Circulating Cell-free DNA as a Prognostic Biomarker in Patients with Advanced *ALK*+ Non–small Cell Lung Cancer in the Global Phase III ALEX Trial

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ABSTRACT

Purpose: We retrospectively assessed prognostic value of circulating cell-free DNA (cfDNA) using data from the phase III ALEX study in treatment-naïve, advanced *ALK*+ non–small cell lung cancer (NSCLC).

Patients and Methods: Patients were randomized to receive twice-daily alectinib 600 mg ($n = 152$) or crizotinib 250 mg ($n = 151$). cfDNA was quantified from baseline plasma samples, with patients stratified into ≤median and >median cfDNA biomarker-evaluable populations (BEP). Effect of cfDNA concentration on outcomes was analyzed using a Cox regression model with treatment group as covariate, and in multivariate analyses.

Results: Median cfDNA concentration in the BEP was 11.53 ng/mL ($n = 276$). A positive correlation was found between cfDNA concentration and number of lesions, organ lesion sites, and tumor size (sum of longest diameter; all $P < 0.0001$). In both treatment arms, patients in the >median BEP were more likely to experience disease progression than the ≤median BEP [alectinib adjusted HR = 2.04; 95% confidence interval (CI), 1.07–3.89; $P = 0.0305$ and crizotinib adjusted HR = 1.83; 95% CI, 1.11–3.00, $P = 0.0169$]. Median progression-free survival was longer with alectinib than crizotinib in both ≤median and >median BEPs ($P < 0.0001$). Overall survival data remain immature; survival probability was lower in the >median versus ≤median BEP in both treatment arms (alectinib HR = 2.52; 95% CI, 1.08–5.88; $P = 0.0333$ and crizotinib HR = 2.63; 95% CI, 1.27–5.47; $P = 0.0096$).

Conclusions: These data suggest that plasma cfDNA concentration may have prognostic value in advanced *ALK*+ NSCLC. Prospectively designed studies are warranted to investigate this finding.

Introduction

Patients whose tumors harbor a rearrangement of the anaplastic lymphoma kinase gene (*ALK*+) account for approximately 5% of all patients with non–small cell lung cancer (NSCLC; refs. 1, 2). Tumor biopsy remains the gold standard for accurate diagnosis and prognosis of NSCLC, but it is associated with inherent drawbacks. These include potential problems with the quantity and quality of the tissue obtained, the invasive nature of the procedure, and selection bias resulting from tumor heterogeneity (3).

Plasma-based testing for molecular genomics has the potential to overcome some of the limitations of tissue sampling, and thereby enable clinicians to offer more effective personalized therapies (3, 4). Furthermore, the clinical utility of circulating cell-free DNA (cfDNA) in serum and plasma has been an area of active research in many medical disciplines. cfDNA is believed to originate from multiple sources, including white blood cells, apoptotic/necrotic tumor cells, living tumor cells, and circulating tumor cells, and is therefore present in both healthy and diseased individuals (5, 6). Increased levels of cfDNA have been observed in patients with cancer and may harbor the genomic alterations present in the original tumor (7).

Previous research has indicated that increased baseline cfDNA concentration is linked with poor prognosis in NSCLC (8–10). Studies have also shown that cfDNA can act as a potential molecular prognostic marker for NSCLC; patients with NSCLC and lymph node metastasis or distant metastasis had higher cfDNA levels, associated with a shorter overall survival (OS; refs. 11–13). However, the correlation of cfDNA with outcomes after treatment with ALK inhibitors has not yet been characterized. Other biomarkers have also shown prognostic value in patients with *ALK*+ NSCLC, such as the presence of a *TP53* comutation which has been associated with poorer outcomes regardless of the systemic therapy received (14, 15).

The phase III, global, randomized ALEX study (NCT02075840) compared two ALK inhibitors, alectinib and crizotinib, in patients with treatment-naïve advanced *ALK*+ NSCLC. Alectinib demonstrated superior efficacy to crizotinib, with a favorable safety profile, at the primary data cutoff (February 9, 2017; ref. 16) and in three subsequent

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

Studies have shown that circulating cell-free DNA (cfDNA) may act as a molecular prognostic marker for non–small cell lung cancer (NSCLC), with higher cfDNA levels associated with poor prognosis. The correlation of cfDNA with outcomes after treatment with ALK inhibitors has not yet been characterized. This retrospective analysis assessed the prognostic potential of cfDNA using data from the phase III ALEX study comparing alectinib and crizotinib in treatment-naïve advanced *ALK*+ NSCLC. A positive correlation was found between cfDNA concentration and number of lesions, organ lesion sites, and tumor size. Patients with ≤median cfDNA had a lower risk of disease progression than those with >median cfDNA, along with a higher probability of survival. Alectinib consistently improved outcomes versus crizotinib, regardless of baseline cfDNA concentration, and reduced the risk of tumor progression by more than 50%. These findings suggest that plasma cfDNA concentration may have prognostic value for the treatment of advanced *ALK*+ NSCLC.

analyses after longer-term follow up; after an additional 10 months (17), 22 months, and 32 months (18). Here, we examine the prognostic potential of total baseline quantitative cfDNA using data from the ALEX study.

