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Predictive and Prognostic Value of *ALK* Gene Rearrangement in Non-Small Cell Lung Cancer

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Abstract

Anaplastic Lymphoma Kinase (*ALK*) is a relatively new as an oncogenic driver and a therapeutic target in Non-Small Cell Lung Cancer (NSCLC); the prognostic and predictive implications of *ALK*-positivity in NSCLC is unclear and no large-scale studies have been reported to date. In the current review, we summarize published data examining the variation in prognostic and predictive effect of *ALK*-positivity on clinical outcomes in NSCLC patients, based on the extent of control of or adjustment for known confounding factors such as smoking status, disease stage, and age by study design or analyses. *ALK* rearrangement in NSCLC did not appear to be predictive of improved outcomes with chemotherapy but was predictive of poor response to EGFR TKI therapy. Overall, *ALK* rearrangement was found to be a negative prognostic factor in NSCLC in studies controlling for known confounding factors. In addition to highlighting the importance of controlling for confounding factors in retrospective studies evaluating outcomes, our review also summarizes evidence of an unmet need in terms of poor response amongst *ALK*-positive NSCLC patients to standard therapies that do not target *ALK*.

Keywords: Anaplastic Lymphoma Kinase (*ALK*); Non-Small Cell Lung Cancer (NSCLC); Survival; Clinical Outcomes; Prognostic; Predictive; Review

Introduction

Anaplastic Lymphoma Kinase (ALK) is relatively new as an oncogenic driver an oncogenic driver and a drug target in Non-Small Cell Lung Cancer (NSCLC); however, little is known about the "natural history" of *ALK*-rearranged NSCLC. Some investigators have speculated that it may represent a more indolent disease [1,2] or be an independent positive prognostic factor [3]. Others have suggested that *ALK* rearrangement may be a negative prognostic factor when controlling for known factors such as age, sex, smoking status, stage/grade, and histology [4-6]. With the advent of *ALK*-specific therapies and crossover in clinical trials, it is unlikely that the natural history of *ALK*-rearranged (*ALK*-positive) NSCLC can be examined in an unbiased manner moving forward. However, a handful of retrospective studies examining the outcomes with conventional therapy in *ALK*-positive NSCLC have been published or presented at scientific meetings. Here we review data from these retrospective studies, exclusive of those involving *ALK* inhibitor therapy, with the goal to evaluate historical survival outcomes and treatment outcomes from chemotherapy, *EGFR* Tyrosine Kinase Inhibitor (TKI) therapy, surgical therapy, and thoracic radiotherapy in *ALK*-positive NSCLC.

Methods

We searched published literature in English in peer-reviewed journals indexed in Pub Med, Google Scholar, and presentations at conferences from July 2007 to Nov 2013 that had an observational study design assessing both the predictive and prognostic value of *ALK* in NSCLC, and that tested for *ALK* status using various diagnostic tests including fluorescent in situ hybridization (FISH), Immunohistochemistry (IHC), or polymerase chain reaction (PCR). A total of 26 publications were identified and 8 were excluded, where two were reported from the same cohort [7,8]. Five studies reported only the outcome or gave a conclusion but did not have enough study description or data details [3,9-13]. Two studies had no confirmed *ALK*-negative comparator groups [1,14]. Aggregate data are summarized, comparing survival outcomes between *ALK*-positive versus *ALK*-negative NSCLC patients followed by an evaluation of responses with current non-*ALK*-targeted therapies (Figure 1). Clinical outcomes considered were Overall Survival (OS), Progression-Free Survival (PFS), Recurrence-Free Survival (RFS), Disease-Free Survival (DFS), Time to Progression (TTP), and Objective Response Rate (ORR). Studies were reviewed with a focus on the use of techniques within the study to control via study design and/or adjust with statistical methods for confounding factors that could impact the outcomes being investigated.

Results

ALK gene rearrangement as a prognostic biomarker in NSCLC

Summary of studies with no reported control of or adjustment for confounding factors: Six studies have examined OS in *ALK*-positive compared with *ALK*-negative, *EGFR* wild type (WT/WT) cases and two other studies examined OS in *ALK*-positive versus *ALK*-negative cases in which *EGFR* status was unknown. With a median follow-up time of 13 months at the time of analysis, Shaw et al reported a median OS of 20 months in *ALK*-positive cases and 16 months in WT/WT cases (p=0.152; Table 1) [15]. In another study, which was an indirect comparison of OS between *ALK*-positive, crizotinib-naive

