OS in patients with ALK+ ALCL ($p = 0.02$; Fig. 6A−C). Since MYC expression was associated with age, common morphologic

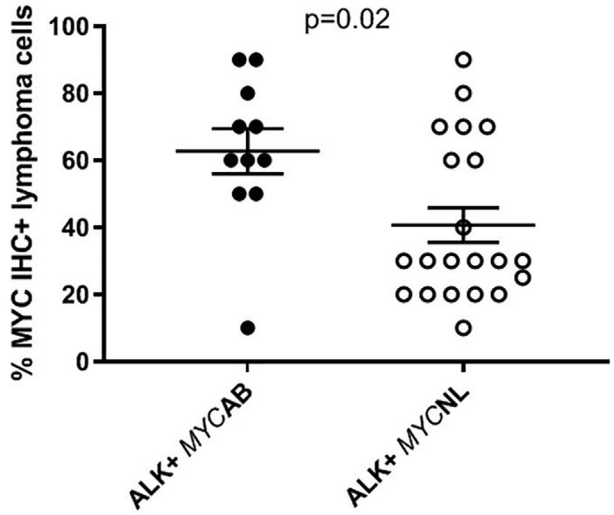


**Fig. 5** MYC expression in ALK+ ALCL was associated with *MYC* genetic aberrations. The percentage of MYC + lymphoma cells in ALK + ALCL cases with *MYC* aberrations was higher than that in the cases without *MYC* aberrations. *MYC*AB, cases with *MYC* aberrations; *MYC*NL, cases without *MYC* aberrations. ALCL, anaplastic large cell lymphoma.

pattern, and increased *MYC* copy number in ALK+ ALCL, the possible roles of these three factors in patient survival were investigated. Age (using 40 years as the cutoff) or morphology (common vs. noncommon pattern) had no impact on OS in patients with ALK+ ALCL ($p = 0.56$ and $p = 0.87$, respectively; Fig. 7A and B). Similarly, there was no significant difference in OS between patients with *versus* without increased *MYC* copy number ($p = 0.55$; Fig. 7C).

## 4. Discussion

Studies of *MYC* dysregulation in T cell lymphomas are very limited. One type of T-cell lymphoma in which MYC expression has been assessed is adult T cell leukemia/lymphoma (ATLL). Mihashi et al. [24] assessed 137 patients and reported that MYC expression (defined as positivity in ≥50% of lymphoma cells by immunohistochemistry) was more common in lymphoma-type (79%) and acute-type (65%) disease as compared with smoldering-type (4%) and chronic-type (9%) disease. In a small study of pediatric ALCL: all 15 ALK+ ALCL cases were MYC+ *versus* 0 of 6 ALK-negative ALCL cases, but MYC cutoff value was not specified [20]. Another more recent study reported that 82% of primary ALK+ ALCL samples were MYC+ by immunohistochemistry; the cutoff used was ≥30% in this study [21]. The variable MYC positivity rate in the literature is explained, at least partially, by the different cutoff values used. In our study, a cutoff value for MYC positivity of ≥50% was used because it identified the subset of patients with shorter OS; therefore 37% of our ALK+ ALCL cases had high MYC expression (positivity in ≥50% lymphoma cells).

**Table 4** High MYC positivity rate in ALK + ALCL with increased *MYC* copy number.

| | No.case | MYC+ (n[%])[a] | MYC- negative (n [%]) | *P* Value |
|---|---|---|---|---|
| Increased *MYC* copy number | s 9 | 8 (89%) | 1(11%) | **0.01** |
| | − 21 | 7 (33%) | 14 (69%) | |

ALCL, anaplastic large cell lymphoma.
[a] ≥50% of lymphoma cells were positive for MYC expression.

A few reports suggest that T cell lymphomas often have increased *MYC* copy number, but rarely carry *MYC* rearrangement. In a small series of cases of Sezary syndrome, 75% of patients had *MYC* gains [25]. Similarly, gains of 8q24 is one of the most frequent chromosomal aberrations in peripheral T cell lymphoma unspecified [26]. In ATLL, MYC expression has been correlated with chromosomal amplification and duplication [24]. In a study of 108 T and NK cell lymphoma cases, all neoplasms with MYC expression lacked *MYC* rearrangement [27].