Patients and Methods

Study design

In the ALEX study, 303 patients were randomized 1:1 to receive twice-daily alectinib 600 mg or crizotinib 250 mg until disease progression, toxicity, withdrawal, or death. Full study details have been described previously (16). Key eligibility criteria included untreated advanced *ALK*+ NSCLC, confirmed centrally by IHC (VENTANA ALK D5F3), and an Eastern Cooperative Oncology Group performance status of 0 to 2. Patients with asymptomatic central nervous system (CNS) metastases were also permitted.

The ALEX 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.

The data presented in this manuscript are taken from the November 30, 2018 data cutoff, after a median follow-up of 37.8 months with alectinib and 23.0 months with crizotinib; and from the November 29, 2019 data cutoff, after a median follow-up of 48.2 months with alectinib and 23.3 months with crizotinib.

Study assessments

Two 5-mL blood samples were taken at baseline from all randomized patients for retrospective pharmacogenomic research. Blood was drawn into K2-EDTA blood collection tubes and centrifuged immediately at 1,500 × *g* for 10 minutes. The plasma layer was carefully transferred into a fresh tube and centrifuged again at 3,000 × *g* for 10 minutes. The plasma samples were then transferred into two storage tubes (plain cap transfer tubes) and stored immediately at −20°C or −70°C. Samples were shipped daily to the central laboratory for long-term storage. All plasma samples were analyzed using a hybrid capture-based NGS assay (FoundationACT, Foundation Medicine Inc.). cfDNA, defined as any volume of DNA detected in plasma, was extracted from baseline plasma samples and the concentration was determined in nanograms per milliliter using the D1000 ScreenTape assay on the 4200 TapeStation (Agilent Technologies; ref. 19). Patients were stratified into ≤median cfDNA and >median cfDNA biomarker-evaluable populations (BEP). Plasma samples were also evaluated for the presence of a *TP53* mutation, which is associated with reduced OS in *ALK*+ NSCLC (20). Circulating tumor DNA was assessed using plasma samples and patients were classified as circulating tumor DNA-positive when any somatic (known or likely status) alteration (short variants, rearrangement, or copy number) was detected, or otherwise as circulating tumor DNA-negative.

Endpoints

Endpoints of the ALEX study, including progression-free survival (PFS), objective response rate (ORR), duration of response (DoR), and OS were determined by investigator assessment. The objective of this exploratory analysis was to examine the prognostic value of cfDNA on PFS, ORR, DoR, and OS.

Statistical analyses

Kaplan–Meier methodology was used to assess the association between cfDNA concentration (stratified by ≤median and >median BEP) and PFS, DoR, and OS. The association between cfDNA concentration and PFS, DoR, and OS was analyzed using a Cox regression model with treatment group as unique covariate, in addition to separate multivariate analyses adjusting for the presence of a *TP53* mutation; measurable/nonmeasurable CNS lesions at baseline; baseline tumor size [measured as the baseline sum of longest diameter (SLD)], number of organ sites, and number of liver lesions. Associations between PFS and *TP53* mutation status and *ALK* rearrangement status according to cfDNA concentration were assessed using an unadjusted, unstratified Cox regression model. The association between cfDNA concentration and ORR was analyzed using a binomial logistic regression model. All regression analyses were performed considering cfDNA as both a categorical and continuous ($\log_2$-transformed) variable. Associations between cfDNA concentration and outcome were analyzed at the time when data for the study endpoints were last reported: at the November 30, 2018 data cutoff for PFS, DoR, and ORR, and at the November 29, 2019 data cutoff for OS.

Data availability statement

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).

Results

Patients

Baseline plasma samples were available for 276 patients; the median cfDNA concentration was 11.53 ng/mL (range 1.6–64.5) for all patients, 11.73 ng/mL in the alectinib treatment arm, and 10.85 ng/mL in the crizotinib treatment arm. Twenty-seven samples were unavailable for analysis. Of 222 (80%) patients with results from the FoundationACT assay, 135 patients (61%) had an *EML4-ALK* rearrangement detected, 10 patients (5%) had an *ALK*

Table 1. Baseline characteristics of the ≤median cfDNA and >median cfDNA BEPs according to treatment group.

	Crizotinib		Alectinib	
	≤median cfDNA (*n* = 72)	>median cfDNA (*n* = 67)	≤median cfDNA (*n* = 66)	>median cfDNA (*n* = 71)
Median age (range), years	54 (18–91)	54 (28–81)	57.5 (28–88)	58 (28–81)
Aged <65 years, *n* (%)	55 (76.4)	51 (76.1)	46 (69.7)	56 (78.9)
Smoking status, *n* (%)				
Active smoker	2 (2.8)	1 (1.5)	5 (7.6)	5 (7.0)
Nonsmoker	48 (66.7)	43 (64.2)	35 (53.0)	48 (67.6)
ECOG PS, *n* (%)				
0 or 1	70 (97.2)	61 (91.0)	66 (100.0)	61 (85.9)
2	2 (2.8)	6 (9.0)	0	10 (14.1)
CNS lesions,[a] *n* (%)	23 (31.9)	28 (41.8)	20 (30.3)	34 (47.9)
TP53-mutant, *n/N* (%)	11/51 (21.6)	21/64 (32.8)	10/40 (25.0)	30/67 (44.8)

Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.
[a]Measurable and nonmeasurable CNS lesions.

rearrangement with a non-*EML4* fusion partner, and 77 patients (35%) had no *ALK* rearrangement detected.