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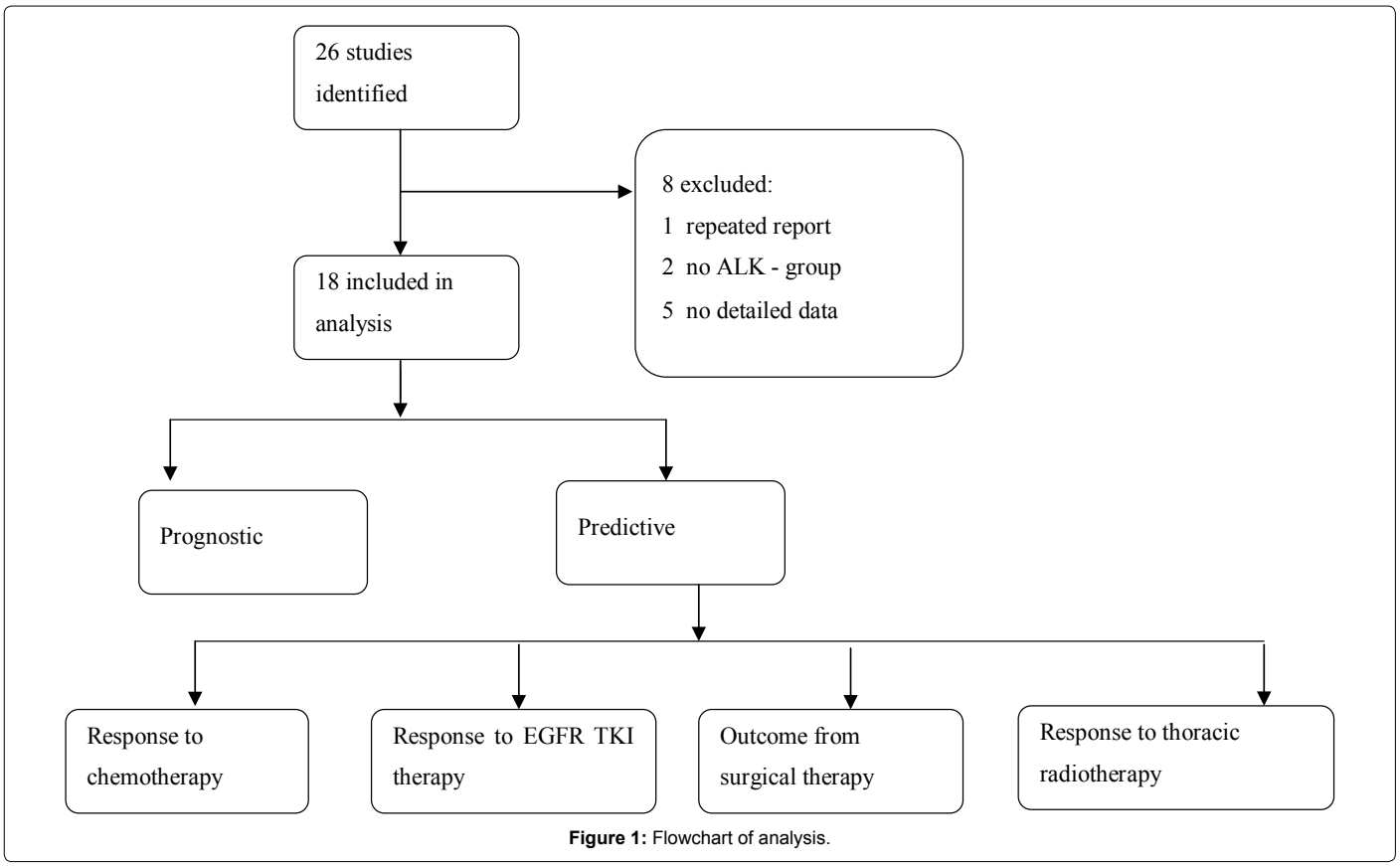

Figure 1: Flowchart of analysis.

cases and WT/WT controls, Shaw et al., reported the median OS from time of metastatic diagnosis as similar for the *ALK*-positive and WT/WT cases at 20 and 15 months, respectively (unadjusted HR 0.77; p=0.244) [2]. Although an additional OS subset analysis conducted on cases ≤ 60 years old who were never- or light-smokers accounted to some degree for age and smoking status and showed median OS of 20 versus 24 months for *ALK*-positive and WT/WT cases, respectively (HR=1.01; p=0.978) [2], it was not formally controlled for all potential confounding variables including histology and treatment. With a median follow-up time of 10.8 months, Takeda et al., reported a median OS of 15.7 months in *ALK*-positive cases and 15.2 months in WT/WT cases (HR=0.83, p=0.591; Table 1) [16]. Wang et al., reported a median OS of 19.27 months in 9 *ALK*-positive cases and 18.93 months in 45 WT/WT cases (p=0.481, Table 1) [17]. Martinez et al., reported a median OS of 4.5 months for WT/WT cases (n=65) versus a median OS not reached for 7 *ALK*-positive cases (p=0.103) and 15.7 months in 13 *EGFR* mutant cases (p=0.018) [8]. No statistically significant differences were found in median OS between groups in all these studies.

Hayashi et al., reported a median OS in locally advanced adenocarcinoma patients of 7.7 months in 3 ALK-positive cases and 42.6 months in 23 WT/WT cases (p=0.007; Table 1) [18]. Fukui et al., selected adenocarcinoma cases who underwent pulmonary resection and reported the 5-year OS rate for *ALK*-positive patients was 81%; whereas, the *ALK*-negative (*EGFR* status unknown) was 77% (p=0.76) [19,20].

In early stage lung cancer patients, Paik et al., reported a median OS in stage I-III NSCLC patients of 97.7 months in *ALK*-positive cases and 78.9 months in *ALK*-negative (*EGFR* status unknown) cases (p=0.10) [19].

