# The Clinical Significance of Activated p-AKT Expression in Peripheral T-cell Lymphoma

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**Abstract**. *Background: The oncogenic PI3K/serine-threonine kinase (PI3K/AKT) pathway is a downstream pathway of B-cell receptor (BCR) signaling pathway and plays a crucial role in the pathogenesis of B-cell lymphoma. However, there have been preclinical data showing PI3K/AKT pathway activation in T-cell lymphoma, with in different mechanisms from those in B-cell lymphoma. In this study, we investigated the impact of p-AKT expression on clinical outcomes of peripheral T-cell lymphoma (PTCL). Materials and Methods: We analyzed 63 patients with PTCL [PTCL-not otherwise specified (NOS), angioimmunoblastic T-cell lymphoma (AITL), anaplastic large cell lymphoma (ALCL) or extranodal natural kiler T-cell lymphoma (NKTCL)]. To define the clinical implications of p-AKT expression in PTCL, we calculated arbitrary units (AUs) by multiplying the intensity and the proportion of p-AKT expression. Results: Based on a cutoff value of the upper limit of the third quartile (Q3) of the AU, 12 patients were classified into the high p-AKT group, while the remaining 51 patients were classified into the low p-AKT group. The overall response rate to frontline chemotherapy was significantly lower in the high p-AKT group than in the low p-AKT group (20.0% vs. 71.1%, p=0.004). The high p-AKT group showed substantially worse overall survival (OS) (median OS=2.3 vs. 25.2 months, p<0.001) and progression-free survival (PFS) (median PFS=1.6 vs. 8.8 months, p<0.001) compared with the low p-AKT group. Multivariate analysis showed that high p-AKT expression remained a significant independent poor prognostic factor for OS (hazard ratio (HR)=7.0; 95% confidence interval (CI)=3.0-16.6; p<0.001) and PFS (HR=6.8; 95% CI=3.0-15.2; p<0.001). Conclusion: PTCL patients with high p-AKT expression showed aggressive clinical courses with significantly worse OS and PFS and a poor chemotherapy response rate. We suggest that targeting the PI3K/AKT pathway may be a promising therapeutic strategy for PTCL.*

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Peripheral T-cell lymphoma (PTCL) is a heterogeneous group of malignancies derived from mature post-thymic T-cells and natural killer (NK) cells. The most common subtypes include PTCL not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), and anaplastic large cell lymphoma (ALCL). A rarer subtype is extranodal NK T-cell lymphoma (NKTCL). Generally, the prognosis for PTCL is poor with conventional cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) chemotherapy, with a 5-year overall survival (OS) of 10-30% (1, 2).

Molecular targeted therapies have achieved great success in treating B-cell lymphoma; such therapies include targeting B-cell surface antigen with rituximab (anti-CD20 monoclonal antibody) (3) and targeting B-cell receptor (BCR) signaling pathway with idelalisib (a selective inhibitor

Table I. *Baseline characteristics of patients.*

| | All patients (n=63) | | Low AKT (n=51) | | High AKT (n=12) | | *p*-Value |
|---|---|---|---|---|---|---|---|
| Median age (range), years | 53 | (18-84) | 54 | (18-83) | 42 | (30-84) | 0.55 |
| Age, no. (%) | | | | | | | |
| 60 years or less | 39 | (61.9) | 30 | (58.8) | 9 | (75.0) | 0.35 |
| Older than 60 years | 24 | (38.1) | 21 | (41.2) | 3 | (25.0) | |
| Sex, no. (%) | | | | | | | |
| Male | 40 | (63.5) | 32 | (62.7) | 8 | (66.7) | 1.00 |
| Female | 23 | (36.5) | 19 | (37.3) | 4 | (33.3) | |
| Pathology, no. (%) | | | | | | | |
| PTCL-NOS | 16 | (25.4) | 13 | (25.5) | 3 | (25.0) | 0.02 |
| AITL | 19 | (30.2) | 19 | (37.3) | 0 | (0.0) | |
| ALCL | 11 | (17.5) | 7 | (13.7) | 4 | (33.3) | |
| NKTCL | 17 | (27.0) | 12 | (23.5) | 5 | (41.7) | |
| Performance status, no. (%) | | | | | | | |
| ECOG 0-1 | 38 | (61.3) | 32 | (64.0) | 6 | (50.0) | 0.51 |
| ECOG 2-4 | 24 | (38.7) | 18 | (36.0) | 6 | (50.0) | |
| Ann Arbor stage, no. (%) | | | | | | | |
| Limited, I-II | 25 | (41.0) | 20 | (40.8) | 5 | (41.7) | 1.00 |
| Advanced, III-IV | 36 | (59.0) | 19 | (59.2) | 7 | (58.3) | |
| No. of ENI, no. (%) | | | | | | | |
| 0 or 1 | 34 | (56.7) | 26 | (54.2) | 8 | (66.7) | 0.53 |
| 2 or more | 26 | (43.3) | 22 | (45.8) | 4 | (33.3) | |
| LDH no. (%) | | | | | | | |
| ULN or below | 23 | (39.7) | 21 | (45.7) | 2 | (16.7) | 0.10 |
| Over ULN | 35 | (60.3) | 25 | (54.3) | 10 | (83.3) | |
| IPI risk group, no. (%) | | | | | | | |
| Low/low intermediate | 28 | (46.7) | 24 | (50.0) | 4 | (33.3) | 0.35 |
| high intermediate/High | 32 | (53.3) | 24 | (50.0) | 8 | (66.7) | |
| PIT risk group, no. (%) | | | | | | | |
| 1-2 | 31 | (51.7) | 26 | (54.2) | 5 | (41.7) | 0.53 |
| 3-4 | 29 | (48.3) | 22 | (45.8) | 7 | (58.3) | |
| B symptom, no. (%) | | | | | | | |
| Negative | 40 | (65.6) | 34 | (69.4) | 6 | (50.0) | 0.31 |
| Positive | 21 | (34.4) | 15 | (30.6) | 6 | (50.0) | |
| Bone marrow involvement, no. (%) | | | | | | | |
| Negative | 44 | (77.2) | 35 | (77.8) | 9 | (75.0) | 1.00 |
| Positive | 13 | (22.8) | 10 | (22.2) | 3 | (25.0) | |
| Primary treatment, no. (%) | | | | | | | |
| CHOP-like regimen | 55 | (87.3) | 46 | (90.2) | 9 | (75.0) | 0.17 |
| Others | 4 | (6.3) | 2 | (3.9) | 2 | (16.7) | |
| No Tx | 4 | (6.3) | 3 | (5.9) | 1 | (8.3) | |
| Response to front-lineTx, no. (%) | | | | | | | |
| CR or PR | 34 | (61.8) | 32 | (71.1) | 2 | (20.0) | 0.004 |
| SD or PD | 21 | (38.2) | 13 | (28.9) | 8 | (80.0) | |

