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ORIGINAL ARTICLE

Concordance Between Tissue *ALK* Detection by Immunohistochemistry and Plasma *ALK* Detection by Next-Generation Sequencing in the Randomized Phase 3 ALEX Study in Patients With Treatment-Naive Advanced *ALK*-Positive NSCLC

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ABSTRACT

Introduction: The Blood First Assay Screening Trial revealed the clinical applicability of blood-based next-generation sequencing to identify patients with *ALK*-positive NSCLC for alectinib treatment. To understand the relationship between tissue-based versus blood-based testing, we retrospectively investigated concordance between VENTANA ALK (D5F3) CDx immunohistochemistry and the FoundationACT (FACT; Foundation Medicine, Inc.) plasma assay, and compared clinical efficacy between phase 3 ALEX study subpopulations.

Methods: Patients with advanced *ALK*-positive (by immunohistochemistry) NSCLC were randomized 1:1 to alectinib 600 mg or crizotinib 250 mg, twice daily. Assessable baseline plasma samples were analyzed for *ALK* positivity by FACT; positive percent agreement with immunohistochemistry was evaluated. Progression-free survival (PFS), duration of response, and objective response rate were compared between intention-to-treat (ITT) and biomarker-evaluable populations, and plasma *ALK*-positive and plasma *ALK*-negative subpopulations.

Results: In the ITT population (303 patients; alectinib, 152; crizotinib, 151), all patients had *ALK*-positive tumors by immunohistochemistry. In the biomarker-evaluable population (149 patients; alectinib, 76; crizotinib, 73), 105 had plasma *ALK*-positive and 44 had plasma *ALK*-negative tumors. Positive percent agreement between immunohistochemistry and FACT was 70.5% (105 of 149; 95% confidence interval: 62.5–77.7). Baseline characteristics were generally balanced, with some exceptions, notably tumor burden. Median PFS in plasma *ALK*-positive and *ALK*-negative patients was 22.4 months and not estimable with alectinib and 7.3 months and 12.9 months with crizotinib, respectively; median duration of response was 25.9 months and not estimable with alectinib and 5.6 months and 11.5 months with crizotinib, respectively.

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Conclusions: Reasonable concordance between FACT and immunohistochemistry was observed; both methods are valuable in identifying *ALK*-positive patients, separately or concurrently. Alectinib was found to have superior PFS in the plasma *ALK*-positive population, as in the ITT population.

Keywords: Alectinib; *ALK*-positive; Concordance; Immunohistochemistry; Next-generation sequencing; NSCLC

Introduction

NSCLC treatment paradigms have changed in the past two decades with the advent of targeted therapies. Clinical practice guidelines recommend incorporation of molecular tumor testing into routine practice to guide clinical care for patients with probable or definite adenocarcinoma.[1,2] Molecular tumor testing generally requires a tissue sample to confirm the primary site and histologic subtype of the cancer.[1,2] Nevertheless, owing to tumor heterogeneity, tissue sampling (an invasive procedure) in one site may not accurately reflect the comprehensive genomic profile of all lesions.[3] Furthermore, adequate tumor tissue may be unavailable due to inaccessibility of lesions and limitations imposed by comorbidities associated with NSCLC, such as emphysema or lung fibrosis.[4,5] Approximately 26% of repeat biopsies fail to provide sufficient material for genomic analysis.[6] Consequently, patients with advanced NSCLC may not receive optimal treatment.[7]

ALK fusions are the driver genetic alteration in approximately 5% of NSCLC.[8,9] *ALK* status of a tumor can be determined in tissue samples using immunohistochemistry (IHC), fluorescence in situ hybridization, polymerase chain reaction, or next-generation sequencing (NGS).[2] The suitability of each assay for identifying patients with *ALK*-positive NSCLC who may benefit from ALK inhibitor therapy is determined by the local prescribing information of each drug.

The global, randomized phase 3 ALEX study (NCT02075840) in patients with treatment-naive advanced *ALK*-positive NSCLC revealed significantly improved progression-free survival (PFS) for the ALK inhibitor alectinib versus crizotinib (stratified hazard ratio [HR] 0.43, 95% confidence interval [CI]: 0.32–0.58) and established alectinib as a preferred first-line treatment option in this setting.[1,10–12] The *ALK*-positive status of patients in ALEX was prospectively determined by the VENTANA ALK (D5F3) CDx IHC assay (Ventana Medical Systems, Oro Valley, AZ).[10] Plasma samples were taken at baseline but not tested during screening.

