Peer review file

Article information: http://dx.doi.org/10.21037/jtd-20-1716

Reviewer A

Comment 1: If possible, I would like authors to add recurrence pattern (local recurrence or distant metastases, metastasis organ, etc).

Reply 1: Thank you for the important suggestion. During the follow-up period, distant metastasis was detected more frequently in patients with ALK IHC positivity, compared to those with other genomic alterations.

Changes in the text: We have added the following sentences in the Results section (Baseline characteristics; see page 10, line 195-199); "Recurrences developed in 32 patients (19.5%) during the follow-up period. Among these patients, 2 patients (1.2%) had only locoregional relapse; 26 patients (15.9%) had distant metastasis alone; and the remainder (4 patients, 2.4%) had both locoregional relapse and distant metastasis."

We have modified the Abstract (Results; see page 4, line 62-64) as follows;

Before: ALK IHC positivity was associated with longer maximal diameter, advanced stage, solid pattern on radiological examination, and solid predominant histologic subtype.

After: ALK IHC positivity was associated with longer maximal diameter, advanced stage, solid pattern on radiological examination, solid predominant histologic subtype, and distant metastasis during follow-up.

We have modified the Results section (Analysis of clinicoradiological and pathological features in relation to genomic alterations; see page 11, line 213-216) as follows;

Before: ALK IHC positivity was associated with longer maximal diameter (P=0.012), solid

lesions on radiological examination (P<0.001), advanced pathologic stage (P=0.009), and solid predominant subtype on histological analysis (P<0.001).

After: ALK IHC positivity was associated with longer maximal diameter (P=0.012), solid lesions on radiological examination (P<0.001), advanced pathologic stage (P=0.009), solid predominant subtype on histological analysis (P<0.001), and distant metastasis during follow-up (P<0.001).

We have modified the Discussion section (see page 12, line 244-246) as follows;

Before: ALK IHC positivity was detected more frequently in patients with large or advanced stage tumors, solid lesions on radiological examination, and solid predominant subtype on histological analysis.

After: ALK IHC positivity was detected more frequently in patients with large or advanced stage tumors, solid lesions on radiological examination, solid predominant subtype on histological analysis, and distant metastasis during follow-up.

We have modified the Discussion section (see page 15, line 303-306) as follows;

Before: We also demonstrated that ALK IHC positivity was independently associated with poor disease-free survival. Even in patients with stage IA or IB disease, recurrence occurred in 50% (2/4) of those positive for ALK IHC.

After: We also demonstrated that ALK IHC positivity was independently associated with poor disease-free survival. ALK IHC positivity was associated with more distant metastasis during follow-up (*Table 3*). Even in patients with stage IA or IB disease, recurrence occurred in 50% (2/4) of those positive for ALK IHC.

We have added the following data in Table 1 and Table 3.

Revised Table 1. Baseline characteristics

Variables	***n* (%)**
Recurrence	32 (19.5)
Locoregional relapse	6 (3.7)
Ipsilateral lung	1 (0.6)
Regional lymph node	5 (3.0)
Distant metastasis	30 (18.3)
Contralateral lung	13 (7.9)
Pleura	7 (4.3)
Brain	7 (4.3)
Bone	6 (3.7)
Liver	1 (0.6)
Kidney	1 (0.6)

Revised Table 3. Clinicoradiopathological features according to genomic alterations

Variables	**Wild type (n = 49)**	**EGFR Mutation positive (n = 95)**	**ALK IHC positive (n = 9)**	**KRAS Mutation positive (n = 11)**	***P-value***
Recurrence					< 0.001
Locoregional relapse	0 (0.0)	0 (0.0)	2 (22.2)	0 (0.0)	
Distant metastasis	3 (6.1)	16 (16.8)	5 (55.6)	2 (18.2)	
Locoregional relapse and distant metastasis	0 (0.0)	4 (4.2)	0 (0.0)	0 (0.0)	

Reviewer B

Although the relationship between the gene mutation status and imaging findings is controversial, the proposal of prognosis prediction by gene mutation status in patients receiving surgical resection for lung cancer is interesting suggestion. However, I would like to suggest the reconsideration for statistical analysis.

