Prevalence and Factors Associated with the Loss of PTEN Expression in Patients with Lung Cancer

Thiva Kiatpanabhikul, M.D.*, Wasakorn Bunyayothin, M.D.**

**Department of Medicine, **Department of Pathology, Charoenkrung Pracharak Hospital, Bangkok 10120, Thailand.*

ABSTRACT

Objectives: Phosphatase and tensin homolog (PTEN) is a major tumor suppressor gene and is involved in cell survival control. PTEN loss of expression (PTEN-) is associated with a poor outcome. Our study investigated the prevalence of *PTEN*- in terms of its characteristics and disease prognosis for lung cancer patients.

Materials and Methods: In total, 167 tissue blocks from lung cancer patients at Chareonkrung Pracharak Hospital between January 2010 and December 2020 were studied through immunohistochemistry staining (IHC) for PTEN expression. The clinicopathological factors, IHC features, and epidermal growth factor receptor (EGFR) status were analyzed in association with PTEN- in term of prognosis and the overall survival (OS).

Result: Adenocarcinoma was the major subtype (85.6%) and most patients (90.6%) were diagnosed at stage IV of lung cancer. The prevalence of PTEN- was 66.5%. A location at the left lower lobe (LLL) location and the absence of tumor-infiltrating lymphocytes (TILs) were significantly associated with PTEN- (p=0.039, p=0.046), while the smoking was likely correlated but not statistically significant (p=0.09). The median OS for PTEN- was not significantly different from PTEN+ (8.88 vs 7.20 months, p=0.38). However, smoking, Eastern cooperative oncology group (ECOG) status and primary symptoms were significantly associated with poorer OS.

Conclusion: The prevalence of PTEN- was higher in our studies. Absent TILs and a LLL location were independent factors associated with PTEN-. However, a right upper lobe (RUL) location with PTEN- tended to have a poor prognosis. Interestingly, better survival was found in active smokers with PTEN-. Further survival studies in cases with no TILs lesions and active smokers in associations PTEN expression and other immune-related biomarkers, such as programmed death–ligand 1 (PD-L1), are warranted.

Keywords: PTEN; immunohistochemistry; tumor infiltrating lymphocytes; lung cancer (Siriraj Med J 2022; 74: 48-63)

INTRODUCTION

Lung cancer is the leading cause of cancer death worldwide, and the second leading cancer in males and females. In 2019, lung cancer was responsible for the death of 1.37 million people in the United States.[1] In Thailand, lung cancer is the third most common cancer in males, accounting for 24.74% of all cancers, and the fourth most common cancer in females, accounting for 7.26% of all cancers, while the most common cancers overall are hepatobiliary cancer in males and breast cancer in females.[2]

Lung cancer can be classified as either small cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC). NSCLC, which is more common (accounting for 85% of lung cancers), can be sub-classified as adenocarcinoma (AC, 40%), squamous cell carcinoma (SC, 25%-30%), and large cell carcinoma (10%-15%).[3,4] Regarding its genetic and molecular basis, the dysregulation of cell growth

Corresponding author: Thiva Kiatpanabhikul
E-mail: mdping143@gmail.com
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ORCID ID: https://orcid.org/0000-0002-5139-5781
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and development may predispose some patients to lung cancer as well as determine its severity.[5] Tumor suppressor genes (TSGs), in particular *PTEN (phosphatase and tensin homolog)*, are considered significant controllers of cell survival and the cell cycle process; thus their dysregulation may lead to the development of several cancers, including lung cancer.[6-10] *PTEN*, located on chromosome 10q23, regulates the cell cycle via the PI3K/AKT pathway. In the development of lung cancer, *PTEN* dysfunction, including its loss of function, protein instability, and somatic mutation, has been noted to be associated with the malignant transformation of lung cells.

Several studies reported that approximately 41.2%-43.7% of lung cancer patients have a loss of *PTEN* expression.[11-13] Moreover, *PTEN* dysregulation was found to be associated with an advanced tumor stage, partly due to the anchorage-independent growth mechanism.[14] In lung cancer patients, the loss of *PTEN* expression *(PTEN-)* made negatively regulates phosphatidylinositol 3 phosphate level in the P13K/AKT pathway[15], and this alteration may be associated with the development of lung cancer.[16] *PTEN* loss of expression *(PTEN-)* was related to characteristics of NSCLC patients, including male, a previous history of smoking, and lung cancer with a poorly differentiated type, increased lymph node involvement, high distant metastasis, and late-stage, and thus it is a factor for a poor prognosis.[17] Further, patients with a combination of the *PTEN-* and *p-AKT+* have a lower 5-year survival rate and median survival time. Though the associations between the loss of *PTEN* expression and SCLC is still unclear. Alterations in the *PTEN* pathway have been regularly reported in SCLC.[18] In the genetic mice model, the *PTEN* inactivation could accelerate the SCLC with more aggressive behavior.[19] *PTEN-* is also an independent poor prognostic factor for NSCLC.[11-13] Furthermore, lung cancer patients with a loss of *PTEN* expression and the positive *epidermal growth factor receptor (EGFR)* mutation may be resistant to chemotherapy and targeted therapy.[20,21]

Consequently, our study aimed to explore the prevalence of *PTEN* loss of expression in Thai lung cancer patients, and also its associated clinical characteristics and possible effects on disease prognosis.

MATERIALS AND METHODS

This retrospective study was approved by the Human Research and Ethics Committees of Bangkok Metropolitan Administration, Bangkok, Thailand. (No. S013h/63_EXP). Informed consent was waived because the study involved anonymous data extraction with no direct patient or public involvement.