The ongoing Blood First Assay Screening Trial (BFAST) (NCT03178552) is the first study to prospectively assign patients to a treatment based on the detection of an oncogenic driver using blood-based NGS as the sole screening method. The aim of BFAST is to evaluate the relationship between blood-based NGS detection of actionable mutations in circulating tumor DNA (ctDNA) and the clinical activity of targeted therapies or immunotherapies in treatment-naive advanced NSCLC without mandatory tissue sampling.[13] BFAST revealed the clinical applicability of blood-based NGS to select patients with *ALK*-positive NSCLC for treatment with alectinib. In this NGS-selected cohort with *ALK*-positive NSCLC, alectinib was found to have similar clinical efficacy to that observed in patients selected based on IHC tissue testing in the ALEX study. However, tissue samples were not mandatory in BFAST, so to further understand the relationship between blood-based and tumor tissue-based testing for *ALK,* we conducted an exploratory, retrospective concordance analysis within the ALEX study.

Here, we present the results of the analysis investigating concordance between the VENTANA ALK (D5F3) IHC CDx tissue assay (used to determine *ALK* status in ALEX) and ctDNA testing from the same patients using the FoundationACT (FACT; Foundation Medicine Inc., Cambridge, MA) assay. In addition, efficacy (primary end point: investigator-assessed PFS; secondary end points: investigator-assessed objective response rate [ORR] and duration of response [DoR]) in different patient subpopulations (intention-to-treat [ITT] versus biomarker-evaluable population [BEP], plasma *ALK*-positive versus plasma *ALK*-negative population) was determined.

Materials and Methods

ALEX Study Design

Full details of the ALEX study have been published previously.[10] Briefly, patients with stage IIIB or IV *ALK*-positive NSCLC (determined centrally using VENTANA ALK [D5F3] CDx IHC tissue assay) were randomized 1:1 to receive alectinib 600 mg twice daily or crizotinib 250 mg twice daily until progressive disease (PD), toxicity, withdrawal, or death. Patients with asymptomatic brain or leptomeningeal metastases were eligible for enrolment. Crossover between treatment arms was not permitted before PD. Further lines of therapy after PD were at the physician's discretion and on the basis of availability.

The study protocol was approved by the Institutional Review Board or ethics committee at each participating

center, and the study was conducted in accordance with the principles of the Declaration of Helsinki, Good Clinical Practice Guidelines, and local laws. Written informed consent was obtained from all patients before enrolment.

ALEX Study Assessments

Patients underwent tumor imaging, including brain scans, at baseline. Tumor response was evaluated every 8 weeks until PD according to Response Evaluation Criteria in Solid Tumors version 1.1. PFS was defined as the time from randomization to confirmed PD or death, whichever occurred first. ORR was defined as the percentage of patients with a complete response or partial response according to Response Evaluation Criteria in Solid Tumors version 1.1. DoR was defined as the time from when the criteria for complete response or partial response were first met to the occurrence of a PFS event. Analyses were based on a clinical cutoff date of November 30, 2018.

Concordance Analysis

Tissue and plasma samples were tested for *ALK* positivity using VENTANA ALK (D5F3) CDx IHC and FACT assays, respectively. The ITT population included all patients randomized to study treatment; all patients had *ALK*-positive tumors by IHC. The BEP included patients in the ITT population with an available plasma sample and valid result, taken at baseline or the day after start of treatment. A valid result was one that passed in-process quality control metrics criteria, excluding those with a failed or no result, insufficient input mass (recommended minimum: 30 ng cell-free DNA), or plasma volume below 2.5 mL. The plasma *ALK*-positive and plasma *ALK*-negative populations comprised all patients in the BEP whose tumors were *ALK* positive or *ALK* negative, respectively, by FACT. Efficacy results were compared between the ITT, BEP, plasma *ALK*-positive, and plasma *ALK*-negative populations.

Concordance was assessed between IHC (tissue) and plasma *ALK* tests. Because all patients enrolled in the study (ITT population) had *ALK*-positive NSCLC by IHC central analysis per protocol inclusion criteria, only positive percent agreement (PPA) between FACT and IHC could be evaluated.

Statistical Analysis

The Kaplan-Meier method was used to estimate the median PFS for each treatment arm with 95% CIs. A stratified Cox proportional-hazards regression model was used to estimate the treatment effect, expressed as a HR together with 95% CI.

The concordance data presented in this manuscript are from an exploratory, retrospective analysis. As ALEX was not designed for the comparison of diagnostic tests, no formal statistical testing was planned; the resultant analyses were therefore not adjusted for multiple testing. Only plasma samples taken at baseline or on the day after the start of treatment were included in the analysis.

Results

Patients

In the ALEX study, 303 patients were randomized to receive alectinib (n = 152) or crizotinib (n = 151) (ITT population), of whom 149 patients (49% of ITT population) were included in the BEP (n = 76 alectinib, n = 73 crizotinib; Table 1). The plasma *ALK*-positive population comprised 105 patients (n = 53 alectinib, n = 52 crizotinib); the plasma *ALK*-negative population included 44 patients (n = 23 alectinib, n = 21 crizotinib; Table 2). Overall, 154 patients (n = 76 alectinib, n = 78 crizotinib) were excluded from the BEP (reasons for exclusion: did not fulfill prespecified sample criteria as defined in the Concordance Analysis section, n = 127; missing samples, n = 17; lack of approval from Human Genetics Resources Administration of China, n = 10) (Supplementary Fig. 1).

