

Original Article

Angioimmunoblastic T-cell lymphoma in Taiwan shows a frequent gain of *ITK* gene

Peir-In Liang¹, Sheng-Tsung Chang², Ming-Yen Lin³, Yen-Chuan Hsieh⁴, Pei-Yi Chu⁵, Chih-Jung Chen^{6,7}, Kai-Jen Lin⁸, Yun-Chih Jung⁹, Wei-Shou Hwang¹⁰, Wen-Tsung Huang¹¹, Wei-Chin Chang¹², Hongtao Ye¹³, Shih-Sung Chuang¹⁴

¹Department of Pathology, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan; ²Department of Pathology, Chi-Mei Medical Center and Department of Nursing, National Tainan Institute of Nursing, Tainan, Taiwan; ³Division of Nephrology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University and Faculty of Renal Care, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; ⁴Department of Pathology, Chi-Mei Medical Center and Department of Medical Laboratory Science and Biotechnology, Chung Hwa University of Medical Technology, Tainan, Taiwan; ⁵Department of Pathology, St. Martin De Porres Hospital, Chiayi and School of Medicine, Fu-Jen Catholic University, New Taipei City, Taiwan; ⁶Department of Surgical Pathology, Changhua Christian Hospital, Changhua, School of Medicine, Chung Shan Medical University, Taichung, Taiwan; ⁷Department of Medical Technology, Jen-Teh Junior College of Medicine, Nursing and Management, Miaoli, Taiwan; ⁸Department of Pathology, E-Da Hospital/I-Shou University, Kaohsiung, Taiwan; ⁹Department of Pathology, Sin-Lau Christian Hospital, Tainan, Taiwan; ¹⁰Division of Hematology and Oncology, Department of Internal Medicine, Chung-Shan Medical University Hospital and School of Medicine, Chung-Shan Medical University, Taichung, Taiwan; ¹¹Division of Hemato-Oncology, Department of Internal Medicine, Chi-Mei Medical Center, Liouying, Tainan, Taiwan; ¹²Department of Pathology, Mackay Medical College and Mackay Memorial Hospital, Mackay Junior College of Medicine, Nursing, and Management, New Taipei City, and Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan; ¹³Department of Histopathology, Royal National Orthopaedic Hospital NHS, Middlesex, United Kingdom and Cancer Hospital of Guangxi Medical University, Nanning, P. R. China; ¹⁴Department of Pathology, Chi-Mei Medical Center, Tainan, Taipei Medical University and National Taiwan University, Taipei, Taiwan

Received July 9, 2014; Accepted August 21, 2014; Epub August 15, 2014; Published September 1, 2014

Abstract: Angioimmunoblastic T-cell lymphoma (AITL) is an aggressive peripheral T-cell lymphoma (PTCL) of follicular helper T-cell origin and is rare in Taiwan. There are overlapping features of AITL and peripheral T-cell lymphoma with a follicular growth pattern (PTCL-F). Around one fifth of PTCL-F exhibits t (5; 9) (q33; q22)/*ITK*-*SYK* chromosomal translocation, which is essentially absent in AITL. We retrospectively investigated 35 cases of AITL from Taiwan with histopathology review, immunohistochemistry, in situ hybridization for Epstein-Barr virus (EBV) and fluorescence in situ hybridization (FISH) for t(5;9)(q33;q22)/*ITK*-*SYK* and correlated the results with overall survival. Twenty-six cases of not otherwise specified PTCL (PTCL-NOS) were also examined by FISH for comparison. Most AITL patients were male (69%) and elderly (median age at 67 years) with frequent bone marrow involvement (53%), high Ann Arbor stages (77%), and elevated serum lactate dehydrogenase (68%). Most cases (80%) showed a typical CD4+/CD8- phenotype and in 90% cases there were scattered EBV-positive B-cells (less than 10% cells). None of these cases showed t(5;9)(q33;q22)/*ITK*-*SYK* translocation by FISH. Gain of *ITK* and *SYK* gene was identified in 38% and 14% tumors, respectively, but both were not associated with overall survival. Performance status < 2 was associated with a better outcome but not the other clinicopathological factors. All PTCL-NOS cases were negative for *ITK*-*SYK* translocation with similar rates (38% and 12%, respectively) of gains at *ITK* and *SYK* loci as that of AITL. In this so far the largest series of AITL from Taiwan, we reported the clinicopathological features and FISH findings on *ITK* and *SYK* genes. We confirmed the absence of t(5;9)(q33;q22)/*ITK*-*SYK* translocation, which may serve as an additional differential diagnostic tool from PTCL-F when present. PTCL-NOS shared a similar pattern of *ITK* and *SYK* gains with AITL. More studies are warranted to elucidate the roles of *SYK* and *ITK* and other genes in the lymphomagenesis of AITL in Taiwan.

Keywords: Angioimmunoblastic T-cell lymphoma, follicular helper T-cell, follicular T-cell lymphoma, *ITK*, peripheral T-cell lymphoma, *SYK*, Taiwan

Introduction

Angioimmunoblastic T-cell lymphoma (AITL), a nodal peripheral T-cell lymphoma (PTCL), is characterized by a systemic disease and polymorphous infiltration with proliferation of high endothelial venules and hyperplasia of the follicular dendritic meshworks [1]. AITL affects mostly elderly patients and the patients frequently present with generalized lymphadenopathy, hepatosplenomegaly, systemic manifestations (such as B symptoms), skin rashes, and polyclonal hypergammaglobulinemia. Immune disorders such as circulating immune complex and hemolytic anemia are not uncommon. The tumor cells of AITL originate from follicular helper T-cells (T_{FH}) in the germinal centers [2] and characteristically express several T_{FH} markers including chemokine CXCL13, cell-surface molecules PD-1/CD279, ICOS and CD200, the adaptor molecule SAP, and the transcription factors Bcl-6 and c-Maf [3-8]. The prognosis of AITL is dismal, with a median survival of less than three years [1].

