RESEARCH ARTICLE

# Retrospective Study of ALK Rearrangement and Clinicopathological Implications in Completely Resected Non-small Cell Lung Cancer Patients in Northern Thailand: Role of Screening with D5F3 Antibodies

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## Abstract

**Background: Anaplastic lymphoma kinase (ALK) gene rearrangement in non-small cell lung cancer (NSCLC) has been intensively studied. The gold standard for ALK detection is FISH, but this is not routinely conducted in clinical practice, so that the IHC method has a role. The aim of this study was to identify the incidence of ALK rearrangement and risk or prognostic factors for ALK positivity using both of IHC and FISH methods. Materials and Methods: From January 2008 to December 2012, 267 completely resected NSCLC patients in Chiang Mai University Hospital were enrolled in this study. Clinical and pathological variables and outcomes of treatment were retrospectively reviewed. IHC and FISH were used to evaluate ALK rearrangement. Sensitivity and specificity of IHC were analyzed. Multivariable analysis was used to identify clinico-pathological correlations with positive results of IHC and clinical outcomes. Results: Twenty-two (8.2%) of 267 specimens were IHC-positive for ALK with intense cytoplasmic staining, whereas only 10 (3.8%) were FISH-positive. Sensitivity, specificity and the positive likelihood ratio with IHC were 80.0%, 94.9%, and 15.8 respectively. Age less than 55 years (RR 4.4, 95%CI 1.78-10.73, p value=0.001) and presence of visceral pleural invasion (VPI) (RR 2.9, 95%CI 1.21-6.78, p value =0.017) were identified as risk factors for ALK rearrangement with FISH. There were no statistically significant differences in other clinical and pathological variables. ALK rearrangement was not a prognostic factor for tumor recurrence or overall survival. Conclusions: The incidences of ALK positivity in completely resected NSCLCs in northern Thailand were 8.2% by IHC and 3.8% by FISH. IHC with mouse monoclonal, Ventana D5F3 antibody can be used as a screening tool before FISH method because of high specificity and high positive likelihood ratio. Age less than 55 years and VPI are risk factors for ALK positivity.**

**Keywords:** ALK rearrangement - lung cancer - complete resection - D5F3 antibody

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## Introduction

Lung cancer is one of the leading causes of cancer-related death. Non-small cell lung carcinoma (NSCLC) accounts for 85 percent of lung cancer cases (Ettinger et al., 2006). In the recent era, multidisciplinary approaches are used for treatment including surgery, radiotherapy, chemotherapy, immunotherapy and targeted therapy. The possible curative treatment is anatomical resection with systematic lymphadenectomy, however it is achieved only for early stage NSCLC. In advanced cases, recently, targeted therapy has a vital role for treatment as first- or second-line treatment. Targeted therapy involves drug for specific gene mutation or abnormal rearrangement such as erotinib or gefitinib for EGFR mutation, which is already approved in advanced NSCLC (Alimujiang et al., 2013; Aydiner et al., 2013; Lee et al., 2013; Zhang et al., 2011). Anaplastic lymphoma kinase (ALK) gene is one of the most important issues in targeted therapy. The ALK gene has been described through chromosomal rearrangements, resulting in the placement of different 5' fusion partners and their associated promoter region upstream of the kinase domain of ALK (Karachaliou and Rosell, 2013). ALK was originally identified in anaplastic large cell lymphoma as a fusion protein to nucleophosmin. (Karachaliou and Rosell, 2013) Echinoderm microtubule-associated protein like 4 (EML4)-ALK was the first targetable fusion oncokinase to be identified in 2 to 13% of NSCLC patients (Soda et al., 2007; Koivunen et al., 2008; Mano, 2008; Takeuchi et al., 2008; Shaw et al.,

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2009; Wong et al., 2009; Takahashi et al., 2010; Paik et al., 2012; Shaw and Engelman, 2013).