Demographic data and baseline characteristics were generally consistent across each population (ITT, BEP, plasma *ALK* positive, and plasma *ALK* negative) and balanced between treatment arms, with some exceptions. Of note, the target lesion median sum of longest diameters was 79.00 mm in the BEP and 68.00 mm in the ITT population, whereas the proportion of patients with more than three lesions was 86% and 77%, respectively, and the percentage of patients with liver lesions was 34% and 23%, respectively. The proportion of patients with more than three lesions at baseline was 96.2% and 92.3% in the alectinib and crizotinib arms in the plasma *ALK*-positive population and 69.6% and 61.9% in the plasma *ALK*-negative population, respectively (Table 2). The percentage of patients who presented with liver lesions at baseline was 27.6% and 39.7% in the alectinib and crizotinib arms in the BEP, 19.7% and 26.5% in the ITT population, 35.8% and 46.2% in the plasma *ALK*-positive population, and 8.7% and 23.8% in the plasma *ALK*-negative population, respectively (Tables 1 and 2).

Concordance Analysis

Since all patients in the ALEX ITT population were *ALK* positive by IHC, only PPA was evaluated. Using *ALK* positivity by IHC as the reference standard, PPA of FACT in plasma was 70.5% (105 of 149 [95% CI: 62.5–77.7]) and comparable between the alectinib (69.7% [95% CI: 58.1–79.8]) and crizotinib (71.2% [95% CI: 59.5–81.2]) arms.

Table 1. Demographic and Baseline Characteristics of the ITT Population and the BEP

	ITT (N = 303)		BEP (n = 149)	
Characteristic	Alectinib (n = 152)	Crizotinib (n = 151)	Alectinib (n = 76)	Crizotinib (n = 73)
Age [median], y (range)	58 (25–88)	54 (18–91)	57 (29–81)	54 (18–91)
<65, n (%)	115 (75.7)	118 (78.1)	61 (80.3)	59 (80.8)
≥65, n (%)	37 (24.3)	33 (21.9)	15 (19.7)	14 (19.2)
Ethnicity, n (%)				
Hispanic/Latino	8 (5.3)	8 (5.3)	6 (7.9)	2 (2.7)
Not Hispanic/Latino	138 (90.8)	136 (90.1)	68 (89.5)	67 (91.8)
Not stated	6 (3.9)	7 (4.6)	2 (2.6)	4 (5.5)
Race, n (%)				
Asian	69 (45.4)	69 (45.7)	33 (43.4)	35 (47.9)
Black/African American	0	4 (2.6)	0	1 (1.4)
Native American	4 (2.6)	0	4 (5.3)	0
Native Hawaiian	1 (0.7)	1 (0.7)	1 (1.3)	0
White	76 (50.0)	75 (49.7)	37 (48.7)	36 (49.3)
Unknown	2 (1.3)	2 (1.3)	1 (1.3)	1 (1.4)
Smoking status, n (%)				
Active smoker	12 (7.9)	5 (3.3)	6 (7.9)	1 (1.4)
Nonsmoker	91 (59.9)	97 (64.2)	48 (63.2)	44 (60.3)
Past smoker	49 (32.2)	49 (32.5)	22 (28.9)	28 (38.4)
ECOG PS, n (%)				
0 or 1	142 (93.4)	141 (93.4)	66 (86.8)	66 (90.4)
2	10 (6.6)	10 (6.6)	10 (13.2)	7 (9.6)
CNS lesions,[a] n (%)				
No	88 (57.9)	93 (61.6)	40 (52.6)	43 (58.9)
Yes	64 (42.1)	58 (38.4)	36 (47.4)	30 (41.1)
Liver lesions, n (%)				
Yes	30 (19.7)	40 (26.5)	21 (27.6)	29 (39.7)
Lesions, n (%)				
1-3	37 (24.3)	34 (22.5)	9 (11.8)	12 (16.4)
>3	115 (75.7)	117 (77.5)	67 (88.2)	61 (83.6)
Target lesion SLD (median), mm (range)	72 (10–206)	64 (14–205)	87 (11–206)	74 (15–205)

[a]Assessed by IRC.
BEP, biomarker-evaluable population; CNS, central nervous system; ECOG PS, Eastern Cooperative Oncology Group performance status; IRC, independent review committee; ITT, intention-to-treat; SLD, sum of longest diameter.