Summary of studies with reported control of or adjustment for confounding factors: Four studies to date have, a priori, matched or controlled for important independent prognostic factors. Three of them suggest or clearly demonstrate a shorter OS or DFS for *ALK*-positive versus *ALK*-negative cases and one stated a prolonged OS in *ALK*-positive cases. The case-matched analysis by JK Lee et al., reported a median OS in stage IIIb-IV cases of 12.23 months in *ALK*-positive (n=23), 29.63 months in *EGFR* mutant (n=46) and 19.33 months in WT/WT (n=46) cases (p=0.001 versus *EGFR* mutant; p=0.127 versus WT/WT) [6]. Yang et al., with selection of never-smoker, adenocarcinoma cases and control for age, sex, stage, and treatment, showed more than a 2-fold greater risk of recurrence or progression within 5 years of diagnosis in *ALK*-positive (n=22) versus *ALK*-negative (*EGFR* status unknown) cases (n=274; p=0.004; Table 1) [4]. In the same study, a higher rate of extra-thoracic metastasis was observed among *ALK*-positive cases compared with *ALK*-negative cases, (HR=2.44, p=0.03); albeit, the number of later stage patients in this analysis was limited (n=13). With selection of never-smokers and comparator groups which were balanced in terms of age, sex, histology, stage and performance status (PS), Kim et al., reported a shorter median OS of 14.3 months in *ALK*-positive cases compared with 33.3 months in *ALK*-negative, *EGFR* WT and KRAS WT (triple WT), and 37.2 months in *EGFR* mutant (*ALK*-negative) cases (p=0.016 for *ALK*-positive versus triple WT) [5]. In the same study, in a multivariate analysis, *ALK*-positivity was associated with a lower OS in patients with resected NSCLC (adjusted HR, 4.162; p=0.005). The authors suggested that *ALK*-positivity may be a negative prognostic factor for early stage NSCLC. Wu et al., examined survival outcomes in lung adenocarcinoma patients with malignant pleural effusions and wild type *EGFR* in which *ALK*-positive and *ALK*-negative comparator groups were balanced in terms of age, sex, smoking history, PS, and treatment, and reported a longer median OS of 14.7

Study	*ALK*+ (N)/Total (N)	Efficacy
Studies with no control or adjustment for confounding factors		
Shaw et al., [15]	17 *ALK*+/96 never/light smokers, stage IV	Median OS: • 20 months in *ALK*+[a] • 32 months in *EGFR* mu (p=0.468 *vs. ALK*+) • 16 months in *ALK*−/*EGFR* WT (*p*=0.152 vs. *ALK*+)
Shaw et al., [2]	36 *ALK*+/356 advanced	Median OS: • 20 months in *ALK*+ • 15 months in *ALK*−/*EGFR* WT (*p*=0.244 *vs. ALK*+)
Takeda et al., [16]	18 *ALK*+/200 advanced non squamous cases	Median OS[†]: • 15.7 months in *ALK*+ • 24.8 months in *EGFR* mu (*p*=0.135 *vs. ALK*+) • 15.2 months in *ALK*−/*EGFR* WT (*p*=0.591 *vs. ALK*+)
Wang et al., [17]	9 *ALK*+/113 stage IV	Median OS: • 19.27 months in *ALK*+ • 23.13 months in *EGFR* mu • 18.93 months in *ALK*−/*EGFR* WT (*p*=0.481 vs. *ALK*+ and *EGFR*
Hayashi et al., [18]	3 *ALK*+/37 locally advanced adeno cases	Median OS: • 7.7 months in *ALK*+ • 67.5 months in *EGFR* mu • 42.6 months in *ALK*−/*EGFR* WT (*p*=0.007 *vs. ALK*+)
Martinez et al., [8]	7 *ALK*+/99 Non squamous, all stages	Median OS: • not reached in *ALK*+[b] • 15.7 months in *EGFR* mu • 4.5 months in *ALK*−/*EGFR* WT (*p* =0.103 *vs. ALK*+)
Paik et al., [19]	28 *ALK*+/735 stage I-III	Median OS: • 97.7 months in *ALK*+ • 78.9 months in *ALK*− (*p*=0.10 *vs. ALK*+)
Fukui et al., [20]	28 *ALK*+/720 Adeno resected cases, all stages	5-year OS rate: • 81% in *ALK*+ • 77% in *ALK*− (*p*=0.76 *vs. ALK*+)
Studies with control or adjustment for confounding factors		
Lee et al., [6]	23 *ALK*+/262 non-squamous *EGFR* WT or TKI non-responders, stage IIIb–IV[b]	Median OS: • 12.23 months in *ALK*+ • 29.63 months in *EGFR* mu (*p*=0.001 *vs. ALK*+) • 19.33 months in WT/WT (*p*=0.127 *vs. ALK*+)
Yang et al., [4]	22 *ALK*+/296 never-smoker, adeno cases[c] 9=stage I/II 7=stage III 6=stage IV	DFS not reported in either *ALK*+ or *ALK*−groups (2-fold greater risk of progression or recurrence within 5 yrs of diagnosis reported in *ALK*+ *vs.ALK*− cases, *p*=0.004)
Kim et al., [5]	13 *ALK*+/229 never-smokers, all stages[d]	Median OS: • 14.3 months in *ALK*+ • 37.2 months in *EGFR* mu (*p*=0.001 *vs. ALK*+) • 33.3 months in *ALK*−/EGFR WT/KRAS WT (*p*=0.016 *vs. ALK*+)
Wu et al., [21]	39 *ALK*+/116 adeno cases, stage IV[e]	Median OS: • 14.7 months in *ALK*+ • 10.3 months in *ALK*−/*EGFR* WT (*p*=0.011 *vs. ALK*+)

[a]7/17 and [b]4/7 *ALK*+ patients enrolled in crizotinib trial; [b]Matched to 46 *EGFR* mu and 46 WT/WT on age at dx, sex, stage, smoking status; [c]Adjusted for age at dx, sex, tumour grade, tx modality; [d]Adjusted for age, sex, stage, tx; [e]Adjusted for age, sex, smoking status, PS, treatment; [†]OS was calculated from the date of chemotherapy; mu, mutated; adeno, adenocarcinoma; dx , diagnosis; tx, treatment; TKI, tyrosine kinase inhibitor; WT, wild type; OS, overall survival; DFS, disease-free survival.

Table 1: Overall Survival.

months in *ALK*-positive cases (*n*=39) compared with 10.3 months in WT/ WT cases (*n*=77, HR=0.53, *p*=0.011) [21]. The authors concluded that ALK translocation is associated with longer overall survival in lung adenocarcinoma *EGFR*–WT patients.

