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ORIGINAL ARTICLE

Blood First Assay Screening Trial (BFAST) in Treatment-Naive Advanced or Metastatic NSCLC: Initial Results of the Phase 2 *ALK*-Positive Cohort

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ABSTRACT

Introduction: The Blood First Assay Screening Trial is an ongoing open-label, multicohort study, prospectively evaluating the relationship between blood-based next-generation sequencing (NGS) detection of actionable genetic alterations and activity of targeted therapies or immunotherapy in treatment-naive advanced or metastatic NSCLC. We present data from the *ALK*-positive cohort.

Methods: Patients aged more than or equal to 18 years with stage IIIB or IV NSCLC and *ALK* rearrangements detected by blood-based NGS using hybrid capture technology (FoundationACT) received alectinib 600 mg twice daily. Asymptomatic or treated central nervous system (CNS) metastases were permitted. Primary end point was investigator-assessed objective response rate (ORR; Response Evaluation Criteria in Solid Tumors version 1.1). Secondary end points were independent review facility-assessed ORR, duration of response, progression-free survival (PFS), overall survival, and safety. Exploratory end points were investigator-assessed ORR in patients with baseline CNS metastases and relationship between circulating biomarkers and response.

Results: In total, 2219 patients were screened and blood-based NGS yielded results in 98.6% of the cases. Of these, 119 patients (5.4%) had *ALK*-positive disease; 87 were enrolled and received alectinib. Median follow-up was 12.6 months (range: 2.6–18.7). Confirmed ORR was 87.4% (95% confidence interval [CI]: 78.5–93.5) by investigator and 92.0% (95% CI: 84.1–96.7) by independent review facility. Investigator-confirmed 12-month duration of response was 75.9% (95% CI: 63.6–88.2). In 35 patients (40%) with baseline CNS disease, investigator-assessed ORR was 91.4% (95% CI: 76.9–98.2). Median PFS was not reached; 12-month investigator-assessed PFS was 78.4% (95% CI: 69.1–87.7). Safety data were consistent with the known tolerability profile of alectinib.

Conclusions: These results reveal the clinical application of blood-based NGS as a method to inform clinical decision-making in *ALK*-positive NSCLC.

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Some of these data were presently presented as an oral presentation at the European Society for Medical Oncology meeting in Barcelona, Spain, September 27 to October 1, 2019; Abstract LBA81_PR.

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Introduction

Standard approaches to biomarker testing in NSCLC use tissue biopsy samples to identify patients who may benefit from targeted therapy and immunotherapy.[1] In patients with newly diagnosed advanced NSCLC, current guidelines recommend testing for targetable genetic alterations.[2,3] Under specific circumstances, the guidelines also recommend considering blood-based testing if insufficient tissue is present.[1] Obtaining sufficient tumor tissue for biomarker testing can be challenging; in western countries, up to 38% of patients with metastatic NSCLC have inadequate tumor samples for comprehensive molecular analysis at diagnosis.[4-6] Repeat biopsies may prolong the diagnostic process, are not feasible in approximately 20% of patients with advanced NSCLC, and almost 25% fail to yield sufficient material for genomic analysis.[7]

Blood-based next-generation sequencing (NGS) could overcome some of the limitations associated with tissue collection and tissue-based testing.[8,9] It offers the advantage of rapid, repeated, and multiplexed biomarker testing in patients unfit for tissue biopsy or those with insufficient tissue material for testing[10] and may shorten the time needed to initiate an appropriate upfront therapy and provide an additional tool for physicians to direct patient treatment. Efforts to develop blood-based diagnostics using a polymerase chain reaction-based approach led to the approval of the cobas EGFR Mutation Test version 2 (Roche Diagnostics) for the detection of *EGFR* mutations in circulating tumor DNA (ctDNA) isolated from plasma.[11] More recently, a blood-based NGS assay was developed and validated to detect tumor mutational burden (TMB) in the blood.[12] Multiplex NGS-based ctDNA assays are currently commercially available, including FoundationOne Liquid CDx (Foundation Medicine), which was recently approved by the Food and Drug Administration as a companion diagnostic for detecting *ALK* rearrangements in metastatic NSCLC.[12–14]

Approximately 5% of NSCLC tumors harbor a chromosomal rearrangement of *ALK*, resulting in oncogenic fusion proteins, most often *EML4-ALK*, which confer constitutively active ALK kinase activity.[15] *ALK* positivity can be defined using immunohistochemistry (IHC), which detects ectopic expression of the ALK protein, or through molecular approaches, such as fluorescence in situ hybridization (FISH), reverse transcriptase-polymerase chain reaction, and NGS, which detect molecular rearrangements in either RNA or DNA. In the phase 3 ALEX trial of alectinib versus crizotinib, *ALK*-positive patients were selected on the basis of IHC and whether sufficient tissue sample was available.[16]

The Blood First Assay Screening Trial (BFAST) was designed to prospectively evaluate the relationship between blood-based biomarkers and clinical activity of targeted therapies or immunotherapy in patients with treatment-naive advanced or metastatic NSCLC who were screened for actionable genetic alterations using only NGS of ctDNA. To date, six cohorts, testing the efficacy and safety of the therapy directed at specific tumor biomarkers, have been initiated (*ALK*-positive, *RET*-positive, biomarker positive by the blood TMB [bTMB] assay, *ROS1*-positive, *BRAF*-positive, and *EGFR* exon 20-positive). Here, we report data from the *ALK*-positive cohort.