Study population

In total, 191 lung cancer patients underwent either transbronchial biopsy (53.9%), transcutaneous biopsy (24.6%), lobectomy (3.6%), or tissue biopsy (lymph node, skin, bone, mass) (18%) at the Charoenkrung Pracharak Hospital between 1 January 2010 and 31 December 2020 and were enrolled in the study. The inclusion criteria were patients aged >15 years old diagnosed with lung cancer by histopathological studies. If there was insufficient tissue for the further analysis of *PTEN* expreesion, the patients were excluded from the study. The patients' demographics and clinical characteristics, including age, gender, smoking, associated symptoms (such as chronic cough, progressive dyspnea, hemoptysis, and chest pain), Eastern cooperative oncology group (ECOG) performance status, tumor size and its primary location, histopathological study, duration of follow-up, and living status, were collected. Ancillary immunohistochemical reports, including *thyroid transcription factor 1 (TTF – 1)* and *epidermal growth factor receptor (EGFR)* mutation, were also collected.

Histopathology and immunohistochemistry (IHC) for PTEN

Formalin-fixed paraffin-embedded (FFPE) blocks were cut 4 μm in thickness by microtomy (Thermo Fisher Scientific HM355S automated microtomy). Slides were stained by hematoxylin and eosin (H&E). All the previous H&E slides were reviewed for confirming the diagnosis and evaluating the tissue adequacy for further *PTEN* immunohistochemical study by two pathologists at pathologists at the Department of Pathology, Charoenkrung Pracharak Hospital, blinded to the patient information and clinical data. The laboratory was certified for Laboratory Academic Standards by The Royal College of Pathologist of Thailand. Histopathological parameters, including histologic subtype and tumor-infiltrating lymphocytes (TILs), were evaluated. Unstained whole-section FFPE slides from 167 FFPE blocks were heated at 70 ^{0}C for 30 min in a hot air oven (Memmert UF55) before running them in a Ventana BenchMark XT automatic sample preparation system (serial number KPXT715667). The IHC staining process included deparaffinization by EZ prep (LOT#G13961) and cell conditioning by CC1 (LOT#G06580) for 56 min and antigen retrieval by a primary peroxidase inhibitor. Rabbit monoclonal primary antibody VENTANA PTEN clone SP218 (LOT#G27111) was used as the primary antibody in this study. Automated incubation was performed for 16 min. OptiView HQ links and OptiView HQ Universal links were mixed to ensure amplified signals, followed

by use of the Ventana OptiView TM Universal DAB Detection Kit. Counterstaining was performed by dyeing with Ventana Hematoxylin II for 12 minutes, followed by bluing reagent for 6 minutes. The oil was removed from each slide by soap and the slide was subsequently dehydrated with 95% ethyl alcohol, absolute alcohol, and xylene, respectively. Finally, a coverslip was placed on the slide and it was then mounted with mounting media.

IHC scoring of PTEN

The IHC score for *PTEN* was modified from the previous literature.[11] The interpretation and scoring of *PTEN* IHC was performed in terms of either the intensity of staining or the average number of positive tumor cells, as independently evaluated by two pathologists. *PTEN* IHC slides were visually scored using a bright field microscope (Olympus BX43) under an objective lens (40x magnification) and eye pieces lens (10x magnification and field number 22). The positive internal control staining included the bronchial epithelial cells and stromal cells. Five 40x high-power fields were selected to includes 200 cell counts. The interpretation of *PTEN* expression involved the cytoplasmic and/or nuclear staining pattern. The average percentage of positive tumor cells is reported as the following: 0 = no tumor cells stained, 1 = 10%-20% of cells stained, 2 = 20%-50% cells stained, and 3 = >50% of cells stained. The intensity of positive cell staining was categorized as follows: 0 = no appreciable staining in the cells, 1 = barely detectable staining as compared with normal stromal cells, 2 = readily appreciable brown staining distinctly marking the cell cytoplasm/or nucleus, and 3 = dark brown staining in the cytoplasm and/or nucleus. *PTEN* IHC scoring was rendered, with regard to the calculated results for the intensity and the average percentage of positive tumor cells, ranging from 0 to 9. A score of 2 or less is defined as negative *PTEN* IHC expression, whereas 3 or greater is defined as positive *PTEN* IHC expression.

Using 200-400x (a 10x eyepieces and a 20-40x objective lens) microscopic magnification, tumor infiltrating lymphocytes (TILs) are the percentages of TILs in the stromal compartment (% stromal TILs), defined as the area of mononuclear cells (including lymphocyte and plasma cell) infiltration, between the cancer cells with no direct contact, in the stromal tissue. The 10% of more stromal TILs is considered positive.[22]

Statistical analysis

Data were analyzed for the prevalence of *PTEN* loss of expression (*PTEN*-) in lung cancer, the associations between *PTEN* loss of expression and the clinicopathological factors, IHC features, and the *EGFR* type status. The parametric statistical analysis was performed using SPSS version 26 software (IBM Corp., Armonk, NY). The patients' demographic and clinical characteristics were expressed as a numbers, percentages, median, and mean and standard deviation (SD). Categorical variables were analyzed by Pearson chi-square test and Fisher's exact test for the normally distributed data. A p-value less than 0.05 was considered statistically significant. Kaplan–Meier analysis and the log-rank test were used to analyze the results from the survival study. Cox proportional hazard regression was applied to determine the *PTEN* loss of expression and the variables affecting the survival status. According to Seol-Bong Yoo *et al.*[13], the prevalence of the *PTEN* loss of expression in NSCLC was 42.4% and was used to calculated the sample size. In this study, there were only 8 patients with SCLC; therefore, only descriptive statistics were applied. Moreover, no prevalence of *PTEN* loss of expression was previously reported.