Data were missing as follows: Performance status (n=1), Ann Arbor stage (n=2), No of extranodal involvement (n=3), Lactic dehydrogenase (n=5), IPI risk groups (n=3), PIT risk groups (n=3), B-symptoms (n=2), BM involvement (n=6), and response to frontline T× (n=8). PTCL-NOS, Peripheral T-cell lymphoma not otherwise specified; AITL, angioimmunoblastic T-cell lymphoma; ALCL anaplastic large cell lymphoma; NKTCL, extranodal natural killer T-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; ENI, extranodal involvement; LDH, lactic dehydrogenase; ULN, upper limit of normal; IPI, International Prognostic Index; PIT, Prognostic Index for T-cell lymphoma; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; Tx, treatment; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

of the delta isoform of phosphatidylinositol-3-kinase (PI3K)) (4, 5) or ibrutinib (an inhibitor of Bruton tyrosine kinase) (6, 7). By contrast, there have been no remarkable advances in molecular targeted therapy for PTCL because of its rarity and heterogeneity of subtypes (8, 9). The notable exception is CD30-positive ALCL treated with brentuximab vedotin in which patients achieved an 86% objective response rate with a durable response (10). Nevertheless, there remains a great need to explore the molecular biology of, and to develop new targeted therapies for, PTCL.

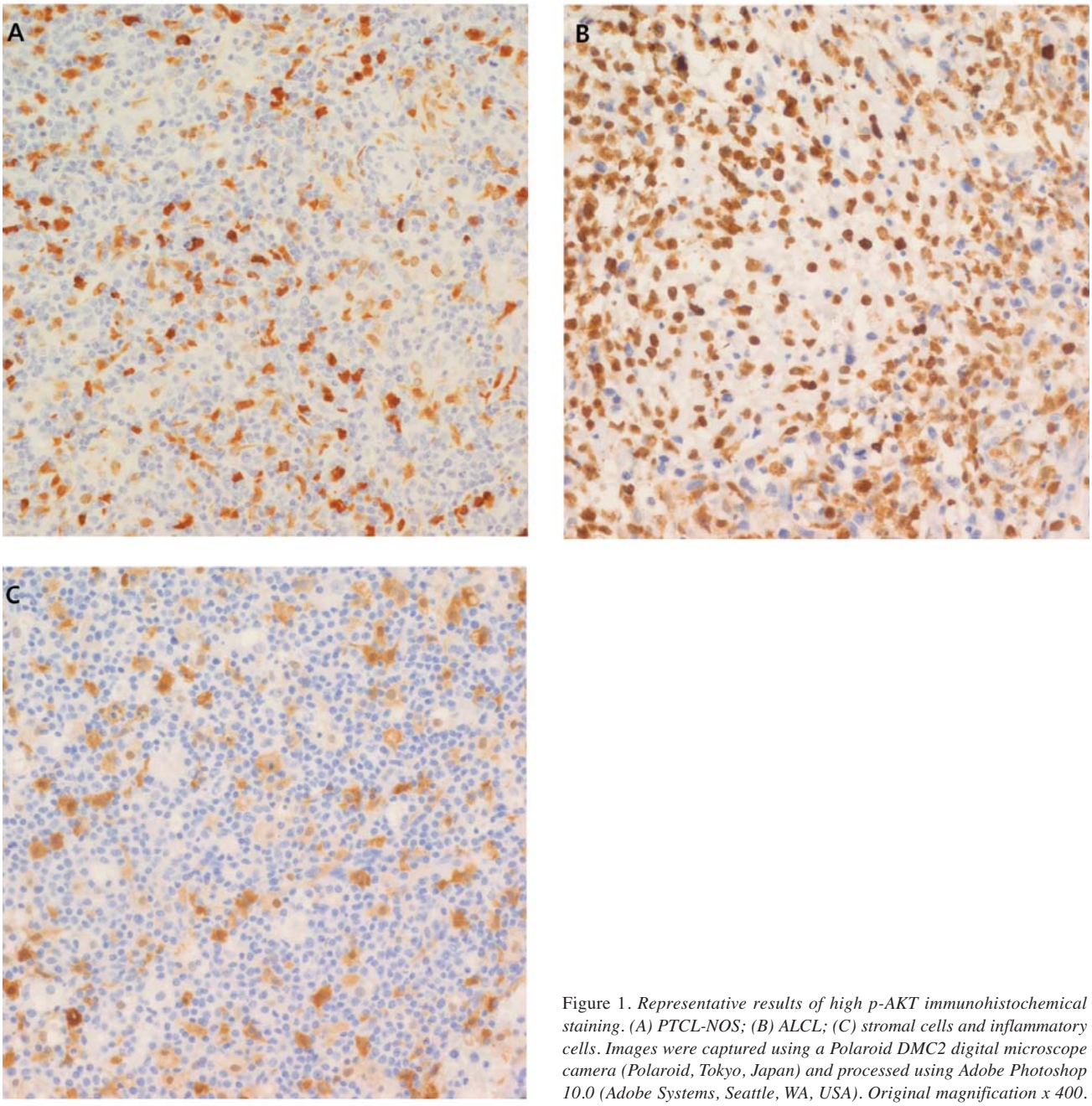


Figure 1. *Representative results of high p-AKT immunohistochemical staining. (A) PTCL-NOS; (B) ALCL; (C) stromal cells and inflammatory cells. Images were captured using a Polaroid DMC2 digital microscope camera (Polaroid, Tokyo, Japan) and processed using Adobe Photoshop 10.0 (Adobe Systems, Seattle, WA, USA). Original magnification x 400.*

The oncogenic PI3K/serine-threonine kinase (PI3K/AKT) pathway is a downstream pathway of the BCR signaling pathway and plays a crucial role in the pathogenesis of cancers (11-13). Activated phosphorylated AKT (p-AKT) has been reported to be abnormally overexpressed and to be an indicator of poor prognosis in patients with diverse solid cancers or B-cell lymphoma (11-16). We note, however, that there are preclinical data showing PI3K/AKT pathway activation in T-cell lymphoma, in different manners from those in B-cell lymphoma (17-22). However, the prognostic role and clinical significance of p-AKT expression in PTCL are not well defined.

To define the clinical significance of PI3K/AKT pathway activation in PTCL, we investigated p-AKT expression in 63 patients with PTCL for whom biopsy specimens were available for immunohistochemical analysis of p-AKT.