ALK gene rearrangement as a predictive biomarker

Six studies published to date report response to platinum-based chemotherapy in *ALK*-positive NSCLC (Table 2) [5,6,15-17,22]. Two of these studies controlled for or matched cases on potential confounding factors, while the other four did not. Four other studies, representing varying degrees of balance or control for confounding factors, reported response to pemetrexed either as a single agent [23,24], or in combination (Table 3) [25,26]. Five studies to date reported the efficacy of *EGFR* TKI therapy in *ALK*-positive NSCLC and four of them showed a 0% response rate [5,6,15,22]. Four studies reported survival outcomes in *ALK*-positive compared with *ALK*-negative patients who underwent surgical resection [5,19,20,27]. One of these studies balanced on clinically relevant factors [5].

Summary of studies with no reported control of or adjustment for confounding factors

Analyses by Koh et al., [22] and Shaw et al., [15] showed similar ORR, PFS or TTP on chemotherapy in retrospectively-identified cohorts of patients with *ALK*-positive versus WT/WT NSCLC cases (Table 2). Koh et al., [22] reported median PFS with first-line platinum-based doublet therapy of 6.2 months in *ALK*-positive (*n*=32) versus 7.3 months in WT/WT cases (*n*=57), with ORR of 18.8% and 40.4%, respectively. Both PFS and ORR were reported as statistically non-significant. This analysis was not balanced for age, with patients in the *ALK*-positive cohort being statistically significantly younger than WT/WT patients (median 49 versus 61 years, respectively; *p*<0.001). Similarly, Shaw et al., reported a median TTP with first-line platinum-

	Chemotherapy regimens			EGFR TKI regimensa		
Study	***ALK*+ (N)**	**Regimen/ Line of treatment**	**Efficacy**	***ALK*+ (N)**	**Response rate**	**Efficacy**
Studies with no control or adjustment for confounding factors						
Shaw et al., [15]	12 *ALK*+ metastatic cases evaluable for chemo	1st line platinum-based chemo	Median TTP reported as "in the range of 8–10 months" across *ALK*+, *EGFR* mu, and WT/WT	10 *ALK*+ stage IV	• 0% in *ALK*+ • 70% in *EGFR* mu • 13% in WT/WT	Median TTP: • 5 months in *ALK*+ • 16 months in *EGFR* mu • 6 months in WT/WT
Koh et al., [22]	32 *ALK*+ advanced adeno cases	1st line platinum-based doublet chemo	Median PFS: • 6.2 months in *ALK*+ • 5.4 months in *EGFR* mu • 7.3 months in WT/WT	16 *ALK*+ advanced adeno	• 0% in *ALK*+ • 50% in *EGFR* mu • 6.9% in WT/WT	Median PFS: • 4.3 weeks in *ALK*+ • 19.6 weeks in *EGFR* mu • 6.0 weeks in WT/WT
Wang et al., [17]	4 *ALK*+ stage IV	1st and 2nd line platinum-based doublet chemo	Median PFS:c • 8.3 months in *ALK*+ • 4.1 months in *EGFR* mu • 4.9 months in WT/WT	9 *ALK*+ stage IV	• 33.3% in *ALK*+ • 46.9% in *EGFR* mu • 16.3% in WT/WT	Median PFS: • 2.1 months in *ALK*+ • 8.8 months in *EGFR* mu • 2.2 months in WT/WT
Takeda et al., [16]	18 *ALK*+ advanced non squamous cases	1st line platinum-based chemo	Median PFS:c • 6.5 months in *ALK*+ • 6.0 months in *EGFR* mu • 4.3 months in WT/WT	Not studied		
Studies with control or adjustment for confounding factors						
Lee et al., [6]	21 *ALK*+ matched to 34 *EGFR* mu and 37WT/WT cases all stage IIIb–IVd	1st line chemob	Median PFS • 3.87 months in *ALK*+ • 4.93 months in *EGFR* mu • 3.73 months in WT/WT	10 *ALK*+ matched to 42 *EGFR* muand 27 WT/WTd	• 0% in *ALK*+ • 80.9% in EGFR mu • 14.8% in WT/WT	Median PFS: • 1.37 months in *ALK*+ • 9.80 months in *EGFR* mu • 2.07 months in WT/WT
Kim et al., [5]	12 *ALK*+ never-smokers, all stagese	1st-line platinum-based chemo	Median PFS: • 5.0 months in *ALK*+ • 7.1 months in *EGFR* mu • 7.2 months in KRAS mu • 5.9 months in WT/WT/WT	8 *ALK*+ never-smokers, all stagese	• 0% in *ALK*+ • 65.5% in *EGFR* mu • 0% in KRAS mu • 10.3% in WT/WT/WT	Median PFS: • 1.6 months in *ALK*+ • 12.8 months in *EGFR* mu (p<0.001 *vs. ALK*+) • 2.1 months in KRAS mu • 6.3 months in WT/WT/WT (p=0.001 *vs. ALK*+)

[a]Line and treatment not stated; [b]all but 2 patients received platinum-based chemotherapy; [c]some of patients received pemetrexed treatment; [d]Matched on age at dx, sex, stage, smoking status; [e]Adjusted for age, sex, histology, PS, stage; mu, mutated; chemo, chemotherapy; adeno, adenocarcinoma; PFS, progression-free survival; TTP, time to progression

Table 2: Median PFS or TTP with Chemotherapy and EGFR TKI Regimens.