Materials and Methods

Study Design and Patients

BFAST (NCT03178552) is an ongoing global, open-label, multicohort study. Eligibility criteria include the following: more than or equal to 18 years of age; unresectable, histologically or cytologically confirmed advanced or metastatic NSCLC not amenable to concomitant chemoradiation; Eastern Cooperative Oncology Group performance status of 0 to 2; life expectancy of more than or equal to 12 weeks; and measurable disease by *Response Evaluation Criteria in Solid Tumors* version 1.1 (RECIST v1.1). Patients with asymptomatic or treated central nervous system (CNS) metastases were eligible. Enrollment was based on blood-based NGS assay results, irrespective of tissue-based results. As BFAST was not designed to evaluate concordance between tissue and blood-based NGS, and to prevent patients without tissue available for testing being unable to join, tissue collection was not mandatory. Tissue collection and central testing for *ALK* status were not required; however, tissue availability for molecular testing and local biomarker test results could be reported by the investigator.

The institutional review board at each study site approved the protocol. The study was performed in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients for initial blood screening and enrollment into a treatment cohort.

Treatment and Assessments

Patients were screened for actionable mutations using comprehensive genomic profiling (Foundation Medicine, Cambridge, MA) by means of hybrid capture-based

NGS assays (FoundationACT [Foundation Medicine), a previous version of FoundationOne Liquid CDx) and a bTMB assay, validated for genomic profiling of ctDNA from the blood.[12–14] FoundationACT is a 62-gene panel analytically validated using reference DNA samples derived from cell lines or synthetic DNA constructs to have 100% sensitivity for fusions or rearrangements with greater than or equal to 0.5% mutation allele frequency in plasma (95% confidence interval [CI]: 77.1–100) and 100% positive predictive value (95% CI: 77.1–100).[13] Turnaround time for blood-based NGS is 10 to 14 calendar days on receipt at the central laboratory. A clinical bridging study was conducted to evaluate the concordance between *ALK* rearrangement status by FoundationACT and FoundationOne Liquid CDx and the clinical efficacy of alectinib in patients with *ALK* rearrangements identified by FoundationOne Liquid CDx (full methods and results can be found in the Appendix).

ALK rearrangement was defined as a chimeric alignment of *ALK* and another location of the genome. Chimeric read pairs must have been separated by greater than or equal to 10 megabase pairs or mapped to different chromosomes. Oncogenic genomic rearrangements must have had chimeric read pairs with a breakpoint in *ALK* and *EML4*, or breakpoint in intron 19 of *ALK* (referred to as "*ALK* positive"). *ALK* fusion copies per milliliter of plasma (*ALK* copy number) were calculated on the basis of the plasma volume used to extract cell-free DNA and the mass of cell-free DNA used for library construction. Variant allele frequency is not a standard output of the clinical trial assay. On the basis of the identified genomic alterations and cohort-specific eligibility criteria, the patients were assigned to the following treatment cohorts: cohort A, *ALK*-positive tumors, alectinib 600 mg orally twice daily; cohort B, *RET*-positive tumors, alectinib 900 mg orally twice daily; cohort C, bTMB-positive tumors, randomized 1:1 to atezolizumab 1200 mg intravenous infusion every 21 days or platinum-based chemotherapy; cohort D, *ROS1*-positive tumors, entrectinib 600 mg orally once daily. Cohorts B, C, and D are closed to enrollment. After the closure of cohort A, cohort E (*BRAF*-positive tumors, atezolizumab 1680 mg every 28 d, vemurafenib 960 mg orally twice daily for 21 days then 720 mg orally twice daily thereafter, and cobimetinib 60 mg orally once daily for 21 out of every 28 days) and cohort F (*EGFR* exon 20-positive tumors, atezolizumab 1200 mg, bevacizumab 15 mg/kg, carboplatin AUC 5, and pemetrexed 500 mg/m^2 by intravenous infusion for 4 or 6 cycles [cycle = 21 days] followed by maintenance treatment with atezolizumab, bevacizumab, and pemetrexed) opened, and enrollment is ongoing.

Tumor assessments were performed at baseline and every 8 weeks throughout the study. Brain magnetic resonance imaging was required at each tumor assessment, regardless of baseline CNS status. Treatment continued until progressive disease (PD), unacceptable toxicity, withdrawal of consent, or death. We report the initial results from the *ALK*-positive cohort only.

