ORIGINAL ARTICLE

Outcomes According to *ALK* Status Determined by Central Immunohistochemistry or Fluorescence In Situ Hybridization in Patients With *ALK*-Positive NSCLC Enrolled in the Phase 3 ALEX Study

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ABSTRACT

Introduction: We retrospectively examined progression-free survival (PFS) and response by *ALK* fluorescence in situ hybridization (FISH) status in patients with advanced *ALK* immunohistochemistry (IHC)-positive NSCLC in the ALEX study.

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Methods: A total of 303 treatment-naive patients were randomized to receive twice-daily alectinib 600 mg or crizotinib 250 mg. *ALK* status was assessed centrally using Ventana ALK (D5F3) CDx IHC and Vysis *ALK* Break Apart FISH Probe Kit. Primary end point is investigator-assessed PFS. Secondary end points of interest are objective response rate and duration.

Results: Investigator-assessed PFS was significantly prolonged with alectinib versus crizotinib in *ALK* IHC-positive and FISH-positive tumors (n = 203, 67%) (hazard ratio [HR] = 0.37, 95% confidence interval [CI]: 0.25–0.56; $p < 0.0001$) and *ALK* IHC-positive and FISH-uninformative tumors (n = 61, 20%) (HR = 0.39, 95% CI: 0.20–0.78) but not in *ALK* IHC-positive and FISH-negative tumors (n = 39, 13%) (HR = 1.33, 95% CI: 0.6–3.2). Objective response rates were higher with alectinib versus crizotinib in *ALK* IHC-positive and FISH-positive tumors (90.6% versus 81.4%; stratified OR = 2.22, 95% CI: 0.97–5.07) and *ALK* IHC-positive and FISH-uninformative tumors (96.0% versus 75.0%; OR = 9.29, 95% CI: 1.05–81.88) but not in *ALK* IHC-positive and FISH-negative tumors (28.6% versus 44.4%; OR = 0.45, 95% CI: 0.12–1.74). Next-generation sequencing was performed in 35 of 39 patients with *ALK* IHC-positive and FISH-negative tumors; no *ALK* fusion was identified in 20 of 35 patients (57.1%) by next-generation sequencing, but 10 of 20 (50.0%) had partial response or stable disease.

Conclusions: Outcomes of patients with *ALK* IHC-positive and FISH-positive and *ALK* IHC-positive and FISH-uninformative NSCLC were similar to those of the overall ALEX population. These results suggest that Ventana ALK IHC is a standard testing method for selecting patients for treatment with alectinib.

Keywords: Alectinib; *ALK*-positive; IHC; FISH; NSCLC

Introduction

Patients with *ALK*-positive NSCLC benefit from treatment with ALK-targeted therapies. The randomized, phase 3, global ALEX study (BO28984, NCT02075840) has established alectinib as the first-line standard-of-care treatment for *ALK*-positive NSCLC.[1–3] Within the ALEX study, patients with advanced *ALK*-positive NSCLC as defined by immunohistochemistry (IHC) were randomized 1:1 to receive alectinib 600 mg twice daily or crizotinib 250 mg twice daily.[2] At an exploratory nonprespecified updated data cutoff, with an additional 10 months of follow-up, median investigator-assessed progression-free survival (PFS) with alectinib was 34.8 months (95% confidence interval [CI]: 17.7–not estimable [NE]) versus 10.9 months (95% CI: 9.1–12.9) with crizotinib (stratified hazard ratio [HR] = 0.43, 95% CI: 0.32–0.58).[3]

Physicians rely on high-quality, robust *ALK* status testing methods to determine optimal therapeutic choices for patients with advanced NSCLC most likely to benefit from ALK tyrosine kinase inhibitor (TKI) treatment. Fluorescence in situ hybridization (FISH) and IHC are the most widely used diagnostic assays to determine *ALK* status, and physicians may use either test in clinical practice.[4–6] Discrepancies between *ALK* IHC and FISH are known to occur,[7–10] and analysis of the clinical outcomes for the discrepant cases is limited,[11–15] especially for patients treated with alectinib.

As central *ALK* FISH testing was conducted retrospectively within the ALEX study, this data set provides a unique opportunity to assess *ALK* IHC-based and *ALK* FISH-based assays in terms of clinical outcomes for patients receiving alectinib or crizotinib. This exploratory analysis examined PFS and response outcomes in patients enrolled in the ALEX study according to *ALK* status determined by central IHC and FISH.