Table II. *Prognostic factors for overall survival by log-rank test in the Kaplan-Meier method.*

| | Overall survival | | |
|---|---|---|---|
| Parameters | Median (95% CI), months | | *p*-Value |
| p-AKT status | | | <0.001 |
| Low p-AKT | 25.2 | (10.7-39.7) | |
| High p-AKT | 2.3 | (1.0-3.6) | |
| Age | | | 0.096 |
| ≤60 years | 14.6 | (2.9-26.3) | |
| >60 years | 11.4 | (0.2-22.6) | |
| Sex | | | 0.624 |
| Female | 18.3 | (10.2-26.5) | |
| Male | 10.6 | (3.8-17.4) | |
| ECOG PS | | | 0.245 |
| ECOG 0-1 | 16.6 | (0.0-34.5) | |
| ECOG 2-4 | 6.0 | (0.0-12.4) | |
| No. of ENI | | | 0.010 |
| 0 or 1 | 44.7 | (22.2-67.2) | |
| 2 or more | 6.0 | (0.7-11.2) | |
| Ann Arbor stage | | | 0.138 |
| Limited, I-II | 29.7 | (0.0-59.7) | |
| Advanced, III-IV | 8.6 | (3.5-13.7) | |
| LDH | | | 0.078 |
| ULN or below | 25.2 | (8.2-42.2) | |
| Over ULN | 7.0 | (1.5-12.6) | |
| IPI risk group | | | 0.003 |
| Low/low intermediate | 30.9 | (0.0-72.2) | |
| High intermediate/high | 5.1 | (0.7-9.5) | |
| PIT risk group | | | |
| 1-2 | 29.7 | (4.8-54.6) | |
| 3-4 | 3.9 | (0.0-10.7) | 0.006 |
| B Symptoms | | | 0.460 |
| Negative | 14.6 | (6.0-23.2) | |
| Positive | 11.4 | (0.0-36.5) | |
| Bone marrow involvement | | | 0.013 |
| Negative | 25.2 | (3.3-47.1) | |
| Positive | 10.5 | (0.0-24.6) | |
| Pathology | | | 0.250 |
| PTCL-NOS | 11.4 | (0.0-26.5) | |
| AITL | 35.5 | (7.4-63.6) | |
| ALCL | 7.0 | (0.0-19.6) | |
| NKTCL | 4.7 | (1.7-7.6) | |
| The use of CHOP- like regimen | | | <0.001 |
| Yes | 16.6 | (1.3-31.8) | |
| No | 0.8 | (0.0-2.2) | |
| Response to front-lineTx | | | <0.001 |
| CR or PR | 44.7 | (8.9-80.5) | |
| SD or PD | 3.7 | (1.4-6.0) | |

ECOG PS, Eastern Cooperative Oncology Group performance status; ENI, extranodal involvement; LDH, lactic dehydrogenase; ULN, upper limit of normal; IPI, International Prognostic Index; PIT, Prognostic Index for T-cell lymphoma; PTCL-NOS, peripheral T-cell lymphoma- not otherwise specified; AITL, angioimmunoblastic T-cell lymphoma; ALCL anaplastic large cell lymphoma; NKTCL, extranodal natural killer T-cell lymphoma; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; Tx, treatment; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Table III. *Multivariate Cox regression analysis for survival.*

| | PFS | | | OS | | |
|---|---|---|---|---|---|---|
| | HR | (95% CI) | *p*-Value | HR | (95% CI) | *p*-Value |
| p-AKT status | | | <0.001 | | | <0.001 |
| Low p-AKT | 1.0 | | | 1.0 | | |
| High p-AKT | 6.8 | (3.0-15.2) | | 7.0 | (3.0-16.6) | |
| Age | | | 0.009 | | | 0.002 |
| ≤60 years | 1.0 | | | 1.0 | | |
| >60 years | 2.4 | (1.2-4.5) | | 3.1 | (1.5-6.5) | |
| Sex | | | 0.480 | | | 0.443 |
| Female | 1.0 | | | 1.0 | | |
| Male | 1.3 | (0.6-2.5) | | 1.3 | (0.7-2.7) | |
| ECOG PS | | | 0.441 | | | 0.114 |
| 0-1 | 1.0 | | | 1.0 | | |
| 2-4 | 1.3 | (0.7-2.5) | | 1.7 | (0.9-3.3) | |
| No. of ENI | | | 0.044 | | | 0.003 |
| 0 or 1 | 1.0 | | | 1.0 | | |
| 2 or more | 2.1 | (1.0-4.2) | | 3.2 | (1.5-6.9) | |
| BM involvement | | | 0.034 | | | 0.932 |
| Negative | 1.0 | | | 1.0 | | |
| Positive | 2.4 | (1.1-5.3) | | 0.9 | (0.4-2.2) | |

HR, Hazard ratio; CI, confidence interval; NA, not available; ECOG PS, Eastern Cooperative Oncology Group performance status; ENI, extranodal involvement; BM, bone marrow.

## Patients and Methods

*Patients.* We retrospectively identified the patients who met the following criteria: (i) pathologically confirmed PTCLs, including 4 subtypes of PTCL-NOS, AITL, ALCL and NKTCL, according to the World Health Organization (WHO) classification; (ii) adequate amount and quality of paraffin-embedded biopsy specimens or unstained slides for immunostaining of p-AKT. Patients with primary central nervous system lymphoma were excluded. Clinical information was obtained by reviewing the medical records of each patient. The Institutional Review Board of the Samsung Medical Center approved this study and the protocol protected the confidentiality of all patients. This study is registered under the ClinicalTrials.gov, number NCT01789060.

*Pathology and immunostaining for p-AKT.* All pathology specimens were reviewed by an experienced hematopathologist (YHK) and classified according to the WHO classification. For p-AKT immunohistochemical staining, antigen retrieval was performed in ER1 buffer (pH 6.0) for 20 min and endogenous peroxidase was blocked for 5 min. After this, the slides were incubated for 15 min with a 1:100 dilution of antibody (no. 9275; Cell Signaling Technology, Beverly, MA, USA) specific for phosphorylated AKT (Thr308) using a BOND-MAX autoimmunostainer (Leica Biosystems, Melbourne, Australia). In every staining set, negative controls were included by omission of primary antibodies and probes. Representative results of p-AKT immunostaining are demonstrated in Figure 1.

*Tissue microarray methods.* p-AKT expression was semiquantitatively assessed based on the intensity of staining and the proportion of p-AKT-stained cells. The intensity of p-AKT

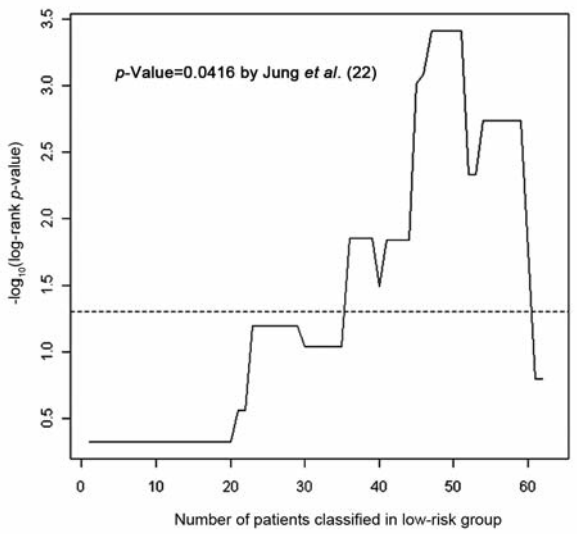


Figure 2. *Graph denotes -log10(log-rank p-value) for all possible cutoff values. The dotted horizontal line represents alpha=0.05. AU was shown to be significantly associated with overall survival by the method of Jung et al. survival.*