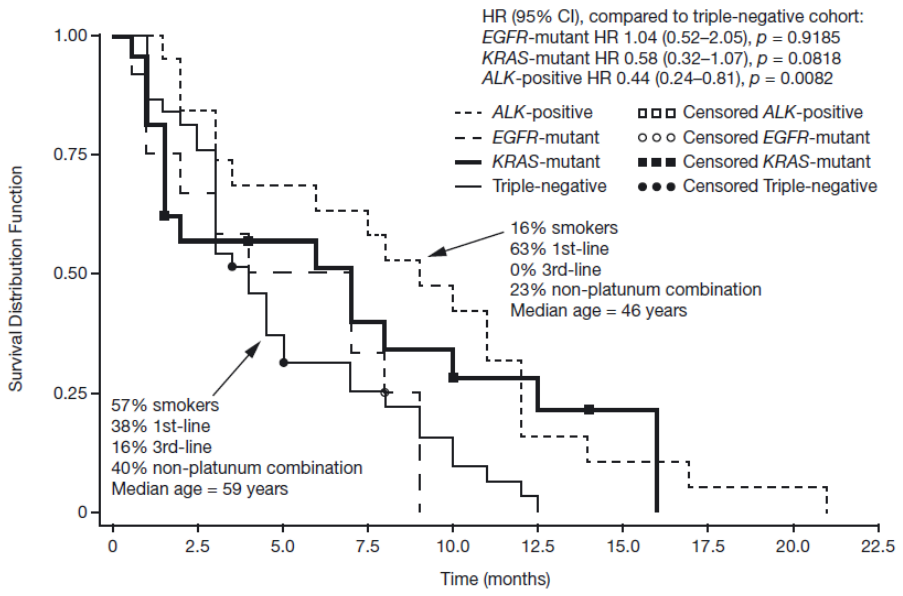

Figure 2: Progression-free survival of patients with NSCLC treated with pemetrexed.

based chemotherapy in the range of 8–10 months for patients with *ALK*-positive, *EGFR* mutant, and WT/WT disease [15]. The chemotherapy ORR in this study was 25% for *ALK*-positive and 35% for WT/WT cases with no statistically significant difference between these groups (p=0.723). This cohort was not balanced for age, sex, smoking history or exposure to *ALK* inhibitor therapy, which would have had an impact on clinical outcome. In addition to these two studies, Takeda et al., reported the median PFS with first-line platinum-based chemotherapy of 6.5 months in 18 *ALK*-positive versus 4.3 months in 151 WT/WT cases (p=0.437), with an ORR of 44% and 39%, respectively [16]. Both

Study	*ALK*+ (N)/*ALK*− (N)	Regimen and Line of Treatment	Efficacy	
			ALK+	*ALK*−
Studies with no control or adjustment for confounding factors				
Camidge et al., [25]	19/37	Pemetrexed alone or in combination any line	Median PFS: • 9 months	Median PFS:[b] • 4 months
Lee et al., [23]	15/37	Pemetrexed mono therapy ≥ 2nd line	Median TTP: • 9.2 months (overall) • 9.2 months (2nd-line) • 6.4 months (≥ 3rd-line)	Median TTP:[a] • 2.9 months (overall) • 2.7 months (2nd-line) • 4.0 months (≥ 3rd-line)
Lee et al., [24]	32/unknown	Pemetrexedmonotherapy ≥ 2nd line	Median PFS: • 4.0 months	Median PFS: • 1.6 months
Shaw et al., [26]	70/112	Platinum-pemetrexed any line	Median PFS: • 7.3 months	Median PFS:[b] • 5.9 months
	58/99	Platinum-pemetrexed any line, ≤ 65 yrs	Median PFS: • 8.1 months	Median PFS:[a] • 6.0 months
	64/45	Platinum-pemetrexed any line, never/light smoking	Median PFS: • 7.3 months	Median PFS:[a] • 7.5 months
	56/44	Platinum-pemetrexed 1st line	Median PFS: • 8.5 months	Median PFS:[b] • 5.4 months
	53/40	Platinum-pemetrexed 1st line, never/light smoking	Median PFS: • 8.5 months	Median PFS:[a] • 7.4 months
	51/75	Pemetrexed alone or no-platinum combination any line	Median PFS: • 5.5 months	Median PFS:[b] • 3.9 months
	41/57	Pemetrexed alone or no-platinum combination any line, ≤ 65 yrs	Median PFS: • 5.1 months	Median PFS:[a] • 4.4 months
	44/34	Pemetrexed alone or no-platinum combination any line, never/light smoking	Median PFS: • 5.5 months	Median PFS:[a] • 5.3 months
	30/27	Pemetrexed alone any line, never/light smoking	Median PFS: • 4.8 months	Median PFS:[a] • 4.6 months
	31/39	Pemetrexed alone or no-platinum combination ≥ 2nd line	Median PFS: • 4.4 months	Median PFS:[b] • 3.8 months

[a]*ALK*− is *ALK*−/EGFR WT; [b]*ALK*− is *ALK*−/*EGFR* WT/*KRAS* WT; 65 years old; PFS, progression-free survival; TTP, time to progression.

Table 3: Response to pemetrexed.

Study	ALK+ (N)/Total(N)	Efficacy
Studies with no control or adjustment for confounding factors		
Paik et al., [19]	28 *ALK*+/735 stage I-III	Median DFS: • 76.4 months in *ALK*+ • 71.3 months in ALK- (*p*=0.66 vs. *ALK*+)
Fukui et al., [20]	28 *ALK*+/720 adeno cases all stages	5-year DFS rate: • 74% in *ALK*+ • 68% in *ALK*- (*p*=0.45 vs. *ALK*+)
Zhou et al., [27]	12 *ALK*+/134 stage IA	5-year DFS rate: • 100% in *ALK*+ • 29.5% in *ALK*- (*p*=0.04 vs. *ALK*+)
	9 *ALK*+/165 stage IIIA	Median DFS: • 6 months in *ALK*+ • 16 months in *ALK*- (*p*=0.0057 vs. *ALK*+)
Studies with control or adjustment for confounding factors		
Kim et al., [5]	11 *ALK*+/119 never-smokers, all stages[a]	Median RFS: • 20.0 months in *ALK*+ • 39.7 months in *EGFR* mu • 21.4 months in KRAS mu • 26.8 months in WT/WT/WT

[a]Adjusted for age, sex, histology, performance status; DFS, disease-free survival; RFS, recurrence-free survival.