The BEP was generally representative of the overall ALEX study population; baseline characteristics were balanced between the treatment arms within the BEP (crizotinib $n = 139$, alectinib $n = 137$). There were more active smokers in the alectinib arm than in the crizotinib arm in both the >median cfDNA BEP (7.0% versus 1.5%, respectively) and the ≤median cfDNA BEP (7.6% versus 2.8%, respectively). In the >median cfDNA BEP, the alectinib arm contained slightly more patients with CNS lesions compared with the crizotinib arm (47.9% versus 41.8%, respectively) and more patients with *TP53* mutations (44.8% versus 32.8%, respectively; **Table 1**). These characteristics were generally more balanced in the ≤median cfDNA BEP.

Biomarker concentration by relevant drug and patient factors

Alectinib exposure (C_{trough}) was not substantially different between the ≤median cfDNA and >median cfDNA BEPs, and there was a trend for wider distribution of a slightly lower exposure in the >median cfDNA BEP (Supplementary Fig. S1). More than 95% of patients achieved alectinib exposures sufficient to maximize PFS, based on previous exposure-response analyses (21, 22). There was a significant difference in median cfDNA concentration in patients with no detectable *ALK* rearrangement in cfDNA (10.91 ng/mL; $n = 77$) and a detectable *ALK* rearrangement in cfDNA (16.37 ng/mL; $n = 145$; $P < 0.0001$).

A trend for slightly higher cfDNA concentration was observed with the presence of measurable/nonmeasurable CNS lesions and *TP53* mutations. Median cfDNA concentration was 12.57 ng/mL in patients with measurable CNS lesions and 10.68 ng/mL in patients without ($P < 0.05$; **Fig. 1A**), and 17.98 ng/mL in patients with *TP53* mutations and 12.10 ng/mL in patients without ($P < 0.001$; **Fig. 1B**). A trend towards higher median cfDNA concentration was seen in patients with liver lesions (**Fig. 1C**; Supplementary Table S1).

A positive correlation was found between cfDNA concentration and the number of measurable/nonmeasurable lesions (Spearman correlation 0.30; $P < 0.0001$; **Fig. 2A**); the number of organ lesion sites (Spearman correlation 0.38; $P < 0.0001$; **Fig. 2B**); and tumor size (SLD) by investigator (Spearman correlation 0.38; $P < 0.0001$; **Fig. 2C**).

Efficacy

Investigator-assessed median PFS was longer for alectinib than for crizotinib in both the ≤median and >median cfDNA BEPs (**Fig. 3A**). In the ≤median cfDNA BEP, median PFS was not estimable (NE; 95% CI, 34.9–NE) in the alectinib arm and 14.8 months (95% CI, 12.7–25.6) in the crizotinib arm (HR = 0.38; 95% CI, 0.23–0.61; $P < 0.0001$). Comparatively, in the >median cfDNA BEP, median PFS was 14.8 months (95% CI, 10.9–40.4) in the alectinib arm and 8.6 months (95% CI, 7.2–10.8) in the crizotinib arm (HR = 0.43; 95% CI, 0.29–0.65; $P < 0.0001$). In the adjusted analysis, the likelihood of experiencing a progression event was lower for patients in the >median cfDNA BEP of the alectinib arm than those in the ≤median cfDNA BEP of the crizotinib arm (HR = 0.46; 95% CI, 0.27–0.77; $P < 0.05$). The biomarker effect (>median cfDNA versus ≤median cfDNA BEP) in the unadjusted analysis in the alectinib arm (HR = 1.94; 95% CI, 1.20–3.15; $P = 0.0071$) and in the crizotinib arm (HR = 2.10; 95% CI, 1.44–3.07; $P = 0.0001$) was similar to the effect observed in the adjusted analysis in the alectinib (HR = 2.04; 95% CI, 1.07–3.89; $P = 0.0305$) and crizotinib arms (HR = 1.83; 95% CI, 1.11–3.00; $P = 0.0169$; Supplementary Table S2). Similar results were observed when cfDNA concentration was analyzed as a continuous value (Supplementary Table S2). Multivariate analysis of covariate effects on PFS by investigator are summarized in **Fig. 4**. cfDNA levels remain significantly associated with PFS after adjusting for variables representative of tumor burden. The higher HR for PFS in the >median cfDNA BEP compared with the ≤median cfDNA BEP was consistent irrespective of the cfDNA cut-off value used (Supplementary Fig. S2). Additionally, in a quartile analysis of cfDNA concentration, the greatest PFS probability in both treatment arms occurred in patients in the 0% to 25% quartile of cfDNA concentration, followed by patients in the 25% to 50%, 50% to 75%, and 75% to 100% quartiles, respectively (Supplementary Fig. S3).