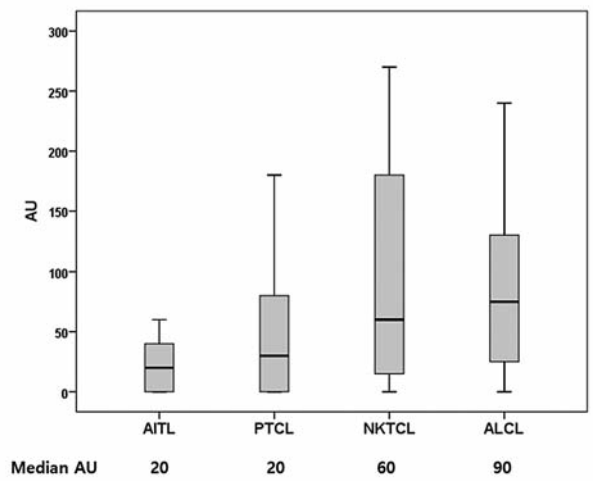


Figure 3. *The boxplots of AU values according to pathologic subtypes. AITL: Angioimmunoblastic T-cell lymphoma; PTCL: peripheral T-cell lymphoma; NKTCL: extranodal natural killer T-cell lymphoma; ALCL: anaplastic large cell lymphoma.*

staining was scored as one of four grades: 0, 1, 2 and 3 depending on whether the dominant staining was negative, weak, moderate or strong. The proportion of p-AKT-positive cells was scored from 0% to 100% as the percentage of total cells that were positively stained, to the nearest 10%. Only p-AKT expression in neoplastic cells was counted, whereas p-AKT expression in background stromal cells and inflammatory cells was not counted. The final p-AKT expression score was expressed as arbitrary units (AUs), calculated by multiplying the intensity score by the proportion score, and ranged from 0 to 300. This scoring system has been successfully applied to evaluate expression of immunohistochemical staining in previous studies, including our series of diffuse large B-cell lymphoma (16, 23). The quantification of p-AKT expression was performed over a short period of time without knowledge of the patient's clinical data.

*Statistical analysis*. Descriptive statistics are reported as proportions and medians. Intergroup comparisons were performed with Fisher's exact test for categorical variables and the Mann-Whitney test for age. Progression-free survival (PFS) was calculated from the date of diagnosis to the first day of disease progression, relapse or death from any cause. Overall survival (OS) was calculated from the date of diagnosis to death. PFS and OS were censored on the last date of follow-up.

Using an appropriate AU value as a cutoff, we partitioned the patients into a high p-AKT group (patients with AUs larger than the chosen cutoff) and a low p-AKT group (patients with AUs smaller than or equal to the chosen cutoff). The association of AUs with OS was tested using the method of Jung *et al*. (24). Survival curves were estimated by the Kaplan-Meier method and the survival distributions between high and low p-AKT groups were compared using the log-rank test. Univariate and multivariate analyses were carried out using the Cox's regression analysis. *p*-Values less than .05 were considered statistically significant and two-sided tests were used in all calculations. Statistical analyses were performed using the statistical software package IBM PASW, version 18.0 (SPSS Inc., Chicago, IL, USA).

## Results

*Patients' characteristics*. A total of 63 patients who were diagnosed with PTCL (PTCL-NOS, AITL, ALCL or NKTCL) between January 1995 and April 2005 for whom biopsy specimens were available for immunohistochemical analysis of p-AKT were included in this study. The baseline characteristics of the patients are summarized in Table I. The median age was 53 years (range=18-84 years) and the male-to-female ratio was 1.7:1.0.

The median p-AKT expression was 30 AUs (range=0-270 AUs) and the expression level was significantly associated with OS when analyzed using the method of Jung *et al*. ($p$=0.0416) (Figure 2) (24). Thus, the upper limit of the third quartile (Q3) of the AU value was 120, which was chosen as the cutoff value in this study. Twelve patients with a p-AKT expression value greater than the cutoff value of 120 AU were classified into the high p-AKT group and the other 51 patients were classified into the low p-AKT group. A higher percentage of NKTCL (41.7%) and ALCL (33.3%) subtypes were included in the high p-AKT group compared with the PTCL-NOS (25.0%) and AITL (0.0%) subtypes. The boxplots of AU values according to pathological subtypes are shown in Figure 3; these show a trend towards higher expression levels in NKTCL and ALCL subtypes