ALK-rearrangement in lung cancer is a unique NSCLC category that is characterized by ALK gene inversion or translocation. ALK-rearranged lung cancer has been considered as a striking response to treatment with a small-molecule inhibitor of ALK(Shaw and Solomon, 2011). However the incidence of NSLCL patients who have EML4-ALK fusion is very low (Kim et al., 2011; Paik et al., 2011).

There are three different methods to determine the ALK status: detection of the protein overexpression by immunohistochemistry (IHC), gene rearrangement by in situ hybridization(ISH), (Kim et al., 2011) and reverse transcription-polymerase chain reaction analysis (RT-PCT) (Marchetti et al., 2013). The IHC method is more difficult than ISH in performing a more reliable quantification of the genomic alteration. FISH has been regarded as most reliable method for detecting ALK rearrangement, however the fluorescent signal rapidly fades over time. Consequently, FISH is not routinely done in clinical practice. Furthermore, it is difficult to detect the overall morphology and tumor heterogeneity (Yoo et al., 2010). RT-PCR also has many disadvantages in its clinical application practice (Marchetti et al., 2013). In the past, using detection of ALK rearrangement was controversial. Some studies have addressed the discordance between FISH and IHC assay (Boland et al., 2009). Kim et al. (2011) observed a good correlation between results obtained using IHC and FISH in a large-scale, single-institution study. Recently many studies reported the efficacy of IHC for detecting ALK rearrangement. Sholl et al. (2013) reported that the ALK IHC using the clone 5A4 was 93% sensitive and 100% specific as compared with FISH using the Vysis ALK Break Apart FISH Probe Kit. To et al. (2013) demonstrated that IHC can effectively detect ALK rearrangement in lung cancer and might provide a reliable and cost-effective diagnostic approach in routine pathologic laboratories for the identification of suitable candidates for ALK-targeted therapy. Studying ALK rearrangement has been intensively studied. The correlation between clinicopathologic features and prognostic implications of anaplastic lymphoma kinase (ALK) gene rearrangement in non-small cell lung cancer (NSCLC) has not been concluded. Thailand has a high prevalence of NSCLC, therefore the Northern Thailand Thoracic Oncology Group (NT-TOG) would like to identify the incidence of ALK rearrangement and prognostic factors for ALK positive in completely resected NSCLC in Northern Thailand. Furthermore, we attempt to identify the diagnostic role of IHC comparing with FISH method.

## Materials and Methods

*Case enrollment*

We reviewed the clinical characteristics and histopathological specimens from patients diagnosed with NSCLC who underwent completely resected anatomical resection with systematic mediastinal lymphadenectomy at Maharaj Nakorn Chiang Mai Hospital (Department of Surgery, Faculty of Medicine, Chiang Mai University), Chiang Mai, Thailand from from January 2008 to December 2012. Patients who did not receive a curative resection and had a previous history of other cancers, pre-surgical chemotherapy or radiotherapy were excluded from this study. Formalin-fixed, paraffin-embedded (FFPE) tissue sections were examined from 267 patients. Clinicopathologic information was reviewed from the patient medical recording system. Histopathologic examination was performed by the same highly experienced pathologist. Pathologic staging was determined according to the IASLC TNM staging classification of NSCLC (Goldstraw, 2009). Histologic subtypes of lung cancer were determined according to World Health Organization classification (Travis, 2004) and International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) International Multidisciplinary Classification of Lung Adenocarcinoma (Travis et al., 2013). Visceral pleural invasion (VPI), intratumoral blood vessel invasion (IVI), intratumoral lymphatic invasion (ILI), neural invasion (NI) were defined as previously described (Tantraworasin et al., 2013). Overall survival was measured from the date of complete resection of lung cancer until the time of death, and disease-free survival was measured from the date of surgery until recurrence or death. Patients with an unknown date of death or recurrence were censored at the time of the last follow up. Disease-free and overall survival rates were compared according to ALK rearrangement. Patients were divided into two groups, ALK positive group and ALK negative group. This study was reviewed and approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

*Construction of the tissue microarray*

Representative core tissue sections were taken from formalin-fixed, paraffin-embedded (FFPE) blocks and arranged in new recipient tissue microarray paraffin blocks, as previously described (Yoo et al., 2010; Ozdemir et al., 2013). Serial sections were cut and fixed with hematoxylin and eosin staining, IHC and FISH was performed. Figure 1 demonstrated the slide of tissue microarray.