When evaluating plasma circulating tumor DNA, we observed a trend for higher baseline cfDNA concentration in patients who were circulating tumor DNA-positive (containing a known or likely alteration) and a trend for lower baseline cfDNA concentration in patients who were circulating tumor DNA-negative (Supplementary Fig. S4). With alectinib, median PFS in the circulating tumor DNA-positive subgroup was 22.4 months (95% CI, 14.6–NE) compared with NE (95% CI, NE–NE) in the circulating tumor DNA-negative subgroup (HR = 2.72; 95% CI, 1.16–6.35; $P = 0.0210$ unadjusted analysis; **Fig. 3B**). With crizotinib, median PFS in the circulating tumor DNA-positive subgroup was 8.8 months (95% CI, 7.3–10.8) compared with 16.6 months (95% CI, 10.8–27.6) in the circulating

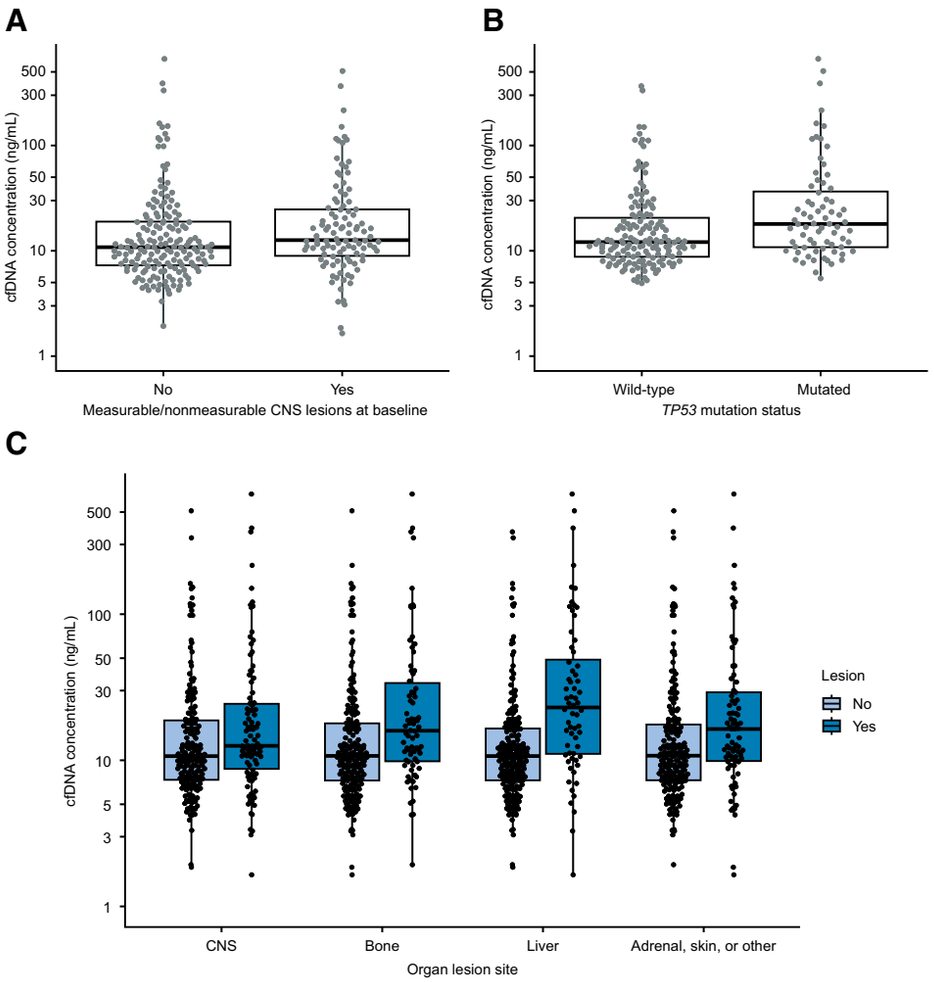


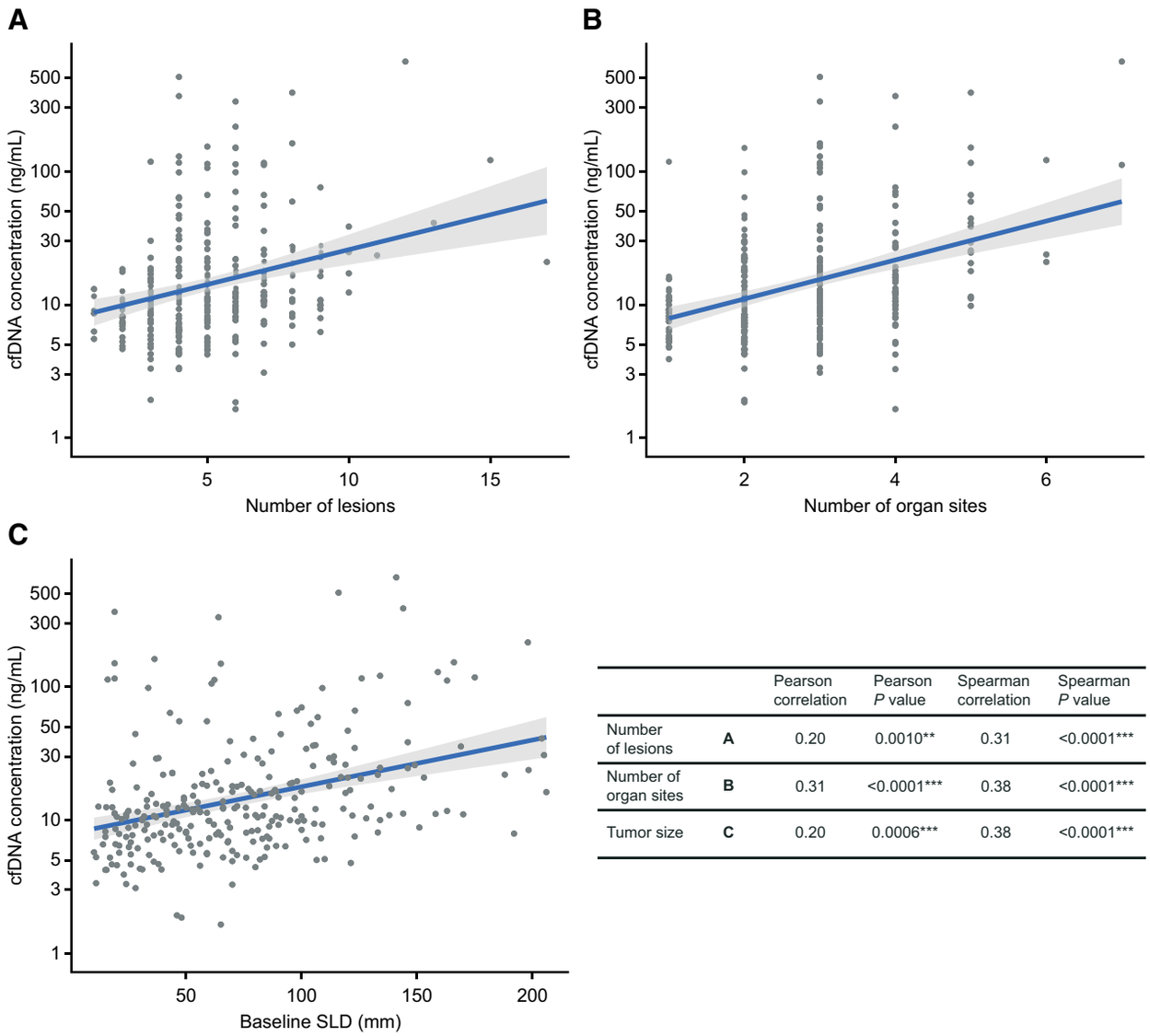
Figure 1.
Correlation of normalized cfDNA concentration with the presence of **A,** Measurable/nonmeasurable CNS lesions, **B,** *TP53* mutation status, and **C,** by organ lesion site (investigator assessment). Central lines represent median values, boxes represent interquartile ranges (IQR) and the top and bottom whiskers extend to the largest and smallest value, respectively, no further than 1.5 times the IQR.

tumor DNA-negative subgroup (HR = 2.03; 95% CI, 1.22–3.37; $P = 0.0066$ unadjusted analysis). A nonsignificant trend for the biomarker effect was also observed in both treatment groups in the adjusted analyses (alectinib HR = 1.95; 95% CI, 0.78–4.83; $P = 0.1506$; and crizotinib HR = 1.52; 95% CI, 0.88–2.63; $P = 0.1323$).