Study End Points

The primary end point was confirmed investigator-assessed objective response rate (ORR), defined as the proportion of patients with a complete response (CR) or partial response (PR) per RECIST v1.1, in two assessments more than or equal to 4 weeks apart. Secondary end points included the following: independent review facility (IRF)-assessed confirmed ORR, investigator- and IRF-assessed duration of response (DoR), clinical benefit rate (CBR), progression-free survival (PFS), overall survival (OS), and safety. Exploratory end points included antitumor effect of alectinib in patients with CNS disease at baseline (determined by investigator-assessed ORR per RECIST v1.1) and the relationship between circulating biomarkers and response.

The following are the secondary end point definitions: DoR, time from confirmed CR/PR to occurrence of a PFS event; CBR, proportion of patients with CR, PR, or stable disease maintained for more than or equal to 24 weeks; PFS, time from randomization to documentation of PD (per RECIST criteria) or death, whichever occurred first; OS, time from first date of treatment to date of death owing to any cause. Safety was assessed by the incidence and severity of adverse events (AEs), graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 and classified according to MedDRA.

Statistical Analysis

Determination of sample size was based on demonstration of data consistency between BFAST (blood-selected patients) and ALEX (tissue-selected patients). Assuming the established ORR found with alectinib in ALEX is 80% (ALEX was ongoing when the BFAST protocol was approved), it was planned to enroll 78 patients to provide an 80% chance that the lower limit of the two-sided 95% CI (using Clopper-Pearson method) around the point estimate of ORR in patients selected by blood-based NGS was greater than 60% (thus preserving 75% of the ORR observed with alectinib in ALEX wherein patients were selected using tissue-based testing). Kaplan-Meier methodology was used to estimate median PFS with corresponding 95% CIs.

Results

Patients

Between November 28, 2017, and September 25, 2018, a total of 2219 patients were screened, and the assay yielded results in 98.6% of the cases (1.4% assay

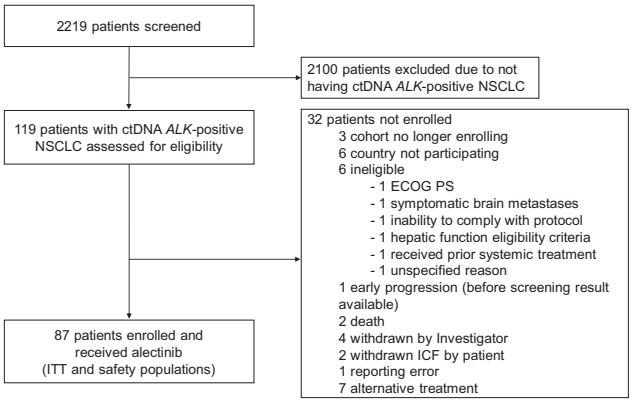

Figure 1. Patient disposition. ctDNA, circulating tumor DNA; ICF, informed consent form; ITT, intent-to-treat; ECOG PS, Eastern Cooperative Oncology Group performance status.

failure); of these, 119 patients had ctDNA *ALK*-positive NSCLC, giving a prevalence in the blood of 5.4%. A total of 32 patients did not enter the *ALK*-positive cohort, mainly owing to ineligibility, most often, not meeting the full treatment cohort criteria, or patients being in a country not participating in the *ALK*-positive cohort (Fig. 1). In total, 87 eligible patients were enrolled at 36 centers in 15 countries; all were included in the intent-to-treat (ITT) and safety populations.

The baseline characteristics of the 87 treated patients are found in Table 1. Median age was 55 years, and 35 patients (40%) had baseline CNS metastases. Of the 87 treated ctDNA *ALK*-positive patients, 65 patients (75%) were reported by the investigator to have a local tissue biomarker test positive for a BFAST alteration (at the time these data were collected, this could have included *ALK* or *RET* fusions as the question in the response system was worded to capture alterations from all cohorts and was not specific to *ALK*), whereas 22 patients (25%) were not: nine (10%) were reported by the investigator to have no tissue available for testing, six (7%) were reported by the investigator to have tissue available but no local biomarker testing was performed, and seven (8%) were reported by the investigator to have a local tissue biomarker test(s) negative for BFAST alterations (Supplementary Fig. 1). Median follow-up time was 12.6 months (range: 2.6–18.7). At the data cutoff (June 1, 2019), 21 patients (24%) had discontinued treatment: 17 (20%) owing to PD, three (3%) due to AEs, and one (1%) owing to symptomatic deterioration.