RESULTS

Among the 191 lung cancer patients enrolled on the study, 24 were excluded because 23 had no available FFPE blocks and one who had inadequate tumor tissue for the IHC study. Subsequently, a total of 167 patients were included in the study for the data analysis. Two pathologists independently interpreted the PTEN IHC staining on each slide. For the first 40 slides, the interpretation kappa values were 0.975 for the inter-observer reliability and 0.95 for the intra-observer reliability, accordingly. For the overall 167 slides, the inter-observer reliability was 0.98. Three discordantly interpreted slide results between the two pathologists were re-evaluated and further discussed until consensus was reached on each slide (positive or negative *PTEN* staining)

The mean age of the patients was 64.8 ± 11.77 years old, with a median age of 65.0 years old (ranging from 32-93 years), with 51.5% having an age ≥65 years old. The majority of patients were male (57.5%). Out of the 167 lung cancers cases, NSCLC was accounted for 159 cases (95.2%) and SCLC 8 cases (4.8%). Lung cancer was the most commonly found in patients who had never smoked group (46.1%), while its incidence in those with a smoking history group, which was active (37.7%), secondhand (6.6%), or former smokers (9.6%), was 53.9%. Clinical symptoms included chronic cough (77.2%), progressive dyspnea (68.3%), hemoptysis (19.8%), and chest pain (24.6%), and the most common ECOG score at the time of diagnosis was 1 (54.5%), respectively. Among

the NSCLC cases, 85.6% of patients had adenocarcinoma subtypes, and 90.6% were diagnosed at an advanced stage (stage IV). Moreover, all 8 SCLC cases were involved extensive disease (ED). Approximately half the patients (57.6%) had a tumor sized 3-7 cm at the greatest diameter. Regarding of the location of the lung lobes, primary lung cancer was mostly located at the right upper lung area (34.1%). Surprisingly, most lung cancer tissues (64.1%) displayed no tumor-infiltrating lymphocytes. The prevalence of PTEN loss of expression was 66.5%. Most lung cancer tissues were stained positive for TTF-1 (77.5%) and Napsin A staining (63.2%). Regarding the EGFR mutation, 46 cases (47.8%) were located on either EXON 19 (68.2%) or EXON 21 (18.2%).

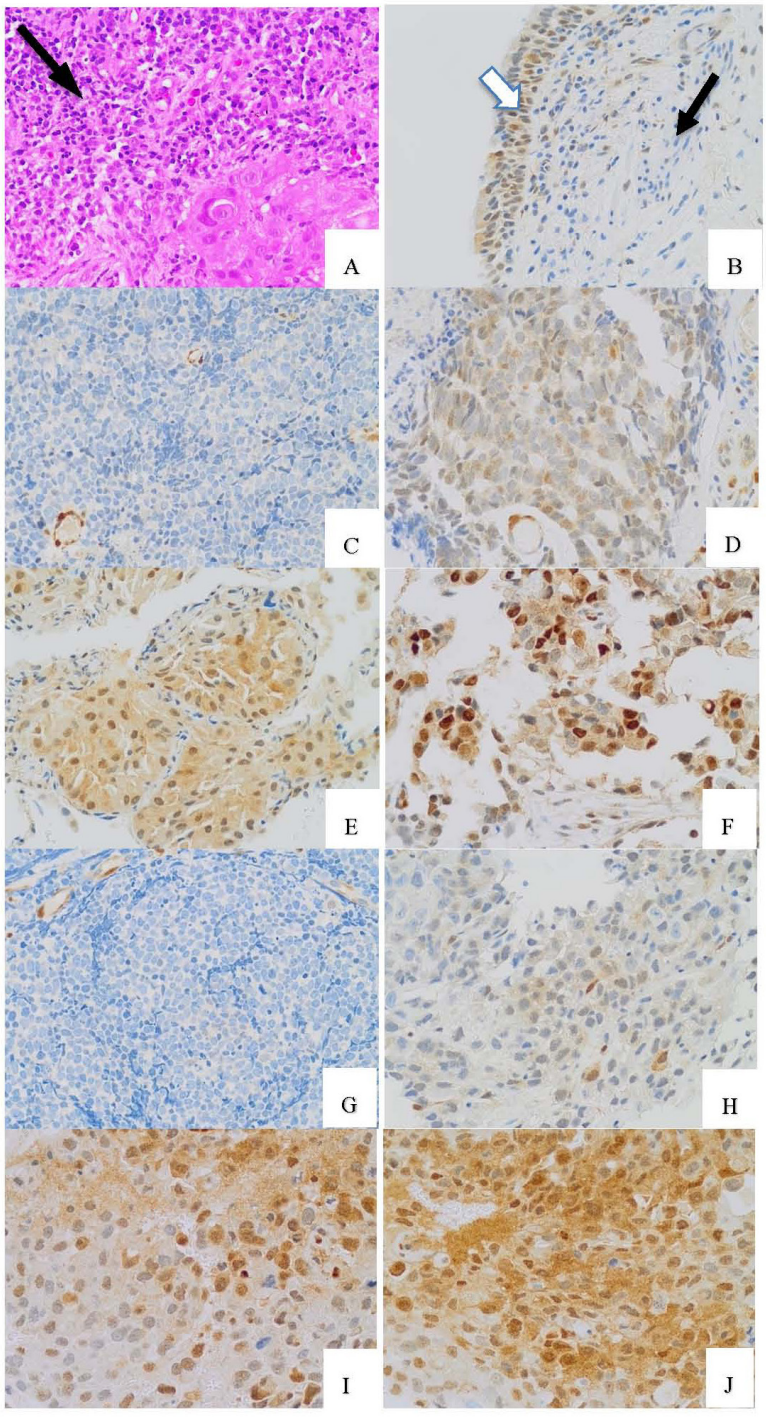

Fig 1. **A:** NSCLC with TILs (dark arrow) (H&E, x40); **B:** Normal bronchial epithelium (white arrow) and stromal cell (dark arrow) with nuclear and cytoplasmic staining of PTEN. PTEN Intensity was categorized as follows: **C:** 0 if no appreciable intensity stain. **D:** 1 if barely stain; **E:** 2 if appreciable brown; **F:** 3 if dark brown stain in the cytoplasm and/or nucleus. The different percentages of PTEN IHC were demonstrated as follows: **G:** no tumor cell staining or 0%; **H:** 10% - 20%, **I:** 20% - 50%; **J:** more than 50% in the NSCLC patients.