Materials and Methods

Study Design and Patients

Full methodology for the ALEX study has been published previously.[2] In brief, patients were at ages greater than or equal to 18 years, with an Eastern Cooperative Oncology Group performance status of 0 to 2, with measurable (Response Evaluation Criteria in Solid Tumors version 1.1 [RECIST v1.1]), previously untreated, advanced *ALK* IHC-positive NSCLC. Patients with asymptomatic brain or leptomeningeal metastases were eligible; previous central nervous system (CNS) radiotherapy was allowed if it was completed greater than or equal to 14 days before enrollment. All eligible patients were randomized 1:1 to receive twice-daily alectinib 600 mg or crizotinib 250 mg until progressive disease (PD), unacceptable toxicity, withdrawal of consent, or death.

The study protocol was approved by the institutional review board or ethics committee at each participating center and complied with Good Clinical Practice guidelines, the principles of the Declaration of Helsinki, and local laws. All patients provided written informed consent.

Diagnostic Assays

At the time of enrollment, *ALK* IHC-positive status was determined in patient samples using the Ventana ALK (D5F3) CDx Assay (Ventana Medical Systems, Tucson, AZ), which was performed at the following central laboratories: HistoGeneX, Belgium, EU; LabCorp/Dianon,

Table 1. Baseline Patient Characteristics in the ALEX Study by *ALK* IHC and FISH Status

Characteristic	*ALK* IHC-Positive (n = 303)[a]		*ALK* IHC-Positive and FISH-Positive (n = 203)		*ALK* IHC-Positive and FISH-Negative (n = 39)	
	Alectinib (n = 152)	Crizotinib (n = 151)	Alectinib (n = 106)	Crizotinib (n = 97)	Alectinib (n = 21)	Crizotinib (n = 18)
Age, y, mean (SD)	56.3 (12.0)	53.8 (13.5)	55.2 (11.9)	52.9 (14.6)	59.5 (10.8)	57.2 (7.2)
Sex, n (%)						
Female	84 (55.3)	87 (57.6)	60 (56.6)	54 (55.7)	8 (38.1)	11 (61.1)
Male	68 (44.7)	64 (42.4)	46 (43.4)	43 (44.3)	13 (61.9)	7 (38.9)
Race, n (%)						
Asian	69 (45.4)	69 (45.7)	52 (49.1)	44 (45.4)	8 (38.1)	8 (44.4)
White	76 (50.0)	75 (49.7)	49 (46.2)	51 (52.6)	13 (61.9)	9 (50.0)
Smoking status, n (%)						
Current smoker	12 (7.9)	5 (3.3)	3 (2.8)	4 (4.1)	5 (23.8)	1 (5.6)
Past smoker	48 (31.6)	48 (31.8)	35 (33.0)	24 (24.7)	9 (42.9)	5 (27.8)
Nonsmoker	92 (60.5)	98 (64.9)	68 (64.2)	69 (71.1)	7 (33.3)	12 (66.7)
ECOG PS, n (%)						
0–1	142 (93.4)	141 (93.4)	99 (93.4)	89 (91.8)	19 (90.5)	18 (100.0)
2	10 (6.6)	10 (6.6)	7 (6.6)	8 (8.2)	2 (9.5)	0
Baseline CNS lesions present, n (%)	64 (42.1)	58 (38.4)	41 (38.7)	32 (33.0)	9 (42.9)	9 (50.0)

[a]For 61 of 303 patients (20%) with an *ALK* IHC-positive result, a valid *ALK* FISH result could not be obtained as the test led to an uninformative FISH result (10.9%), or because insufficient or no tumor tissue was available (9.2%).
CNS, central nervous system; ECOG PS, Eastern Cooperative Oncology Group performance status; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.

US; Q2 Solutions, Singapore, APAC; and Q2 Solutions, Beijing, People's Republic of China (for the People's Republic of China only). The samples were scored as IHC-positive or IHC-negative according to the manufacturer's scoring algorithm. Additional samples from randomized patients were retrospectively tested in the same central laboratories for *ALK* gene rearrangements using the U.S. Food and Drug Administration-approved Vysis ALK Break Apart FISH Probe Kit (Abbott Molecular, Des Plaines, IL). Samples were classified as FISH-positive or FISH-negative according to the manufacturer's instructions.