Comment 1: Authors showed that ALK gene mutation is associated with relapse free survival. However, ALK gene mutation is also associated with diameter and solidity of tumor, which are considered predicter for patient prognosis. Therefore, if the relationship between ALK gene mutation and relapse free survival is due to diameter and solidity of tumor, the significance and novelty of the result seems to be unclear.

Reply 1: We also understand the reviewer #2's concern. In this study, ALK IHC positivity was associated with longer maximal diameter (P=0.012), solid lesions on radiological examination (P<0.001), advanced pathologic stage (P=0.009), and solid predominant subtype on histological analysis (P<0.001). As the reviewer #2 pointed out, the size of the tumor, solidity, pathologic stage, and histology subtype could also influence on the recurrence-free survival. Therefore, we performed the multivariable analysis to identify independent prognostic factors for recurrence-free survival after adjusting for the effects of these variables. Consequently, ALK IHC positivity was independently associated with poor recurrence-free survival on multivariable analysis. As we have already described in the Discussion section, even in patients with stage IA or IB disease, recurrence occurred in 50% (2/4) of those positive for ALK IHC. Therefore, we think that the poor prognosis of ALK IHC positivity in this study is a significant finding.

Changes in the text: NA

Comment 2: In addition, authors explained that variables with P value of less than 0.05 in univariate analysis were selected as independent variables in multivariate analysis (" Univariable and multivariable analyses with Cox proportional-hazards regression model were carried out using variables with P<0.05 on univariable analysis to identify risk factors for recurrence after surgery"). However, Solidity, Histologic subtype, and T factor (P value was less than 0.05 in these factors) were not selected as independent variables in multivariate analysis. I think the association between ALK gene mutation and relapse free survival should be analyzed adjusting for these variables including Solidity, Histologic subtype, and T factor.

Reply 2: Thank you for the important comment. In this study, to identify risk factors for recurrence-free survival after adjusting for the effects of the possible confounders, multivariable Cox proportional-hazards regression analysis with stepwise selection model was performed using variables with P<0.10 on univariable analysis (solidity, ALK IHC, histologic subtype, pathologic T stage, and pathologic N stage) and clinically relevant variables (age, sex, and smoking status). However, the administration of adjuvant chemotherapy could be related to advanced stage of the disease and it was excluded from the multivariable analysis to avoid collinearity. During stepwise variable selection, variables with P<0.10 were entered and those with P≥0.05 were removed (1). As a result, ALK IHC positivity and nodal involvement were identified as independent risk factors for recurrence after surgery.

Changes in the text: We have modified the Methods section (Statistical analysis; see page 9, line 170-175) to clarify this point as follows;

Before: "Univariable and multivariable analyses with Cox proportional-hazards regression model were carried out using variables with P<0.05 on univariable analysis to identify risk factors for recurrence after surgery."

After: "Multivariable analyses with Cox proportional-hazards regression and stepwise

selection models were carried out using variables with P<0.10 on univariable analysis and clinically relevant variables (age, sex, and smoking status) to identify independent risk factors for recurrence after surgery. During stepwise variable selection, variables with P<0.10 on univariable analysis and clinically relevant variables were entered and those with P≥0.05 were removed (1).”

Reference:

1. Collett D. Modelling survival data in medical research: CRC press; 2015.

Comment 3: Authors should present how long they observed patients (median follow up duration) and how many patients showed relapse after receiving surgery in Baseline characteristics in Results.

Reply 3: Thank you for the comment.