TABLE 1. Demographics, clinical characteristics, and histopathological and immunohistochemical studies of the patients with lung cancer.

Characteristic variables	Number	Percentage (%)
Gender (n = 167)		
Male	96	57.5
Female	71	42.5
Age (years) (n = 167)		
≥65	86	51.5
<65	81	48.5
Smoking (n = 167)		
Never smoked	77	46.1
Active smoker	63	37.7
Secondhand smoker	11	6.6
Former smoker	16	9.6
Chronic cough (n = 167)	129	77.2
Hemoptysis (n = 167)	134	80.2
Progressive dyspnea (n = 167)	114	68.3
Weight loss (n = 167)	100	59.9
Chest pain (n = 167)	41	24.6
ECOG status (n = 167)		
0	8	4.8
1	91	54.5
2	36	21.6
3	21	12.6
4	11	6.6
Histological type of lung cancer (n = 167)		
NSCLC	159	95.2
SCLC	8	4.8
NSCLC subtype (n = 159)		
Adenocarcinoma	143	89.9
Squamous cell carcinoma	12	7.5
Large cell neuroendocrine carcinoma	2	1.3
Other (e.g. carcinosarcoma)	2	1.3
Tumor size (n = 167) (cm.)		
<3	15	9.3
3–7	95	58.6
>7	52	32.1
Location of the primary tumor (n = 167)		
RUL	57	34.1
RML	20	12
RLL	31	18.6
LUL	32	19.1
LLL	26	15.6
Center	1	0.6

TABLE 1. Demographics, clinical characteristics, and histopathological and immunohistochemical studies of the patients with lung cancer. (Continue)

Characteristic variables	Number	Percentage (%)
Tumor-infiltrating lymphocytes (TILs) (n = 167)		
Present	60	35.9
Histological pattern (n = 167)		
Acinar	89	53.3
Papillary	8	4.8
Solid	61	36.5
Lepidic	9	5.4
Degree of differentiation (n = 167)		
Moderate	157	94
Poor	10	6
PTEN expression (n = 167)		
Loss of expression	111	66.5
TTF-1 IHC (n = 142)		
Positive staining	110	77.5
EGFR mutation (n = 46)	22	47.8
Exon 19	15	68.2
Exon 20	1	4.5
Exon 21	4	18.2
Double mutation	2	9.1
Treatment (1st line)		
1st line chemotherapy	79	47.3
1st line tyrosine kinase inhibitor (TKI)	12	7.2
1st line surgery	3	1.8
1st line radiotherapy	15	9
no definite treatment/palliative care	58	34.7
Living status		
Alive	23	13.77
Dead	144	86.23

Abbreviations: ECOG = Eastern Cooperative Oncology Group performance status, NSCLC = non-small cell lung cancer, SCLC = small cell lung cancer, PTEN = phosphatase and tensin homolog, TTF-1 = thyroid transcription factor 1, EGFR = epidermal growth factor receptor, RUL=Right upper lung, RML=Right middle lung, RLL=Right lower lung, LUL=Left upper lung, and LLL=Left lower lung

Correlation between PTEN expression and survival time

In terms of the correlation between the *PTEN* status and clinical outcome, *PTEN*- was less common in lung cancer that was primarily located at the left lower lobe (LLL), compared to at the right upper lobe (RUL) (p = 0.039, OR = 0.36), and in the absence of TILs (p = 0.045, OR = 1.96). Whereas an age <65 years old and smoking were likely correlated with the *PTEN* status (p = 0.056, OR = 0.22 and p = 0.089, OR = 1.75, respectively).

According to the multivariate analysis, the absence of TILs (p = 0.017, adjusted OR = 2.5), location at the LLL (p = 0.026, adjusted OR = 0.297), and age <65 years old (p = 0.04, adjusted OR = 0.47) were independent factors correlated with the PTEN loss of expression. In addition, SCLC and smoking behavior were also marginally significantly associated with the PTEN loss of expression (p = 0.054 and 0.089, respectively).

The median follow-up time was 8.04 months (range, 0.01–94.80). Most patients (65.3%) had received specific treatments (including 1st line chemotherapy (47.3%), 1st line radiotherapy (13.8%), 1st line tyrosine kinase inhibitor (7.2%), or surgery for primary cancer (1.8%)), while the remaining 34.7% had received no aggressive treatment due to their poor baseline status. The median overall survival (mOS) was 8.88 months, with 2- and 5-year overall survival rates of 19.7% and 7.4%, respectively. Of note, 27 patients (16.17%) had CNS/spine metastasis at the 1st diagnosis.

TABLE 2. Association among clinical status, immunohistochemical study, *EGFR* mutation of primary lung cancer, and PTEN expression.

Clinicopathological, immune-molecular features	Total n (%)	PTEN (+) n (%)	PTEN (-) n (%)	P-value
Gender				
Male	96 (57.5)	33 (58.9)	63 (56.8)	0.79
Female	71 (42.5)	23 (41.1)	48 (43.2)	
Age				
≥65	86 (51.5)	23 (41.1)	63 (56.8)	0.056
<65	81 (48.5)	33 (58.9)	48 (43.2)	
Smoking				
No smoking	77 (46.1)	31 (55.4)	46 (41.4)	0.089
History of smoking	90 (53.9)	25 (44.6)	65 (58.6)	
Chronic cough				
Yes	129 (77.2)	41 (73.2)	88 (79.3)	0.37
No	38 (22.8)	15 (26.8)	23 (20.7)	
Progressive dyspnea				
Yes	114 (68.3)	39 (69.6)	75 (67.6)	0.786
No	53 (31.7)	17 (30.4)	36 (32.4)	
Hemoptysis				
Yes	33 (19.8)	12 (21.4)	21 (18.9)	0.70
No	134 (80.2)	44 (78.6)	90 (81.1)	
Weight loss				
Yes	100 (59.9)	34 (60.7)	66 (59.5)	0.88
No	67 (40.1)	22 (39.3)	45 (40.5)	
Chest pain				
Yes	41 (24.6)	15 (26.8)	26 (23.4)	0.63
No	126 (75.4)	41 (73.2)	85 (76.6)	
Size				
<3 cm	15 (9.3)	2 (3.6)	13 (12.3)	0.12
3–7 cm	95 (58.6)	39 (69.6)	56 (52.8)	
>7 cm	52 (32.1)	15 (26.8)	37 (34.9)	