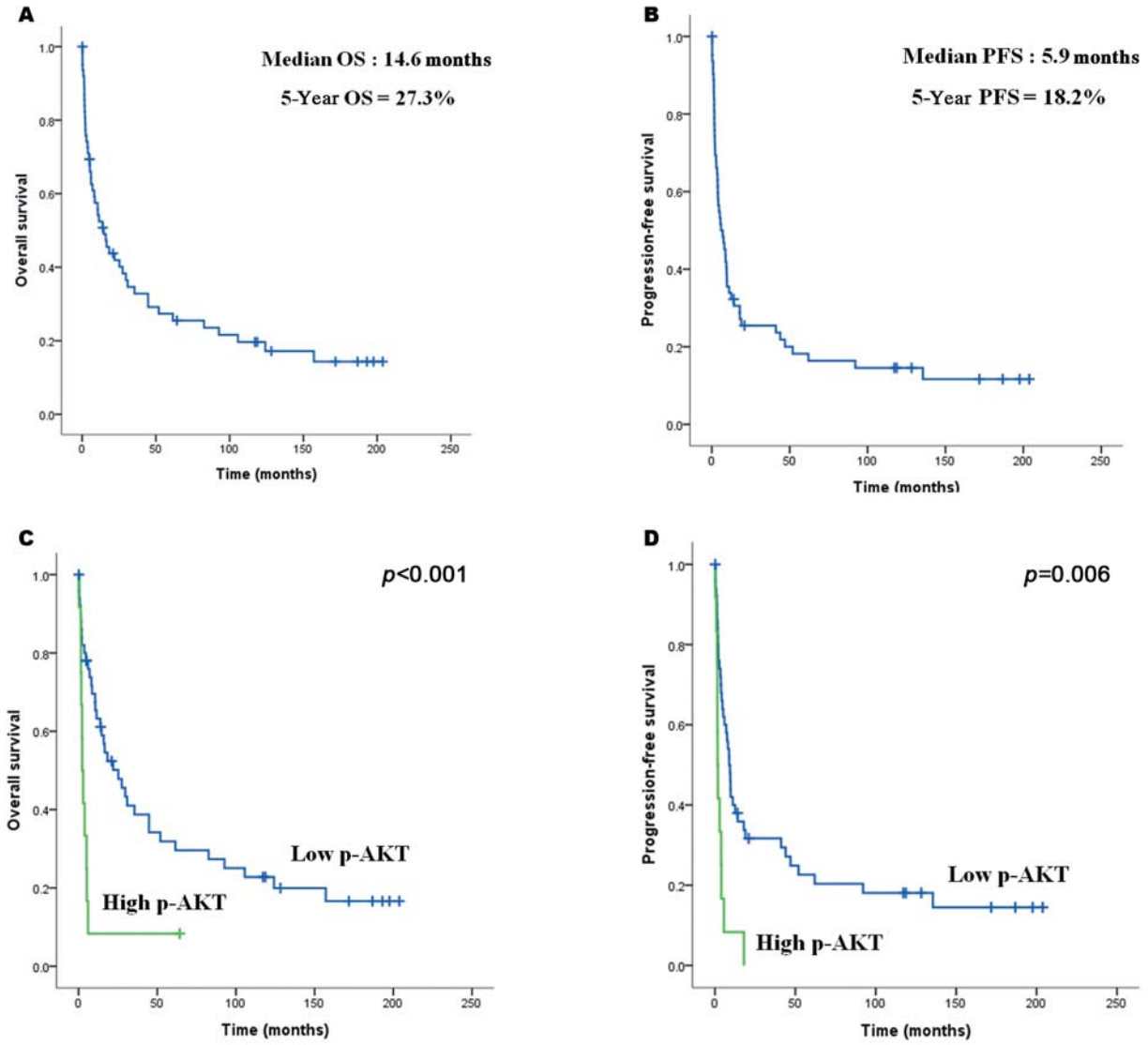


Figure 4. *Overall (OS) and progression-free (PFS) survival of the patients according to the p-AKT expression status.*

compared with AITL and PTCL-NOS subtypes. The overall response rate to frontline treatment was significantly lower in the high p-AKT group than in the low p-AKT group (20.0% *vs*. 71.1%, $p$=0.004).

*Prognostic value of p-AKT expression for survival*. After a median follow-up of 169.6 months (range=98.4-221.8 months), the median OS was 14.6 months 95% confidence interval (CI)=6.4-22.8 months and the 5-year OS rate was 27.3% (95% CI=21.4-33.2%) (Figure 4A). The 5-year OS rates according to pathological subtypes were 25.0%, 39.4%, 34.1% and 12.5% for the PTCL-NOS, AITL, ALCL and NKTCL subgroups, respectively.

Table II shows the results of survival analysis for OS. The high p-AKT group had substantially poorer OS compared with the low p-AKT group (median OS=2.3 months *vs*. 25.2 months, respectively; $p$<0.001) (Table II, Figure 4C). There were also significant differences in OS according to extranodal involvement (0 or 1 node *vs*. 2 or more), International Prognostic Index (IPI) risk group (low/low intermediate *vs*. high intermediate/high), Prognostic Index for PTCL-U (PIT) risk group (1 or 2 *vs*. 3 or 4) and bone marrow involvement (negative *vs*. positive) (Table II).

We also performed a subgroup analysis based on pathological subtypes. Even though there were small numbers of patients in the subgroups, analysis of the ALCL subtype

showed that high p-AKT expression was significantly associated with poorer OS compared with the low p-AKT expression group (median OS=1.7 *vs*. 105.6 months, respectively; $p$=0.012) (Figure 5A). Subgroup analysis of the NKTCL subtype also showed a trend for poorer OS in the high p-AKT group compared with the low p-AKT group (median OS=2.3 *vs*. 8.1 months, respectively; $p$=0.104) (Figure 5C).

*Prognostic factor analyses*. The clinical factors associated with worse OS in the univariate analysis were high p-AKT expression ($p$=0.001), PIT risk group of 3 or 4 ($p$=0.007), IPI risk group of high intermediate/high ($p$=0.004) and 2 sites of extranodal involvement ($p$=0.011). Multivariate analysis showed that high p-AKT expression remained as a significant independent poor prognostic factor for OS hazard ratio (HR)=7.0; 95% CI=3.0-16.6; $p$<0.001) and PFS (HR=6.8; 95% CI=3.0-15.2; $p$<0.001). Other independent prognostic factors for poor OS were age >60 years (HR=3.1; 95% CI=1.5-6.5; $p$=0.002) and 2 or more sites of extranodal involvement (HR=3.2; 95% CI=1.5-6.9; $p$=0.003) (Table III).

## Discussion

This study revealed that a substantial percentage of patients with PTCL had p-AKT overexpression and patients with high p-AKT expression had significantly worse OS and PFS, as well as a poor chemotherapy response rate. These results strongly suggest that targeting the PI3K/AKT pathway may be a novel approach to treating PTCL.

Among the notable advances in molecular targeted therapy for B-cell lymphoma, the most recent advance targets the BCR signaling pathway in B-cell lymphoma. Several reports have shown that the PI3K/AKT pathway plays a key role in the pathogenesis of diverse B-cell lymphomas and is associated with poor prognosis (15, 16, 25-27). It is noteworthy that subsequent trials have shown that idelalisib has impressive efficacy and an acceptable toxicity profile in treating diverse B-cell hematological malignancies, including chronic lymphocytic leukemia (CLL) (4, 28), indolent B-cell lymphoma (5, 29), and mantle cell lymphoma (MCL) (30). Ibrutinib also showed impressive clinical data in treating CLL and MCL (7, 31). However, there are limited data about the activation of the PI3K/AKT pathway in PTCL. Cai *et al*. reported recently that p-AKT overexpression may be an independent prognostic factor for poor OS in Chinese patients with PTCL, even though the optimum cutoff value and method to quantify immunohistochemical p-AKT expression have not been validated (32). In this study, using the AUs from immunohistochemical staining, we also confirmed that p-AKT overexpression was an indicator of poor prognosis in patients with PTCL.

Previous studies have described the PI3K/AKT pathway activation in T-cell lymphoma and implied the clinical significance of the PI3K/AKT pathway activation beyond BCR signaling pathway. In ALCL, preclinical studies have shown that the PI3K/AKT pathway, which is activated by nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) fusion oncoprotein, plays a critical role in the molecular pathogenesis of ALCL (17, 20, 21, 33). Moreover, preclinical models in NKTCL also have shown that AKT signaling plays a role in tumor cell survival and the molecular pathogenesis of NKTCL (18, 19, 22). It is noteworthy that analysis of the ALCL subgroup showed that high p-AKT expression compared with low p-AKT expression remained a significant indicator of poor prognosis despite the small numbers of patients (median OS=1.7 *vs*. 105.6 months; $p$=0.012) (Figure 5A). Analysis of the NKTCL subgroup also showed a trend toward poor prognosis in patients with high p-AKT expression compared with low p-AKT expression (median OS=2.3 *vs*. 8.1 months; $p$=0.104) (Figure 5B). We believe that these findings support the preclinical data of the PI3K/AKT pathway activation in T-cell lymphoma and suggest that targeting the PI3K/AKT pathway may be a new therapeutic approach, especially for ALCL and NKTCL. However, our findings are based on small sample sizes and these findings must be confirmed in a larger cohort with various subtypes of PTCL.

Our unpublished data demonstrated that PTCL is generally associated with a higher incidence of p-AKT positivity than B-cell lymphoma (Figure 6). The percentages of patients with 10% or more cells positive for p-AKT at any intensity in the PTCL group [ALCL (10/11, 91%), NKTCL (13/17, 76%), PTCL-NOS (10/16, 63%) and AITL (10/19, 53%)] were higher than those of patients with B-cell lymphoma (150/246, 61%), marginal zone B-cell lymphoma (5/16, 31%), Burkitt's lymphoma (3/14, 21%) or mantle cell lymphoma (0/10, 0%). The exact mechanisms responsible for the more prevalent p-AKT overexpression in PTCL compared with B-cell lymphoma are not clear. However, NPM-ALK-associated PI3K/AKT pathway activation in ALCL or Epstein-Barr virus-associated PI3K/AKT activation in NKTCL may be involved (22, 34).