Exploratory Analysis: PFS Results

The treatment effect of alectinib versus crizotinib with respect to PFS in the ITT population and the BEP was similar (HR = 0.43 [95% CI: 0.32–0.58] and HR = 0.41 [95% CI: 0.27–0.61], respectively). Median investigator-assessed PFS in the BEP was 27.9 months (95% CI: 12.9–not estimable [NE]) with alectinib versus 9.0 months (95% CI: 7.2–10.8) with crizotinib (Fig. 1). In alectinib-treated patients, median PFS was 22.4 months (95% CI: 12.9–38.7) versus NE (95% CI: 3.6–NE) in the plasma *ALK*-positive and plasma *ALK*-negative populations, respectively (Fig. 2). Median PFS with crizotinib was 7.3 months (95% CI: 6.1–9.6) versus 12.9 months (95% CI: 7.5–18.4) in the plasma *ALK*-positive and plasma *ALK*-negative populations, respectively (Fig. 2). The PFS HR with alectinib versus crizotinib was 0.37 (95% CI: 0.23–0.59) in the plasma *ALK*-positive population and 0.50 (95% CI: 0.22–1.11) in the plasma *ALK*-negative population (Fig. 2). The PFS rate in the ITT population versus the BEP from years 1 to 4 in both treatment arms is found in Supplementary Figure 2.

Exploratory Analysis: ORR Results

Investigator-assessed ORR by treatment arm was similar between the ITT population and the BEP. With alectinib, 82.9% (ITT) and 84.2% (BEP) of patients achieved an objective response; ORR was 94.3% (95% CI: 84.3–98.8) in plasma *ALK*-positive patients and 60.9% (95% CI; 38.5–80.3) in plasma *ALK*-negative patients (Table 3). In the crizotinib arm, 75.5% (ITT) and 74.0% (BEP) of patients achieved an objective response (Table 3). The ORR with crizotinib was 80.8% (95% CI: 67.5–90.4) for plasma *ALK*-positive patients and 57.1% (95% CI: 34.0–78.2) for plasma *ALK*-negative patients.

Exploratory Analysis: DoR Results

Median investigator-assessed DoR was similar between the ITT population (alectinib, NE [95% CI: 29.8–

Table 2. Demographic and Baseline Characteristics of the Plasma *ALK*-Positive and Plasma *ALK*-Negative Populations

	Plasma *ALK* Positive (n = 105)		Plasma *ALK* Negative (n = 44)	
Characteristic	Alectinib (n = 53)	Crizotinib (n = 52)	Alectinib (n = 23)	Crizotinib (n = 21)
Age [median], y (range)	56 (29–81)	54 (18–91)	58 (42–74)	59 (30–79)
<65, n (%)	44 (83.0)	42 (80.8)	17 (73.9)	17 (81.0)
≥65, n (%)	9 (17.0)	10 (19.2)	6 (26.1)	4 (19.0)
Ethnicity, n (%)				
Hispanic or Latino	5 (9.4)	1 (1.9)	1 (4.3)	1 (4.8)
Not Hispanic or Latino	47 (88.7)	48 (92.3)	21 (91.3)	19 (90.5)
Not stated	1 (1.9)	3 (5.8)	1 (4.3)	1 (4.8)
Race, n (%)				
Asian	25 (47.2)	25 (48.1)	8 (34.8)	10 (47.6)
Black or African American	0	1 (1.9)	0	0
Native American	3 (5.7)	0	1 (4.3)	0
Native Hawaiian	1 (1.9)	0	0	0
White	23 (43.4)	26 (50.0)	14 (60.9)	10 (47.6)
Unknown	1 (1.9)	0	0	1 (4.8)
Smoking status, n (%)				
Active smoker	2 (3.8)	0	4 (17.4)	1 (4.8)
Nonsmoker	39 (73.6)	31 (59.6)	9 (39.1)	13 (61.9)
Past smoker	12 (22.6)	21 (40.4)	10 (43.5)	7 (33.3)
ECOG PS, n (%)				
0 or 1	46 (86.8)	46 (88.5)	20 (87.0)	20 (95.2)
2	7 (13.2)	6 (11.5)	3 (13.0)	1 (4.8)
CNS lesions,[a] n (%)				
No	30 (56.6)	33 (63.5)	10 (43.5)	10 (47.6)
Yes	23 (43.4)	19 (36.5)	13 (56.5)	11 (52.4)
Liver lesions, n (%)				
Yes	19 (35.8)	24 (46.2)	2 (8.7)	5 (23.8)
Lesions, n (%)				
1–3	2 (3.8)	4 (7.7)	7 (30.4)	8 (38.1)
>3	51 (96.2)	48 (92.3)	16 (69.6)	13 (61.9)
Target lesion SLD (median), mm (range)	90 (15–206)	79 (18–205)	57 (11–198)	71 (15–146)

[a]Assessed by IRC.
CNS, central nervous system; ECOG PS, Eastern Cooperative Oncology Group performance status; IRC, independent review committee; SLD, sum of longest diameter.