TABLE 2. Association among clinical status, immunohistochemical study, *EGFR* mutation of primary lung cancer, and PTEN expression. (Continue)

Clinicopathological, immune-molecular features	Total n (%)	PTEN (+) n (%)	PTEN (-) n (%)	P-value
Primary site				
RUL (0)	57 (34.1)	17 (30.4)	40 (36)	0.31
RML (1)	20 (12)	5 (8.9)	15 (13.5)	
RLL (2)	31 (18.6)	8 (14.3)	23 (20.7)	
LUL (3)	32 (19.2)	12 (21.4)	20 (18)	
LLL (4)	26 (15.6)	14 (25)	12 (10.8)	
Central (5)	1 (0.6)	-	1 (0.9)	
ECOG status				
0	8 (4.8)	1 (1.8)	7 (6.3)	0.49
1	91 (54.5)	30 (53.6)	61 (55)	
2	36 (21.6)	14 (25)	22 (19.8)	
3	21 (12.6)	7 (12.5)	14 (12.6)	
4	11 (6.6)	4 (7.1)	7 (6.3)	
Type				
NSCLC	159 (95.2)	56 (100)	103 (92.8)	0.05
SCLC	8 (4.8)	-	8 (7.2)	
Stage (NSCLC)				
I	4 (2.5)	1 (1.8)	3 (2.9)	0.21
II	4 (2.5)	1 (1.8)	3 (2.9)	
III	7 (4.4)	4 (7.3)	3 (2.9)	
IV	144 (90.6)	49 (89.1)	95 (91.3)	
Stage (small cell)				
Extensive disease	8 (100)	0 (0)	8 (100)	-
NSCLC				
Adenocarcinoma	143 (89.9)	47 (83.9)	96 (93.2)	0.12
Squamous cell CA	12 (7.5)	6 (10.7)	6 (5.8)	
Large cell / NE	2 (1.3)	1 (1.8)	1 (1)	
Other	2 (1.3)	2 (3.6)	-	
TILs				
Yes	60 (35.9)	26 (46.4)	34 (30.6)	0.045
No	107 (64.1)	30 (53.6)	77 (69.4)	
TTF-1				
Yes	110 (77.5)	39 (79.6)	71 (76.3)	0.66
No	32 (22.5)	10 (20.4)	22 (23.7)	
EGFR mutation				
Yes	22 (47.8)	11 (55)	11 (42.3)	0.39
No	24 (52.2)	9 (45)	15 (57.7)	
EGFR mutation				
EXON 18	-	-	-	0.79
EXON 19	15 (68.2)	8 (72.7)	7 (63.6)	
EXON 20	1 (4.5)	-	1 (9.1)	
EXON 21	4 (18.2)	2 (18.2)	2 (18.2)	
Double mutation	2 (9.1)	1 (9.1)	1 (9.1)	
Treatment				
1st line chemotherapy	79 (86.8)	27 (87.1)	52 (86.7)	0.003
1st line (TKI)	12 (13.2)	4 (12.9)	8 (13.3)	

Abbreviations: ECOG = Eastern Cooperative Oncology Group performance status, NSCLC = non-small cell lung cancer, SCLC = small cell lung cancer, PTEN = phosphatase and tensin homolog, TTF-1 = thyroid transcription factor 1, EGFR = epidermal growth factor receptor, RUL=Right upper lung

Patients who had a history of smoking, chronic cough, progressive dyspnea, no hemoptysis, chest pain, weight loss, larger tumor size, and lower ECOG status had a lower mOS time than in the opposite group (p = 0.003, 0.005, 0.015, 0.008, 0.03, 0.001, 0.001, and 0.001, respectively). Nevertheless, gender, age, a subtype of lung cancer (adenocarcinoma vs squamous cell subtypes) and primary brain/spine metastasis, presence of TILs, TTF-1, and *EGFR* mutation showed no significant difference in the mOS time between the comparative populations (p >0.05).

In all lung cancer patients, the mOS was 8.88 months (ranging from 0.01 to 94.8 months). There was no significant difference in mOS between the *PTEN*+ and *PTEN*– groups in NSCLC (7.20 vs. 8.88 months) (p = 0.38) and also no significant difference in mOS between the *PTEN*+ and *PTEN*– groups in adenocarcinoma (7.20 vs 9.96 months) (p = 0.23) (Fig 2.1 and 2.2).

TABLE 3. Association among the clinicopathologic, immune-molecular features and median overall survival (mOS) and hazard ratio (HR) (n = 167).

Clinicopathological, immune-molecular features	Total n (%)	mOS month	95% CI n (%)	P-value	Hazard ratio	95% CI n (%)	P-value
Gender							
Male	96 (57.5)	7.68	5.54–9.82	0.368	reference	-	-
Female	71 (42.5)	13.63	8.91–18.35		0.86	0.62–1.20	0.369
Age							
≥65	86 (51.5)	8.64	4.86–12.42	0.256	reference	-	-
<65	81 (48.5)	9.96	7.25–12.67		0.823	0.60–1.15	0.258
Smoking							
No smoking	77 (46.1)	14.57	8.83–20.31	0.003	reference	-	-
History of smoking	90 (53.9)	6.36	4.62–8.10		1.64	1.18–2.29	0.004
Chronic cough							
No	38 (22.8)	19.2	13.94–24.46	0.005	1.75	1.17–2.62	-
Yes	129 (77.2)	7.2	5.20–9.2		reference	-	0.006
Progressive dyspnea							
No	53 (31.7)	18.84	12.39–25.29	0.015	reference	-	-
Yes	114 (68.3)	7.2	4.44–9.96		1.55	1.08–2.20	0.016
Hemoptysis							
No	134 (80.2)	7.68	4.89–10.47	0.008	reference	-	-
Yes	33 (19.8)	10.08	0–20.67		0.55	0.35–0.86	0.009
Weight loss							
No	67 (40.1)	18.24	9.70–26.78	0.001	reference	-	-
Yes	100 (59.9)	7.08	4.57–9.59		1.77	1.25–2.49	0.001
Chest pain							
No	126 (75.4)	9.96	5.92–14	0.03	reference	-	-
Yes	41 (24.6)	7.2	2.93–11.47		1.54	1.04–2.28	0.032