When the association between *TP53* comutation status and the biomarker effect of cfDNA concentration on PFS was analyzed, a longer median PFS was seen in the ≤median cfDNA BEP than the >median cfDNA BEP in both the alectinib and crizotinib arms, irrespective of *TP53* mutation status (Supplementary Fig. S5). When cfDNA concentration was considered as a continuous variable, a similar biomarker effect (longer median PFS with lower cfDNA concentration) was observed, which was significant in both treatment arms (Supplementary Table S3). A similar trend was seen when the association between detection of an *ALK* rearrangement in cfDNA and the biomarker effect of cfDNA concentration on PFS was analyzed (Supplementary Fig. S6); a longer median PFS was seen in the ≤median cfDNA BEP compared with the >median cfDNA BEP in both the alectinib and crizotinib arms, irrespective of the detection of an *ALK* rearrangement. When cfDNA concentration was considered as a continuous variable, the biomarker effect on PFS was significant across all comparisons in both treatment groups (Supplementary Table S4).

ORR by investigator was numerically higher in the ≤median cfDNA BEP compared with the >median cfDNA BEP in both treatment arms (**Table 2**). In the ≤median cfDNA BEP, ORR was 88.7% in the alectinib arm and 80.3% in the crizotinib arm; in the >median cfDNA BEP, ORR was 86.6% in the alectinib arm and 72.3% in the crizotinib arm. No significant difference was seen between the ≤median cfDNA and >median cfDNA BEPs in either the alectinib ($P = 0.9191$) or crizotinib ($P = 0.3720$) arms. Similar results for ORR were observed when cfDNA was analyzed as continuous value (Supplementary Table S5). Median DoR by investigator in the ≤median cfDNA BEP was NE in the alectinib arm (95% CI, NE–NE) compared with 18.4 months in the crizotinib arm (95% CI, 11.3–25.8); in the >median cfDNA BEP, median DoR was 29.6 months (95% CI, 12.9–NE) in the alectinib arm compared with 7.0 months (95% CI, 5.5–11.1) in the crizotinib arm (**Fig. 3C**).

