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ORIGINAL ARTICLE

Early Circulating Tumor DNA Dynamics and Efficacy of Lorlatinib in Patients With Treatment-Naive, Advanced, *ALK*-Positive NSCLC

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ABSTRACT

Introduction: Circulating tumor DNA (ctDNA) has been used as a biomarker for prognostication and response to treatment. Here, we evaluate ctDNA as a potential biomarker for response to lorlatinib, a third-generation ALK tyrosine kinase inhibitor in patients with treatment-naive,

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Drs. Soo and Martini contributed equally and share first authorship.

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advanced, *ALK*-positive NSCLC in the ongoing phase 3 CROWN study (NCT03052608).

Methods: Molecular responses were calculated using mean variant allele frequency (VAF), longitudinal mean change in VAF (dVAF), and ratio to baseline. Efficacy assessments (progression-free survival [PFS] and objective response rate) were paired with individual patient ctDNA and analyzed for association.

Results: Compared with baseline, mean VAF at week 4 was decreased in both treatment arms. Considering all detected somatic variants, a reduction in dVAF ($\leq$0) was associated with a longer PFS in the lorlatinib arm. The hazard ratio (HR) for a dVAF less than or equal to 0 versus more than 0 was 0.50 (95% confidence interval [CI]: 0.23–1.12) in the lorlatinib arm. A similar association was not observed for crizotinib (HR = 1.00, 95% CI: 0.49–2.03). Comparing molecular responders with nonresponders, patients treated with lorlatinib who had a molecular response had longer PFS (HR = 0.37, 95% CI: 0.16–0.85); patients treated with crizotinib who had a molecular response had similar PFS as those without a molecular response (HR = 1.48, 95% CI: 0.67–3.30).

Conclusions: In patients with treatment-naive, advanced, *ALK*-positive NSCLC, early ctDNA dynamics predicted better outcome with lorlatinib but not with crizotinib. These results suggest that ctDNA may be used to monitor and potentially predict efficacy of lorlatinib treatment.

Keywords: Lorlatinib; ALK; Tyrosine kinase inhibitor; Non–small cell lung cancer; Circulating tumor DNA

Introduction

Lorlatinib is a highly potent, brain-penetrant, third-generation *ALK* tyrosine kinase inhibitor (TKI) that was found to have broad activity against *ALK* resistance mutations compared with other ALK inhibitors.[1,2] In a global phase 1/2 study (NCT01970865), lorlatinib was found to have clinically meaningful antitumor activity after failure of previous ALK inhibitor therapy, either first generation, second generation, or both.[3,4] Lorlatinib has also resulted in clinically meaningful improvement in progression-free survival (PFS) and a higher frequency of intracranial response versus crizotinib in patients with treatment-naive, advanced, *ALK*-positive NSCLC in the randomized, global, phase 3 CROWN study (NCT03052608), leading to approval of lorlatinib for this indication by the U.S. Food and Drug Administration and many other regulatory agencies.[5]

Circulating tumor DNA (ctDNA), consisting of small fragments of naked DNA shed in the bloodstream by tumor cells and obtained from minimally invasive blood samples, may represent a convenient option to identify biomarkers not only for patient selection but also for prognostication, monitoring of response to treatment, and detection of recurrence/progression through serial monitoring.[6–8] In patients with NSCLC treated with osimertinib, an EGFR TKI, the absence of detectable mutant *EGFR* in the plasma at baseline and clearance of detectable mutant *EGFR* in the plasma at weeks 3 and 6 were associated with improved PFS.[9–11] Additional new and ongoing studies are evaluating the role of ctDNA monitoring across various treatment modalities and disease settings.[12–16] In patients with NSCLC, recent studies, including a study of lorlatinib, have prospectively included ctDNA and cell-free DNA analyses for monitoring therapy response and detection of disease progression.[13,14,17]

We report a prospectively planned analysis of the CROWN study to evaluate the association between early ctDNA dynamics, assessed by two different methods, and clinical efficacy to identify potential early biomarkers of response to lorlatinib in patients with treatment-naive, advanced, *ALK*-positive NSCLC. We present the impact of *ALK* alteration dynamics alone or in the presence of other gene alterations and methods to evaluate the impact of changing levels of ctDNA on clinical outcomes in response to lorlatinib.

Materials and Methods

Patients and Study Design

The study design, objectives, and eligibility criteria of the CROWN study, which enrolled patients with treatment-naive, advanced, *ALK*-positive NSCLC, have been published previously and are discussed briefly here.[5] Patients were randomly assigned (1:1) to receive either oral lorlatinib 100 mg daily or oral crizotinib 250 mg twice daily in the course of the study in 28-day cycles. The primary end point was PFS, determined by the time between randomization and Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1–defined disease progression (evaluated by blinded independent central review [BICR]) or death from any cause.