*Immunohistochemistry*

FFPE tissues were sectioned at a thickness of four micrometers and stained with the antibody for ALK (mouse monoclonal, D5F3 antibody (Ventana) using a

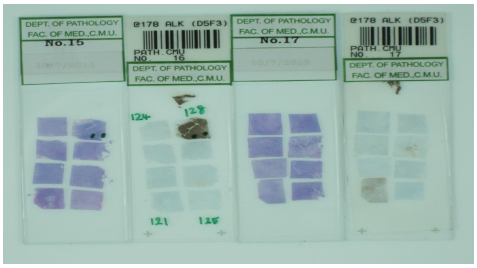


**Figure 1. Slide of Tissue Microarray with ALK Positive in IHC (Dark-Brown Color)**

Ventana automated immunostainer (Ventana Medical Systems, Tucson, AS) by optiview detection system as previously described (Paik et al., 2011). The results from this method were reported as positive or negative. Positive result referred to strongly positive cell staining (dark-brown color) as shown in Figure 1 and negative result referred to none, mild or moderate positive cell staining. Fluorescence *in situ* hybridization (FISH).

FISH was performed on the FFPE tumor tissues using a break-apart probe specific to the ALK locus (Vysis LSI ALK Dual Color, break-apart rearrangement probe; Abbott Molecular, Abbott Park, IL) according to the manufacturer's instructions. FISH positive is defined as those presenting more than 15% split signals or an isolated red signal (IRS) in tumor cells (Kim et al., 2011) as shown in Figure 3. Dual-probe hybridization was performed with a three micrometer-thick FFPE sections using the LSI ALK Dual Color Probe, which hybridizes to the 2p23 band with spectrum Orange (red) and Spectrum Green on either side of the ALK gene breakpoint (Abbott Molecule). The 4, 6-diamidino-2-phenylindole (DAPI) II are applied for the nuclei counterstaining. The signals for each probe were evaluated under a microscope equipped with a triple-pass filter (DAPI/Green/Orange; Abbott Molecular) and an oil immersion objective lens. The FISH tests were performed with unknown results of the IHC for ALK (Paik et al., 2012).

*Statistical analysis*

The data were analyzed using the Stata statistical package (Release 11, 2011; Stata Corporation, College Station, TX). Sensitivity, specificity, accuracy, positive predictive value, negative predictive value and likelihood ratio of positive IHC comparing with FISH were calculated. Continuous data were presented as mean and standard deviation or median with 50$^{th}$ percentiles according to data distribution and were analyzed using student t-test or wilcoxon rank-sum test. Categorical data were presented as frequency and percent and were analyzed using the Fisher-exact test. Univariable and multivariable risk regression analysis were used for controlling confounding factors and for identifying risk factor of ALK positive. Cut-off points of some variables such as age were determined by the maximal likelihood method for achieving the best discrimination between patients with ALK-Fish negative and ALK-FISH positive. Any risk factors with p values <0.1 in the univariable analyses and other potential clinical confounders associated with ALK-positive were selected to be included in the multivariable analysis. The Cox proportional hazards model was used to estimate the hazard ratio (HR) for disease-free and overall survival in patients with ALK positive. All the p values are based on a two-sided hypothesis. The p value less than 0.05 was considered significant.