The 5-year OS data from ALEX (data cutoff: November 29, 2019) used for this analysis remain immature. In the ≤median cfDNA BEP, median OS was NE (95% CI, NE–NE) in both treatment arms. Comparatively, in the >median cfDNA BEP, median OS was NE (95% CI, 27.7–NE) in the alectinib arm and 21.4 months (95% CI, 16.9–47.7) in the crizotinib arm (**Fig. 3D**). Survival probability was lower in the >median cfDNA BEP compared with the ≤median cfDNA BEP, in both the alectinib arm (HR = 2.58; 95% CI, 1.37–4.86; $P = 0.0033$) and the crizotinib arm (HR = 3.53; 95% CI, 1.99–6.28; $P < 0.0001$). This was consistent with the findings of the multivariate analysis in both the alectinib arm (HR = 2.52; 95%



		Pearson correlation	Pearson *P* value	Spearman correlation	Spearman *P* value
Number of lesions	A	0.20	0.0010**	0.31	<0.0001***
Number of organ sites	B	0.31	<0.0001***	0.38	<0.0001***
Tumor size	C	0.20	0.0006***	0.38	<0.0001***

Figure 2.

Correlation of normalized cfDNA concentration and **A,** number of lesions, **B,** number of organ sites, and **C,** tumor size (investigator assessment). Blue lines represent hazard functions as determined by Cox multivariate analyses. Grey shaded areas represent 95% CIs. ctDNA, circulating tumor DNA.

CI, 1.08–5.88; $P = 0.0333$) and the crizotinib arm (HR = 2.63; 95% CI, 1.27–5.47; $P = 0.0096$).

Discussion

In this retrospective analysis of the ALEX study, the number of lesions, organ lesion sites, and tumor size positively correlated with cfDNA concentration; patients with ≤median cfDNA had a lower risk of disease progression than those with >median cfDNA, along with a higher probability of survival. These data suggest that cfDNA concentration could potentially be used as a prognostic indicator and surrogate marker for tumor and overall disease burden, although it should be noted that *ALK* rearrangement was not detected in cfDNA in 35% of patients in this analysis. Reasons for these false negative results are currently unclear and do not appear to correlate with patient demographic or disease characteristics, but they may be related to the lower concentrations of cfDNA in patient samples where *ALK* rearrangement was not detected in cfDNA.

Alectinib consistently improved PFS, ORR, and DoR versus crizotinib, regardless of BEP subgroup, and reduced the risk of tumor progression by more than 50%. Additional analyses showed that patients in the "poor prognosis" group (>median cfDNA) treated with alectinib had either a similar median PFS (unadjusted analysis) or a longer median PFS (adjusted analysis) than patients in the "good prognosis" group (≤median cfDNA) treated with crizotinib. These data support alectinib as a preferred first-line treatment option for advanced *ALK*+ NSCLC (23, 24). Compared with results from the intent-to-treat (ITT) population at the same data cutoff, the PFS of patients in the >median BEP was lower in both treatment arms (18). Although OS data remain immature, OS was NE for the alectinib