Follicular variant of peripheral T-cell lymphoma (PTCL-F) is a subtype of PTCL with a follicular growth pattern. PTCL-F resembles AITL both histologically and immunophenotypically with a T_{FH} immunophenotypic profile. Furthermore, both diseases may present synchronously or metachronously in the same patient, suggesting a close relationship [9]. Interleukin 2-inducible T-cell kinase (*ITK*) gene on chromosome 5 encodes for a tyrosine kinase and plays an important role in T-cell proliferation and differentiation. Chromosomal translocation t(5;9) (q33;q22) involving *ITK* gene and the spleen tyrosine kinase (*SYK*) gene on chromosome 9 has been reported in around one fifth of patients with PTCL-F but is extremely rare in AITL [9-12].

In our previous studies, the frequency of AITL among lymphomas in Taiwan increased from 0.5% in the period of 1989-1998 to 3% during 2005-2007, while the relative frequency of T-cell lymphomas among non-Hodgkin lymphoma remained stationary at 18% [13-15]. Recently, Lin et al. investigated 31 cases of AITL from Taiwan and found that the initial presentation with fever, advanced stage, and failure to achieve complete remission (CR) were independent adverse factors for overall survival (OS) with multivariate analysis [16]. To date, there is no molecular study on the alteration of *ITK* and *SYK* genes on AITLs from Taiwan. In this retro-

spective study we investigated the clinicopathological features, Epstein-Barr virus (EBV) status, and possible roles of *ITK* and *SYK* genes in this so far the largest series of AITL from Taiwan.

Materials and methods

Patients

We searched the lymphoma database for cases of AITL and not otherwise specified PTCL (PTCL-NOS) at Chi-Mei Medical Center, Tainan, Taiwan from 1998 to 2010. All lymphoma cases were diagnosed and classified according to the World Health Organization (WHO) criteria [1]. This study was approved by the Institutional Review Board of Chi-Mei Medical Center.

Histopathology and immunophenotyping

The original and newly cut HE sections of all cases were reviewed and the diagnosis confirmed by at least two of us (P.-I.L. S.-T.C, and S.-S.C). For immunohistochemistry, formalin-fixed paraffin embedded (FFPE) sections of 4 μ m thickness were used with the labeled streptavidin-biotin peroxidase method (Super Sensitive™ Link-Label HRP Detection Systems, BioGenex Laboratories, San Ramon, CA., U.S.A.) and an antigen-retrieval technique was applied as needed for each antibody. The antibodies used were CD3, CD8, CD20, bcl-6, Ki67 (DakoCytomation, Glostrup, Denmark), CD4, CD5, CD10 (Novocastra, Newcastle upon Tyne, U.K.), bcl-2, bcl-6, and IRF4/MUM1 (DakoCytomation, Glostrup, Denmark), and PD-1 (Cell Marque, Uden, The Netherlands). Appropriate positive controls were used in all stains.

In-situ hybridization for EBV

We performed in situ hybridization for EBV-encoded mRNA (EBER) in an autostainer (Bond MAX, Vision BioSystems Ltd., Mount Waverley, Australia) using a polymer-based detection system (Bond™ Polymer Refine Detection, Vision BioSystems Ltd.) with an EBV specific probe (Bond™ ISH EBER Probe) and 3, 3'-Diaminobenzidine (DAB) as chromogen.

Fluorescence in situ hybridization (FISH)

We selected the following bacterial artificial clones for identifying *ITK*-*SYK* rearrangements: RP11-563G12 and RP11-51G20 flanking *SYK* for *SYK* translocations, RP11-31B18 and RP11-47012 flanking *ITK* for *ITK* translocations. We directly labeled the probes with Spectrum Ora-

AITL FISH with *ITK* and *SYK*

Table 1. Pertinent clinicopathological data and FISH findings of the 35 patients with angioimmunoblastic T-cell lymphoma

Case no./ Sex/Age	PS	LDH	Pattern	CD4/ CD8	CD10	B-cell proliferation.	EBER	Stage	FISH (<i>ITK</i> / <i>SYK</i>)*	Treatment	Outcome (m)
1/M/62	NA	NA	3	N/P	N	Absent	< 1%	NA	2Co/2Co	CEOP	DOD (8)
2/M/62	NA	Elevated	3	P/N	P	Present	1-10%	II	2Co/2Co	CEOP, Mitoxantrone & Etoposide, IME	DOD (36)
3/F/84	NA	Elevated	2	P/N	P	Present	< 1%	III	2Co/2Co	COP	DOD (12)
4/M/77	NA	Normal	2	P/N	N	Present	1-10%	III	2Co/2Co	COP	DOD (1)
5/M/71	NA	Elevated	3	P/N	P	Absent	< 1%	NA	2Co/2Co	NA.	DOD (5)
6/F/57	NA	Normal	3	N/P	N	Absent	1-10%	NA	20% 3Co/2Co	Supportive	LTF (0.5)
7/F/68	2	Elevated	2	P/N	P	Present	1-10%	IIB	10% 3Co/2Co	CEOP	AWD (18)
8/F/62	1	Elevated	2	P/N	N	Absent	1-10%	III	2Co/2Co	Supportive	AWD (1)
9/F/80	2	Elevated	2	P/N	P	Absent	< 1%	III	2Co/2Co	Supportive	DOD (1)
10/M/69	1	Normal	1	P/N	P	Absent	1-10%	IV	2Co/2Co	Cyclophosphamide	DOD (26)
11/M/68	0	Normal	3	P/N	P	Absent	1-10%	III	50% 3Co/2Co	CHOP, VIP	DURD (13)
12/F/82	3	Elevated	3	P/N	N	Absent	1-10%	IV	10% 3Co/2Co	Cyclophosphamide	DOD (2)
13/M/76	3	NA	3	ND	N	Present	1-10%	III	30% 3Co/20% 3Co	Supportive	DOD (1)
14/F/78	NA	Normal	3	ND	N	Absent	ND	II	70% 3-4Co/20% 3Co	Cyclophosphamide	DOD (19)
15/M/50	3	NA	2	P/N	P	Present	1-10%	IV	2Co/2Co	CEOP, ESHAP, Hyper-CVAD.	DOD (95)
16/M/64	3	NA	3	N/N	P	Present	1-10%	II	2Co/2Co	Supportive	DOD (1)
17/M/62	0	NA	3	N/N	P	Absent	1-10%	NA	2Co/2Co	None	LTF (0.5)
18/F/76	NA	Elevated	3	P/N	P	Absent	ND	III	10% 3Co/2Co	COP (reduced dose)	DOD (5)
19/F/46	NA	Elevated	2	P/N	P	Present	< 1%	IV	20% 3Co/2Co	hyper-CVAD	NED (67)
20/M/73	NA	Elevated	3	N/N	N	Absent	< 1%	III	20% 3Co/2Co	CHOP	NED (59)
21/M/54	NA	Elevated	3	P/N	P	Absent	> 10%	IVB	10% 3Co/20% 3Co	Vincristine	DOD (5.5)
22/M/60	2	Elevated	3	P/N	N	Absent	1-10%	IVB	2Co/2Co	CEOP	DOD (4)
23/M/61	3	Elevated	2	P/N	P	Present	> 10%	IV	2Co/2Co	Cyclophosphamide	DOD (3)
24/M/63	0	Elevated	2	P/N	N	Present	1-10%	II	2Co/2Co	CEOP	AWD (32)
25/M/74	1	Normal	3	P/N	N	Absent	1-10%	II	2Co/2Co	CHOP (reduced dose)	NED (14)
26/M/63	1	Elevated	1	ND	N	Absent	ND	IVB	2Co/2Co	Radiotherapy, CHOP, ICE	DOD (67.5)
27/M/59	NA	NA	3	P/N	P	Absent	1-10%	NA	2Co/2Co	Unknown regimen	DOD (61)
28/M/67	NA	NA	3	P/N	P	Absent	ND	NA	2Co/2Co	Unknown regimen	DOD (11.5)
29/M/60	2	Elevated	3	N/P	N	Present	> 10%	IVB	2Co/2Co	CHOP	DOD (5)
30/M/68	NA	NA	3	P/N	N	Absent	1-10%	NA	2Co/30% 3Co	None	LTF (0.5)
31/M/56	1	Normal	2	P/N	P	Present	1-10%	IV	10% 3Co/2Co	CHOP	DOD (39)