NE]; crizotinib, 11.1 mo [95% CI: 7.9–13.0]) and the BEP (alectinib, 36.9 mo [95% CI: 20.6–NE]; crizotinib, 7.3 mo [95% CI: 5.5–11.1]; Table 4). In the BEP (treatment arms combined), median DoR was 11.1 months (95% CI: 7.4–14.8) in the plasma *ALK*-positive population and NE (95% CI: 14.5–NE) in the plasma *ALK*-negative population (Table 4). Similar results were observed with alectinib in the BEP (Table 4); median DoR was 25.9 months (95% CI: 11.9–38.7) in the plasma *ALK*-positive population and NE (95% CI: NE) in the plasma *ALK*-negative population. With crizotinib, median DoR was 5.6 months (95% CI: 5.5–9.2) and 11.5 months (95% CI: 6.9–14.8) in the plasma *ALK*-positive and plasma *ALK*-negative populations, respectively.

In the ITT population and the BEP, 46.8% and 51.6% of alectinib-treated patients who achieved an objective response experienced PD or death compared with 79.8% and 85.2% of crizotinib-treated patients, respectively (Table 4). In the BEP (treatment arms combined), 69 of 92 patients (75.0%) with an objective response progressed or died in the plasma *ALK*-positive population compared with 10 of 26 patients (38.5%) in the plasma *ALK*-negative population (Table 4). With alectinib, 32 of 50 patients (64.0%) with an objective response in the plasma *ALK*-positive population and 1 of 14 patients (7.1%) with an objective response in the plasma *ALK*-negative population progressed or died (Table 4). With crizotinib, the rate of PD or death was 88.1% in the plasma *ALK*-positive population and 75.0% in the plasma *ALK*-negative population (Table 4).

Discussion

Guidelines recommend testing for several targetable gene alterations; however, tissue testing for several biomarkers can be challenging, especially for patients with limited tissue availability or when repeat biopsies are not feasible.[6,7] Many biomarkers for oncogenic driver

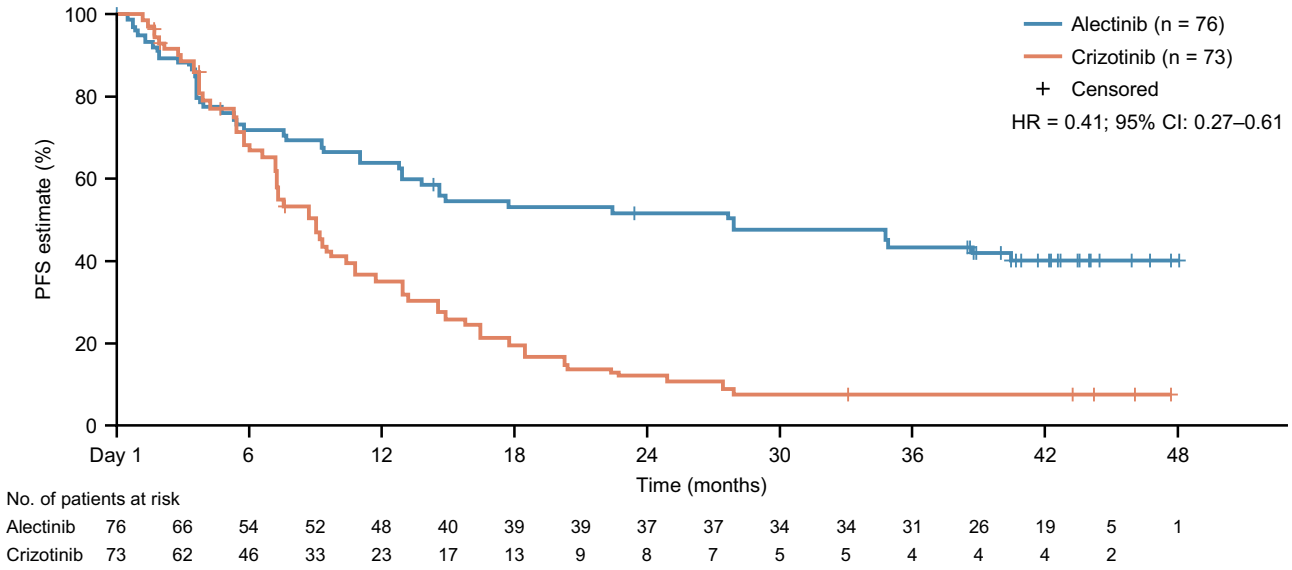

Figure 1. Kaplan-Meier plot of investigator-assessed PFS in the BEP. BEP, biomarker-evaluable population; CI, confidence interval; HR, hazard ratio; PFS, progression-free survival.

mutations, some with low prevalence, have been identified in lung cancer.[14] Consequently, extensive molecular testing for these biomarkers is required to determine the optimal treatment for each patient.[1] Implementing blood-based NGS analysis would provide a comprehensive, less-invasive method of analyzing multiple tumor molecular alterations simultaneously, which may help overcome some limitations of tissue-based, single-biomarker testing.[2] Moreover, ctDNA analyzed using blood-based NGS represents the molecular profile of multiple lesion