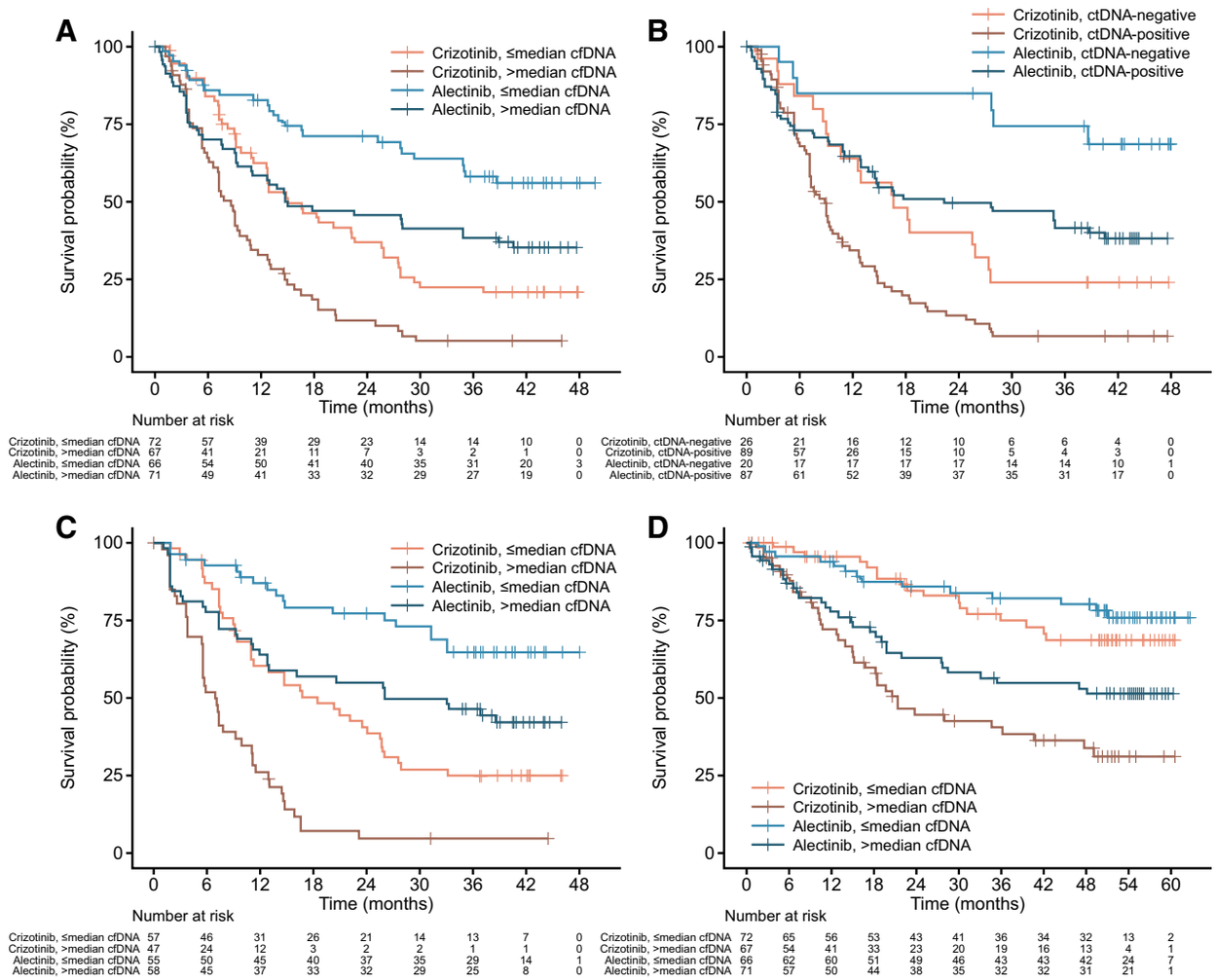


Figure 3.

Kaplan–Meier analysis of **A,** PFS by median cfDNA BEP, **B,** PFS by circulating tumor DNA status, **C,** DoR by median cfDNA BEP, and **D,** OS by median cfDNA BEP. PFS and DoR were investigator-assessed.

treatment arm in both arms of the BEP and the ITT, and the crizotinib arm of the >median BEP had a numerically lower OS than the ITT (18). At the present stage, the diagnosis and treatment of cancer is greatly dependent on molecular detection of tumor-derived genes, but tumor tissue biopsies are limited by their invasiveness, selection bias for heterogeneity, and inability to monitor the ongoing evolution of a tumor. Although this analysis focuses on the prognostic value of cfDNA, from a diagnostic perspective, cfDNA can be extracted from blood and measured, and so may overcome some of the limitations seen with using tissue samples. However, the practical application of the use of cfDNA data in a clinical setting is still not widely understood.

One step towards improving our understanding of the clinical utility of cfDNA will be to assess whether temporal changes in cfDNA levels during the course of ALK inhibitor monotherapy can be correlated with radiologic progression and other key trial endpoints, in order to monitor the progression of *ALK*+ NSCLC. Data from other studies have suggested that changes in cfDNA concentration during the course of lung cancer treatment may not correlate well with radiologic CT responses (8, 9). In the present analysis, patients with ≤median cfDNA at baseline had a numerically higher ORR than patients with >median cfDNA at baseline, which was more pronounced in the crizotinib arm. This is in line with the concept that patients with higher cfDNA levels may have a higher tumor burden and belong to a worse prognostic patient group. We also noted a slight trend for higher cfDNA concentrations in patients with CNS lesions, which may be a further indicator of higher overall tumor burden.

Another consideration for understanding the potential clinical value of cfDNA is the optimal cut-off threshold for quantification. The median value of 11.53 ng/mL was used in this study to stratify patients into separate BEPs, but this cutoff may not be applicable to other patient populations. Indeed, the cut-off thresholds of plasma cfDNA varied widely in a meta-analysis investigating the association

Treatment arm	Covariate	Subgroup	*n*	HR	95% CI	*P* value
Crizotinib	cfDNA (categorical)	≤Median	51			
		>Median	64	1.83	(1.11–3.00)	0.0169
Crizotinib	Measurable/nonmeasurable CNS lesions at baseline	No	70			
		Yes	45	1.75	(1.09–2.82)	0.0217
Crizotinib	*TP53* mutation status	Wild type	83			
		Mutated	32	1.63	(1.00–2.67)	0.0515
Crizotinib	Baseline SLD (cm)			0.97	(0.91–1.04)	0.4048
Crizotinib	Number of organ sites			1.25	(0.98–1.61)	0.0770
Crizotinib	Number of liver lesions by INV	No	81			
		Yes	34	0.99	(0.53–1.82)	0.9621
Alectinib	cfDNA (categorical)	≤Median	40			
		>Median	67	2.04	(1.07–3.89)	0.0305
Alectinib	Measurable/nonmeasurable CNS lesions at baseline	No	62			
		Yes	45	1.02	(0.51–2.02)	0.9554
Alectinib	*TP53* mutation status	Wild type	67			
		Mutated	40	2.42	(1.36–4.32)	0.0027
Alectinib	Baseline SLD (cm)			1.06	(1.00–1.12)	0.0513
Alectinib	Number of organ sites			1.31	(0.95–1.79)	0.0947
Alectinib	Number of liver lesions by INV	No	84			
		Yes	23	0.44	(0.20–1.00)	0.0496