TABLE 3. Association among the clinicopathologic, immune-molecular features and median overall survival (mOS) and hazard ratio (HR) (n = 167). (Continue)

Clinicopathological, immune-molecular features	Total n (%)	mOS month	95% CI n (%)	P-value	Hazard ratio	95% CI n (%)	P-value
ECOG status							
0	8 (4.8)	35.16	22.83–47.49	<0.01	reference	-	-
1	91 (54.5)	14.52	10.19–18.85		2.28	0.92–5.66	0.076
2	36 (21.6)	3.79	1.40–6.18		2.36	2.08–13.84	0.001
3	21 (12.6)	5.88	2.63–9.13		5.99	2.21–16.23	<0.001
4	11 (6.6)	4.6	0–9.52		3.92	1.30–11.84	0.016
Primary site							
RUL (0)	57 (34.1)	16.03	9.81–22.26	0.024	reference	-	-
RLL (2)	31 (18.6)	9.24	5.91–12.57		0.96	0.59–1.54	0.86
LUL (3)	32 (19.2)	3.79	2.01–5.57		1.60	1.01–2.54	0.046
LLL (4)	26 (15.6)	5.58	1.19–10.57		1.90	1.16–3.09	0.01
Type							
Adenocarcinoma	143 (92.3)	9.24	6.49–11.99	0.26	reference	-	-
Squamous cell CA	12 (7.7)	11.64	0.03–23.25		0.68	0.34–1.34	0.27
Brain/spine metastasis							
No	140 (83.8)	9.24	6.39–12.09	0.31	reference	-	-
Yes	27 (16.2)	6.72	3.60–9.84		1.254	0.81–1.95	0.32
Size							
<3 cm	15 (9.3)	20.88	3.61–39.15	<0.01	0.45	0.23–0.88	0.02
3–7 cm	95 (58.6)	11.28	7.44–15.12		reference	-	-
>7 cm	52 (32.1)	5.28	3.23–7.33		2.13	1.46–3.10	<0.001
TILs							
Yes	60 (35.9)	7.08	1.36–12.80	0.68	reference	-	-
No	107 (64.1)	8.88	5.9–11.86		1.08	0.76–1.52	0.67
TTF-1							
No	32 (22.5)	10.08	6.75–13.41	0.88	reference	-	-
Yes	110 (77.5)	7.92	4.68–11.16		1.03	0.68–1.56	0.88
PTEN							
No	56 (33.5)	7.2	1.97–12.43	0.38	reference	-	-
Yes	111 (66.5)	8.8	6.62–11.14		0.85	0.60–1.22	0.38
EGFR							
No	24 (52.2)	7.44	4.62–10.26	0.41	reference	-	-
Yes	22 (47.8)	14.52	11.99–17.05		0.76	0.39–1.46	0.41

Abbreviations: ECOG = Eastern Cooperative Oncology Group performance status, TILs = tumor-infiltrating lymphocytes, TTF-1 = thyroid transcription factor 1, PTEN = phosphatase and tensin homolog, EGFR = epidermal growth factor receptor.