Detection of ALK Fusions by Next-Generation Sequencing

ALK fusions were classified using a hybrid-capture, next-generation sequencing (NGS) test method using proprietary computational algorithms that enabled variant calls to be accurately detected by discriminating sequencing artifacts from real mutations. Plasma samples were analyzed using the FoundationACT platform, and tissue samples were analyzed using FoundationONE, as previously described.[16–18]

Efficacy Assessments

The primary study end point of the ALEX study was investigator-assessed PFS, defined as the time from randomization to documented PD (RECIST v1.1) or death, whichever occurred first. Secondary end points included independent review committee-assessed PFS, objective response rate (ORR), duration of response (DoR), and CNS efficacy (time to CNS progression, CNS ORR, and CNS DoR). ORR was defined as the percentage of patients with a complete response or partial response (PR) according to RECIST v1.1. DoR was defined as the time from when the criteria for complete response or PR were first met to the occurrence of a PFS event. CNS end points were analyzed in patients with or without baseline CNS disease and in patients with baseline CNS disease with or without previous radiotherapy.

Statistical Analysis

Time-to-event summaries were estimated using Kaplan-Meier methodology, with 95% CI for the median computed using the Clopper-Pearson method. Stratified HRs were estimated by Cox regression, in which the stratification factors were Eastern Cooperative Oncology Group performance status (0 or 1 versus 2), race (Asian versus non-Asian), and presence of baseline CNS metastases by independent review committee (yes versus no). PFS was evaluated in the intent-to-treat (ITT) population, which comprised all randomized patients. ORR was determined in the response-assessable population, which included all patients with measurable disease at baseline according to the investigator.

Table 2. *ALK* Status by IHC and FISH in the ALEX Study

Result, n (%)	Alectinib (n = 152)	Crizotinib (n = 151)	Total (n = 303)
IHC			
Positive	152 (100.0)	151 (100.0)	303 (100.0)
FISH			
Positive	106 (69.7)	97 (64.2)	203 (67.0)
Negative	21 (13.8)	18 (11.9)	39 (12.9)
Uninformative[a]	25 (16.4)	36 (23.8)	61 (20.1)

[a]Uninformative FISH result, or insufficient adequate tissue or no tissue available for FISH test.
FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.

Results

ALK Status

In total, 303 patients with advanced *ALK* IHC-positive NSCLC were randomized to treatment in the ALEX study. Baseline patient characteristics were balanced between the alectinib and crizotinib arms in the overall *ALK* IHC-positive population (Table 1).

Overall, 242 of 303 patients (79.9%) had a valid *ALK* FISH result, of whom 203 patients (83.9%) had *ALK* IHC-positive and FISH-positive tumors and 39 (16.1%) had *ALK* IHC-positive and FISH-negative tumors (alectinib, n = 21; crizotinib, n = 18; Table 2). For 61 of 303 patients (20.1%) with an *ALK* IHC-positive result, a valid *ALK* FISH result could not be obtained as the test led to an uninformative FISH result (10.9%), or because insufficient or no tumor tissue was available (9.2%). An imbalance in smoking status was observed in the *ALK* IHC-positive and FISH-negative subgroup, with approximately double the proportion of current or past smokers in the alectinib arm (66.7%) relative to the crizotinib arm (33.4% Table 1).

Efficacy Outcomes by ALK Status

***Investigator-Assessed* PFS.** At the exploratory nonprespecified updated data cutoff (December 1, 2017), with a median follow-up of 27.8 months with alectinib and 22.8 months with crizotinib, the HR for investigator-assessed PFS in patients with ALK IHC-positive and FISH-positive tumors was 0.37 (95% CI: 0.25–0.56, median = 34.8 mo [95% CI: 27.8–NE] for alectinib versus 12.6 mo [95% CI: 9.1–14.8] for crizotinib) (Fig. 1*A*). These findings were consistent with both the primary ITT analysis of ALEX and the updated ITT analysis of PFS in the ALK IHC-positive population.[2,3]

The HR for investigator-assessed PFS in patients with *ALK* IHC-positive and FISH-uninformative results (n = 61) was 0.39 (95% CI: 0.20–0.78; median = 22.4 mo [95% CI: 11.1–NE] with alectinib versus 9.8 mo [95% CI: 7.5–14.6] with crizotinib) (Fig. 1*B*), which was also similar to the overall ALEX ITT population PFS.[2,3]

In patients with *ALK* IHC-positive and FISH-negative tumors (n = 39), the HR for PFS was 1.33 (95% CI: 0.6–3.2), the Kaplan-Meier curves crossed, and median PFS times were low for both alectinib (3.8 mo [95% CI: 1.9–NE]) and crizotinib (7.4 mo [95% CI: 2.7–22.1]) (Fig. 1*C*). Of note, the number of patients at risk was very small in these nonprespecified subgroups.