Before participation, patients provided written informed consent. The independent ethics committee or institutional review board at each site approved the protocol, which complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, Good Clinical Practice guidelines, the Declaration of Helsinki, and local laws.

Plasma Collection and Molecular Profiling

Blood specimens for ctDNA profiling were collected in K2EDTA-coated tubes at baseline, week 4 (d 1 of cycle

2), week 24 (d 1 of cycle 7), and the end of treatment or patient withdrawal. Plasma cell-free DNA extraction and analysis of ctDNA were performed at a central laboratory using a validated, commercially available 74-gene ctDNA next-generation sequencing assay,[18] as previously described.[19,20] Mean variant allele frequencies (VAFs) were calculated at baseline, week 4, and week 24 for each patient. Additional details are included in the Supplementary Methods.

Measurement of Molecular Response

For each sample, somatic single-nucleotide variants, insertion-or-deletion variants, and fusions were used to calculate the mean and maximum VAF and total variant count. Patients with no ctDNA detected (NCTD) at baseline and on treatment (wk 4 and/or wk 24) were analyzed separately. Two methods were used to evaluate molecular response, comprising (1) absolute change in VAF (dVAF), calculated as $(mean\ VAF_{week\ n}) - (mean\ VAF_{BL})$, with n = 4 or 24 and BL as baseline; a dVAF less than or equal to 0 indicated decreased or no change in ctDNA over time (dVAF was separately calculated for *ALK*-specific variants and for any somatic variants); and (2) relative change in VAF or molecular response, previously used with immunotherapy,[12,21] on the basis of the ratio of mean VAF on treatment (wk 4 or wk 24) to mean VAF at baseline. Molecular response was defined as a ratio less than 50% of mean VAF on treatment (wk 4 or wk 24) to mean VAF at baseline; non-response was defined as a ratio more than or equal to 50%.[12,21] Responders who achieved complete clearance of ctDNA at 4 or 24 weeks (molecular response 0%) were further classified as "cnleared." Clearance was defined as complete disappearance of detectable ctDNA. Additional details are included in the Supplementary Methods.

Measurements of Efficacy Based on Molecular Response

End points evaluated were PFS and objective response rate (ORR) on the basis of BICR and RECIST 1.1. Tumor measurements during the study were performed at screening and every 8 weeks and continued until progression. Tumor measurement at week 8 (after baseline to d 56 ± 15 days) was paired with ctDNA analysis of the plasma sample collected at week 4 (d 1 of cycle 2). Efficacy assessments at week 24 (after baseline to d 168 ± 15 days) were paired with the plasma sample collected at week 24 (d 1 of cycle 7).

We first assessed the association between the reduction in tumor burden (considering all measurable lesions and using the longest diameter for nonnodal lesions and the short axis for nodal lesions) compared with baseline and on-treatment mean VAF reduction. A more complex molecular response approach was used to explore the association between molecular response on the basis of ctDNA dynamics and RECIST response or outcome after treatment with lorlatinib.[12] We evaluated the association between molecular response on the basis of ctDNA at week 4 and objective radiologic RECIST response assessed during the study at week 8. Objective radiologic RECIST response (complete response [CR], partial response [PR], stable disease [SD]) was evaluated as a ratio of mean VAF on treatment to mean VAF at baseline of less than 50% (molecular responders) or more than or equal to 50% (molecular nonresponders [MNRs]). For those classified as molecular responders, we evaluated whether molecular response on the basis of ctDNA assessment at week 4 was associated with PFS. Molecular responders were further subdivided between patients who achieved complete clearance of ctDNA at week 4 (molecular responder cleared [MRC]) and patients who had remaining ctDNA detectable at week 4 (molecular responder not cleared [MRNC]). Responses in patients who had NCTD at baseline and remained with NCTD at week 4 were also assessed.

Statistical Analysis

The intention-to-treat population included all patients who had undergone randomization. The ctDNA population encompassed all patients from the safety analysis set who had more than or equal to 1 screening blood-based biomarker assessment on the basis of the Guardant multigene panel data. The molecular analysis set included all patients with assessable on-treatment mean VAF. Statistical parameters were described previously.[5,20] Additional details are included in the Supplementary Methods.