In terms of preclinical and early clinical data for the inhibition of the PI3K/AKT pathway in patients with PTCL, Martin-Sanchez *et al*. reported promising preclinical data showing that cell arrest and apoptosis were observed in PTCL cell lines treated with PI3K inhibitors, especially when PI3K inhibition was combined with mitogen-activated protein kinase kinase (MEK) inhibition (35). Our group also reported the results of a phase I trial showing that a mammalian target of rapamycin (mTOR) inhibitor (everolimus), which blocks downstream signal transduction of the PI3K/AKT pathway, was safe and active in PTCL patients when combined with CHOP chemotherapy (36). The *in vitro* evidence showing PI3K/AKT pathway activation in

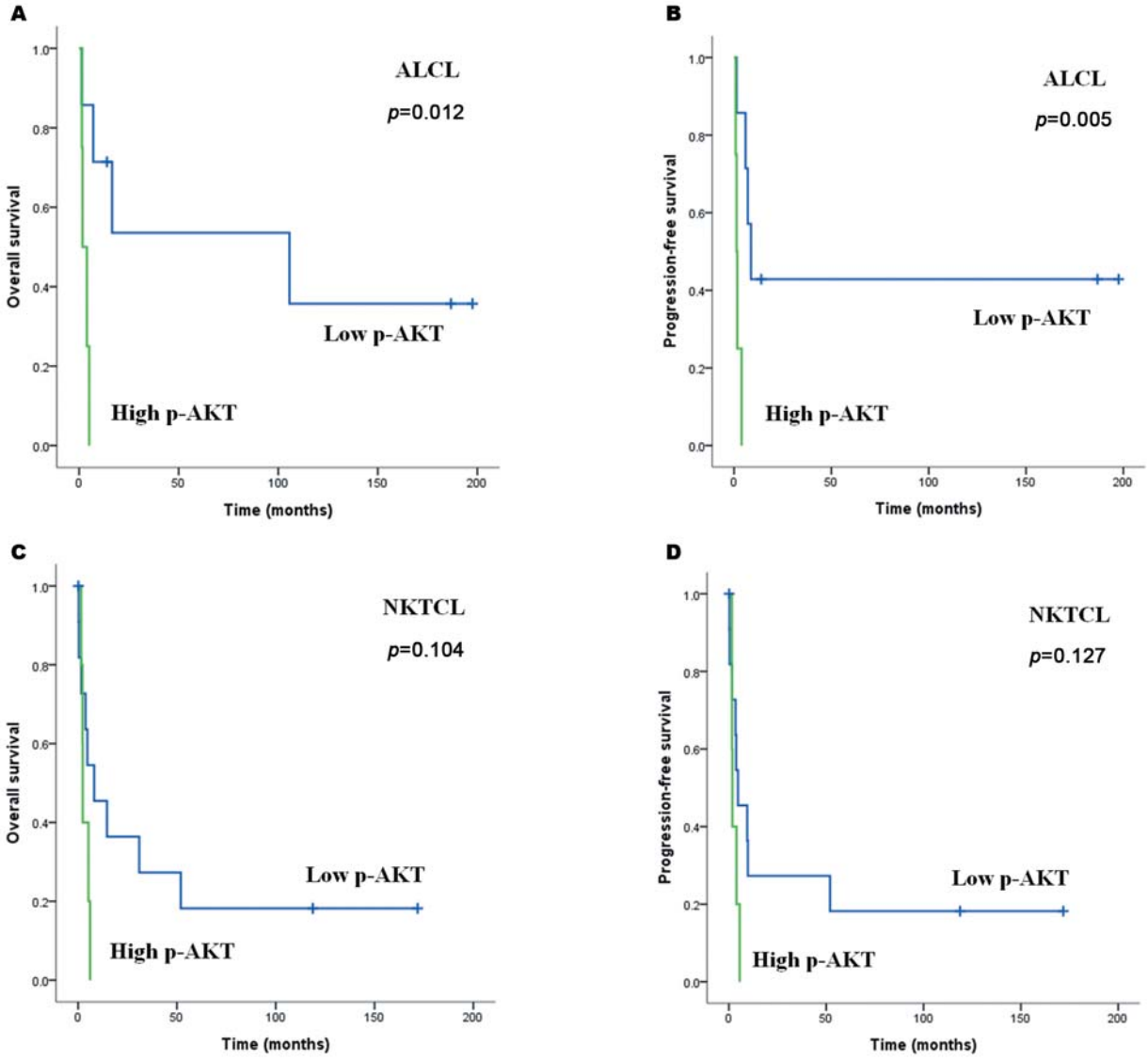


Figure 5. *The impact of p-AKT expression status on overall (A, C) and progression-free (B, D) survival in anaplastic large cell lymphoma (ALCL) (A, B) and extranodal natural killer T-cell lymphoma (NKTCL) (C, D) subtypes.*

ALCL and NKTCL, the preclinical and early clinical data for the effectiveness of targeting PI3K/AKT pathway in PTCL, as well as more prevalent p-AKT expression associated with PTCL compared with B-cell lymphoma lead us to suggest that targeting the PI3K/AKT pathway in PTCL may be a promising therapeutic strategy in a different manner from targeting the BCR signaling pathway in B-cell lymphoma.

In conclusion, survival was significantly shorter in patients with PTCL with high p-AKT expression compared with patients with low p-AKT expression. More effective treatment approaches are needed for this subset of patients with PTCL. We suggest that inhibition of the PI3K/AKT pathway by using PI3K inhibitors, AKT inhibitors and/or dual PI3K-mTOR inhibitors may be a promising therapeutic strategy for PTCL.

## Conflicts of interest

The Authors declare no competing financial interests.