Objective Response Rate

Higher ORRs were noted with alectinib versus crizotinib in patients with *ALK* IHC-positive and FISH-positive tumors: 90.6% (n = 96) versus 81.4% (n = 79), respectively (stratified OR = 2.22, 95% CI: 0.97–5.07; Table 3). This was similar to the ITT population in the ALEX study[2] and to the updated analysis of ALEX in the *ALK* IHC-positive population (ORR = 82.9% alectinib versus 75.5% crizotinib).[3] Low rates of PD were observed in both treatment groups: 1.9% alectinib versus 3.1% crizotinib.

In the *ALK* IHC-positive and FISH-uninformative subgroup, the ORR for alectinib and crizotinib was 96.0% (n = 24) and 75.0% (n = 27), respectively (stratified OR = 9.29, 95% CI: 1.05–81.88). PD rates were low, at 0% for alectinib and 8.3% for crizotinib (Table 3).

In samples from patients with *ALK* IHC-positive and FISH-negative results, 28.6% of the patients (n = 6) responded to alectinib and 44.4% of the patients (n = 8) responded to crizotinib (stratified OR = 0.45, 95% CI: 0.12–1.74; Table 3). PD rates were higher than those in the *ALK* IHC-positive and FISH-positive subgroup, at 28.6% and 22.2% for alectinib and crizotinib, respectively.

To determine *ALK* fusion status in the 39 patients with *ALK* IHC-positive and FISH-negative results (regardless of treatment arm), we performed targeted NGS using tumor tissue and plasma samples (tissue and plasma samples, n = 17; tissue only samples, n = 9; plasma only samples, n = 9). Data were available for 35 of 39 patients; no *ALK* fusion was identified in 20 (57.1%) of these patients, with *EML4-ALK* fusion detected in 15 patients (42.9%). In the *ALK* fusion-positive subgroup by NGS (n = 15), 46.7% of the patients (n = 7) responded to treatment (either crizotinib or alectinib); 20.0% (n = 3) had PD. Of note, in the *ALK*