Changes in the text: We have added the information about the follow-up duration and recurrence in Table 1 as follows;

Revised Table 1. Baseline characteristics

Variables	***n* (%) or median (IQR)**
Follow-up duration after surgery, months	50.0 (29.5-53.0)
Recurrence	32 (19.5)
Locoregional relapse	6 (3.7)
Ipsilateral lung	1 (0.6)
Regional lymph node	5 (3.0)
Distant metastasis	30 (18.3)
Contralateral lung	13 (7.9)
Pleura	7 (4.3)

Brain	7 (4.3)
Bone	6 (3.7)
Liver	1 (0.6)
Kidney	1 (0.6)

We have also added the following sentences in the Results section (Baseline characteristics; see page 10, line 195-199); “The median follow-up period after surgery was 50.0 (IQR 29.5-53.0) months. Recurrences developed in 32 patients (19.5%) during the follow-up period. Among these patients, 2 patients (1.2%) had only locoregional relapse; 26 patients (15.9%) had distant metastasis alone; and the remainder (4 patients, 2.4%) had both locoregional relapse and distant metastasis (*Table 1*).”

Reviewer C

The authors assess the impact of clinicoradiopathological features on the prognosis of pts with resected adenocarcinoma. The study is retrospective. Overall, the interesting part is about the prognostic relevance of ALK status at multivariate analysis.

Comment 1: In the abstract I would add that solidity was not independently associated with prognosis (in the tile there's "radio"). Why in the conclusion the authors state that solidity help predict the prognosis (not significant at multivariate analysis).

Reply 1: We have modified the manuscript as Reviewer C recommended.

Changes in the text: We have added the following sentence in the Abstract (Results; see page 4, line 68); “However, solidity was not an independent risk factor for recurrence.”

We have modified the Abstract (Conclusions; see page 4, line 69-71) as follows;

Before: “Radiological features are associated with genomic alterations in patients with resected lung adenocarcinoma. Genomic alterations and solidity of the lesions could help to

predict the prognosis of early lung adenocarcinoma."

After: "Genomic alterations are associated with clinicoradiopathologic features in patients with resected lung adenocarcinoma. Identifying genomic alterations could help to predict the prognosis of early-stage lung adenocarcinoma."

We have modified the Conclusions section (see page 15-16, line 324-326) as follows;

Before: "Radiological features are associated with genomic alterations in patients with resected early- lung adenocarcinoma. Genomic alterations and solidity of the lesions could help in predicting the prognosis of early-stage lung adenocarcinoma."

After: "Genomic alterations are associated with clinicoradiopathologic features in patients with resected lung adenocarcinoma. Identifying genomic alterations could help to predict the prognosis of early-stage lung adenocarcinoma."

Comment 2: Methods, patient selection and clinical assessment: was surgery radical meaning negative margins in all cases? was lymphnode sampling always performed? please specify both.

Reply 2: Thank you for your comment. In this study, all study patients who underwent surgical treatment achieved R0 resection and more than 95% of study patients received lymph node dissection or sampling.

Changes in the text: We have added this information in the Results section (Baseline characteristics; see page 10, line 190-192) and Table 1 as follows; "All patients included in this study achieved R0 resection and 158 patients (96.3%) received lymph node dissection or sampling."

Revised Table 1. Baseline characteristics

Variables	*n* (%)
Surgery	
Extent of resection	
Wedge resection	18 (11.0)
Segmentectomy	24 (14.6)
Lobectomy	122 (74.4)
Mediastinal lymph node dissection or sampling*	158 (96.3)
R0 resection	164 (100.0)

*Mediastinal lymph node dissection was performed in 145 patients (88.4%).

Comment 3: Methods, EGFR and KRAS mutations and ALK IHC: what algorithm did the authors use for ALK IHC? Pleae specify the reported staining to confirm ALK positivity. I understood that "strong" is 2+ or 3+. However current algorithms suggest that while no further testing is required for 3+, 2+ is a kind of grey zone where additional tests (FISH or NGS) are required in order to confirm ALK-positivity. Can the authors support the algorithm they used (2+ automatically positive) with any reference? see also the paragraph genomic alterations.