AITL FISH with *ITK* and *SYK*

32/F/70	1	NA	2	P/N	N	Absent	ND	NA	10% 3Co/2Co	CHOP	DOD (26)
33/M/69	0	Elevated	2	P/N	P	Present	1-10%	IIIB	2Co/2Co	CAV	DURD (2.5)
34/M/79	NA	NA	3	P/N	P	Present	1-10%	NA	50% 3Co/60% 3Co	Unknown regimen	AWD (6)
35/F/60	NA	NA	3	P/N	N	Present	1-10%	III	2Co/2Co	None	LTF (0.5)

Abbreviations: AWD, alive with disease; BM, bone marrow involvement; CAV, cyclophosphamide, adriamycin, vincristine; CEOP, cyclophosphamide, epirubicin, vincristine, and prednisolone; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisolone; COP, cyclophosphamide, vincristine, prednisolone; DURD, died of unrelated disease; DOD, died of disease; ESHAP, etoposide, methylprednisolone, high dose ARA-C, cisplatin; F, female; FU, follow-up; Hyper-CVAD, hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone; ICE, ifosfamide, carboplatin, and etoposide; IME, ifosfamide, methotrexate, and etoposide; LDH, lactate dehydrogenase; LTF, loss to follow-up; M, male; N, negative; NA, not available; ND, not done; NED, no evidence of disease; P, positive; PS, performance status; &A relapse case with unspecified previous history. *FISH, Co, number of copies; NS, no signal.

nge and Spectrum Green-dUTP using nick translation (Vysis/Abbott Laboratories Ltd., Maidenhead, UK.). FISH study was performed on deparaffinized FFPE sections of 4 μ m thickness as previously described [17]. Co-localization of the orange and green signals in over 10% cells examined were considered as presence of t (5; 9) (q33; q22)-*ITK*/*SYK* translocation. Gain of *ITK* and *SYK* genes was defined as equal to or greater than 3 signals (copies) in over 10% cells examined.

Statistical analysis

Medical records of the patients were reviewed. OS was measured from the date of diagnosis to the date of last follow-up or death. Categorical data were described by counts and percentages and were compared by using Fisher's exact test. Continuous variables with skewed distribution were described by median and interquartile range; comparisons of them were performed by Mann-Whitney U test. Kaplan-Meier survival curves were drawn and log rank test was used to compare the difference between survival curves. Univariate and multivariate Cox proportional hazard regression analyses were also performed to get the crude and the adjusted hazard ratio of demographic and clinicopathological factors for survival. Data analyses were done by using SPSS for Windows, version 17.0 (SPSS Inc., Illinois, U.S.A.). *P* values less than 0.05 were considered statistically significant.

Results

Patients and clinical features

We identified 35 AITL cases including 25 in-house (Cases no. 1-25) and 10 consultation cases (Cases no. 26-35; to S.-S.C.). **Table 1** summarizes the pertinent clinicopathological features. Of the 35 patients, 24 were male and 11 were female with a M:F ratio of 2.2:1. The median age was 67 years old (range, 46-84). All cases presented as nodal diseases. Seventeen of 25 patients (68%) tested had elevated lactate dehydrogenase (LDH) level. Performance status score of 2 or below was noted in 73% patients. Cutaneous and bone marrow involvement occurred in 15% and 58% of patients, respectively. Of the 26 patients with complete staging, 6 patients (23%) were at Ann Arbor stage II disease, and 10 each at stage III and IV, with high-stage diseases accounting for 77% patients.

Therapy and outcome

Of the 31 AITL patients with complete clinical follow-up information (range, 1 to 95 months), 24 received chemotherapy with all but one by cyclophosphamide-based therapies. Two patients died of disease within one month with only supportive treatment. The treatment modality of Case 5 was not available and he died in 5 months. Only one patient received an additional radiotherapy. None of the patients received hematopoietic stem-cell transplantations. The median and mean survival times were 10.5 months and 20.5 months, respectively. Twenty-four patients died including two due to unrelated diseases after a follow-up period of 1 to 95 months. Six patients were alive, including 2 in complete remission and 4 with disease. Excluding the two patients (Case no. 11 and 33) who died of unrelated reasons, the disease-related 1-yr, 2-yr, and 5-yr survival rates were 54%, 40%, and 17%, respectively.