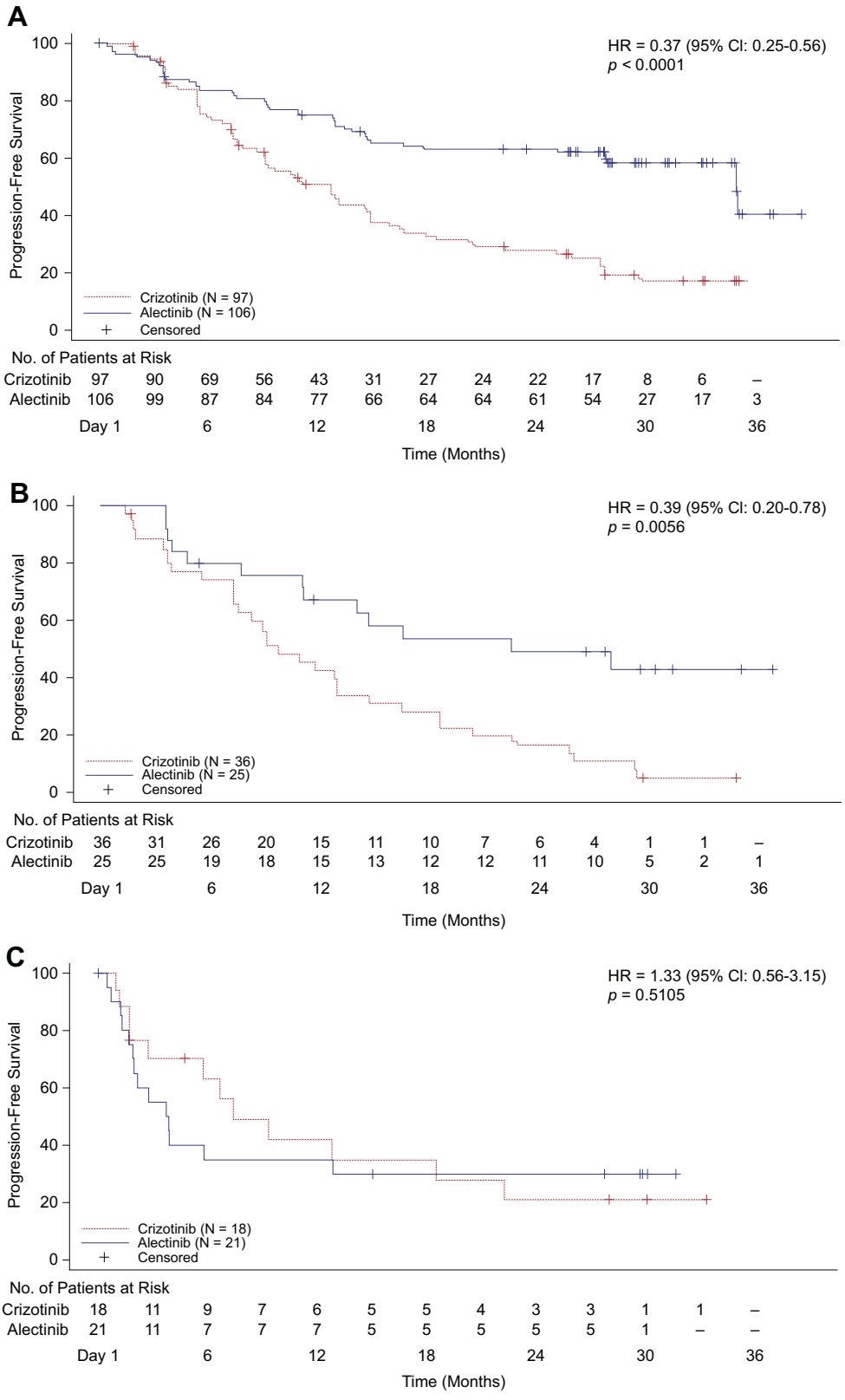

Figure 1. Investigator-assessed progression-free survival: (*A*) *ALK* IHC-positive and FISH-positive NSCLC; (*B*) *ALK* IHC-positive and FISH-uninformative NSCLC; and (*C*) *ALK* IHC-positive and FISH-negative NSCLC. CI, confidence interval; FISH, fluorescence in situ hybridization; HR, hazard ratio; IHC, immunohistochemistry.

Table 3. Objective Response Rate According to *ALK* Status (Response-Assessable Population)

ORR	Alectinib	Crizotinib	Stratified OR (95% CI)
ALK IHC-positive and FISH-positive	n = 106	n = 97	–
ORR, n (%)	96 (90.6)	79 (81.4)	2.22 (0.97-5.07)
CR	6 (5.7)	3 (3.1)	–
PR	90 (84.9)	76 (78.4)	–
SD	4 (3.8)	14 (14.4)	–
PD	2 (1.9)	3 (3.1)	–
Missing or unassessable	4 (3.8)	1 (1.0)	–
ALK IHC-positive and FISH-uninformative[a]	n = 25	n = 36	–
ORR, n (%)	24 (96.0)	27 (75.0)	9.29 (1.05-81.88)
CR	0	0	–
PR	24 (96.0)	27 (75.0)	–
SD	0	5 (13.9)	–
PD	0	3 (8.3)	–
Missing or unassessable	1 (4.0)	1 (2.8)	–
ALK IHC-positive and FISH-negative	n = 21	n = 18	–
ORR, n (%)	6 (28.6)	8 (44.4)	0.45 (0.12-1.74)
CR	1 (4.8)	0	–
PR	5 (23.8)	8 (44.4)	–
SD	5 (23.8)	5 (27.8)	–
PD	6 (28.6)	4 (22.2)	–
Missing or unassessable	4 (19.0)	1 (5.6)	–

[a]Uninformative FISH result, or insufficient adequate tissue or no tissue available for FISH test.
CI, confidence interval; CR, complete response; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease.

fusion-negative subgroup (n = 20), a PR was observed in 15.0% of the patients (n = 3) and stable disease in 35.0% of the patients (n = 7).

Duration of Response

In line with the results from the ITT population of ALEX, DoR was significantly longer with alectinib than with crizotinib in patients with *ALK* IHC-positive and FISH-positive tumors: HR = 0.34, 95% CI: 0.22–0.53; $p < 0.0001$. Median DoR was 33.1 months (95% CI: 31.3–NE) with alectinib versus 11.1 months (95% CI: 7.4–14.7) with crizotinib (Table 4).

In patients with *ALK* IHC-positive and FISH-uninformative NSCLC, median DoR was similar to the *ALK* IHC-positive and FISH-positive group with 26.1 months for alectinib and 9.1 months with crizotinib (HR = 0.37, 95% CI: 0.17–0.80).

Median DoR was NE in patients with *ALK* IHC-positive and FISH-negative results receiving either crizotinib or alectinib (Table 4). Individual DoR ranged from 1.8 to 29.9 months with alectinib (one patient 1.8 mo, five patients > 24.0 mo) and from 0 to 31.5 months with crizotinib (one patient 0 mo, three patients 24.0 mo) (Fig. 2).