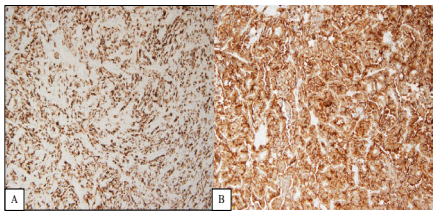


**Figure 2. Poorly Differentiated Adenocarcinoma with ALK Positive by IHC (A). Micropapillary Type of Invasive Adenocarcinoma with ALK Strongly Positive by IHC (B)**

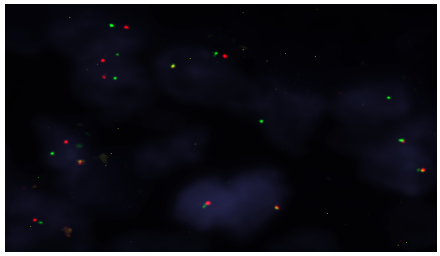

**Figure 3. FISH Positive is Defined as those Presenting more than 15% Split Signals or an Isolated Red Signal (IRS) in Tumor Cells**

## Results

In this study, 267 completely resected NSCLC were included. The patients composed of 156 (58.4%) men and 111 (41.6%) women. The histology was adenocarcinoma in 165 (61.8%) patients, squamous cell carcinoma in 72 (27.0%) patients, and other NSCLC in 30 (11.2%) patients. The pathologic stage was I in 37 (13.9%) patients, II in 47 (17.6%) patients, and III in 45 (16.9%) patients. At the time of the analysis, the mean follow-up time was 32.4 months. Fifty five percent (146/267) of the total NSCLC patients were still alive at the time of the analysis. The result of both methods for ALK analysis was shown in Table 1. Twenty-two (8.2%) of 267 specimens were IHC-positive of ALK with intense cytoplasmic staining, whereas, 10 (3.8%) of 267 specimens were FISH-positive of ALK. Sensitivity, specificity, accuracy, positive predictive value, negative predictive value and positive likelihood ratio of IHC were 80.0%, 94.9%, 87.5%, 38.1%, 99.2% and 15.8 respectively as shown in Table 2.

Clinicopathological data and treatment outcomes comparing between ALK-FISH positive (AP) group and ALK-FISH negative (AN) group was shown in Table 3-6. The mean age of the AP group was significantly lower than those in the AN group (51.8 versus 62.7, p value <0.001) especially, when using 55 years as a cut-off point

**Table 1. The Results of Both Tests**

| IHC | FISH | | |
|---|---|---|---|
| | 0 | 1 | Total |
| 0 | 244 | 2 | 246 |
| 1 | 13 | 8 | 21 |
| Total | 257 | 10 | 267 |

**Table 2. Diagnostic Test of IHC**

| IHC | % | 95%CI |
|---|---|---|
| Sensitivity | 80 | 75.20-84.80 |
| Specificity | 94.94 | 92.31-97.57 |
| Accuracy | 87.47 | 74.34-100.00 |
| Positive predictive value | 38.1 | 32.27-43.92 |
| Negative predictive value | 99.19 | 98.11-100.26 |
| Likelihood ratio positive | 15.82 | |