Reply 3: Thank you for your valuable comment. During the study period (2009-2016), ALK IHC assay using clone 5A4 antibody was performed for a resected lung adenocarcinoma as a screening test. *ALK* break-apart FISH was performed as a confirmation test when the recurrence of the disease developed and the administration of ALK-TKI was considered. Therefore, in this study, 7 of 9 ALK IHC-positive cases (2+ or 3+) were further evaluated by *ALK* FISH at the time of recurrence. Of 7 cases examined, 6 cases were positive for *ALK* FISH (>15% rearranged signals). However, one case positive for ALK IHC (3+) was negative for *ALK* FISH. In 2016, Korean National Health Insurance did not reimburse the use of ALK-TKI in the situation where *ALK* FISH was not confirmed. The patient received

cytotoxic chemotherapy instead of ALK-TKI owing to this financial issue. Unfortunately, the patient expired due to the progression of disease. Recent studies revealed that ALK IHC was better predictor for ALK inhibition than *ALK* FISH (1-3). In the previous studies, *ALK* discordant (IHC-positive / FISH-negative) cases showed *ALK* gene amplification (2) and 13 (100%) of 13 discordant (IHC-positive / FISH-negative) cases responded to ALK-TKI (3). Therefore, the discrepancies between IHC and FISH data seem to be associated with biological events rather technical issues. In that reason, recent NCCN guidelines also acknowledged FDA-approved ALK IHC (D5F3 CDx Assay) as a stand-alone test, not requiring confirmation by FISH (4). ALK IHC positivity is an important predictive biomarker for ALK-TKI response and we use it as a surrogate marker for *ALK* genomic alterations. We have modified the manuscript to clarify this point.

References:

1. van der Wekken AJ, Pelgrim R, 't Hart N, et al. Dichotomous ALK-IHC Is a Better Predictor for ALK Inhibition Outcome than Traditional ALK-FISH in Advanced Non-Small Cell Lung Cancer. *Clin Cancer Res*. 2017;23(15):4251-4258.

2. Ilie MI, Bence C, Hofman V, et al. Discrepancies between FISH and immunohistochemistry for assessment of the ALK status are associated with ALK 'borderline'-positive rearrangements or a high copy number: a potential major issue for anti-ALK therapeutic strategies. *Ann Oncol*. 2015;26(1):238-244.

3. Cabillic F, Hofman P, Ilie M, et al. ALK IHC and FISH discordant results in patients with NSCLC and treatment response: for discussion of the question-to treat or not to treat?. *ESMO Open*. 2018;3(6):e000419.

4. NCCN Clinical Practice Guidelines in Oncology, Non-Small Cell Lung Cancer Version 6.2020. https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf

Changes in the text:

We have modified the Methods section (EGFR and KRAS mutations and ALK IHC; see page 8, line 153-158) as follows;

Before: As there was a good correlation between the results of ALK IHC and fluorescence in situ hybridization (FISH), ALK IHC positivity was regarded as a surrogate marker for *ALK* rearrangement (1).

After: Although it was well known that there was a good correlation between the results of ALK IHC and fluorescence in situ hybridization (FISH) (1), *ALK* discordant (IHC-positive / FISH-negative) cases were also reported (2, 3). In the previous studies, *ALK* discordant (IHC-positive / FISH-negative) cases showed *ALK* gene amplification (2) and responsiveness to ALK-TKI (3). In this study, ALK IHC positivity was regarded as a surrogate marker for *ALK* gene rearrangement or amplification (1).

We have also added the following sentence in the Discussion section (see page 13-14, line 272-283) as follows; "For ALK IHC, in this study, 7 of 9 IHC-positive cases (2+ or 3+) were further evaluated by *ALK* FISH at the time of recurrence. Of 7 cases examined, 6 cases were positive for *ALK* FISH (>15% rearranged signals). However, one case positive for ALK IHC (3+) was negative for *ALK* FISH and received cytotoxic chemotherapy instead of ALK-TKI due to a financial issue. Unfortunately, the patient expired due to the progression of the disease. In the previous studies, *ALK* discordant (IHC-positive / FISH-negative) cases were related to *ALK* gene amplification (2) and 13 (100%) of 13 discordant (IHC-positive / FISH-negative) cases responded to ALK-TKI (3). Therefore, the discrepancies between IHC and FISH data seem to be associated with biological events rather technical issues. Recent NCCN guidelines also acknowledged FDA-approved ALK IHC (D5F3 CDx Assay) as a stand-alone test, not requiring confirmation by FISH (4).