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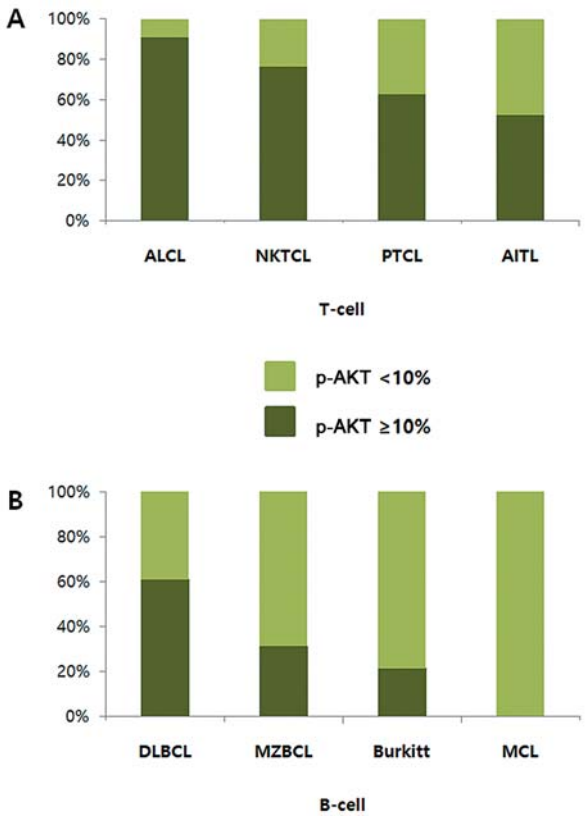


Figure 6. *Percentage of patients 10% or more p-AKT expression according to pathologic subtypes: (A) T-cell lymphomas; (B) B-cell lymphomas.*

## References


1 Abouyabis AN, Shenoy PJ, Sinha R, Flowers CR and Lechowicz MJ: A systematic review and meta-analysis of front-line anthracycline-based chemotherapy regimens for peripheral T-cell lymphoma. ISRN Hematol *2011*: 623924, 2011.

2 Vose J, Armitage J, Weisenburger D and International TCLP: International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. J Clin Oncol *26*: 4124-4130, 2008.

3 Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, Morel P, Van Den Neste E, Salles G, Gaulard P, Reyes F, Lederlin P and Gisselbrecht C: CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med *346*: 235-242, 2002.

4 Furman RR, Sharman JP, Coutre SE, Cheson BD, Pagel JM, Hillmen P, Barrientos JC, Zelenetz AD, Kipps TJ, Flinn I, Ghia P, Eradat H, Ervin T, Lamanna N, Coiffier B, Pettitt AR, Ma S, Stilgenbauer S, Cramer P, Aiello M, Johnson DM, Miller LL, Li D, Jahn TM, Dansey RD, Hallek M and O'Brien SM: Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. N Engl J Med *370*: 997-1007, 2014.

5 Gopal AK, Kahl BS, de Vos S, Wagner-Johnston ND, Schuster SJ, Jurczak WJ, Flinn IW, Flowers CR, Martin P, Viardot A, Blum KA, Goy AH, Davies AJ, Zinzani PL, Dreyling M, Johnson D, Miller LL, Holes L, Li D, Dansey RD, Godfrey WR and Salles GA: PI3Kdelta inhibition by idelalisib in patients with relapsed indolent lymphoma. N Engl J Med *370*: 1008-1018, 2014.

6 O'Brien S, Furman RR, Coutre SE, Sharman JP, Burger JA, Blum KA, Grant B, Richards DA, Coleman M, Wierda WG, Jones JA, Zhao W, Heerema NA, Johnson AJ, Izumi R, Hamdy A, Chang BY, Graef T, Clow F, Buggy JJ, James DF and Byrd JC: Ibrutinib as initial therapy for elderly patients with chronic lymphocytic leukaemia or small lymphocytic lymphoma: an open-label, multicentre, phase 1b/2 trial. Lancet Oncol *15*: 48-58, 2014.

7 Wang ML, Rule S, Martin P, Goy A, Auer R, Kahl BS, Jurczak W, Advani RH, Romaguera JE, Williams ME, Barrientos JC, Chmielowska E, Radford J, Stilgenbauer S, Dreyling M, Jedrzejczak WW, Johnson P, Spurgeon SE, Li L, Zhang L, Newberry K, Ou Z, Cheng N, Fang B, McGreivy J, Clow F, Buggy JJ, Chang BY, Beaupre DM, Kunkel LA and Blum KA: Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. N Engl J Med *369*: 507-516, 2013.

8 Feugier P, Van Hoof A, Sebban C, Solal-Celigny P, Bouabdallah R, Ferme C, Christian B, Lepage E, Tilly H, Morschhauser F, Gaulard P, Salles G, Bosly A, Gisselbrecht C, Reyes F and Coiffier B: Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: a study by the Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol *23*: 4117-4126, 2005.

9 Pfreundschuh M, Kuhnt E, Trumper L, Osterborg A, Trneny M, Shepherd L, Gill DS, Walewski J, Pettengell R, Jaeger U, Zinzani PL, Shpilberg O, Kvaloy S, de Nully Brown P, Stahel R, Milpied N, Lopez-Guillermo A, Poeschel V, Grass S, Loeffler M, Murawski N and MabThera International Trial G: CHOP-like chemotherapy with or without rituximab in young patients with good-prognosis diffuse large-B-cell lymphoma: 6-year results of an open-label randomised study of the MabThera International Trial (MInT) Group. Lancet Oncol *12*: 1013-1022, 2011.

10 Pro B, Advani R, Brice P, Bartlett NL, Rosenblatt JD, Illidge T, Matous J, Ramchandren R, Fanale M, Connors JM, Yang Y, Sievers EL, Kennedy DA and Shustov A: Brentuximab vedotin (SGN-35) in patients with relapsed or refractory systemic anaplastic large-cell lymphoma: results of a phase II study. J Clin Oncol *30*: 2190-2196, 2012.

11 Davies MA: Regulation, role, and targeting of Akt in cancer. J Clin Oncol *29*: 4715-4717, 2011.

12 Courtney KD, Corcoran RB and Engelman JA: The PI3K pathway as drug target in human cancer. J Clin Oncol *28*: 1075-1083, 2010.

13 Wendel HG, De Stanchina E, Fridman JS, Malina A, Ray S, Kogan S, Cordon-Cardo C, Pelletier J and Lowe SW: Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. Nature *428*: 332-337, 2004.

14 Uddin S, Hussain AR, Siraj AK, Manogaran PS, Al-Jomah NA, Moorji A, Atizado V, Al-Dayel F, Belgaumi A, El-Solh H, Ezzat A, Bavi P and Al-Kuraya KS: Role of phosphatidylinositol 3'-kinase/AKT pathway in diffuse large B-cell lymphoma survival. Blood *108*: 4178-4186, 2006.

15 Hasselblom S, Hansson U, Olsson M, Toren L, Bergstrom A, Nilsson-Ehle H and Andersson PO: High immunohistochemical expression of p-AKT predicts inferior survival in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. Br J Haematol *149*: 560-568, 2010.

16 Hong JY, Hong ME, Choi MK, Kim YS, Chang W, Maeng CH, Park S, Lee SJ, Do IG, Jo JS, Jung SH, Kim SJ, Ko YH and Kim WS: The impact of activated p-AKT expression on clinical outcomes in diffuse large B-cell lymphoma: a clinicopathological study of 262 cases. Ann Oncol *25*: 182-188, 2014.

17 Bai RY, Ouyang T, Miething C, Morris SW, Peschel C and Duyster J: Nucleophosmin-anaplastic lymphoma kinase associated with anaplastic large-cell lymphoma activates the phosphatidylinositol 3-kinase/Akt antiapoptotic signaling pathway. Blood *96*: 4319-4327, 2000.

18 Chow C, Liu AY, Chan WS, Lei KI, Chan WY and Lo AW: AKT plays a role in the survival of the tumor cells of extranodal NK/T-cell lymphoma, nasal type. Haematologica *90*: 274-275, 2005.