**Table 3. Patient Characteristics between the Two Groups**

| Characteristics | ALK-4 Positive (n=10) 3.8% | ALK-4 Negative (n=257) 96.2% | p value |
|---|---|---|---|
| Age, Mean±SD | 51.8±5.9 | 62.7±10.2 | <0.001 |
| ≤55 years | 8 (80.0) | 57 (22.2) | <0.001 |
| >55 years | 2 (20.0) | 200 (77.8) | |
| Gender | | | 0.099 |
| Female | 7 (70.0) | 104 (40.7) | |
| Male | 3 (30.0) | 153 (59.5) | |
| Smoking | | | 0.085 |
| Never smoked | 4 (40.0) | 60 (23.3) | |
| Stopped smoking | 4 (40.0) | 179 (69.6) | |
| Active smoking | 2 (20.0) | 14 (5.4) | |
| Passive smoker | 0 | 4 (1.6) | |
| Family history of lung cancer | 3 (30.0) | 15 (5.8) | 0.023 |
| Underlying disease | | | |
| Chronic lung disease | 0 | 42 (16.3) | 0.371 |
| Diabetic mellitus | 0 | 30 (11.7) | 0.609 |
| Essential hypertension | 1 (10.0) | 91 (35.4) | 0.172 |
| Dyslipidemia | 1 (10.0) | 36 (14.0) | 1.000 |
| Symptoms | | | |
| Hemoptysis | 6 (60.0) | 93 (36.2) | 0.181 |
| Chronic cough | 5 (50.0) | 109 (42.4) | 0.748 |
| Poor appetite | 1 (10.0) | 32 (12.5) | 1.000 |
| Significant weight loss | 1 (10.0) | 69 (26.9) | 0.463 |
| Chest pain | 1 (10.0) | 22 (8.6) | 0.600 |
| Dyspnea | 1 (10.0) | 44 (17.1) | 1.000 |
| Asymptomatic | 3 (30.0) | 94 (36.6) | 0.752 |
| Present with brain metastasis | 0 | 6 (2.3) | 1.000 |
| Treatment modalities | | | |
| Surgical procedures | | | 0.199 |
| Lobectomy | 8 (80.0) | 225 (87.5) | |
| Bilobectomy (RUL and RML) | 0 | 7 (2.7) | |
| Bilobectomy (RLL and RML) | 1 (10.0) | 21 (8.2) | |
| Pneumonectomy | 1 (10.0) | 4 (1.6) | |
| Chemotherapy | | | 0.296 |
| No chemotherapy | 3 (30.0) | 133 (51.8) | |
| Adjuvant chemotherapy | 7 (70.0) | 111 (43.2) | |
| Neoadjuvant chemotherapy | 0 | 13 (5.0) | |

**Table 4. Histopathologic Reports**

| Covariates | ALK-4 Positive n (%) | ALK-4 Negative n (%) | p value |
|---|---|---|---|
| Histologic types | | | 0.159 |
| Adenocarcinoma | 5 (50.0) | 160 (62.3) | |
| Squamous cell carcinoma | 2 (20.0) | 70 (27.2) | |
| Others* | 3 (30.0) | 27 (10.5) | |
| Tumor grading | | | 0.584 |
| Well differentiated | 3 (30.0) | 84 (33.9) | |
| Moderately differentiated | 5 (50.0) | 109 (43.9) | |
| Poorly differentiated | 1 (10.0) | 45 (18.2) | |
| Undifferentiated | 1 (10.0) | 10 (4.0) | |
| Pathological staging | | | 0.431 |
| IA | 0 | 37 (14.4) | |
| IB | 2 (20.0) | 45 (17.5) | |
| IIA | 1 (10.0) | 44 (17.1) | |
| IIB | 2 (20.0) | 34 (13.2) | |
| IIIA | 4 (40.0) | 73 (28.4) | |
| IIIB | 1 (10.0) | 4 (1.6) | |
| IV | 0 | 20 (7.8) | |
| Maximal tumor diameter(cm) | 5.0±1.8 | 4.9±2.6 | 0.872 |
| Nodal involvement | | | 0.225 |
| Node negative | 4 (40.0) | 148 (57.6) | |
| N 1 group | 1 (10.0) | 43 (16.7) | |
| N 2 group | 5 (50.0) | 66 (25.7) | |
| Tumor necrosis | 1 (10.0) | 110 (42.8) | 0.049 |
| Visceral pleural invasion | 4 (40.0) | 53 (20.6) | 0.228 |
| Neural invasion | 1 (10.0) | 10 (3.9) | 0.348 |
| Intratumoral lymphatic invasion | 8 (80.0) | 217 (84.4) | 0.660 |
| Intratumoral blood vessel invasion | 4 (40.0) | 110 (42.8) | 1.000 |
| High ERCC1 expression | 3 (30.0) | 95 (36.9) | 0.750 |
| High RRM1 expression | 3 (30.0) | 97 (37.7) | 0.748 |
| EGFR mutation | | | 0.343 |
| Axon 19 deletion | 0 | 18 (18.0) | |
| L858R | 0 | 17 (17.0) | |
| T790M | 0 | 1 (1.0) | |
| Wild type | 6 | 64 (64.0) | |