Efficacy

Overall, 76 of 87 patients attained a confirmed response (all PRs), giving an ORR by investigator of 87.4% (95% CI: 78.5–93.5) (Table 2). This was consistent with IRF-assessed ORR, with confirmed responses in 80 of 87 patients (92.0%, 95% CI: 84.1–96.7), including CR in 11 patients (12.6%). Investigator-assessed CBR was 81.6% (95% CI: 71.9–89.1) (Table 2). Median DoR was not reached; with a 12-month investigator-confirmed DoR of 75.9% (95% CI: 63.6–88.2). Confirmed ORR by the investigator was 91.4% (95% CI: 76.9–98.2) and 84.6% (95% CI: 71.9–93.1) in the patients with and without baseline CNS disease, respectively. The investigators reported that 22 of 87 patients (25%) did not have a positive tissue test result for a BFAST alteration, including *ALK*, yet of those, 18 of 22 (82%) responded to alectinib.

At the data cutoff, median PFS was not reached (Fig. 2*A*), with 20 events (23%) recorded. Kaplan-Meier estimated that 6- and 12-month investigator-assessed PFS rates were 90.7% (95% CI: 84.5–96.8) and 78.4% (95% CI: 69.1–87.7), respectively. Corresponding IRF values were 89.5% (95% CI: 93.0–96.0) and 74.5% (95% CI: 65.0–84.0), respectively. A total of 13 patients (14.9%) had died at the data cutoff, with 6- and 12-month survival rates of 97.7% (95% CI: 94.6–100) and 86.8% (95% CI: 79.6–94.1), respectively.

Biomarker Analyses

In total, 84% (73 of 87) of ctDNA *ALK*-positive patients had tumors harboring an *EML4-ALK* fusion (Table 1). The most common *EML4* variants were V1 (29%) and V3 (28%). Other fusion partners or rearrangements included *CLIP4* (n = 2); *AAK1*, *DCTN1*, *EPS8*, *ERCC8*, *ETV6*, *KIF5B*, *PGM2*, *STRN*, and *TMEM178A* (n = 1 each); fusion partner not identified (n = 3). Overall, 44% (38 of 87) of the patients had a *TP53* mutation and 5% (4 of 87) had an *NF1* mutation, which was consistent with the screening cohort (Fig. 3). Mutations in *KRAS* and *EGFR* were mutually exclusive to *ALK* fusions (Supplementary Table 1).

Median bTMB at baseline was two mutations (range: 0–21). Three of 87 (3%) patients had bTMB greater than or equal to 16 mutations,[12] and all achieved confirmed responses to alectinib. There was no association between response to alectinib and bTMB ($p = 0.7661$) (Supplementary Fig. 2*A*). The copies per milliliter of plasma of *ALK* fusion present in ctDNA (median = 5.56, range: 0.43–686.28) was not associated with response by Mann-Whitney ($p = 0.2979$) or t test ($p = 0.2834$) (Supplementary Fig. 2*B*). Patients with a very high allele fraction (>150 copies per mL) all had objective responses (n = 4), and 20 of 21 patients with greater than 25 *ALK*-positive copies per milliliter achieved a response.

Additional analyses were performed to evaluate the association between clinical outcome and individual biomarkers (*EML4* versus non-*EML4* fusions, *EML4* V3 versus *EML4* V1, *TP53* status, *ALK* allele frequency, bTMB, and local tissue test status [Table 2 and Fig. 2*B*, *D*,

Table 1. Patient Baseline and Molecular Characteristics

Characteristics	BFAST *ALK*-Positive Cohort (N = 87)
Baseline characteristics	
Median age, y (range)	55.0 (25-82)
Sex, n (%)	
Male	35 (40)
Female	52 (60)
Race, n (%)	
Asian	29 (33)
Non-Asian	48 (55)
Unknown	10 (12)
ECOG PS, n (%)	
0–1	82 (94)
2	5 (6)
Smoking status, n (%)	
Active smoker	5 (6)
Nonsmoker	50 (58)
Past smoker	32 (37)
Disease stage, n (%)	
IIIB	5 (6)
IV	82 (94)
Histologic type, n (%)	
Adenocarcinoma	81 (93)
Other	4 (5)
Missing	2 (2)
CNS metastases at baseline, n (%)	
Yes	35 (40)
No	52 (60)
Molecular characteristics	
EML4 fusions, n (%)	73 (84)
EML4 V1	25 (29)
EML4 V2	12 (14)
EML4 V3	24 (28)
EML4 V4	12 (14)
Non-*EML4* fusions, n (%)	14 (16)
Fusion partner not identified	3 (3)
CLIP4	2 (2)
AAK1	1 (1)
DCTN1	1 (1)
EPS8	1 (1)
ERCC8	1 (1)
ETV6	1 (1)
KIF5B	1 (1)
PGM2	1 (1)
STRN	1 (1)
TMEM178A	1 (1)
TP53 status, n (%)	
WT *TP53*	49 (56)
MUT *TP53*	38 (44)
ALK allele frequency (cut by median of 5.56 copies per mL), n (%)	
Lower than median	44 (51)
Higher than median	43 (49)
bTMB (cut by median of bTMB = 2), n (%)	
Lower than median	46 (53)
Higher than median	41 (47)

BFAST, Blood First Assay Screening Trial; bTMB, blood tumor mutational burden; CNS, central nervous system; ECOG PS, Eastern Cooperative Oncology Group performance status.