Table 4: Median DFS or RFS with Surgical Therapy.

PFS and ORR were reported as statistically non-significant. Similarly, this analysis was not balanced for age, with patients in the *ALK*-positive cohort being statistically significantly younger than WT/WT patients (median 46 versus 64 years, respectively; $p<0.001$). The regimens were also not balanced between the comparator groups. The proportion of patients in *ALK*-positive and WT/WT groups treated with platinum plus pemetrexed were 39% and 17%, respectively ($p=0.049$). These would have had an impact on clinical outcome. Wang et al., reported median PFS with first-line and second-line platinum-based doublet therapy of 8.3 months in *ALK*-positive versus 4.9 months in WT/WT cases ($p=0.25$) [17]. The chemotherapy ORRs were 25.0% and 32.4%, respectively ($p=0.762$). Both ORR and PFS were not statistically significant. This study was not balanced in age, smoking status and other important prognostic factors between *ALK*-positive and WT/WT groups.

Response to pemetrexed-based therapy: Camidg et al., Lee et al., and Lee et al., each retrospectively studied *ALK*-positive NSCLC response to pemetrexed-based therapy, yielding similar results (Table 3) [23-25]. Lee et al., examined single-agent pemetrexed response in second-line therapy or beyond and reported an overall median TTP of 9.2 months (95% CI 4.65-13.74 months) in *ALK*-positive ($n=15$) versus 2.9 months (95% CI of 0.51- 5.28 months) for WT/WT ($n=37$) and 1.4 months (95% CI of 1.27-1.52 months) in *EGFR* mutant cases ($n=43$, $p=0.001$) [23]. In the treatment-line stratified multivariate analysis, *ALK*-positivity was an independently significant predictor for TTP (HR=0.44; $p=0.005$). However, there were imbalances in the *ALK*-positive versus WT/WT groups in this cohort with respect to: (1) the majority (60%) of *ALK*-positive cases were in their third line of therapy or beyond compared with 29.7% of WT/WT cases and (2) the median age of *ALK*-positive cases was the youngest at 52 years. The authors did take the line of therapy into consideration by stratifying TTP by second or ≥ 3 lines of therapy; however, this step reduced the *ALK*-positive sample sizes to 6 and 9 patients, respectively [23].

Camidge et al., assessed response to pemetrexed as single agent or in combination in metastatic NSCLC across all lines of therapy. A median PFS of 9 months (95% CI 3-12 months) in 19 *ALK*-positive cases compared with 4 months (95% CI 3-5 months) in 37 triple WT cases was observed [25]. Similar to the analysis of Lee et al., the only significant

variable associated with prolonged PFS in a multivariate analysis was *ALK*-positivity (HR=0.36; p=0.0051). Important imbalances in the comparator groups in this analysis are depicted in Figure 2, Camidge et al., were careful to state that multiple other confounding factors may contribute to the longer PFS observed in the *ALK*-positive group [25].

Lee et al., examined single-agent pemetrexed response in second-line therapy or beyond and reported a median PFS of 4.0 months (95% CI 2.2-5.8 months) in 32 ALK-positive cases compared with 1.6 months (95% CI 1.0-2.2 months) in WT/WT cases [24]. Compared with the *ALK*-negative patients, *ALK*-positive patients were significantly younger (median age, 62 versus 50 years, respectively), with a higher proportion of never or light smokers (32.9% versus 73.9%, respectively; p=0.002). The authors stated that the difference in PFS between the *ALK*-positive and WT/WT cases should be interpreted cautiously.

Recently, Shaw et al., studied *ALK*-positive NSCLC response to pemetrexed-based therapy stratified for treatment line, age, smoking status, and different regimen [26]. The authors assessed response to pemetrexed-platinum combination regimen and reported the median PFS of 7.3 months in *ALK*-positive versus 5.9 months in WT/WT cases (p=0.182). Although an additional PFS subgroup analysis was conducted on first line treatment patients age ≤ 65 years old with never/light smoking history accounted to some degree for confounders; it was not formally controlled for all potential confounding variables in the same sub group (Table 3). Only in the subset of patients who received pemetrexed–platinum combination as first line treatment, was there a statistically significant difference in median PFS among *ALK*-positive and WT/WT patients (p=0.018). All other subsets had no statistically significant differences between *ALK*-positive and WT/WT groups. The authors also examined single-agent pemetrexed or no-platinum combination response and reported a median PFS of 5.5 months in *ALK*-positive cases compared with 3.9 months in WT/WT cases (p=0.409). An additional PFS subgroup analysis was conducted on second or third line treatment, age ≤ 65 years old, never/light smoking history and pemetrexed alone with never/light smoking history (Table 3). In all the subsets of patients who received single-agent pemetrexed or no-platinum combination, no statistically significant differences were found in median PFS between *ALK*-positive and WT/WT groups.