*Other cell types include Adenocarcinoma *in situ*, large cell carcinoma, neuroendocrine tumor, adenoid cystic carcinoma, metastasis, mucoepidermoid carcinoma, lymphoepithelioma-like carcinoma, adenosquamous cell carcinoma; #Nodal positive refer to presenting of malignant cell in any node level (1-14)

**Table 5. Outcomes of Treatment**

| Variables | ALK-4 Positive n (%) | ALK-4 Negative n (%) | p value |
|---|---|---|---|
| Tumor recurrence | 7 (70.0) | 133 (51.8) | 0.340 |
| Overall mortality | 3 (30.0) | 118 (45.9) | 0.520 |
| First site of recurrence | | | 0.560 |
| Lung | 3 (42.9) | 49 (36.8) | |
| Pleura | 0 | 4 (3.0) | |
| Bone | 2 (28.6) | 13 (9.8) | |
| Brain | 0 | 26 (19.6) | |
| Liver | 0 | 2 (1.5) | |
| Others | 2 (28.6) | 28 (21.1) | |
| Number of recurrent sites | | | 0.146 |
| Single site | 5 (71.4) | 121 (91.0) | |
| Multiple site | 2 (28.6) | 12 (9.0) | |

(p value <0.001). Thirty percent of patients in the AP group (three of 10, one of first degree relative and two of second degree relative) had a family history of lung cancer whereas only 5.8 percent in the AN group (p=0.023). There were no statistically significant differences in other patient characteristics, histopathologic data, treatment modalities, and clinical outcomes including overall survival and tumor recurrence between both groups as shown in Table 3-5. Visceral pleural invasion presented higher in the ALK-positive patients. The median follow up time of this cohort was 22.4 months. The mean overall survival was 44.7 months for the ALK-negative patients versus 41.8 months for the ALK-positive patient cases (p=0.754). The mean disease-free survival was 37.7 months in the ALK-negative patients *vs* 37.2 months in the ALK positive patients (p=0.954). No one who had EGFR mutation had ALK rearrangement. Univariable risk regression analysis identified that age less than 55 years (Risk ratio (RR) of 12.4, 95% confidence interval (CI) of 2.71-57.07, p value of <0.001), family history of lung cancer (RR of 5.9, 95%CI of 1.67-21.01, p value of 0.006) and multiple sites metastasis (RR of 4.5, 95%CI of 1.06-19.32, p value of 0.042) were risk factors for ALK-positivity as shown in Table 6. Multivariable risk regression analysis demonstrated that age less than 55 years (RR of 9.4, 95%CI of 2.07-42.58, p value of 0.004) and family history of lung cancer (RR of 7.9, 95%CI of 2.12-29.41, p value of 0.002) were significant risk factors for ALK-positivity as shown in Table 6.

A multivariable analysis using a Cox proportional hazards model compared overall survival and tumor recurrence between ALK-positive and -negative patients. After adjusting for nodal involvement and tumor staging, ALK-positivity was not associated with overall survival

**Table 6. Univariable & Mutivariable Risk Ratio (RR) and 95% Confidence Interval (CI) of ALK-Positivity for Parameters with Clinical and Statistical Significance**