Table 4. Duration of Response According to *ALK* Status (Response-Assessable Population)

DoR	Alectinib	Crizotinib
ALK IHC-positive and FISH-positive	n = 106	n = 97
Median DoR, mo (95% CI)	33.1 (31.3-NE)	11.1 (7.4-14.7)
Stratified HR (95% CI)	0.34 (0.22-0.53), $p < 0.0001$	
ALK IHC-positive and FISH-uninformative[a]	n = 25	n = 36
Median DoR, mo (95% CI)	26.1 (9.4-NE)	9.1 (6.6-12.9)
Stratified HR (95% CI)	0.37 (0.17-0.80), $p = 0.0087$	
ALK IHC-positive and FISH-negative	n = 21	n = 18
Median DoR, mo (95% CI)	NE (NE)	NE (7.4-NE)
Stratified HR (95% CI)	0.24 (0.02-2.62), $p = 0.2181$	

[a]Uninformative FISH result, or insufficient adequate tissue or no tissue available for FISH test.
CI, confidence interval; DoR, duration of response; FISH, fluorescence in situ hybridization; HR, hazard ratio; IHC, immunohistochemistry; NE, not estimable.

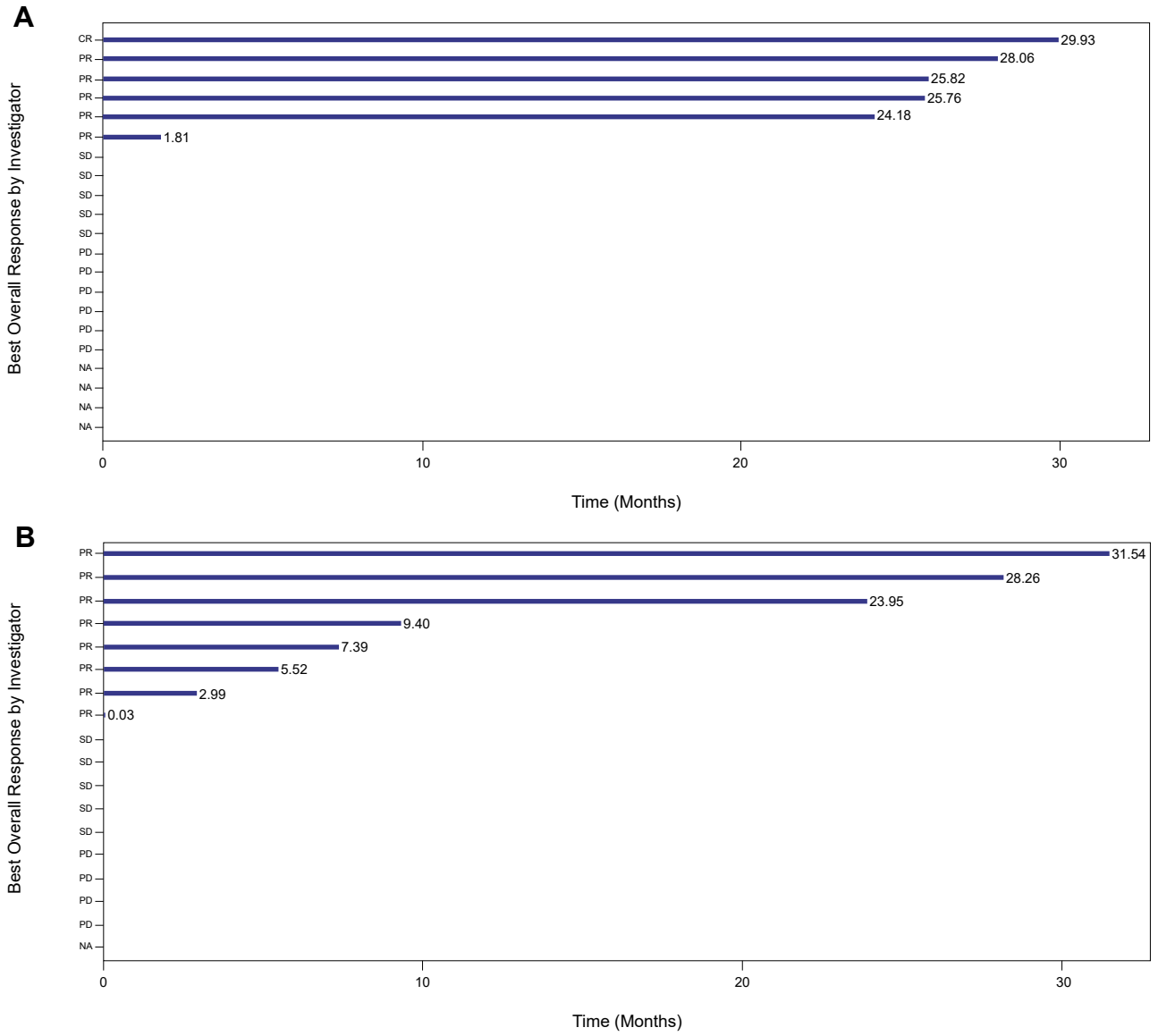

Figure 2. Best individual overall response in patients with *ALK* IHC-positive and FISH-negative NSCLC treated with (*A*) alectinib or (*B*) crizotinib (response-assessable population). CR, complete response; FISH, fluorescence in-situ hybridization; IHC, immunohistochemistry; NA, not assessable; PD, progressive disease; PR, partial response; SD, stable disease.