Response to EGFR TKI therapy: Koh et al., reported a median PFS with *EGFR* TKI therapy of 4.3 weeks in *ALK*-positive (n=16) versus 6.0 weeks in WT/WT (n=29) and 19.6 weeks in *EGFR* mutant (n=18) cases (p<0.001) [22]. Shaw et al., measured median TTP as 5 months in *ALK*-positive, 6 months in WT/WT, and 16 months in *EGFR* mutant cases (p=0.004 for *ALK*-positive versus *EGFR* mutant; Table 2) [15]. Wang et al., reported a median PFS of 2.1 months in *ALK*-positive versus 2.2 months in WT/WT and 8.80 months in *EGFR* mutant cases (p=0.696 for *ALK*-positive versus WT/WT; p=0.032 for *ALK*-positive versus *EGFR* mutant) [17]. As mentioned above, these three studies had imbalances relative to age and smoking status, which may have confounded median PFS/TTP estimates. Additionally, in the study of Wang et al, there were only 9*ALK*-positive cases among the patients received *EGFR* TKI therapy.

Outcome from surgical therapy: With selection of surgically resected stage I-III NSCLC patients, Paik et al., reported a median DFS of 76.4 months in *ALK*-positive and 71.3 months in *ALK*-negative (EGFR status unknown) cases (p=0.66) [19]. The authors suggested *ALK*-positivity may not be a predictive factor in the early (surgically resectable) stages of NSCLC. However, this analysis was not balanced for gender, age, smoking status and histology. Fukui et al., selected adenocarcinoma patients with primary lung cancer who underwent pulmonary resection and reported the 5-year DFS rate for *ALK*-positive patients as 74%; whereas, the DFS rate for *ALK*-negative (*EGFR* status unknown) was 68% (p=0.45) [20]. No significant difference was observed between the *ALK*-positive and *ALK*-negative groups. The analysis was not balanced for age and smoking history. Zhou et al., analyzed outcomes of patients with NSCLC who underwent radical surgical resection stratified into specific clinical stages [27]. In stage IA, *ALK*-positive cases had significantly longer DFS than *ALK*-negative (*EGFR* status unknown) cases (5-year DFS rate, 100% versus 29.5%, p=0.04). In stage IIIA, *ALK*-positive patients had poorer DFS than *ALK*-negative patients (median DFS, 6 months versus 16 months, p=0.0057, Table 4). In a multivariate analysis, the *ALK*-positivity was the only significant variable associated with poor survival in stage IIIA NSCLC (HR=4.0, p<0.001). Although stratified subset analysis accounted for stage to some degree; it was not controlled for age and gender.

Response to thoracic radiotherapy: Hayashi et al., reported outcomes of patients with locally advanced adenocarcinoma NSCLC who underwent thoracic radiotherapy (TRT) alone or together with chemotherapy. The median PFS with TRT in 3 *ALK*-positive cases was 4.2 months compared with 18.6 months in 23 WT/WT and 13.1 months in 11 *EGFR* mutant cases (p=0.037 for ALK-positive versus WT/WT) [18]. The authors concluded the *ALK ALK*-positive patients had a poorer outcome after TRT treatment. However, this study was not matched or controlled for important prognostic factors. Additionally, there were only 3 *ALK*-positive patients who treated with TRT.

Summary of studies with control of or adjustment for confounding factors: In order to account for imbalances in clinical or patient characteristics between patient subgroups, a few investigators matched cases or applied statistical adjustment (control) in their survival analyses, accounting for age, sex, smoking status, histology, and stage of disease (Table 2).

Response to chemotherapy: Lee et al., retrospectively identified stage IIIb-IV cases of non-squamous histology and created a case cohort of *ALK*-positive cases matched 2:1 to both *EGFR* mutant and WT/WT cases on age at diagnosis, sex, stage, and smoking status. They observed a median PFS with first-line chemotherapy (none of which included pemetrexed) of 3.87 months in *ALK*-positive versus 4.93 months in *EGFR* mutant (p=0.825), and 3.73 months in WT/WT NSCLC cases (p=0.474) [6]. The first-line chemotherapy ORRs in this study were 28.6%, 32.4%, and 35.1% for *ALK*-positive, *EGFR* mutant, and WT/WT groups, respectively (p=0.857 versus *EGFR* mutant; p=0.695 versus WT/WT).

Kim et al., [5] examined outcomes in NSCLC patients who were never-smokers, controlling for age, sex, histology, and PS in a multivariate analysis. In this analysis, the ORR to first-line platinum-based chemotherapy was 0% for *ALK*-positive (n=12) and 23% for triple WT cases (n=61). The difference in ORRs between groups was not statistically significant, nor was the median PFS estimate of 5.0 months for *ALK*-positive and 5.9 months for triple WT cases. The comparator groups, particularly the *ALK*-positive and triple WT cases, in this study were well-balanced in terms of age, sex, histology, PS, stage, and smoking status. Thus, even though unadjusted statistically, the Kaplan–Meier survival curves in this study represent well-balanced comparisons.

Response to EGFR TKI therapy: The matched case cohort analysis by Lee et al., [6] demonstrated a median PFS of 1.37 months in *ALK*-positive versus 2.07 months in WT/WT and 9.80 months in *EGFR* mutant cases (p=0.037 for *ALK*-positive versus WT/WT; p<0.001 for *ALK*-positive versus *EGFR* mutant). In the analysis by Kim et al., [5],

median PFS with *EGFR* TKI therapy in *ALK*-positive cases was 1.6 months compared with 6.3 months in WT/WT and 12.8 months in *EGFR* mutant cases ($p<0.001$ for *ALK*-positive versus *EGFR* mutant; $p=0.001$ for *ALK*-positive versus WT/WT; Table 2). Thus, these results corroborate the findings of Shaw et al., Koh et al., and Wang et al., and show worse response to *EGFR* TKI therapy in *ALK*-positive NSCLC cases.