C, and *E*]). No significant association was found between ORR and any of these biomarkers (Table 2). Nevertheless, patients with wild-type *TP53* had significantly better PFS than those with comutated *TP53* and *ALK* (six versus 14 events, hazard ratio = 0.28, 95% CI: 0.11–0.74; Fig. 2*C*).

Safety

Median alectinib treatment duration was 11.1 months (range: 0–18), and median dose intensity was 99.9% (range: 41.4–100.1). Serious AEs occurred in 24% of the patients and were considered treatment related in 6% of the patients (Table 3). Grade 3 or 4 AEs were reported in 34% of the patients; the most frequent events were dyspnea (n = 6), anemia (n = 4), asthenia (n = 3), and constipation, pneumonia, respiratory tract infection, and elevations of alanine aminotransferase, aspartate transaminase, bilirubin, and blood creatine phosphokinase (all n = 2). Supplementary Table 2 lists AEs occurring in at least 10% of the patients. There was one fatal AE, reported as an unexplained death, considered by the investigator to be unrelated to the study treatment. The patient died unexpectedly while hospitalized four days after permanently discontinuing alectinib; no autopsy was performed. AEs leading to treatment discontinuation, dose reduction, or dose interruption occurred in 7%, 8%, and 31% of the patients, respectively.

Discussion

BFAST is the first trial to use blood-based NGS prospectively as the sole method of identifying patients with NSCLC with an actionable genetic alteration. The *ALK*-positive cohort met its primary end point, with an investigator-assessed confirmed ORR of 87.4%, supported by an IRF-assessed confirmed ORR of 92.0%, meeting the target of greater than or equal to 75% of the confirmed ORR found in ALEX.[17] These data are consistent with first-line trials with other ALK inhibitors (Supplementary Table 3). The 5.4% prevalence of *ALK* in the screening population is close to that reported in the literature (5%) and higher than that in previous cohorts undergoing blood-based NGS.[18,19]

The baseline characteristics in the BFAST *ALK*-positive cohort are comparable to those in the patients in the pivotal phase 3 ALEX study.[16] Data from ALEX led to the approval of alectinib as first-line treatment of advanced *ALK*-positive NSCLC, detected by IHC in tumor samples, with superior investigator-assessed PFS for alectinib versus crizotinib.[20] Median duration of follow-up in BFAST (12.6 mo) is shorter than that for alectinib at the primary analysis of ALEX (18.6 mo).[16] Although median PFS was not reached in BFAST, the 12-month–predicted event-free PFS rate (investigator-assessed) was 78%,

Table 2. ORR Summary for the *ALK*-Positive Cohort and ORR and PFS in Biomarker Subgroups (Total N = 87 for All Groups)

ALK-Positive Cohort	Investigator	IRF
Confirmed responders, n (%)	76 (87.4)	80 (92.0)
95% CI	78.5–93.5	84.1–96.7
Complete response, n (%)	0	11 (12.6)
95% CI	0.0–4.2	6.5–21.5
Partial response, n (%)	76 (87.4)	69 (79.3)
95% CI	78.5–93.5	69.3–87.3
Stable disease, n (%)	10 (11.5)	5 (5.7)
95% CI	5.7–20.1	1.9–12.9
Progressive disease, n (%)	1 (1.1)	1 (1.1)
95% CI	0.0–6.2	0.0–6.2
Not assessable, n (%)	0	0
95% CI	–	–
Missing,[a] n (%)	–	1 (1.1)
Clinical benefit rate,[b] n (%)	71 (81.6)	69 (79.3)
95% CI	71.9–89.1	69.3–87.3
ORR for patients with baseline CNS disease, n (%)	32 (91.4)	–
95% CI	76.9–98.2	–