Histopathology and immunohistochemistry (IHC) study

Table 1 summarizes the pertinent histopathological and immunophenotypic findings with a representative case in **Figure 1**. Histopathologically, pattern 1 morphology was present in only 2 (5%) cases, while patterns 2 and 3 accounted for 12 (34%) and 21 (60%) cases, respectively. B-cell proliferation, characterized by the presence of sheets of large B lymphocytes, was observed in 15 (43%) cases; none was diagnosed as concurrent large B-cell lymphoma. B-cell proliferation was more frequently associated with cases with patterns 1 or 2 than with pattern 3 (64% vs. 29%, *P* = 0.036). Immunohistochemically, 26 (81%) of 32 cases showed the typical CD4-positive CD8-negative phenotype. Cases without the typical CD4/CD8 phenotype were seen in pattern 3 tumors but not in patterns 1 or 2 (*p* = 0.02). CD10 expression was observed in 19 (54%) cases. Interestingly, 6 of the 16 CD10-negative cases that stained for PD-1 were all positive. The Ki-67 labeling indices of these cases ranged from 10% to 70%, with a median and a mean value of 50% and 45%, respectively.

EBER study

EBER study was performed in 30 cases. In six (20%) cases EBER-positive signals accounted for less than 1% cells. In 21 (70%) and 3 cases, EBER positive cells accounted for 1-10% and

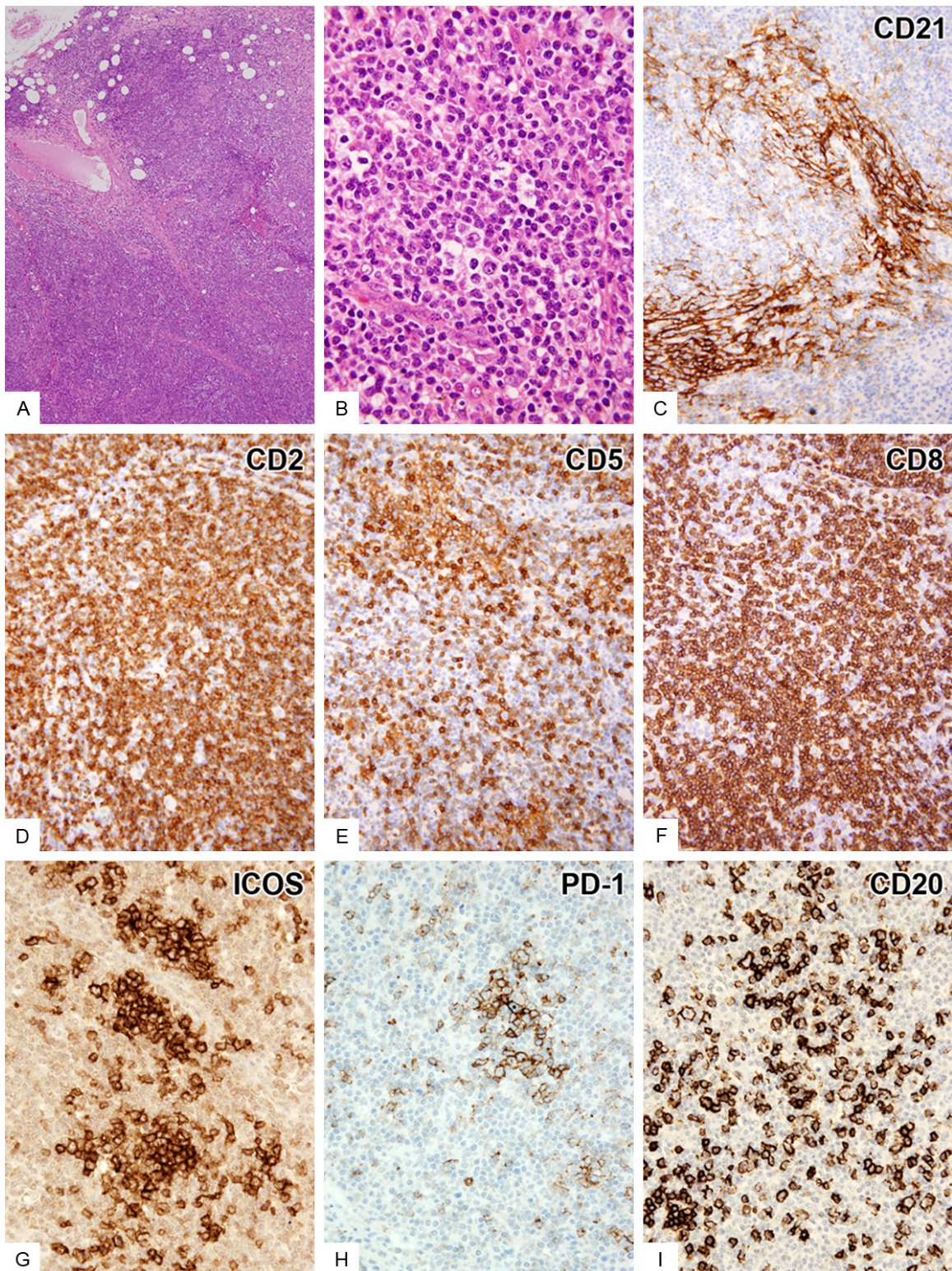


Figure 1. A representative case (Case no. 29) in a 60-year-old male. A. Effacement of the nodal architecture with vaguely nodular lymphoid infiltrate and extension to extranodal adipose tissue. B. A polymorphous infiltrate comprising many clear cells and high-endothelial venules. C. CD21 immunostaining highlights the hyperplastic follicular dendritic meshworks. D-I. Immunohistochemically, the tumor cells express CD2, CD3, CD5, CD8, ICOS, and PD-1 with focal proliferation of CD20-positive large cells.