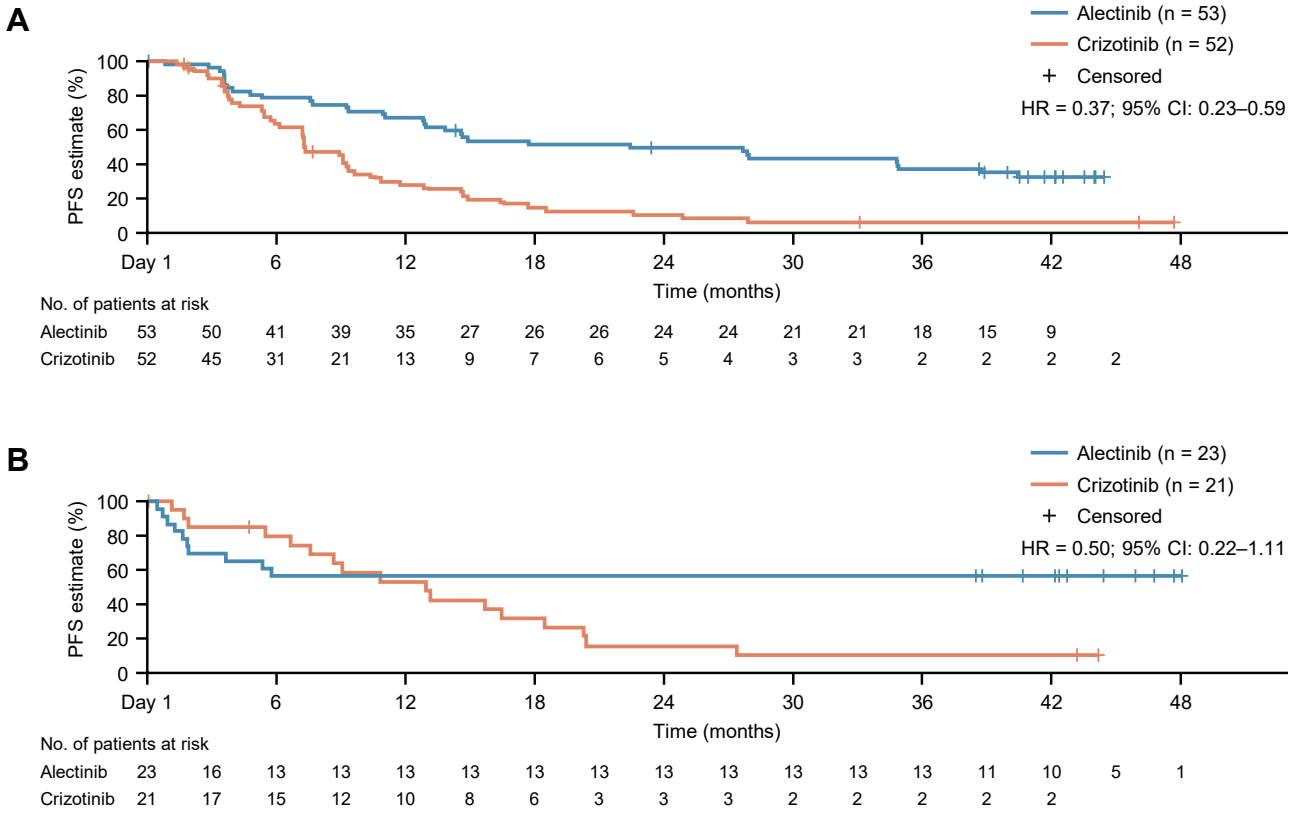

Figure 2. Kaplan-Meier plot of investigator-assessed PFS with alectinib versus crizotinib in (*A*) the plasma *ALK*-positive and (*B*) the plasma *ALK*-negative population. CI, confidence interval; HR, hazard ratio; PFS, progression-free survival.

Table 3. Investigator-Assessed Response in the ITT Population, the BEP, and the Plasma *ALK*-Positive and Plasma *ALK*-Negative Population by Treatment Arm