Biomarker Subgroups	ORR, n (%)		PFS Events, n[c]	
EML4 fusions	*EML4* (n = 73)	non-*EML4* (n = 14)	*EML4* (n = 73)	non-*EML4* (n = 14)
	65 (89)	11 (79)	15	5
	OR = 2.22 (95% CI: 0.44-9.1)		HR = 0.51 (95% CI: 0.18-1.39)	
EML4 variants	*EML4* V3 (n = 24)	*EML4* V1 (n = 25)	*EML4* V3 (n = 24)	*EML4* V1 (n = 25)
	22 (92)	22 (88)	6	3
	OR = 1.5 (95% CI: 0.23-12.24)		HR = 2.06 (95% CI: 0.51-8.25)	
TP53 status	WT *TP53* (n = 49)	MUT *TP53* (n = 38)	WT *TP53* (n = 49)	MUT *TP53* (n = 38)
	44 (90)	32 (84)	6	14
	OR = 1.65 (95% CI: 0.46-6.18)		HR = 0.28 (95% CI: 0.11-0.74)	
ALK allele frequency (cut by median of 5.56 copies per mL)	Lower than median (n = 44)	Higher than median (n = 43)	Lower than median (n = 44)	Higher than median (n = 43)
	37 (84)	39 (91)	8	12
	OR = 0.54 (95% CI: 0.13-1.95)		HR = 0.59 (95% CI: 0.24-1.45)	
bTMB (cut by median of bTMB = 2)	Lower than median (n = 46)	Higher than median (n = 41)	Lower than median (n = 46)	Higher than median (n = 41)
	39 (85)	37 (90)	7	13
	OR = 0.6 (95% CI: 0.15-2.16)		HR = 0.45 (95% CI: 0.18-1.13)	
Local tissue test status[d]	Other (n = 22)	Tissue positive for BFAST alteration[e] (n = 65)	Other (n = 22)	Tissue positive for BFAST alteration[e] (n = 65)
	18 (82)	58 (89)	6	14
	OR = 0.54 (95% CI: 0.15-2.27)		HR = 1.38 (95% CI: 0.53-3.59)	

[a]Patients were classified as missing if postbaseline response assessments were not available.
[b]Defined as patients with confirmed complete response, partial response, or stable disease maintained for more than or equal to 24 weeks.
[c]Median PFS not available.
[d]Local tissue testing was self-reported by investigator sites if tissue-based local molecular testing was completed.
[e]BFAST alterations were *ALK*+, *RET*+, or bTMB.
BFAST, Blood First Assay Screening Trial; bTMB, blood tumor mutational burden; CI, confidence interval; CNS, central nervous system; HR, hazard ratio; IRF, independent review facility; MUT, mutated; ORR, objective response rate; PFS, progression-free survival; WT, wild type.

compared with 68% from the primary analysis of ALEX.[16] Confirmed ORR for patients with baseline CNS disease in BFAST was 91.4%. Although this analysis did not include a formal assessment of intracranial response, results can be considered in the context of ALEX, wherein CNS ORR was 85.7% in the patients with measurable baseline CNS disease who had received previous radiotherapy.[21]

The safety profile of alectinib in BFAST was consistent with that in previous phase 3 trials[16,22,23] and post-marketing experience. There were low rates of AEs leading to dose reduction (8%) or discontinuation (7%); comparative data from ALEX were 16% and 11%, respectively.[16] A higher rate of alectinib dose interruptions was observed in BFAST (31%) relative to ALEX (19%), likely owing to more frequent liver function test monitoring shortly after