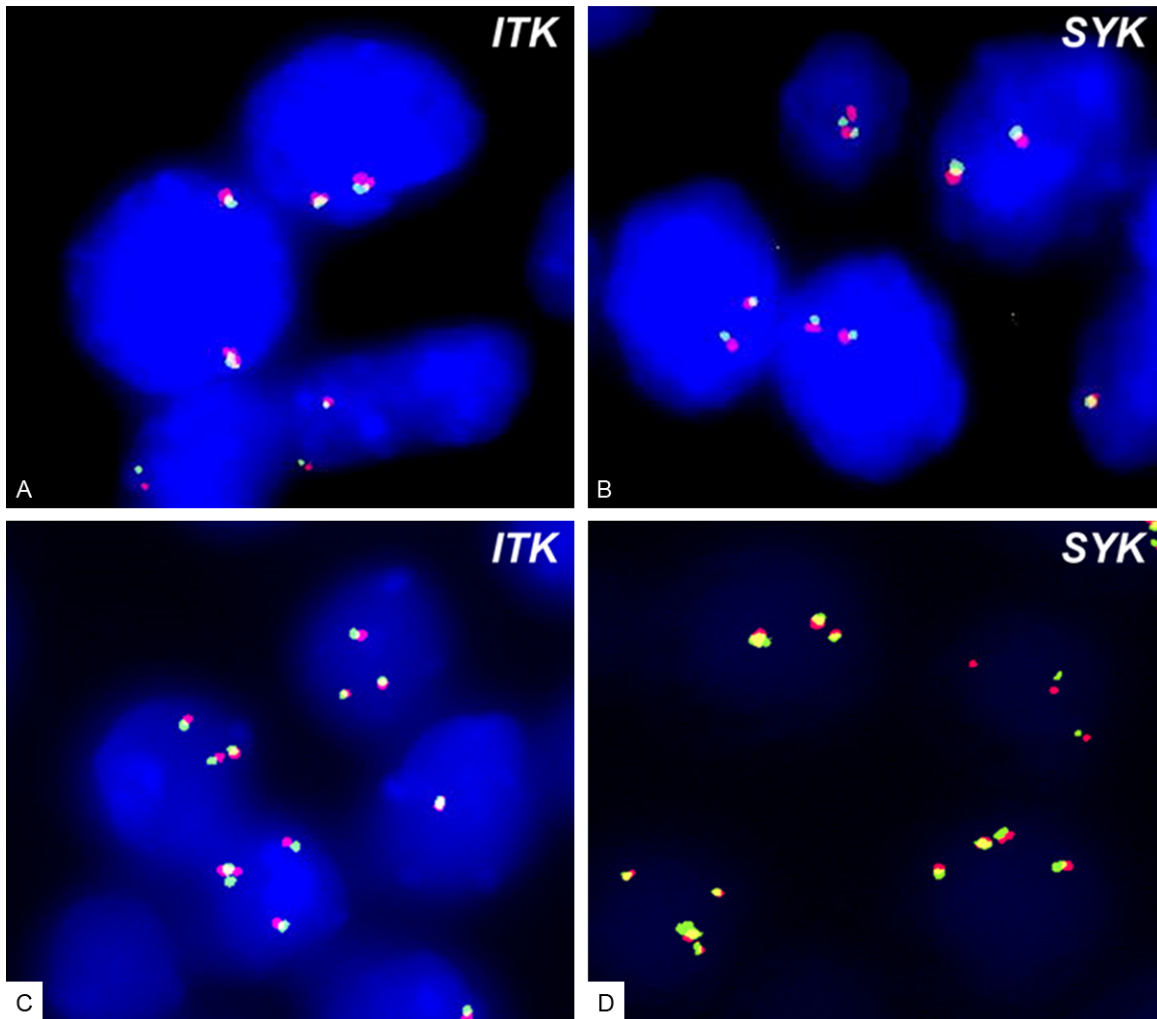


Figure 2. Representative FISH images using dual colour break-apart probes with a normal pattern of 2 copies of *ITK* (A) and *SYK* (B) genes, respectively. (C) A case showing 3 copies of *ITK* gene. (D) A case displaying 3-4 copies of *SYK* gene.

over 10% cells, respectively, corresponding to the cells in B-cell areas.

Fluorescence in situ hybridization (FISH) study

FISH study for t(5;9)(q33;q22)-*ITK*/*SYK* translocation were successfully performed in all AITL cases, which were all negative for t(5;9)(q33;q22)-*ITK*/*SYK* translocation. Gain of *ITK* and *SYK* gene was observed in 37% (13 of 35 cases), and 14% (5 of 35 cases), respectively (**Figure 2**). Cases with gain of *SYK* gene was more frequently seen in those with concurrent gain of *ITK* gene (45% vs. 80%, $P = 0.037$) and only in those with pattern 3 but not in patterns 1 or 2 ($P = 0.043$). Gain of *ITK* gene was more frequently observed in female patients (64% vs. 25%, $P = 0.028$).

A total of 26 cases of PTCL-NOS were randomly selected for comparison. FISH study for t(5;9)(q33;q22)-*ITK*/*SYK* translocation were successfully performed in all PTCL-NOS cases. All cases were negative for t(5;9) translocation. Gains of *ITK* and *SYK* were noted in 38% (10/26) and 12% (3/26) cases, respectively, nearly at the same rates as that of the AITL cases.

Survival analysis

Univariate analysis (**Table 2**) showed that performance status equal to or less than 1 at the diagnosis correlated with better OS ($P < 0.001$). Other clinicopathological factors, including histological patterns (patterns 1 or 2 vs. pattern 3), presence of a B-cell proliferation, CD4 vs.

AITL FISH with *ITK* and *SYK*

Table 2. Univariate analysis of the clinicopathological variables on overall survival of AITL patients

Parameter		No. of cases [§]	Overall survival	
			No. of event	P-value
Gender	Male	22	18	0.671
	Female	9	7	
Age	≤ 60	6	5	0.241
	> 60	25	20	
Ann Arbor Stage	II	6	4	0.652
	III-IV	21	18	
Performance status	< 2	14	11	< 0.001
	≥ 2	6	6	
Histological pattern	Pattern 1/2	14	11	0.186
	Pattern 3	17	14	
EBER	Negative (< 1%)	6	4	0.569
	Positive (> 1%)	20	16	
B-cell proliferation	Absent	17	14	0.755
	Present	14	11	
CD4+/CD8-	Typical immunoprofile	25	20	0.648
	Non-typical immunoprofile	4	3	
<i>SKY</i> gene	Gain	4	3	0.393
	No gain	26	21	
<i>ITK</i> gene	Gain	12	9	0.683
	No gain	19	16	
Ki-67 labeling index	< 40%	12	11	0.854
	≥ 40%	15	10	

[§]Only the patients (n = 31) with at least one month of follow-up were included for analysis with Cases no. 6, 17, 30, and 35 being excluded.