19 Huang Y, de Reynies A, de Leval L, Ghazi B, Martin-Garcia N, Travert M, Bosq J, Briere J, Petit B, Thomas E, Coppo P, Marafioti T, Emile JF, Delfau-Larue MH, Schmitt C and Gaulard P: Gene expression profiling identifies emerging oncogenic pathways operating in extranodal NK/T-cell lymphoma, nasal type. Blood *115*: 1226-1237, 2010.

20 Rassidakis GZ, Feretzaki M, Atwell C, Grammatikakis I, Lin Q, Lai R, Claret FX, Medeiros LJ and Amin HM: Inhibition of Akt increases p27-Kip1 levels and induces cell cycle arrest in anaplastic large cell lymphoma. Blood *105*: 827-829, 2005.

21 Slupianek A, Nieborowska-Skorska M, Hoser G, Morrione A, Majewski M, Xue L, Morris SW, Wasik MA and Skorski T: Role of phosphatidylinositol 3-kinase-Akt pathway in nucleophosmin/anaplastic lymphoma kinase-mediated lymphomagenesis. Cancer Res *61*: 2194-2199, 2001.

22 Yamanaka Y, Tagawa H, Takahashi N, Watanabe A, Guo YM, Iwamoto K, Yamashita J, Saitoh H, Kameoka Y, Shimizu N, Ichinohasama R and Sawada K: Aberrant overexpression of microRNAs activate AKT signaling *via* down-regulation of tumor suppressors in natural killer-cell lymphoma/leukemia. Blood *114*: 3265-3275, 2009.

23 David O, Jett J, LeBeau H, Dy G, Hughes J, Friedman M and Brody AR: Phospho-Akt overexpression in non-small cell lung cancer confers significant stage-independent survival disadvantage. Clin Cancer Res *10*: 6865-6871, 2004.

24 Jung SH, Wieand S and Cha SS: A statistic for comparing two correlated markers which are prognostic for time to an event. Stat Med *14*: 2217-2225, 1995.

25 Rudelius M, Pittaluga S, Nishizuka S, Pham TH, Fend F, Jaffe ES, Quintanilla-Martinez L and Raffeld M: Constitutive activation of Akt contributes to the pathogenesis and survival of mantle cell lymphoma. Blood *108*: 1668-1676, 2006.

26 Dal Col J, Zancai P, Terrin L, Guidoboni M, Ponzoni M, Pavan A, Spina M, Bergamin S, Rizzo S, Tirelli U, De Rossi A, Doglioni C and Dolcetti R: Distinct functional significance of Akt and mTOR constitutive activation in mantle cell lymphoma. Blood *111*: 5142-5151, 2008.

27 Herishanu Y, Perez-Galan P, Liu D, Biancotto A, Pittaluga S, Vire B, Gibellini F, Njuguna N, Lee E, Stennett L, Raghavachari N, Liu P, McCoy JP, Raffeld M, Stetler-Stevenson M, Yuan C, Sherry R, Arthur DC, Maric I, White T, Marti GE, Munson P, Wilson WH and Wiestner A: The lymph node microenvironment promotes B-cell receptor signaling, NF-kappaB activation, and tumor proliferation in chronic lymphocytic leukemia. Blood *117*: 563-574, 2011.

28 Brown JR, Byrd JC, Coutre SE, Benson DM, Flinn IW, Wagner-Johnston ND, Spurgeon SE, Kahl BS, Bello C, Webb HK, Johnson DM, Peterman S, Li D, Jahn TM, Lannutti BJ, Ulrich RG, Yu AS, Miller LL and Furman RR: Idelalisib, an inhibitor of phosphatidylinositol 3 kinase p110delta, for relapsed/refractory chronic lymphocytic leukemia. Blood, *123*: 3390-7, 2014.

29 Flinn IW, Kahl BS, Leonard JP, Furman RR, Brown JR, Byrd JC, Wagner-Johnston ND, Coutre SE, Benson DM, Peterman S, Cho Y, Webb HK, Johnson DM, Yu AS, Ulrich RG, Godfrey WR, Miller LL and Spurgeon SE: Idelalisib, a selective inhibitor of phosphatidylinositol 3-kinase-delta, as therapy for previously treated indolent non-Hodgkin lymphoma. Blood, *123*: 3406-13, 2014.

30 Kahl BS, Spurgeon SE, Furman RR, Flinn IW, Coutre SE, Brown JR, Benson DM, Byrd JC, Peterman S, Cho Y, Yu A, Godfrey WR and Wagner-Johnston ND: Results of a phase I study of idelalisib, a PI3Kdelta inhibitor, in patients with relapsed or refractory mantle cell lymphoma (MCL). Blood, *123*: 3398-405, 2014.

31 Byrd JC, Furman RR, Coutre SE, Flinn IW, Burger JA, Blum KA, Grant B, Sharman JP, Coleman M, Wierda WG, Jones JA, Zhao W, Heerema NA, Johnson AJ, Sukbuntherng J, Chang BY, Clow F, Hedrick E, Buggy JJ, James DF and O'Brien S: Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med *369*: 32-42, 2013.

32 Cai QQ, Deng HX, Xie D, Lin TX and Lin TY: Phosphorylated AKT protein is overexpressed in human peripheral T-cell lymphomas and predicts decreased patient survival. Clin Lymph Myeloma Leuk *2*: 106-112, 2012.

33 Chiarle R, Voena C, Ambrogio C, Piva R and Inghirami G: The anaplastic lymphoma kinase in the pathogenesis of cancer. Nat Rev Cancer *8*: 11-23, 2008.

34 Jeon YK, Park CH, Kim KY, Li YC, Kim J, Kim YA, Paik JH, Park BK, Kim CW and Kim YN: The heat-shock protein 90 inhibitor, geldanamycin, induces apoptotic cell death in Epstein-Barr virus-positive NK/T-cell lymphoma by Akt down-regulation. J Pathol *213*: 170-179, 2007.

35 Martin-Sanchez E, Rodriguez-Pinilla SM, Sanchez-Beato M, Lombardia L, Dominguez-Gonzalez B, Romero D, Odqvist L, Garcia-Sanz P, Wozniak MB, Kurz G, Blanco-Aparicio C, Mollejo M, Alves FJ, Menarguez J, Gonzalez-Palacios F, Rodriguez-Peralto JL, Ortiz-Romero PL, Garcia JF, Bischoff JR and Piris MA: Simultaneous inhibition of pan-phosphatidylinositol-3-kinases and MEK as a potential therapeutic strategy in peripheral T-cell lymphomas. Haematologica *98*: 57-64, 2013.

36 Kim SJ, Kang HJ, Kim JS, Eom HS, Huh J, Ko YH, Lee J, Yim DS, Lee SY, Park WS, Yang WI, Lee SS, Suh C and Kim WS: A phase I study of everolimus and CHOP in newly diagnosed peripheral T-cell lymphomas. Invest New Drugs *31*: 1514-1521, 2013.

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