References:

1. Paik JH, Choe G, Kim H, et al. Screening of anaplastic lymphoma kinase rearrangement by immunohistochemistry in non-small cell lung cancer: correlation with fluorescence in situ hybridization. J Thorac Oncol 2011;6:466-72.

2. Ilie MI, Bence C, Hofman V, et al. Discrepancies between FISH and immunohistochemistry for assessment of the ALK status are associated with ALK 'borderline'-positive rearrangements or a high copy number: a potential major issue for anti-ALK therapeutic strategies. *Ann Oncol*. 2015;26(1):238-244.

3. Cabillic F, Hofman P, Ilie M, et al. ALK IHC and FISH discordant results in patients with NSCLC and treatment response: for discussion of the question-to treat or not to treat?. *ESMO Open*. 2018;3(6):e000419.

4. NCCN Clinical Practice Guidelines in Oncology, Non-Small Cell Lung Cancer Version 6.2020. https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf

We have also modified the Results section as follows (Genomic alterations; see page 10, line 202-207);

Before: With regard to genomic alterations, patients had *EGFR* mutation (95/164, 57.9%), *ALK* rearrangement (9/164, 5.5%), and *KRAS* mutation (11/164, 6.7%). In terms of subtypes of *EGFR* mutation, 47 patients (47/95, 49.5%) were positive for the L858R point mutation and 41 patients (41/95, 43.2%) were positive for an exon 19 deletion. Patients with *ALK* rearrangement had an IHC score of 2 (6/9, 66.6%) or 3 (3/9, 33.3%), and all patients with a *KRAS* mutation had a missense mutation in codon 12 (11/11, 100%).

After: With regard to genomic alterations, patients had *EGFR* mutation (95/164, 57.9%), ALK IHC positivity (9/164, 5.5%), and *KRAS* mutation (11/164, 6.7%). In terms of subtypes of *EGFR* mutation, 47 patients (47/95, 49.5%) were positive for the L858R point mutation

and 41 patients (41/95, 43.2%) were positive for an exon 19 deletion. Patients with ALK IHC positivity had an IHC score of 2 (6/9, 66.6%) or 3 (3/9, 33.3%), and all patients with a *KRAS* mutation had a missense mutation in codon 12 (11/11, 100%).

Comment 4: Risk factors for recurrence after surgery, last paragraph: if solidity was not significant at multivariat analysis I would not consider putting a figure).

Reply 4: We have removed Figure 3B (formerly named Figure 2B) in the revised manuscript as Reviewer C recommended.

Changes in the text:

We have also revised the Results section (Risk factors for recurrence after surgery; see page 12, line 235-239) as follows;

Before: "The disease-free survival rate according to genomic alterations was significantly lower in patients with ALK IHC positivity than in those with other genomic alterations (P<0.001); the median disease-free survival was 24.0 months (95% CI=15.2–32.8) in patients with ALK IHC positivity and not reached in those with other genomic alterations (*Figure 2A*). In terms of solidity, the disease-free survival rate was significantly lower in patients with solid lesions compared to those with subsolid lesions (P<0.001); median disease-free survival was not reached regardless of solidity (*Figure 2B*)."

After: "The disease-free survival rate according to genomic alterations was significantly lower in patients with ALK IHC positivity than in those with other genomic alterations (P<0.001); the median disease-free survival was 24.0 months (95% CI=15.2–32.8) in patients with ALK IHC positivity and not reached in those with other genomic alterations (*Figure 3).*"