CD8 immunoprofile, Ki-67 labeling index, and EBV status were not statistically significant. Gain of either *ITK* or *SYK* genes was not associated with OS ($P = 0.736$ and 0.428 , respectively).

Discussion

We reported the clinicopathological and FISH findings in this so far the largest series of AITL from Taiwan. The clinicopathological features including old age, male predominance, a T_{FH} phenotype, and a poor prognosis were in agreement with prior studies. We identified PS score as an important prognostic factor in OS. Furthermore, we confirmed the previous study that AITL was negative for t(5;9)(q33;q22)-*ITK*/*SYK* translocation [9-12]. Instead we found that 38% and 14% of the AITL cases exhibited gains of *ITK* and *SYK* genes, respectively. Interestingly such genetic alterations occurred nearly at the same rate in our cohort of 26 examples of PTCL-NOS, suggesting that these two diseases

may share some common genetic features. However, such genetic alterations were not associated with OS in our cohort of AITL patients.

Although the relative frequency of T and NK/T-lymphoma in Taiwan, up to 18% among non-Hodgkin lymphoma, is higher than that in the West, AITL is rare and comprises only 0.5 to 2.4% of all lymphoma cases in various institutes [13, 14, 16, 18-20]. The clinical features of this lymphoma have not been characterized until recently by Lin et al. who reported a retrospective study of 31 patients from northern Taiwan [16]. Interestingly,

our data, mainly cases from southern Taiwan, were essentially the same as that study in terms of male predominance (2.2:1 vs. 2.1:1), high-stage disease (68% vs. 63%), elevated LDH (68% vs. 63%) and 2-yr survival (40% vs. 39%), except that our patients were slightly less elderly (mean age at 67 vs. 74 years).

PTCL-F, a variant of PTCL-NOS in the WHO classification, is characterized by neoplastic T-cells localized within the germinal centers, distributed within the marginal zone with a perifollicular distribution, or confined in the expanded mantle zones of the follicles [21]. PTCL-F closely resembles AITL histologically, and shares a similar T_{FH} -immunoprofile. Furthermore, both diseases may occur synchronously or metachronously in the same patient, indicating a close relationship. In contrast to AITL in which t(5;9)(q33;q22)-*ITK*/*SYK* translocation is extremely rare, one fifth of PTCL-F cases exhibit this translocation. However, recently Attygalle et al. demonstrated an exceptional AITL case

exhibiting $t(5;9)(q33;q22)$ -*ITK/SYK* translocation, providing an additional link between these two lymphoma types [12]. In our study, no such translocation was identified, either in the 35 cases of AITL or 26 cases of PTCL-NOS. Although the frequency of this translocation is not high in PTCL-F, its presence may serve as a surrogate favoring PTCL-F when the differential is AITL.

The growth patterns of AITL have been classified into 1, 2, or 3, representing those with hyperplastic, regressed or effaced germinal centers, respectively [22]. Pattern 1 is rarely seen and mimics a reactive process, thus is easily misdiagnosed as reactive lymphoid hyperplasia. Very recently, Tan et al. compared 30 cases each of pattern 1 AITL and reactive lymphoid hyperplasia from Singapore and found that the former patients were older, more frequently non-Chinese and with nodal presentation. The former could be distinguished from the latter by an aberrant immunarchitecture [23]. In addition, AITL patients with pattern 1 morphology had a better OS than those with pattern 2 [24]. With disease progression, tumors with pattern 1 morphology may evolve to pattern 3 with an interval ranging from 2 to 168 months [22]. Noteworthy, secondary lymphomas such as classic Hodgkin lymphoma and diffuse large B-cell lymphoma may complicate tumors with patterns 2/3 morphology but not pattern 1 [22]. In our series, there were only two cases (Case no. 10 and 26) with pattern 1 morphology and the case number was too small for comparison with those with other patterns. Our Case no. 10 was initially misdiagnosed as reactive hyperplasia and was included as one of the cases in the study of histological evolution of AITL by Attygalle et al. (Case no. 2 in that series) [22]. Misdiagnosis of AITL is not infrequent, especially by general pathologists inexperienced in hematopathology. Tan et al. observed a 48% discordant rate, occurring more frequent in those with pattern 1 (4 out of 7 cases) morphology than pattern 3 (7 out of 17 cases). Pathologists should pay particular attention to nodal lesions with atypical morphological features of germinal centers and take into account the clinical context, particularly for elderly patients with generalized lymphadenopathy, B symptoms and elevated LDH to avoid misdiagnosis, especially those with AITL of pattern 1 morphology.

The poor outcome of patients with AITL may be due to a high frequency of advanced disease at diagnosis, old age, and frequent presence of infectious complications; all these factors render these patients unsuitable for chemotherapy with curative intent. From the observation of 157 patients in the GELA trial, Mourad et al. showed that male patients, anemia (Hb < 120 g/dL), and presence of mediastinal lymphadenopathy were factors adversely affecting the overall survival of patients with AITL [25]. In recent years, gene expression profiling indicated T_{FH} origin of AITL and revealed substantial components of the molecular signature of AITL are contributed by the follicular dendritic cells, B-cell, and other stromal components [26-28]. Accordingly, Iqbal et al. constructed a molecular prognosticator for AITL that appears to be largely related to the microenvironmental signature [28]. They subsequently applied their molecular classifier for AITL and were able to reclassify 14% of PTCL-NOS as AITL, which were initially not pathologically diagnosed as AITL because of the absence of certain key morphologic or immunohistochemical features. They found that in AITL, high expression of several signatures associated with the tumor microenvironment was significantly associated with outcome and a combined prognostic score was predictive of survival in an independent cohort [29]. Future studies on AITL may aim at reversing the immunosuppressive microenvironment to promote antitumor immunity and the suppression of the proliferation of EBV-transformed B cells [29].