| | Risk ratio | 95%CI | p value |
|---|---|---|---|
| Univariable risk ratio (RR) | | | |
| Age <55 years | 12.4 | 2.71-57.07 | 0.001 |
| Women | 3.3 | 0.87-12.40 | 0.080 |
| Family history of lung cancer | 5.9 | 1.67-21.01 | 0.006 |
| Never smoking | 2.1 | 0.62-7.26 | 0.234 |
| Staging of lung cancer | 1.2 | 0.83-1.65 | 0.372 |
| Adenocarcinoma | 0.3 | 0.08-1.20 | 0.089 |
| Visceral pleural invasion | 2.5 | 0.72-8.41 | 0.152 |
| Intratumoral vascular invasion | 0.9 | 0.26-3.10 | 0.861 |
| Intratumoral lymphatic invasion | 0.7 | 0.16-3.39 | 0.705 |
| Tumor recurrence | 2.1 | 0.56-8.01 | 0.270 |
| Multiple sites recurrence | 4.5 | 1.06-19.32 | 0.042 |
| Overall mortality | 0.5 | 0.14-1.96 | 0.331 |
| Bone metastasis | 2.4 | 0.54-10.78 | 0.246 |
| Brain metastasis | 0.6 | 0.08-4.45 | 0.599 |
| High ERCC1 expression | 0.7 | 0.20-2.79 | 0.656 |
| High RRM1 expression | 0.7 | 0.19-2.71 | 0.622 |
| Failed first-line chemotherapy | 3.6 | 1.05-12.12 | 0.041 |
| Mutivariable risk ratio (RR) | | | |
| Age <55 years | 9.4 | 2.07-42.58 | 0.004 |
| Female | 2.3 | 0.62-8.22 | 0.216 |
| Family history of lung cancer | 7.9 | 2.12-29.41 | 0.002 |
| Multiple sites recurrence | 2 | 0.41-9.62 | 0.389 |
| Failed first-line chemotherapy | 3.1 | 0.73-13.47 | 0.126 |

**Table 7. Multivariable Hazard Ratios (HR) and 95% Confidence Interval (CI)**

| Covariates | Univariable analysis | | Multivariable analysis | |
|---|---|---|---|---|
| | HR (95%CI) | p value | HR (95%CI) | p value |
| Overall survival, adj. for nodal involvement and stage of disease | | | | |
| Age | | | | |
| ≤70 | Reference | | Reference | |
| >70 | 1.5 (1.00-2.28) | 0.047 | 1.6 (1.02-2.48) | 0.041 |
| Gender | | | | |
| Female | Reference | | Reference | |
| Male | 1.7 (1.13-2.42) | 0.010 | 1.2 (0.75-1.78) | 0.508 |
| Smoking status | | | | |
| Never smoked | Reference | | Reference | |
| Stopped smoking | 2.6 (1.53-4.43) | <0.001 | 2.2 (1.17-4.00) | 0.014 |
| Active smokers | 2.9 (1.26-6.47) | 0.012 | 3.0 (1.13-7.78) | 0.028 |
| ALK rearrangement | | | | |
| Negative | Reference | | Reference | |
| Positive | 0.8 (0.24- 2.37) | 0.629 | 0.7 (0.19-2.25) | 0.504 |
| Tumor recurrence, adj. for nodal involvement and stage of disease | | | | |
| Age | | | | |
| ≤70 | Reference | | Reference | |
| >70 | 1.1 (0.71-1.61) | 0.755 | 1.1 (0.69-1.65) | 0.784 |
| Gender | | | | |
| Female | Reference | | Reference | |
| Male | 1.0 (0.74-1.45) | 0.845 | 1.0 (0.67-1.47) | 0.965 |
| Smoking status | | | | |
| Never smoked | Reference | | Reference | |
| Stopped smoking | 1.3 (0.88-1.97) | 0.175 | 1.4 (0.89-2.28) | 0.141 |
| Active smokers | 1.4 (0.63-2.98) | 0.421 | 1.1 (0.44-2.86) | 0.809 |
| ALK rearrangement | | | | |
| Negative | Reference | | Reference | |
| Positive | 2.2 (1.02-4.69) | 0.045 | 3.2 (1.30-8.11) | 0.012 |

(HR of 0.8, 95%CI of 0.24-2.37, p value of 0.504), however, it was a significantly adverse prognostic factor of tumor recurrence (HR of 3.2 95%CI of 1.30-8.11, p value of 0.012) as shown in Table 7. These results suggest that ALK rearrangement may be a prognostic factor of tumor recurrence in completely resected NSCLC of any stage.