Discussion

Discrepancies between IHC and FISH pose a challenge for physicians when determining *ALK* status before selecting the optimal ALK TKI therapy for patients with advanced NSCLC. A number of reasons for the discrepancies between the two methods have been proposed, including technical aspects of the assays and biological features of *ALK*-positive tumors.[7–9]

Nonspecific staining, poor fixation, or insensitive detection methods can produce false IHC results.[9] False FISH results, be that positive or negative, can occur as a result of a number of preanalytical factors or atypical break-apart signal profiles, with discrepant IHC or FISH findings often associated with FISH "borderline"-positive samples (<15% split-positive cells).[8–10] In addition, whereas levels of IHC ALK positivity have been reported to correlate with FISH *ALK* positivity in some series,[19] it is not possible to correlate IHC intensity with FISH positivity or negativity given the binary readout of the Ventana IHC assay. Although a screening strategy on the basis of ALK IHC is nowadays considered standard, these data add to the debate about the need for confirmatory testing.

In this exploratory analysis of patients with advanced *ALK* IHC-positive NSCLC enrolled in the ALEX study, 83.9% (203 of 242) of the patients with a valid FISH result had *ALK* IHC-positive and FISH-positive tumors

and 16.1% had *ALK* IHC-positive and FISH-negative tumors (a valid *ALK* FISH result could not be obtained for 20.1% of 303 *ALK* IHC-positive patients, either because no sample was available for FISH testing or the test was inconclusive). The concordance between IHC and FISH that we observed in ALEX is in line with the 85% (154 of 182) positive concordance rate reported in the PROFILE 1014 study of first-line crizotinib versus platinum-doublet chemotherapy for advanced *ALK*-positive NSCLC.[20] The level of discordance for IHC-positive and FISH-negative cases in PROFILE 1014 was also similar to ours, at 15%.[20] Furthermore, in a large systematic review and meta-analysis including 11,806 NSCLC cases from 42 individual studies, the concordance rate between ALK IHC and FISH in patients with *ALK* IHC-positive disease was 80.5% (95% CI: 73.3–86.1).[21]

We found that the HR for investigator-assessed PFS was 0.37 (95% CI: 0.25–0.56) in patients with *ALK* IHC-positive and FISH-positive disease, which is consistent with the primary ITT analysis of ALEX (stratified HR = 0.47, 95% CI: 0.34–0.65, $p < 0.001$).[2] The HR of 0.37 is consistent with that reported in the open-label, randomized phase 3 J-ALEX study of alectinib versus crizotinib in Japanese patients with advanced NSCLC, which required patients to have tumors that were both *ALK* IHC-positive and FISH-positive at enrollment (HR = 0.34, 99.7% CI: 0.17–0.71).[22] For patients with *ALK* IHC-positive and FISH-uninformative disease, the HR for investigator-assessed PFS was 0.39 (95% CI: 0.20–0.78), with a median PFS of 22.4 months (95% CI: 11.1–NE) with alectinib, which is consistent with the *ALK* IHC-positive and FISH-positive subgroup and the primary ITT analysis. Therefore, an uninformative *ALK* FISH test result should not prevent physicians from treating patients with alectinib if the patient's tumor is determined to be *ALK* positive by another approved method, such as Ventana ALK (D5F3 CDx Assay) IHC.

In contrast to these findings, the HR for investigator-assessed PFS in patients enrolled in ALEX with *ALK* IHC-positive and FISH-negative disease was 1.33 (95% CI: 0.6–3.2). ORR was also lower in this subgroup than in patients with *ALK* IHC-positive and FISH-positive disease. In some cases, patients with discordant *ALK* IHC and FISH results derived a clinical benefit from treatment with alectinib. However, the small sample size may prevent us from making a conclusive remark on efficacy.