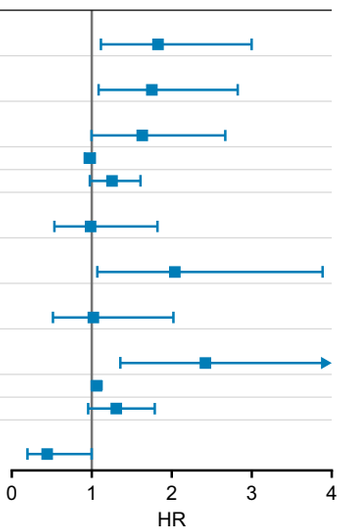


Figure 4.

Multivariate analysis of covariate effects on PFS by investigator. Unstratified Cox regression model adjusted for: cfDNA (categorical), measurable/nonmeasurable CNS lesions at baseline, *TP53* mutation status, baseline SLD (cm), number of organ sites, and number of liver lesions by investigator. INV, investigator.

between survival and baseline cfDNA level in patients with lung cancer (25). Nevertheless, the correlation of PFS by cfDNA quartiles in the present analysis (Supplementary Fig. S3), in addition to the consistent HR observed between high and low cfDNA concentration subgroups across different cut-off values (Supplementary Fig. S2), showed that the prognostic value of cfDNA was observed independent of the cutoff. Results were consistent when the effects of cfDNA concentration were analyzed as either a categorical or continuous variable. Our observation of a similar prognostic effect using baseline circulating tumor DNA has also been reported in other tumor types (26, 27). This is consistent with our findings and suggests that cfDNA may have value as a surrogate marker of tumor burden.

When considering detection of other potential predictive markers that can be identified in cfDNA, we observed a trend towards longer PFS with lower cfDNA concentrations in patients with and without a *TP53* comutation, across both treatment arms. Similarly, the biomarker effect of cfDNA concentration on PFS was evident in patients with and without detectable *ALK* rearrangements, with a trend for longer PFS seen in patients with lower cfDNA concentrations. The prognostic effect of cfDNA concentration is therefore retained when other biomarkers are considered in parallel, but the trends were also only consistently significant when cfDNA concentration was considered as a continuous variable. It is also worth noting that patients with a *TP53* comutation or detectable *ALK* rearrangement had higher cfDNA concentrations; as such, these biomarkers may not be independent. When adjusting for other variables representing tumor burden, such as measurable/nonmeasurable CNS lesions at baseline, tumor size (measured as the baseline sum of longest diameter), number of organ sites, and number of liver lesions, the prognostic effect of cfDNA remains in both arms indicating that cfDNA may be an independent prognostic marker and not only a possible surrogate for tumor burden.

Table 2. Investigator-assessed ORRs for alectinib- and crizotinib-treated patients by median cfDNA BEP.

Treatment	BEP	*n*	ORR	*P* value[a]
Crizotinib	≤median cfDNA	71	80.3	0.3720
	>median cfDNA	65	72.3	
Alectinib	≤median cfDNA	62	88.7	0.9191
	>median cfDNA	67	86.6	

[a]*P* value from hypothesis testing of whether the frequency of response is identical in all of the biomarker subgroups calculated using the Pearson χ^2 test.

Although the prognostic value of *TP53* comutation on treatment outcomes has been previously reported, these are the first data correlating cfDNA concentration (alone or in the context of other biomarkers) with treatment outcomes (8–10, 14, 15). Our findings suggest that cfDNA may have the potential to identify patients that are likely to have a worse prognosis and may derive less benefit from ALK inhibitor monotherapy. ALK inhibitors have greatly improved outcomes for patients with *ALK*+ NSCLC (17, 18, 28, 29). Identification of a patient subgroup with a poorer prognosis will be important to develop treatment strategies to overcome the negative prognostic effect by intensification of treatment beyond ALK inhibitor monotherapy; for example, by combination with chemotherapy or immunologic therapy.

It is important to note that this was a retrospective analysis of data emerging from a clinical study. ALEX was not designed or powered to investigate the impact of cfDNA on efficacy with ALK inhibitors. The results of this work are considered exploratory and would need to be validated in a prospectively designed study with balanced arms for cfDNA values to confirm observed effects.

In summary, the results of this analysis suggest that plasma cfDNA concentration may have prognostic value in patients with advanced *ALK*+ NSCLC. These exploratory findings warrant further investigation in prospectively designed studies.

Authors' Disclosures

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Authors' Contributions

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