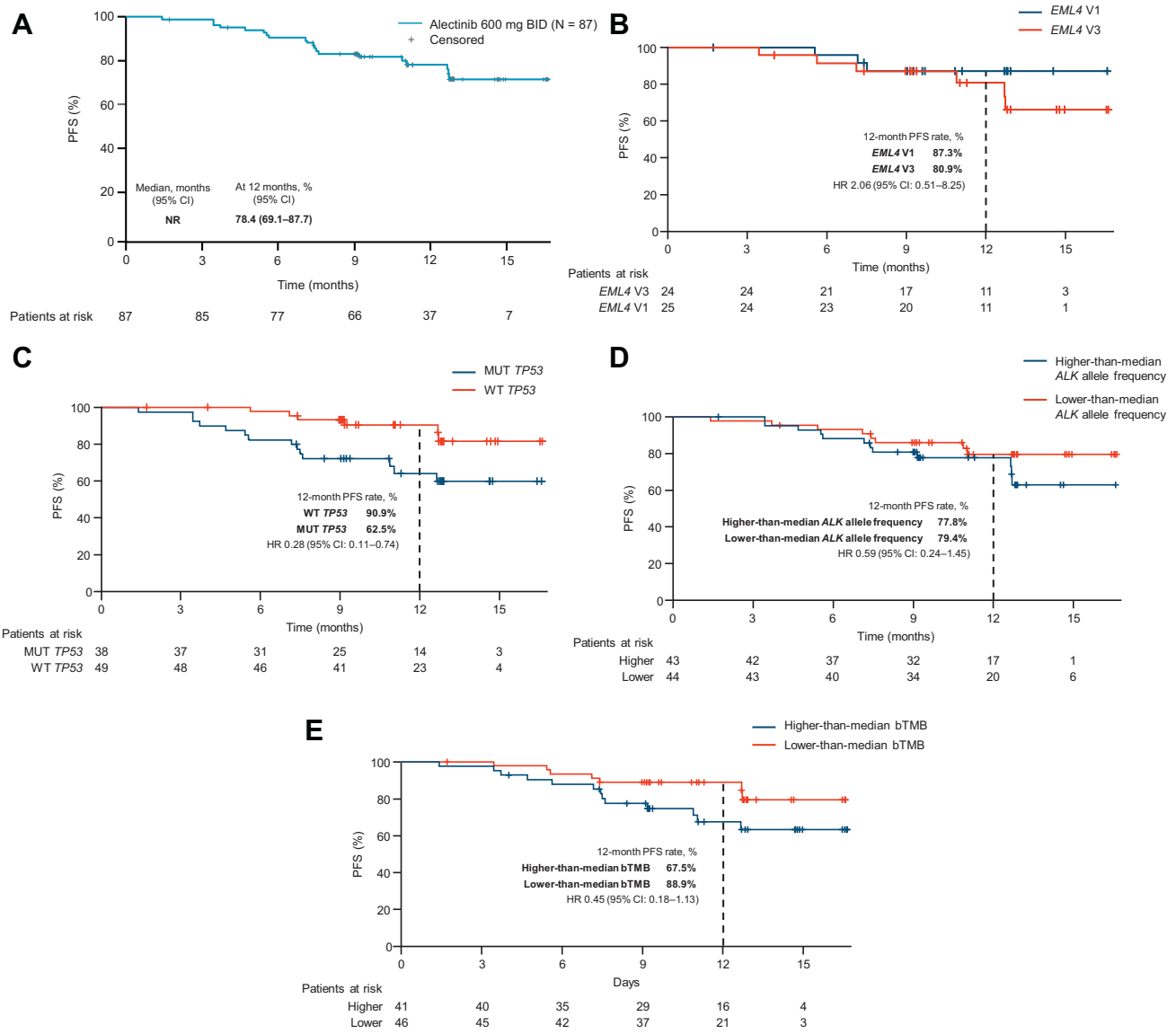

Figure 2. Kaplan-Meier plot of investigator-assessed progression-free survival in (*A*) the *ALK*-positive cohort (N = 87), (*B*) patients with *EML4* V3 or *EML4* V1, (*C*) patients with and without a co-occurring *TP53* mutation, (*D*) patients with higher-than-median or lower-than-median *ALK* allele frequency (cut by median of 5.56 copies per mL), and (*E*) patients with higher than median or lower than median bTMB (cut by median of bTMB = 2). bTMB, blood tumor mutational burden; CI, confidence interval; HR, hazard ratio; MUT, mutation; NR, not reached; PFS, progression-free survival; WT, wild type.

treatment initiation. There was one fatal AE, which occurred when the patient was no longer taking alectinib and was considered unrelated to the study treatment.

BFAST was designed to evaluate whether a blood-based NGS assay could select patients for targeted therapy and to reveal clinical applicability in a blood-selected, tissue-agnostic patient population, which is representative of up to 38% of the patients with NSCLC who lack sufficient tissue for comprehensive molecular testing.[5,6] Tissue collection and central testing were not required for study eligibility, and the study was not designed to evaluate tissue-plasma concordance. Although most patients in the *ALK*-positive cohort had tissue available for local biomarker analysis, this was not the case for all patients, and several patients were reported to have inadequate or insufficient tissue for biomarker testing. This highlights the role for blood-based assays in selecting patients for targeted therapy, as previously suggested by Akhoundova et al.[24]

The numerically higher investigator-assessed confirmed ORR reported in BFAST (87.4% [95% CI: 78.5–93.5]) versus ALEX (71.7% [95% CI: 63.8–78.7]) suggests that patient populations selected by means of blood-based NGS assay and tissue-based IHC/FISH may differ slightly.[17] Patients with *ALK*-positive NSCLC by IHC, but negative by FISH, made up 13% of the ALEX ITT