There were some limitations of our study, including a relatively small number of cases, a long study period that spanning 13 years, and a proportion of the patients with incomplete clinical data, particularly the consultation cases, and heterogeneous treatment modality. All these factors made the evaluation of treatment effect and the evaluation of various prognosticators difficult. Nonetheless, this is the largest series of AITL patients from Taiwan and we characterized the clinicopathological features and genetic alterations of *ITK* and *SYK* in this rare lymphoma. Future national and prospective studies with a homogeneous treatment modality are warranted.

Acknowledgements

This work was supported by the research grant CLFR10137 from Liouying Chi Mei Hospital,

Tainan, Taiwan and grant no. 30960122 from National Natural Science Foundation of China, Beijing, and Distinguished Experts Guangxi Special Funding Support, Guangxi, P. R. China.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hongtao Ye, Department of Histopathology, Royal National Orthopaedic Hospital NHS, Brockley Hill, Stanmore, Middlesex HA7 4LP, United Kingdom. Tel: 44-(020) 8909-5347; E-mail: hongtao.ye@rnoh.nhs.uk; Dr. Shih-Sung Chuang, Department of Pathology, Chi-Mei Medical Centre, 901 Chung-Hwa Road, Yung-Kang District, Tainan 710, Taiwan. Tel: 886-6-281-2811 Ext. 53686; Fax: 886-6-251-1235; E-mail: cmh5301@mail.chimei.org.tw

References

[1] Dogan A, Gaulard P, E.S. J, Ralfkiaer E and Muller-Hermelink HK. Angioimmunoblastic T-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW, editors. WHO classification of tumours of haemtopoietic and lymphoid tissues. Lyon: IARC; 2008. pp. 309-311.

[2] Grogg KL, Attygalle AD, Macon WR, Remstein ED, Kurtin PJ and Dogan A. Angioimmunoblastic T-cell lymphoma: a neoplasm of germinal-center T-helper cells? *Blood* 2005; 106: 1501-1502.

[3] Bisig B, Thielen C, Herens C, Gofflot S, Travert M, Delfau-Larue MH, Boniver J, Gaulard P and de Leval L. c-Maf expression in angioimmunoblastic T-cell lymphoma reflects follicular helper T-cell derivation rather than oncogenesis. *Histopathology* 2012; 60: 371-376.

[4] Dorfman DM and Shahsafaei A. CD200 (OX-2 membrane glycoprotein) is expressed by follicular T helper cells and in angioimmunoblastic T-cell lymphoma. *Am J Surg Pathol* 2011; 35: 76-83.

[5] Marafioti T, Paterson JC, Ballabio E, Chott A, Natkunam Y, Rodriguez-Justo M, Plonquet A, Rodriguez-Pinilla SM, Klapper W, Hansmann ML, Pileri SA, Isaacson PG, Stein H, Piris MA, Mason DY and Gaulard P. The inducible T-cell co-stimulator molecule is expressed on subsets of T cells and is a new marker of lymphomas of T follicular helper cell-derivation. *Haematologica* 2010; 95: 432-439.

[6] Ree HJ, Kadin ME, Kikuchi M, Ko YH, Suzumiya J and Go JH. Bcl-6 expression in reactive follicular hyperplasia, follicular lymphoma, and angioimmunoblastic T-cell lymphoma with hyperplastic germinal centers: heterogeneity of

intrafollicular T-cells and their altered distribution in the pathogenesis of angioimmunoblastic T-cell lymphoma. *Hum Pathol* 1999; 30: 403-411.

[7] Roncador G, Garcia Verdes-Montenegro JF, Tedoldi S, Paterson JC, Klapper W, Ballabio E, Maestre L, Pileri S, Hansmann ML, Piris MA, Mason DY and Marafioti T. Expression of two markers of germinal center T cells (SAP and PD-1) in angioimmunoblastic T-cell lymphoma. *Haematologica* 2007; 92: 1059-1066.

[8] Yu H, Shahsafaei A and Dorfman DM. Germinal-center T-helper-cell markers PD-1 and CXCL13 are both expressed by neoplastic cells in angioimmunoblastic T-cell lymphoma. *Am J Clin Pathol* 2009; 131: 33-41.

[9] Huang Y, Moreau A, Dupuis J, Streubel B, Petit B, Le Gouill S, Martin-Garcia N, Copie-Bergman C, Gaillard F, Qubaja M, Fabiani B, Roncador G, Haioun C, Delfau-Larue MH, Marafioti T, Chott A and Gaulard P. Peripheral T-cell lymphomas with a follicular growth pattern are derived from follicular helper T cells (TFH) and may show overlapping features with angioimmunoblastic T-cell lymphomas. *Am J Surg Pathol* 2009; 33: 682-690.

[10] Streubel B, Vinatzer U, Willheim M, Raderer M and Chott A. Novel t(5;9)(q33;q22) fuses *ITK* to *SYK* in unspecified peripheral T-cell lymphoma. *Leukemia* 2006; 20: 313-318.

[11] Feldman AL, Sun DX, Law ME, Novak AJ, Attygalle AD, Thorland EC, Fink SR, Vrana JA, Caron BL, Morice WG, Remstein ED, Grogg KL, Kurtin PJ, Macon WR and Dogan A. Overexpression of Syk tyrosine kinase in peripheral T-cell lymphomas. *Leukemia* 2008; 22: 1139-1143.

[12] Attygalle AD, Feldman AL and Dogan A. *ITK/SYK* translocation in angioimmunoblastic T-cell lymphoma. *Am J Surg Pathol* 2013; 37: 1456-1457.

[13] Chuang SS, Lin CN and Li CY. Malignant lymphoma in southern Taiwan according to the revised European-American classification of lymphoid neoplasms. *Cancer* 2000; 89: 1586-1592.

[14] Lu D, Lin CN, Chuang SS, Hwang WS and Huang WT. T-cell and NK/T-cell lymphomas in southern Taiwan: a study of 72 cases in a single institute. *Leuk Lymphoma* 2004; 45: 923-928.

