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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 progressionfree survival (PFS) and response by *ALK* fluorescence in

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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 < 1000.0001) 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 IHCpositive 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.

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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 non-prespecified 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 <i>ALK</i> IHC and FISH Status | | | | | | | | |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|--|--|--|--|--|--|--|
| ALK IHC-Positive $(n = 303)^a$ ALK IHC-Positive and FISH-Positive $(n = 203)$ ALK IHC-Positive FISH-Negative $(n = 203)$ | ALK IHC-Positive and FISH-Negative $(n = 39)$ | | | | | | | |
| $\begin{array}{c cccc} Alectinib & Crizotinib \\ Characteristic & (n = 152) & (n = 151) \\ \end{array} \begin{array}{c ccccc} Alectinib & Crizotinib \\ (n = 106) & (n = 97) \\ \end{array} \begin{array}{c cccccc} Alectinib & C \\ (n = 21) & (n = 21) \\ \end{array} \begin{array}{c ccccccccccccccccccccccccccccccccccc$ | rizotinib $n = 18)$ | | | | | | | |
| 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 | 7.2 (7.2) | | | | | | | |
| Sex, n (%) | | | | | | | | |
| Female 84 (55.3) 87 (57.6) 60 (56.6) 54 (55.7) 8 (38.1) 12 | (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 | 2 (66.7) | | | | | | | |
| ECOG PS, n (%) | | | | | | | | |
| 0-1 142 (93.4) 141 (93.4) 99 (93.4) 89 (91.8) 19 (90.5) 18 | 3 (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) | | | | | | | |

^aFor 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 IHCpositive 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 FISHpositive 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-assesable 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) | | | |

^aUninformative FISH result, or insufficient adequate tissue or no tissue available for FISH test.

 $\ensuremath{\mathsf{FISH}}$, 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 investigatorassessed 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 FISHpositive 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 study² 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* fusionpositive 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) | _ | | |

^aUninformative 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 FISHuninformative 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* IHCpositive 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) | $0.24 \ (0.02 - 2.62), \ p = 0.2181$ | | | | |

^aUninformative 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 platinumdoublet 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 IHCpositive 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 IHCpositive 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 IHCpositive 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 IHCpositive 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 investigatorassessed PFS in patients enrolled in ALEX with *ALK* IHCpositive 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* IHCpositive 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 formalinfixed, 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 NGSnegative 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 FISHnegative cases that cannot be resolved by tissue-based NGS.

In summary, outcomes of patients with ALK IHCpositive 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

February 2021

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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References

- National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology non-small cell lung cancer, version 6.2020. https://www.nccn.org/professionals/ physician_gls/pdf/nscl_blocks.pdf. Accessed July 7, 2020.
- 2. Peters S, Camidge DR, Shaw AT, et al. Alectinib versus crizotinib in untreated ALK-positive non-small-cell lung cancer. *N Engl J Med*. 2017;377:829-838.
- **3.** Camidge DR, Dziadziuszko R, Peters S, et al. Updated efficacy and safety data and impact of *EML4-ALK* fusion variant on the efficacy of alectinib in untreated *ALK*-positive advanced non-small cell lung cancer in the global phase III ALEX study. *J Thorac Oncol.* 2019;14:1233-1243.
- 4. Planchard D, Popat S, Kerr K, et al. Correction to Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2018;29(suppl 4):iv192-iv237.
- 5. Leighl NB, Rekhtman N, Biermann WA, et al. Molecular testing for selection of patients with lung cancer for epidermal growth factor receptor and anaplastic lymphoma kinase tyrosine kinase inhibitors: American Society of Clinical Oncology endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology Guideline. J Clin Oncol. 2014;32:3673-3679.
- 6. Lindeman NI, Cagle PT, Aisner DL, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. J Mol Diagn. 2018;20:129-159.
- 7. Cabillic F, Gros A, Dugay F, et al. Parallel FISH and immunohistochemical studies of ALK status in 3244 non-small-cell lung cancers reveal major discordances. *J Thorac Oncol.* 2014;9:295-306.
- 8. 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;26:238-244.
- 9. Yatabe Y. ALK Fish and IHC: you cannot have one without the other. *J Thorac Oncol*. 2015;10:548-550.
- Mattsson JSM, Brunnström H, Jabs V, et al. Inconsistent results in the analysis of *ALK* rearrangements in nonsmall cell lung cancer. *BMC Cancer*. 2016;16:603.
- Shan L, Jiang P, Xu F, et al. BIRC6-ALK, a novel fusion gene in ALK break-apart FISH-negative lung adenocarcinoma, responds to crizotinib. *J Thorac Oncol*. 2015;10:e37-e39.
- Zanwar S, Noronha V, Joshi A, et al. Efficacy of crizotinib in ALK mutant non-small cell lung cancers that are positive by IHC but negative by FISH compared to FISH positive cases. *Indian J Cancer*. 2017;54:678-680.
- 13. 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 nonsmall cell lung cancer. *Clin Cancer Res.* 2017;23:4251-4258.

- 14. 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:e000419.
- **15.** Letovanec I, Finn S, Zygoura P, et al. Evaluation of NGS and RT-PCR methods for ALK rearrangement in European NSCLC patients: results from the European Thoracic Oncology Platform Lungscape Project. *J Thorac Oncol.* 2018;13:413-425.
- Foundation Medicine FoundationOne[®]CDx. https://www. foundationmedicine.com/genomic-testing/foundationone-cdx. Accessed February 3, 2020.
- **17.** Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol*. 2013;31:1023-1031.
- **18.** Clark TA, Chung JH, Kennedy M, et al. Analytical validation of a hybrid capture-based next-generation sequencing clinical assay for genomic profiling of cell-free circulating tumor DNA. J Mol Diagn. 2018;20:686-702.
- **19.** Blackhall FH, Peters S, Bubendorf L, et al. Prevalence and clinical outcomes for patients with ALK-positive resected stage I to III adenocarcinoma: results from the

European Thoracic Oncology Platform Lungscape Project. *J Clin Oncol.* 2014;32:2780-2787.

- 20. Thorne-Nuzzo T, Williams C, Catallini A, et al. A sensitive ALK immunohistochemistry companion diagnostic test identifies patients eligible for treatment with crizotinib. *J Thorac Oncol.* 2017;12:804-813.
- 21. Pyo JS, Kang G, Sohn JH. ALK immunohistochemistry for ALK gene rearrangement screening in non-small cell lung cancer: a systematic review and meta-analysis. *Int J Biol Markers*. 2016;31:e413-e421.
- 22. Hida T, Nokihara N, Kondo M, et al. Alectinib versus crizotinib in patients with ALK-positive non-small-cell lung cancer (J-ALEX): an open-label, randomised phase 3 trial. *Lancet*. 2017;390:29-39.
- 23. Beadling C, Wald AI, Warrick A, et al. A multiplexed amplicon approach for detecting gene fusions by next-generation sequencing. *J Mol Diagn*. 2016;18:165-175.
- 24. McLeer-Florin A, Duruisseaux M, Pinsolle J, et al. ALK fusion variants detection by targeted RNA-next generation sequencing and clinical responses to crizotinib in ALK-positive non-small cell lung cancer. *Lung Cancer*. 2018;116:15-24.
- 25. Dacic S, Villaruz LC, Abberbock S, Mahaffey A, Incharoen P, Nikiforova MN. ALK FISH patterns and the detection of ALK fusions by next generation sequencing in lung adenocarcinoma. *Oncotarget*. 2016;7:82943-82952.