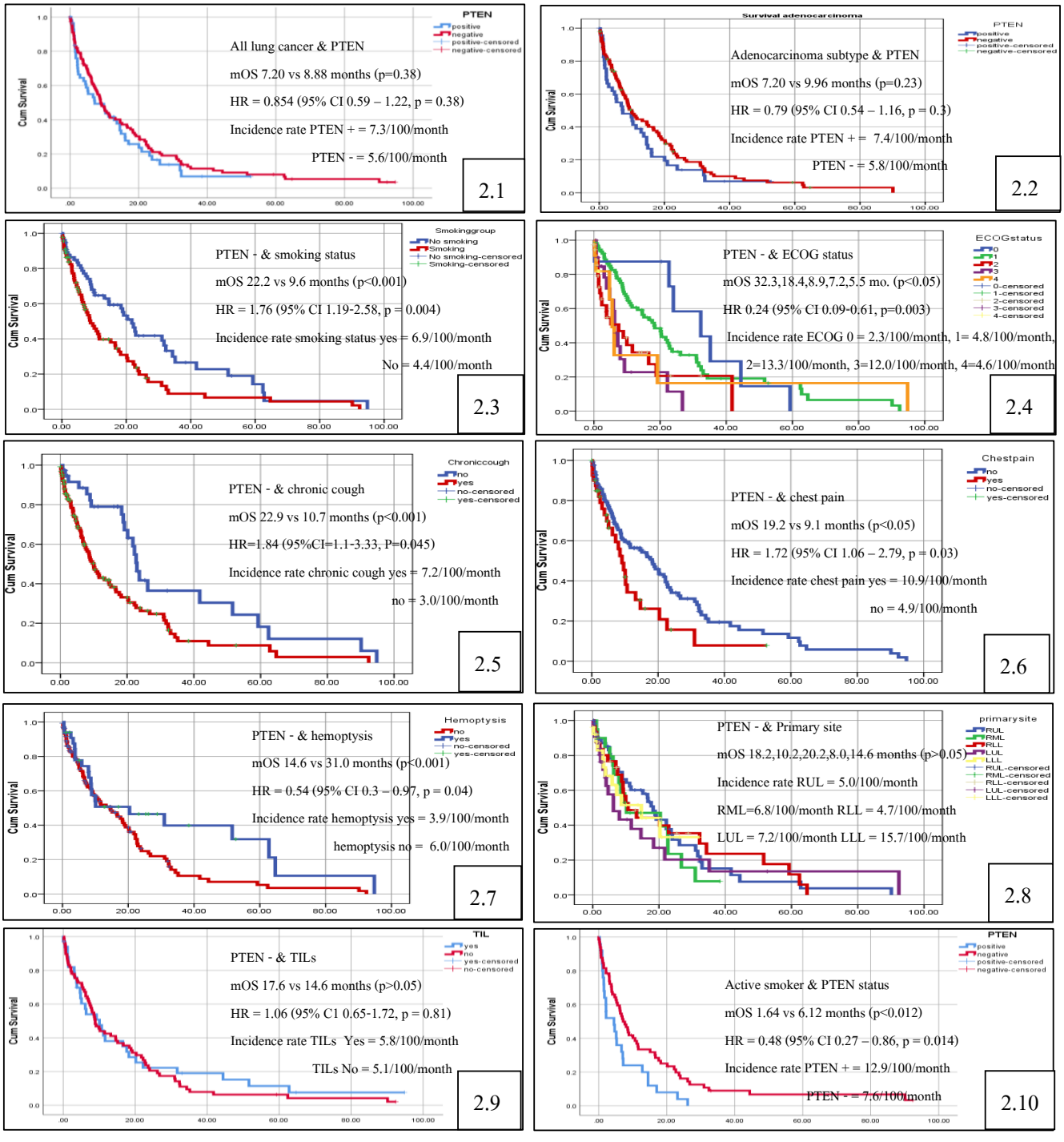

Fig 2. The Kaplan–Meier graph demonstrating the mOS of the patients with lung cancer **(2.1)**, adenocarcinoma subtypes **(2.2)**, and active smoking **(2.10)** in association with the *PTEN* status. **Fig 2.3–2.9** display the mOS of *PTEN*- correlated with the smoking status, EGOG status, present symptom (chronic cough, progressive dyspnea, hemoptysis), primary site, TILs.

In the subgroup analysis of *PTEN*– patients, the mOS was significantly decreased with the presence of smoking (Fig 2.3). high ECOG status (Fig 2.4), chronic cough (Fig 2.5), chest pain (Fig 2.6) and hemoptysis (Fig 2.7). Nevertheless, there was no significant difference in the mOS depending on the primary tumor site (Fig 2.8). Fig 2.9 reveals that the mOS in the no TILs feature was longer than the mOS in the TILs group, but was no statistical difference. Among the active smokers, the mOS was longer in the *PTEN*- group than in the *PTEN*+ group (Fig 2.10). The mOS in either presence or absence of EGFR mutation was not significantly different (7.44 vs 14.52 months) (p = 0.41).

DISCUSSION

As the function of *PTEN* is to inhibit the Akt kinase activity in the kinase/Akt/mTOR pathway, *PTEN* deletion results in high levels of activated Akt, which brings out the G1 cell cycle and possible progress to pathogenesis of the NSCLC and subsequent metastasis.[23] Furthermore, the absence of *PTEN* creates an immunosuppressive microenvironment, thus facilitating tumor growth and metastasis.[24] *PTEN*- creates an immunosuppressive microenvironment.[25] On the contrary, the presence of CD8+ T-lymphocyte infiltration is correlated with a better prognosis in several cancers.[26] The expression of *PTEN* protein could also block the programmed cell death receptor-1 (PD-1). Consequently, several studies have reported the expression of *PD-1* is related with the expression of *PTEN*.[27]

Our study is the first to report the overall prevalence of *PTEN*- in Thai patients with lung cancer (66.5%), with a prevalence of 64.8% in NSCLC patients and 100% in SCLC patients. Previously, the prevalence of *PTEN*- in NSCLC patients was reported to be approximately 24%-59.86%[11,13,28-32], In the literature, adenocarcinoma (AC) is the most common subtype of NSCLC, representing approximately 40% of patients.[33] However, in this study, the prevalence of AC subtypes was very high (84.4% in males and 87.3% in females), compared to the reported prevalence according to a SEER study in 2018[34] and in the 2015 report of the National Cancer Research Institute of Thailand[2], which to be reported a rate of approximately 50%-60%.

Of note, the percentages of AC and *PTEN*- in our study population were relatively high. Possible explanations for this include the higher cut-off score of 3 and higher we employed, similar to the study of Tang *et al.* in NSCLC patients[11], whereas many previous studies utilized lower cut-off scores of 1-2.[11,35-40] Table 4 described the PTEN expression in lung cancer patients from each study and their correlated parameters.

TABLE 4. The studies of the PTEN loss of expression in lung cancer patients and their correlated clinical parameters.

References	Histology	Finding	PTEN score	Number of patients	Related clinical parameters with PTEN-
Soria et al.[28]	NSCLC	Protein loss	Intensity - absence	24% (30/125)	No related parameters
Chang et al.[41]	NSCLC	Protein loss	< 3	59.86% (173/289)	LN metastasis, smoking status, low survival rate
Yoo et al.[42]	NSCLC	Protein loss	No data	42.4% (122/288)	SC, smoking status, low progression free survival
Scrima et al.[43]	NSCLC	Protein loss	0-25%	39% (41/104)	SC subtype
Tang et al.[11]	NSCLC	Protein loss	0-2	46.1% (47/102)	Poor survival of p-Akt^{S473} positive
Goncharak et al.[32]	NSCLC	Protein loss	0-2	41.4% (43/104)	Advanced disease, LN metastasis, low survival rate
Kim et al.[37]	AD, SC	Protein loss	0-2	37.4% (34/91)	High histological grade, pathological T stage, N stage, short survival in AD
Thiva et al., These study	NSCLC	Protein loss	<3	64.8% (103/159)	Absence of TILs, poor location at LLL, age <65 years, smoking