## Discussion

The main results of our study were as follows: 1) IHC with mouse monoclonal, clone 5A4, Ventana D5F3 antibody can be used for screening ALK rearrangement before using FISH to confirm diagnosis because of high specificity, high negative predictive value and high likelihood ratio (LR) of positivity; 2) Completely resected NSCLC patients with age less than 55 years had higher risk for ALK rearrangement comparing to those with age more than 55 years; 3) Completely resected NSCLC patients with family history of lung cancer had higher risk for ALK rearrangement comparing to those without VPI; 4) ALK rearrangement was not prognostically significant for overall survival but affected the tumor recurrence in completely resected NSCLC of any stage.

A gold standard to determine ALK rearrangement has not been concluded. Recently, there are two well established methods for diagnostic analyses in clinical practice, IHC and FISH (Savic et al., 2008). The advantage of the FISH method is an availability of a validated kit with standard procedures such as Abbott Vysis (ALK Break Apart FISH Probe Kit, Abbott Molecular Inc., certificated by FDA) and reliable for use in clinical trials, (Yi et al., 2011; Marchetti et al., 2013) however it is still technically challenging and costly. IHC is easily used and costly but lacks dedicated kits and standard procedures. Minca et al. (2013) reported that IHC with D5F3 antibody demonstrated 100% sensitivity and specificity (95%CI, 0.86 to 1.00 and 0.97 to 1.00, respectively) for ALK detection on 249 specimens. Although IHC in our study did not show a 100% sensitivity and specificity and low positive predictive value, the negative predictive value and likelihood ratio of IHC positive are very high (99.1% and 15.82 respectively). Therefore, IHC with D5F3 is a valuable screening tool before testing with FISH method.

Paik et al. (2012) studied 735 completely resected NSCLC patients. They found that ALK rearrangement was not prognostically significant for disease-free survival or overall survival; their results are the same as our studies. However, their studies found some different results. They reported ALK-rearranged lung cancer showed a lower tumor stage (T1) in NSCLC (p=0.020), whereas it tended to harbor lymph node metastasis in adenocarcinoma (p=0.090). Furthermore, they found that ALK rearrangement was more frequently observed in women, adenocarcinoma, and those who never smoked in surgically resectable NSCLC patients, but no gender difference was observed in the adenocarcinoma or in the subgroup that never-smoked. Our study found that ALK rearrangement is a prognostic factor of tumor recurrence in completely resected NSCLC, which has not been previously reported. Adjuvant chemotherapy may be beneficial in this setting.

Our study found that patients with ALK rearrangement were significantly younger, like a previous report (Zhong et al., 2013). Furthermore, many recent studies found that ALK rearrangements are more likely of significance in young women (Paik et al., 2012; Li et al., 2013; Zhong et al., 2013). Shaw et al. (2009) found that patients with ALK rearrangement were more likely to be men (p=0.039),

contrary to our results. Previously, many studies found that ALK rearrangement had been variably detected in both smokers and nonsmokers and suggested a lack of association between smoking history and presence of ALK rearrangement (Rikova et al., 2007; Koivunen et al., 2008; Shinmura et al., 2008). However in recent studies, they found that ALK rearrangement is strongly associated with never/light smoking history (Paik et al., 2012; Conde et al., 2013; Li et al., 2013; Zhong et al., 2013). Besides young women and non or light smokers, adenocarcinoma is also significantly higher in ALK rearrangement patients (Paik et al., 2012; Conde et al., 2013; Li et al., 2013; Martinez et al., 2013) like our study, adenocarcinoma was predominantly found in 63.6% of ALK rearrangement patients. Our study found that a family history of lung cancer is a risk factor of ALK rearrangement which was not previously reported. Therefore, ALK rearrangement may have a genetic heredity.

In conclusion, the incidence of ALK positive in completely resected NSCLC in Northern Thailand is 8.2%by the IHC method and 3.8% by the FISH method. IHC with clone D5F3 antibody can be used as screening tool. Age less than 55 years and family history of lung cancer are risk factors of ALK-FISH positive. Moreover, ALK rearrangement is a prognostic factor of tumor recurrence in completely resected NSCLC of any stage.

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