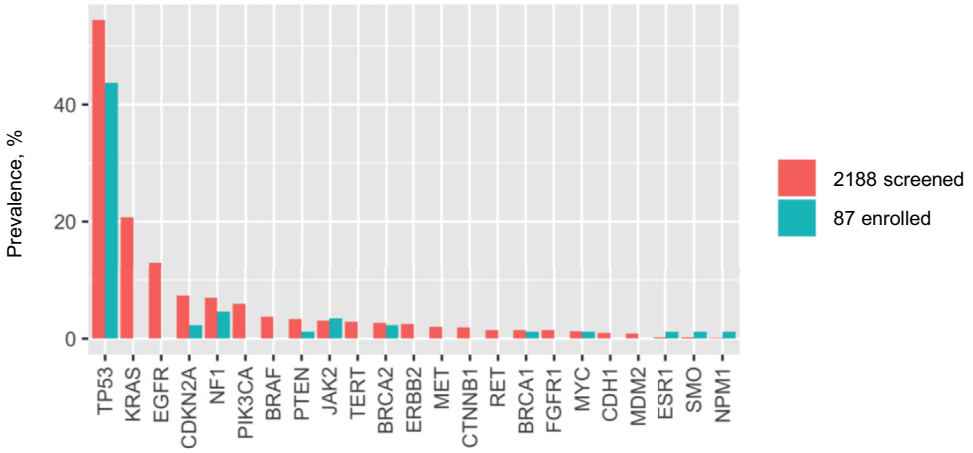

Figure 3. Prevalence of known and likely mutations in the *ALK*-positive cohort (N = 87) as detected by the FoundationACT assay (all samples had *ALK* fusions) and in the screening population (N = 2188 assay results available). Prevalence for each gene was calculated as the percent of enrolled patients who had one or more driver alterations (short variant, rearrangement, or copy number alteration) detected in the screening sample. Only the most prevalent genes/mutations are depicted (>1% in either the screening or enrolled population).

population, and these patients derived less clinical benefit than those with concordant results.[25] These data suggest biologically and clinically distinct subtypes of *ALK*-positive NSCLC: patients with discordant IHC/FISH results may have more heterogeneous tumors with fewer *ALK*-driven tumor cells, whereas patients with concordant results may have more homogenous, *ALK*-driven tumors. An alternative hypothesis is that some *ALK*-positive tumors by FISH may not actually result in expression of a functional ALK fusion.[26] These data may also reflect the inherent differences in diagnostic accuracies of technologies used to detect protein versus DNA fusion or differences between analytes. We hypothesize that the blood-based NGS assay used in BFAST may potentially have higher specificity than non-NGS tissue testing. Alternatively, *ALK*-positive tumors detectable by blood-based NGS may be more sensitive to systemic therapies with ALK inhibitors.

Blood-tissue concordance was investigated by retrospective testing of ALEX plasma samples with FoundationACT and comparing against the IHC results (VENTANA ALK [D5F3] CDx IHC tissue assay) (Roche data on file). In ALEX, all patients had tumors that were *ALK* IHC positive and approximately half of the ITT population had plasma available (the study was not prospectively designed to evaluate blood-tissue concordance). These investigators found 70.5% concordance between IHC and blood-based NGS and observed a longer median PFS with alectinib versus crizotinib in both plasma-*ALK*–positive and plasma-*ALK*–negative populations. Taken together with BFAST, this suggests that both or either IHC or blood-based NGS can be used to select patients for treatment.

Preliminary biomarker analyses in BFAST revealed a trend toward association between *ALK* fusions and *TP53* mutations as a poor prognostic factor for efficacy. This association was reported previously in patients with *ALK/TP53*-comutated tumors, wherein PFS and OS were significantly shorter compared with *TP53* wild-type tumors.[27,28] Further analyses from BFAST relating to other genomic co-mutations and their clinical impact are ongoing.

There are limitations to a blood-based NGS detection of *ALK*-positive disease. Because the use of ctDNA as a substrate depends on tumor shedding into the blood, patients whose tumors shed to a lesser extent and patients with low tumor burden may not be assessable by this method and could result in a higher false-negative rate than tissue testing. Small tissue biopsies are required for NSCLC diagnosis; however, with blood-based NGS becoming more widely adopted, availability

Table 3. Safety Summary

Event	BFAST *ALK*-Positive Cohort (N = 87)
All-grade AEs, n	715
Patients with at least one, n (%)	
AE	87 (100)
Serious AE	21 (24)
Grade 3 or 4 AE	30 (34)
Related serious AE	5 (6)
Fatal AE[a]	1 (1)
AEs leading to, n (%)	
Treatment discontinuation	6 (7)
Dose reduction	7 (8)
Dose interruption	27 (31)

[a]Reported term of unexplained death, considered by the investigator to be unrelated to study treatment, which was withdrawn 4 days before death.
AE, adverse event; BFAST, Blood First Assay Screening Trial.

and turnaround times will likely improve, thereby enabling access to targeted therapies for patients who might otherwise only receive conventional cytotoxic chemotherapy because they were not able to have a biopsy for molecular analysis, or the analysis was not delivered on time for any reason.

A constraint of the current analysis is that the limited follow-up time and number of events did not allow for precise secondary end point estimates, such as median PFS. Nevertheless, primary data indicate that blood-based NGS represents a less invasive diagnostic tool that all patients should be able to access, in particular patients unable to provide tissue or those with insufficient tissue for *ALK* testing. Further analyses of the *ALK*-positive cohort are planned once survival end points are more mature.

First-line detection of *ALK* fusions using a validated blood-based NGS assay in BFAST predicts for high ORR and significant clinical benefit in patients with metastatic NSCLC receiving alectinib. These results reveal the clinical application of blood-based NGS as a method to inform clinical decision-making in *ALK*-positive disease.

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 *Journal of Thoracic Oncology* at www.jto.org and at https://doi.org/10.1016/j.jtho.2021.07.008.

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