Moreover, we preferred the detection of *PTEN* expression by the IHC method over *PTEN* mRNA study as the method is more sensitive for studying overall survival. Further, in the meta-analysis, NSCLC patients with *PTEN* loss of expression had an unfavorable prognosis, whereas the results could not be demonstrated when the PTEN mRNA method was utilized.[24]

In our study, patients with hemoptysis had a longer mOS than asymptomatic patients (p = 0.008). Thus, hemoptysis might prompt both patients and doctors' concerns, thus allowing early investigation, followed by prompt treatment, eventually extending the overall mOS period. Furthermore, similar to the study of Port *et al*., our study indicated that a smaller tumor size and lower ECOG performance status were correlated with a prolonged mOS.[44] Moreover, no significant difference in mOS was observed between the age groups and genders, corresponding with another study by Franceschini *et al*.[45]

In NSCLC, no significant difference was seen in mOS between *PTEN*– and *PTEN*+ (p = 0.38). Though most studies expected a lower mOS in NSCLC patients with *PTEN* - [31,35-38,40,42,46-50], some studies reported a longer mOS.[51] The differences could be possibly due to different races and ethnicities, techniques to detect *PTEN* loss of expression (including polymerase chain reaction, fluorescence *in situ* hybridization, IHC), and cut-off score in IHC. Notably, in our study, a higher proportion of AC was observed, and this NSCLC subtype is more responsive to treatment.

Interestingly, all the SCLC patients in our study were at an extensive stage with *PTEN*-, compared with those with the NSCLC subtype (p = 0.05). The mOS of SCLC was short, only 5.28 months (range. 0.96-9.61). Regarding previous reports on the prevalence of SCLC, possible SCLC-Y accounted for approximately 10% of all four subtypes. However, only in the SCLC-Y subtype is the oncogenesis related to the mTOR pathway and associated with *PTEN* loss of expression. Presumably, all the SCLC cases in our study were likely to be the SCLC-Y subtype. However, further studies to analyze the subtypes of the SCLC are required to demonstrate the actual prevalence of each subtype in Thai patients.

In this study, there were no significant differences in mOS in patients with smoking history, a more advanced stage, and squamous cell type with *PTEN*- status (p = 0.089, 0.237, and 0.053); though previous studies demonstrated a correlation between smoking status[41,52,53], stage[13], and squamous cell subtype[13,31], with *PTEN*-. In the PTEN- group in our study, patients with a smoking history had a longer mOS than those with a negative smoking history (p = 0.012; 6.12 vs 1.68 months, HR (*PTEN* +/-) = 0.48, 95%CI 0.27-0.86, p = 0.014). Chang *et al*. also reported an unclear association between the *PTEN* status and smoking behavior.[41] Of note, the frequency and intensity of smoking are known risk factors for lung cancer, but these were not extensively included in the analysis due to the limited data availability in the medical records.

Interestingly, our study is the first to report that the location of the primary tumor at the RUL was significantly correlated with *PTEN*-, compared to at the LLL location (p = 0.03). Patients with the primary site at the RUL also had a significantly longer mOS than at the LUL and LLL locations (16.03 months vs. 3.79 months and 5.58 months (p = 0.024) (LUL:HR 1.60, 95%CI 1.01–2.54, p = 0.046 and LLL:HR = 1.90, 95%CI 1.16–3.09, p = 0.01). For the primary location of NSCLC, Lee *et al*. reported that a lower lobe group had a higher mortality rate than non-lower lobe ones (48.6% and 40.3%, p <0.0001) with less frequent *EGFR* mutation.[54] Lung cancer with the primary location at the upper lobe may contain different immunomolecular processes that might interfere with the survival outcomes. These hypotheses probably lead to the need for personalized medicine.

In the *PTEN*+ group, the location of the primary site was significantly associated with the mOS, of which the RUL location had a longer mOS than the LLL location. However, this association was not found in the *PTEN*- group. Nevertheless, in the *PTEN*+ group, the primary location of the tumor at the RUL had a longer mOS than at the LLL location [32.4 months vs 14.8 months (p = 0.0001), HR 3.14, 95%CI 1.52–6.52, p = 0.002]. Therefore, further studies on the *PTEN* expression using the IHC of the patients with the primary location of lung cancer at the upper lobe may reveal its prognostic capability.

For the histopathological studies, the *PTEN*- was found to be associated with the negative findings of TILs (p = 0.045). In our study, there was no significant difference in mOS using the TILs status (p = 0.67). On the contrary, Schalper *et al*. and Gao *et al*. reported better survival outcomes in NSCLC and triple-negative breast cancer with the presence of TILs, respectively.[55,56] In our mOS study in NSCLC, no TILs feature was not a prognostic factor, probably not only the level of activated Akt in the mTOR pathway, influencing cellular survival, and other tumor suppressor functions, such as chromosome integrity and DNA repair[57], take part in the survival process. Other confounding factors, such as a small sample size number, the method used to identify *PTEN*, and scoring of the *PTEN* expression by IHC, were influential in these studies.

CONCLUSION

The prevalence of the PTEN loss of expression in NSCLC in our study population was quite a bit higher than in previous studies. The pathological no TILs feature and RUL location were found to be independent factors associated with the PTEN loss of expression. The small cell subtype and the smoking group were nearly significantly related to negative *PTEN* staining. Smoking status, all symptoms, ECOG status, RUL location, and tumor size >7 cm were found to play unfavorable prognostic roles in the overall survival in NSCLC. In the subgroup study, PTEN loss of expression with RUL location tended to involve a poor prognosis. Interestingly, a better survival outcome was shown in the active smoker group with *PTEN* negative. Further survival study of the no TILs pathological status and active smoker subgroups in terms of PTEN expression and other immune-related biomarkers, such as IHC for programmed death–ligand 1 (PD-L1), with an adequate sample size and proper study design is warranted.

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