[15] Chuang SS. Significant increase in the relative frequency of follicular lymphoma in Taiwan in the early 21st century. *J Clin Pathol* 2008; 61: 879-880.

[16] Lin HN, Liu CY, Hong YC, Pai JT, Yang CF, Yu YB, Hsiao LT, Chiou TJ, Liu JH, Gau JP, Tzeng CH and Chen PM. Clinical features and prognostic factors of angioimmunoblastic T-cell lympho-

- ma in Taiwan: a single-institution experience. *Leuk Lymphoma* 2010; 51: 2208-2214.
- [17] Ye H, Liu H, Attygalle A, Wotherspoon AC, Nicholson AG, Charlotte F, Leblond V, Speight P, Goodlad J, Lavergne-Slove A, Martin-Subero JI, Siebert R, Dogan A, Isaacson PG and Du MQ. Variable frequencies of t(11;18)(q21;q21) in MALT lymphomas of different sites: significant association with CagA strains of *H pylori* in gastric MALT lymphoma. *Blood* 2003; 102: 1012-1018.
- [18] Chang KC, Huang GC, Jones D, Tsao CJ, Lee JY and Su JJ. Distribution and prognosis of WHO lymphoma subtypes in Taiwan reveals a low incidence of germinal-center derived tumors. *Leuk Lymphoma* 2004; 45: 1375-1384.
- [19] Lee MY, Tan TD, Feng AC and Liu MC. Clinicopathological analysis of 598 malignant lymphomas in Taiwan: seven-year experience in a single institution. *Am J Hematol* 2006; 81: 568-575.
- [20] Chen YP, Jones D, Chen TY and Chang KC. Epstein-Barr virus present in T cells or B cells shows differential effects on hemophagocytic symptoms associated with outcome in T-cell lymphomas. *Leuk Lymphoma* 2014 Feb 17; [Epub ahead of print].
- [21] Pileri SA, Weisenberger DD, Sng I, E.S. J, Ralfkiaer E, Nakamura S and Muller-Hermelink HK. Peripheral T-cell lymphoma, not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW, editors. WHO classification of tumours of haemtopoietic and lymphoid tissues. Lyon: IARC; 2008. pp. 306-309.
- [22] Attygalle AD, Kyriakou C, Dupuis J, Grogg KL, Diss TC, Wotherspoon AC, Chuang SS, Cabecadas J, Isaacson PG, Du MQ, Gaulard P and Dogan A. Histologic evolution of angioimmunoblastic T-cell lymphoma in consecutive biopsies: clinical correlation and insights into natural history and disease progression. *Am J Surg Pathol* 2007; 31: 1077-1088.
- [23] Tan LH and Tan SY. Aberrant immunarchitecture distinguishes hyperplastic germinal centres in pattern 1 angioimmunoblastic T-cell lymphoma from reactive follicles. *Hematol Oncol* 2013 Nov 19; [Epub ahead of print].
- [24] Tan LH, Tan SY, Tang T, Lim ST, Tan D, Lim LC, Kam GL, Loh TP, Tao M and Koay ES. Angioimmunoblastic T-cell lymphoma with hyperplastic germinal centres (pattern 1) shows superior survival to patterns 2 and 3: a meta-analysis of 56 cases. *Histopathology* 2012; 60: 570-585.
- [25] Mourad N, Mounier N, Briere J, Raffoux E, Delmer A, Feller A, Meijer CJ, Emile JF, Bouabdallah R, Bosly A, Diebold J, Haioun C, Coiffier B, Gisselbrecht C, Gaulard P; Groupe d'Etude des Lymphomes de l'Adulte. Clinical, biologic, and pathologic features in 157 patients with angioimmunoblastic T-cell lymphoma treated within the Groupe d'Etude des Lymphomes de l'Adulte (GELA) trials. *Blood* 2008; 111: 4463-4470.
- [26] de Leval L, Rickman DS, Thielen C, Reynies A, Huang YL, Delsol G, Lamant L, Leroy K, Briere J, Molina T, Berger F, Gisselbrecht C, Xerri L and Gaulard P. The gene expression profile of nodal peripheral T-cell lymphoma demonstrates a molecular link between angioimmunoblastic T-cell lymphoma (AITL) and follicular helper T (TFH) cells. *Blood* 2007; 109: 4952-4963.
- [27] Piccaluga PP, Agostinelli C, Califano A, Carbone A, Fantoni L, Ferrari S, Gazzola A, Gloghini A, Righi S, Rossi M, Tagliafico E, Zinzani PL, Zupo S, Baccarani M and Pileri SA. Gene expression analysis of angioimmunoblastic lymphoma indicates derivation from T follicular helper cells and vascular endothelial growth factor deregulation. *Cancer Res* 2007; 67: 10703-10710.
- [28] Iqbal J, Weisenburger DD, Greiner TC, Vose JM, McKeithan T, Kucuk C, Geng H, Deffenbacher K, Smith L, Dybkaer K, Nakamura S, Seto M, Delabie J, Berger F, Loong F, Au WY, Ko YH, Sng I, Armitage JO, Chan WC; International Peripheral T-Cell Lymphoma Project. Molecular signatures to improve diagnosis in peripheral T-cell lymphoma and prognostication in angioimmunoblastic T-cell lymphoma. *Blood* 2010; 115: 1026-1036.
- [29] Iqbal J, Wright G, Wang C, Rosenwald A, Gascoyne RD, Weisenburger DD, Greiner TC, Smith L, Guo S, Wilcox RA, Teh BT, Lim ST, Tan SY, Rimsza LM, Jaffe ES, Campo E, Martinez A, Delabie J, Braziel RM, Cook JR, Tubbs RR, Ott G, Geissinger E, Gaulard P, Piccaluga PP, Pileri SA, Au WY, Nakamura S, Seto M, Berger F, de Leval L, Connors JM, Armitage J, Vose J, Chan WC, Staudt LM; Lymphoma Leukemia Molecular Profiling Project and the International Peripheral T-cell Lymphoma Project. Gene expression signatures delineate biological and prognostic subgroups in peripheral T-cell lymphoma. *Blood* 2014; 123: 2915-2923.