Nevertheless, in agreement with reports of an isolated number of *ALK* IHC and FISH discordant cases,[11–14] we observed that a proportion of patients with *ALK* IHC-positive and FISH-negative NSCLC achieved an ORR with ALK TKI treatment (28.6% alectinib, 44.4% crizotinib). Individual DoR ranged up to 31 months (29.9 mo alectinib, 31.5 mo crizotinib) in responding patients, which was similar to the DoR achieved in the *ALK* IHC-positive and FISH-positive subgroup. However, as a subpopulation, these patients had a relatively lower ORR and shorter median PFS than the other subgroups, which may suggest that responding patients in this subgroup are true *ALK* IHC-positive but false *ALK* FISH-negative. Furthermore, this *ALK* IHC-positive and FISH-negative subgroup may represent an ALK patient population with FISH scores just below the FISH cutoff, which could be a possible reason for discordance, but this is not based on data from our cohort. Nonresponders may either be truly ALK fusion-negative, have high ALK protein expression or *ALK* gene copy number in the absence of *ALK* rearrangements, or may not respond for other reasons than ALK fusion negativity, because even in the *ALK* IHC-positive and FISH-positive subgroup, 10% to 20% of the patients did not respond to ALK inhibitors. It is also possible that the lower ORR achieved with alectinib versus crizotinib in this patient subgroup may be because of the small sample size and imbalances with regards to smoking status. The question is how best to identify patients who will derive benefit from ALK inhibitors in the small group of patients with discordant *ALK* IHC and FISH results.

One way to resolve discordant ALK cases is to perform NGS, or alternatively a reverse-transcriptase polymerase chain reaction assessment, to detect *ALK* gene mutations and fusions. A number of NGS platforms are available, including a highly multiplexed polymerase chain reaction amplicon-based targeted NGS method that detects both known and novel *ALK* fusions in formalin-fixed, paraffin-embedded tissue samples.[23] The use of targeted NGS has already proven beneficial in clarifying cases of discordant *ALK* IHC or FISH results in patients with lung cancer.[24,25] McLeer-Florin et al.[24] reported high sensitivity and specificity of NGS compared with IHC and FISH for the detection of *ALK* fusions in 76 patients with NSCLC that was discordant by IHC and FISH. In addition, Dacic et al.[25] analyzed the detection of *ALK* fusions using NGS versus IHC and FISH in 28 patients with discordant *ALK* FISH-positive NSCLC. No significant association between response to crizotinib and FISH patterns was found, but NGS fusion-positive and IHC-positive cases were associated with a higher response rate than NGS fusion-negative cases ($p = 0.016$).[25] In line with these earlier reports, we also observed a higher response rate in NGS fusion-positive cases compared with NGS fusion-negative cases within the *ALK* IHC-positive and FISH-negative subgroup. Three patients with *ALK* IHC-positive, FISH-negative and NGS-negative disease responded to an *ALK* TKI, and seven had stable disease. The reason for their response remains unclear, but it could be because of tumor heterogeneity or complex *ALK* rearrangements that are not detected by FISH or NGS.

It should be noted that our data are hypothesis generating and are limited by the exploratory nature of the analysis and the low number of patients in the different *ALK* subgroups, especially the *ALK* IHC-positive and FISH-negative subgroup. More than one-third of the responders in the *ALK* IHC-positive and FISH-negative subgroup did not have NGS results available. A certain degree of discordance is inevitable when using different IHC and FISH diagnostic tests, and this cannot always be resolved by NGS. Thus, careful consideration should be taken to identify the optimal treatment for patients with discordant IHC and FISH results. RNA-based or plasma circulating tumor DNA *ALK* fusion detection methods should be considered for *ALK* IHC-positive and FISH-negative cases that cannot be resolved by tissue-based NGS.

In summary, outcomes of patients with *ALK* IHC-positive and FISH-positive and *ALK* IHC-positive and FISH-uninformative NSCLC were similar to those of the overall ALEX population, which was selected prospectively by ALK IHC only. Even in patients with discordant results (*ALK* IHC-positive and FISH-negative NSCLC), more than 40% were positive for *ALK* fusion by NGS, and objective response to ALK TKI therapy was observed. These results suggest that Ventana ALK IHC alone is a standard testing method that is sufficient for selecting patients for treatment with alectinib, as supported by multiple treatment guidelines.[1,4] Nevertheless, for the small subset of patients in whom discordant ALK results are observed, DNA- or RNA-based NGS or circulating tumor DNA-based diagnostic methods should be considered to resolve these cases and enable optimal care for the patients.

Data Sharing

Qualified researchers may request access to individual patient-level data through the clinical study data request platform (https://vivli.org/). Further details on Roche's criteria for eligible studies are available here (https://vivli.org/members/ourmembers/). For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here (https://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/our_commitment_to_data_sharing.htm).

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