	ITT (N = 303)		BEP (n = 149)		Plasma *ALK* Positive (n = 105)		Plasma *ALK* Negative (n = 44)	
n (%) 95% CI	Alectinib (n = 152)	Crizotinib (n = 151)	Alectinib (n = 76)	Crizotinib (n = 73)	Alectinib (n = 53)	Crizotinib (n = 52)	Alectinib (n = 23)	Crizotinib (n = 21)
ORR	126 (82.9) 76.0–88.5	114 (75.5) 67.8–82.1	64 (84.2) 74.0–91.6	54 (74.0) 62.4–83.6	50 (94.3) 84.3–98.8	42 (80.8) 67.5–90.4	14 (60.9) 38.5–80.3	12 (57.1) 34.0–78.2
CR	9 (5.9) 2.7–10.9	5 (3.3) 1.1–7.6	5 (6.6) 2.2–14.7	0 0.0–4.9	3 (5.7) 1.2–15.7	0 0.0–6.9	2 (8.7) 1.1–28.0	0 0–16.1
PR	117 (77.0) 69.5–83.4	109 (72.2) 64.3–79.2	59 (77.6) 66.6–86.4	54 (74.0) 62.4–83.6	47 (88.7) 77.0–95.7	42 (80.8) 67.5–90.4	12 (52.2) 30.6–73.2	12 (57.1) 34.0–78.2
Stable disease	9 (5.9) 2.7–10.9	24 (15.9) 10.5–22.7	3 (3.9) 0.8–11.1	12 (16.4) 8.8–27.0	1 (1.9) 0.1–10.1	7 (13.5) 5.6–25.8	2 (8.7) 1.1–28.0	5 (23.8) 8.2–47.2
PD	8 (5.3) 2.3–10.1	10 (6.6) 3.2–11.8	5 (6.6) 2.2–14.7	5 (6.8) 2.3–15.3	0 0.0–6.7	2 (3.8) 0.5–13.2	5 (21.7) 7.5–43.7	3 (14.3) 3.1–36.3
Missing or unassessable	9 (5.9)	3 (2)	4 (5.3)	2 (2.7)	2 (3.8)	1 (1.9)	2 (8.7)	1 (4.8)

BEP, biomarker-evaluable population; CI, confidence interval; CR, complete response; ITT, intention to treat; ORR, objective response rate; PD, progressive disease; PR, partial response.

sites and may better detect tumor heterogeneity than localized, tissue-based testing from a single sample.[3]

Initial results from the *ALK*-positive cohort of BFAST revealed the clinical utility of blood-based NGS in a tissue-agnostic patient population selected by blood-based NGS.[13] The primary end point of the *ALK*-positive cohort (confirmed ORR by investigator, 87.4%) was achieved; an independent review facility-confirmed ORR of 92.0% was reported.[13] These data are consistent with the investigator-assessed ORR of 82.9% observed with alectinib in ALEX[15] and reveal the clinical utility of blood-based NGS as a method to inform clinical decision-making in *ALK*-positive NSCLC. However, BFAST was not designed to assess concordance between IHC and NGS testing.

The ALEX concordance analysis presented here is retrospective and exploratory, using data from the BEP, which comprised approximately 50% of the ITT population. These results, consistent with those from BFAST, suggest that tissue- and blood-based detection of *ALK* fusions are viable and complementary options to select patients for treatment with alectinib. However, as can be observed from the results, plasma testing may fail to detect *ALK*-positive tumors, because this method relies heavily on tumors shedding ctDNA into the blood stream. We found a PPA of 70.5% between the VENTANA IHC and FACT plasma assays (as all patients had *ALK*-positive tumors by IHC, per study protocol, only PPA could be analyzed). This suggests that in the event of a negative *ALK* result using plasma testing, reflex testing using a tissue test should be considered where feasible as a response was observed in the plasma *ALK*-negative population, as outlined in the technical information for FoundationOne Liquid CDx, the Food and Drug Administration–approved successor to FACT.[16]

The PPA result may have been affected by the comparison of two different analytes, protein and ctDNA (IHC versus blood-based NGS), as the recent concordance analysis between fluorescence in situ hybridization and IHC, where both assays used tumor samples from ALEX patients, reported a PPA of 83.9%.[17] In addition, other factors, such as the kinetics of the targeted analyte or nature of the genetic alteration may have played a role. Differences in interpretation of results may also lead to discordance, especially for samples close to the assay limit of detection. Detection of oncogenic drivers in plasma, such as *ALK* rearrangements or *EGFR* point mutations, may be affected by the level of ctDNA shedding from the tumor or background cfDNA.[18,19] Similar to our observations, a PPA of 79% and 68%, respectively, was found in a comparison of blood- and tissue-based testing for the detection of Ex19del and L858R *EGFR* mutations in the phase 3 osimertinib trial, FLAURA.[20]

An important advantage of NGS assays is that multiple gene alterations can be assessed simultaneously.[2] With the increasing number of Food and Drug Administration–approved targeted therapies, an assay that can detect targetable alterations in multiple genes is preferred over conducting multiple separate tests for individual gene alterations, as the latter can deplete tissue obtained from biopsies, meaning rare biomarkers may not be tested for and patients may not receive targeted therapy.[2]

In this analysis, investigator-assessed ORR was similar between the alectinib and crizotinib arms in the ITT population. However, for both treatment arms, ORR appeared to be numerically higher in the plasma *ALK*-positive population, whereas PFS appeared to be

Table 4. Investigator-Assessed DoR in the ITT Population, the BEP, and the Plasma *ALK*-Positive and Plasma *ALK*-Negative Populations