Chromosomal structural alterations including gains of 8q affecting *MYC* were reported in an early study of an ALCL cell line [28]. In our study, increased *MYC* copy number was present in almost 30% of cases, but *MYC* rearrangement was rare (3%) [23]. Among our cases with increased *MYC* copy number, 62% (5/8) were due to *MYC* copy gain and/or amplification, whereas 38% (3/8) were due to polysomy 8, suggesting that *MYC* copy gain/amplification is the main mechanism leading to increased *MYC* copy number.

In one study, activation of ALK fusion protein induced overexpression of *MYC*, supporting dysregulation of *MYC* as a potential downstream target of ALK signaling [20]. Although the expression level of MYC varied in each of our cases, all cases showed some extent of MYC expression even in the group without increased *MYC* copy number, possibly induced by ALK fusion protein. We also found an association between MYC expression and increased *MYC* copy number, suggesting that increased *MYC* copy number is a mechanism underlying high MYC expression in ALK+ ALCL. Therefore, high *MYC* copy number may act in synergy with ALK fusion protein. Interestingly, 33% of cases without increased *MYC* copy number had MYC expression level of ≥50% (MYC+ using 50% as cut-off), suggesting that other factors (not *MYC* copy number) may also play a role in regulating MYC expression.

In the study by Mihashi et al. [24], ATLL patients with ≥50% MYC expression had significantly worse prognosis than those with <50% MYC expression. Furthermore, in 4 of 7 patients with smoldering or chronic-type ATLL that transformed to acute-type, MYC expression increased from <50% to ≥50%, suggesting that MYC expression in ATLL

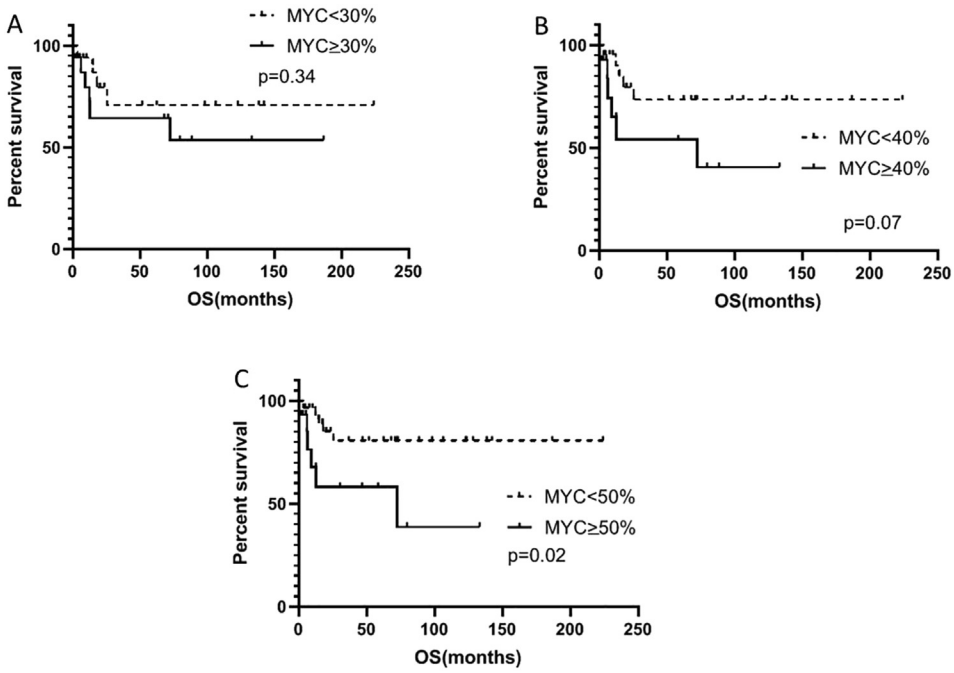


**Fig. 6** MYC expression in ≥50% lymphoma cells correlated with overall survival (OS) in patients with ALK+ ALCL. (A) 30% as the cutoff value; (B) 40% as the cutoff value; (C) 50% as the cutoff value. ALCL, anaplastic large cell lymphoma.