Outcome from surgical therapy: Kim et al., examined outcomes in surgically resected NSCLC patients who were never-smokers, controlling for age, sex, histology, stage, and PS in comparator groups [5]. The median RFS with radical surgery in *ALK*-positive cases was 20.0 months compared with 26.8 months in WT/WT and 39.7 months in *EGFR* mutant cases ($p=0.344$). Although in a multivariate analysis, *ALK*-positivity was associated with a lower OS in patients with resected NSCLC (adjusted HR, 4.162; $p=0.005$) and the authors suggested that *ALK*-positivity may be a negative prognostic factor for early stage NSCLC.

Discussion and Conclusion

Lessons learned from the *ALK* NSCLC experience emphasize the importance of early molecular and clinical epidemiology research needed to understand the prognostic and predictive value of candidate biomarkers or drug targets. Overall, studies that controlled for potential confounding factors either by study design or in the analyses suggest worse or equivalent prognosis for *ALK*-positive NSCLC cases. Only one analysis, studied by Wu et al., concluded that *ALK* rearrangement is a favorable predictive factor for OS in *ALK*-positive NSCLC [21]. One observation in this study was the percentage of cases who received platinum-based doublet chemotherapy, second- or subsequent-line therapy, pemetrexed treatment and *EGFR* TKI therapy were all higher in the *ALK*-positive group than in the *ALK*-negative group even though individually there was no significant treatment difference between *ALK*-positive cases and *ALK*-negative cases. These multiple favorable factors, when combined, in the *ALK*-positive group could skew the overall results; in addition, the absence of significant differences could have possibly been due to too small a sample size.

Both non-balanced and balanced/controlled analyses demonstrated statistically significantly shorter PFS or TTP amongst *ALK*-positive (including *EGFR* WT or *EGFR* unknown but *EGFR* TKI resistant) cases compared with *EGFR* mutant and WT/WT cases treated with *EGFR* TKI therapy. All studies except one reported a 0% ORR to *EGFR* TKI therapy in *ALK*-positive cases (Table 2). Although a small total number of patients were studied, these data suggest that *ALK* is a negative predictive factor for *EGFR* TKI therapy outcomes in *ALK*-positive NSCLC. All studies included in this review, regardless of whether they were balanced/controlled or not, suggested that PFS with platinum-based chemotherapy might not be significantly influenced by *ALK* status in patients with NSCLC. Although in two of the studies the patients received pemetrexed as a platinum partner, the difference between *ALK*-positive and WT/WT were not found to be statistically significant. Therefore, *ALK* rearrangements might not be a predictive marker for PFS with conventional platinum-based chemotherapy. It is important to note though, that sample size which could influence statistical significance of comparisons is a limitation in most of these studies.

The analysis by Camidge et al., exploring PFS with pemetrexed-based therapy in groups defined by *ALK*, *EGFR*, and KRAS status has important imbalances to consider. Specifically, the *ALK*-positive and triple WT groups represented considerably different treatment populations, with 63% of the former being 1st-line-treated patients versus 38% of the latter. There were also third-line treatment patients in the triple WT group (16%) but none in the *ALK*-positive group in addition to differences in smoking status and age. Thus, the median PFS difference observed of 9 months versus 4 months for *ALK*-positive versus triple WT cases can, at least in part, be explained by a differential prognosis of the two groups based upon the above-mentioned factors. The study by Lee et al., however, did demonstrate some degree of balance between patient and clinical characteristics for *ALK*-positive and WT/WT cases treated with single-agent pemetrexed; however, there were still important imbalances for age and line of therapy, with more *ALK*-positive patients being younger and in a third- or greater line of therapy [23]. With stratifying by important clinical characteristics such as line of therapy, age, smoking status, and different regimen, the study by Shaw et al., stated similar PFS between *ALK*-positive and WT/WT groups with pemetrexed-based therapy [26]. None of the studies examining response to pemetrexed matched cases or controlled for known confounding factors when comparing the survival curves. Thus, despite the multivariate analyses identifying *ALK* as the only significant predictive variable in response to pemetrexed, the point estimates of median TTP or PFS and the accompanying Kaplan–Meier curves do not account for these same potentially confounding variables used in the multivariate analyses.

Retrospective studies to date on *ALK*-positive NSCLC highlight the importance of controlling for already known independent prognostic or predictive factors when trying to evaluate the impact of a new NSCLC biomarker. For *ALK*-positive NSCLC, opposing conclusions can be drawn from aggregate balanced/controlled versus non-balanced analysis. Smoking status can be an important confounding factor. For example, across several previous studies, the hazard ratio (HR) for survival is consistently lower for never-smokers versus smokers (HR across studies approximately 0.8) [28-32]. Thus, smoking status is a critical factor to consider in analyses assessing the effect of a new biomarker on NSCLC treatment response or survival.

Combining all published analyses of observational data to date, with the understanding of limitations of small sample sizes, degree of control of confounding factors, and retrospective study designs, *ALK* rearrangement in NSCLC appears to be (1) not predictive of improved outcomes with standard chemotherapy, (2) predictive of poor response to *EGFR* TKI therapy, and (3) not a favorable prognostic factor in NSCLC [2,4-6,15,22]. In fact, the majority of controlled or case matched analyses suggest that *ALK* positivity is a negative prognostic factor in NSCLC [4-6]. Thus, the example of *ALK* translocation serves as a good model of the need for attention to careful control or adjustment for clinically relevant confounding factors when evaluating the prognostic and predictive value of newly identified candidate biomarkers in NSCLC.

Conflict of interest statement

Kimary Kulig is a former employee of Pfizer Inc. Shrividya Iyer is a current employee of Pfizer. Yi Wang and Ping Yang have declared no conflicts of interest.

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Author Contributions

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literature search, and review. Kimary Kulig, Yi Wang, Shrividya Iyer, and Ping Yang contributed to data analysis interpretation, manuscript writing, and final approval of the manuscript.

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