	ITT (N = 303)	
DoR	Alectinib (n = 152)	Crizotinib (n = 151)
Patients analyzed, n	126	114
Patients with event,[a] n (%)	59 (46.8)	91 (79.8)
DoR (median), mo (95% CI)	NE (29.8–NE)	11.1 (7.9–13.0)
	BEP (n = 149)	
	Alectinib (n = 76)	Crizotinib (n = 73)
Patients analyzed, n	64	54
Patients with event,[a] n (%)	33 (51.6)	46 (85.2)
DoR (median), mo (95% CI)	36.9 (20.6–NE)	7.3 (5.5–11.1)
	BEP Alectinib and crizotinib arms combined (n = 149)	
	Plasma *ALK* positive (n = 105)	Plasma *ALK* negative (n = 44)
Patients analyzed, n	92	26
Patients with event,[a] n (%)	69 (75.0)	10 (38.5)
DoR (median), mo (95% CI)	11.1 (7.4–14.8)	NE (14.5–NE)
	BEP Alectinib arm only (n = 76)	
	Plasma *ALK* positive (n = 53)	Plasma *ALK* negative (n = 23)
Patients analyzed, n	50	14
Patients with event,[a] n (%)	32 (64.0)	1 (7.1)
DoR (median), mo (95% CI)	25.9 (11.9–38.7)	NE (NE)
	BEP Crizotinib arm only (n = 73)	
	Plasma *ALK* positive (n = 52)	Plasma *ALK* negative (n = 21)
Patients analyzed, n	42	12
Patients with event,[a] n (%)	37 (88.1)	9 (75.0)
DoR (median), mo (95% CI)	5.6 (5.5–9.2)	11.5 (6.9–14.8)

[a]Event defined as PD or death.
BEP, biomarker-evaluable population; CI, confidence interval; DoR, duration of response; ITT, intention to treat; NE, not estimable; PD, progressive disease.

numerically longer in the plasma *ALK*-negative population. Similarly, with alectinib, DoR appeared to be numerically longer in the plasma *ALK*-negative versus the plasma *ALK*-positive population. These findings may be explained by the disparity in tumor burden and visceral involvement (sum of longest diameters, lesions, and liver lesions) found in plasma *ALK*-positive compared with plasma *ALK*-negative patients, likely representing a population with worse overall prognosis. Reasons for this potential disparity in PFS and DoR in plasma *ALK*-positive versus plasma *ALK*-negative patients are unclear but could be due to the small patient numbers in each subgroup. It has been suggested that increased ctDNA shed identifies a subset of patients with worse prognosis.[21] Larger data sets are required to confirm this observation.

In the phase 3 FLAURA trial, similar to the results presented here, the absence of an *EGFR* mutation in plasma was associated with prolonged PFS, potentially owing to lower tumor burden in these patients compared with those harboring a detectable *EGFR* mutation in plasma.[20] It is worth noting that some patients who may derive benefit from targeted treatments such as ALK or EGFR inhibitors may not be identified using a blood-based assay, so a tissue sample would be required for reflex testing, as found in this analysis. However, because all patients included in ALEX were *ALK* positive by IHC, this concordance analysis does not include patients who were *ALK* negative by IHC but *ALK* positive by blood-based NGS.

A limitation of this analysis is its exploratory, retrospective nature. The ALEX study was not designed to compare diagnostic tests; therefore, no formal statistical testing was planned. Furthermore, some populations contained a limited sample size because the ALEX study was not designed with these subpopulations in mind. Optimal plasma volumes were not collected for all patients, and samples with lower than required volumes were not selected for analysis, as defined in the Concordance Analysis section.

In conclusion, in this analysis, the blood-based NGS assay FACT revealed a 70.5% PPA compared against protein-based VENTANA ALK (D5F3) CDx IHC, comparable with other ctDNA genotyping assays. When taken together with results from BFAST, this analysis indicates that blood-based NGS is an additional, important option to test patients for targetable biomarkers and is a valuable diagnostic tool to direct optimal treatment choice for patients with advanced *ALK*-positive NSCLC.[16] Finally, this analysis reveals that plasma *ALK*-positive patients can derive clinical benefit from ALK inhibitors and that in this patient population, as in the ITT population, alectinib demonstrated superior PFS compared with crizotinib.

CRediT Authorship Contribution Statement

Johannes Noé: Conceptualization, Investigation, Writing – original draft, Writing – review and editing, Supervision.

Walter Bordogna, Venice Archer, Vlatka Smoljanovic: Conceptualization, Writing – review and editing.

Magalie Hilton: Methodology, Formal analysis, Writing original draft, Writing – review and editing.

Ryan Woodhouse: Conceptualization, Methodology, Writing – review and editing.

Simonetta Mocci: Conceptualization, Methodology, Writing – review and editing.

Shirish M. Gadgeel: Investigation, Writing – review and editing.

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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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2022.100341.

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