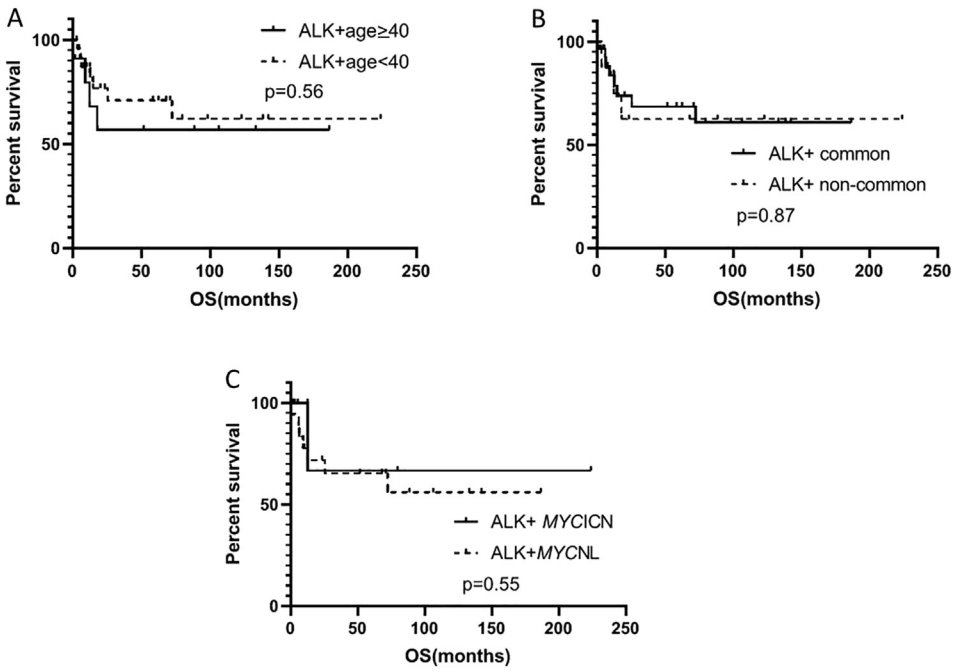


**Fig. 7** Age (A), morphologic pattern (B), and increased *MYC* copy number (C) had no prognostic impact on overall survival (OS) in patients with ALK+ ALCL. ICN, cases with increased copy number; NL, cases without increased copy number. ALCL, anaplastic large cell lymphoma.

is associated with aggressive behavior and inferior patient prognosis. In the cohort of ALK+ ALCL cases we present, MYC expression in ≥50% of cells was associated with a shorter OS. Although ≥40% is a well-accepted cutoff value for MYC positivity in DLBCL, our data in combination with the results from the ATLL study by Mihashi et al. [24] suggest that ≥ 50% might be a more appropriate cutoff value to define MYC positivity in T cell lymphomas

because it stratified the patients with a worse outcome. Despite its association with MYC expression, increased *MYC* copy number did not affect patient OS in our cohort.

Because MYC expression was also associated with older age and common morphologic pattern in this study, we investigated the possible prognostic roles of age and morphology. In general, ALK+ ALCL patients have a better prognosis than those with ALK-negative ALCL [29–33]. However, in some studies, ALK expression has not been identified as an independent prognostic factor in multivariate analysis, mainly because of the correlation between younger age and ALK positivity. If the analysis was limited to patients younger than 40 years old, patients with ALK-negative ALCL have relatively good outcomes, similar to those with ALK+ ALCL, suggesting that age might be a major factor driving the difference in outcomes [34]. However, in the cohort we present, age had no prognostic importance.

In childhood ALK+ ALCL patients, the presence of a small cell/lymphohistiocytic component was significantly associated with a high risk of failure in multivariate analysis [35]. In our cohort composed of predominantly adult patients, the MYC-negative ALK+ ALCL cases more often had a noncommon morphologic pattern, but these patients had better OS than patients with MYC+ neoplasms. As expected, there was no significant difference in OS between our patients with common *versus* noncommon morphologic patterns.

In conclusion, we assessed MYC expression in 46 patients with ALK+ ALCL and compared the clinicopathologic features and outcome between patients with MYC+ *versus* MYC-negative tumors. In addition, we also investigated *MYC* aberrations and its relationship with MYC expression. We found that MYC expression was associated with older age, common morphologic pattern, and increased *MYC* copy number. Among the cases with increased *MYC* copy number, a large subset had *MYC* copy gain/amplification and a small subset had polysomy 8. MYC expression, but not increased *MYC* copy number, correlated with shorter OS. Our data suggest that MYC expression is an adverse prognostic factor and may be useful in stratifying or predicting the prognosis of patients with ALK+ ALCL.

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