Results

Analysis Population

The data cutoff was March 20, 2020. Plasma samples for analysis in this study were available from 263 of 291 treated patients (90.4%) at baseline; samples from 20 patients enrolled in the People's Republic of China were not available at the time of this analysis, and eight patients did not provide baseline samples. Of the 263 patients included in the current analysis, 134 were enrolled in the lorlatinib arm and 129 in the crizotinib arm. Nevertheless, five patients in the lorlatinib arm and four in the crizotinib arm had baseline samples that failed the analysis (e.g., were uninformative, not analyzed); the failure rate of plasma genotyping was 3.4%. Paired samples (baseline and wk 4 or 24) were available for most of the patients (235 of 263 [89.4%] at

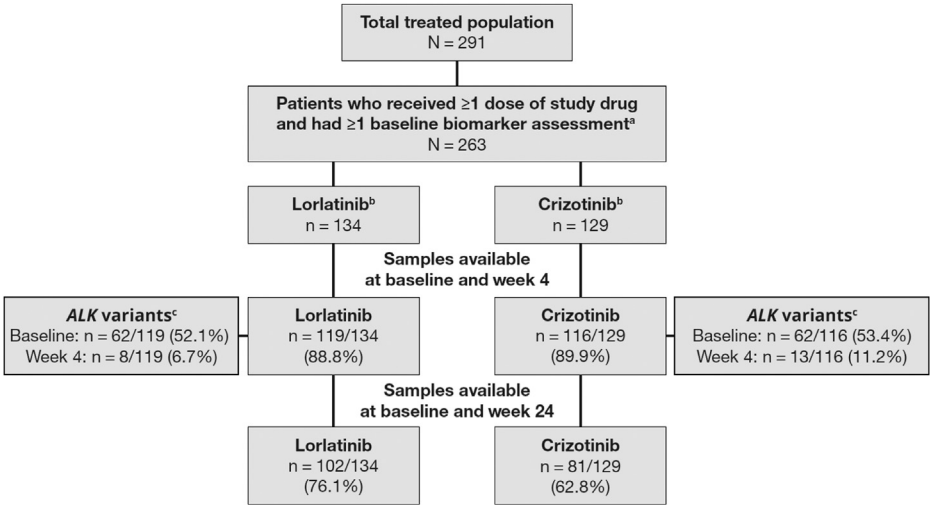


Figure 1. CONSORT diagram. [a]Plasma samples prospectively collected for ctDNA analysis at baseline; patients enrolled in the People's Republic of China (n = 20) were not included in this analysis. [b]Five patients in the lorlatinib arm and four patients in the crizotinib arm had samples that failed analysis, were uninformative, or were not analyzed. [c]*ALK* fusions and/or variants. ctDNA, circulating tumor DNA.

wk 4 and 183 [69.6%] at wk 24) (Fig. 1). Because most patients were included in these baseline analyses, patient characteristics were similar to those published for the overall study population (Supplementary Table 1). Although *ALK* fusions were the main alterations detected in this treatment-naive patient population, 20 *ALK* mutations and one deletion were detected at baseline in the plasma of 12 patients, and five and seven mutations were detected in the lorlatinib and crizotinib arms, respectively (Supplementary Table 2).

ctDNA at Baseline and Its Association With Tumor Burden

Plasma samples were successfully assessed and returned genotyping results for 129 patients in the lorlatinib arm and 125 in the crizotinib arm. Overall, ctDNA was detected in samples from 188 of 254 patients (74.0%) with measurable lesions at baseline (92 patients [71.3%] in the lorlatinib arm and 96 [76.8%] in the crizotinib arm); no ctDNA was detected in samples from 33 (25.6%) and 26 (20.8%) patients, respectively. Measurable lesions could not be detected in seven patients, who were excluded from this analysis. Patients with NCTD tended to have a lower tumor burden at baseline (Supplementary Fig. 1). Mean tumor size (SD) by BICR at screening was 70.5 (±37.4) mm in the ctDNA-detected group and numerically lower, 51.0 (±39.3) mm, in the NCTD group for lorlatinib-treated patients and, similarly, 72.4 (±47.2) and 52.8 (±30.3) mm, respectively, for crizotinib-treated patients. At baseline, 131 patients had ctDNA with a detectable *ALK* alteration (mutation and/or fusion) (Supplementary Table 2).

On-Treatment ctDNA Dynamics

Analysis of on-treatment ctDNA dynamics was performed on a subgroup of 174 patients (66.2%) (96 [71.6%] in the lorlatinib arm, 78 [60.5%] in the crizotinib arm) for whom samples were available and analyzed successfully at baseline, week 4, and week 24 (Supplementary Table 2).

In patients with *ALK* fusions and/or variants, treatment with lorlatinib or crizotinib was associated with a rapid reduction in mean VAF in patients with a best overall response (BOR) of CR/PR. Most patients achieved clearance at either week 4 or week 24 (Fig. 2*A*). When looking at the VAF for all the somatic variants detected in each sample, the pattern of overall ctDNA was more variable. Compared with those with *ALK* fusions and/or variants, fewer patients achieved clearance in either week 4 or week 24 in both the lorlatinib and crizotinib arms (Fig. 2*B*).

Association of Molecular Response With RECIST Response and Clinical Outcome

First, we assessed the association between reduction in tumor burden versus baseline and on-treatment mean VAF reduction. The reduction in VAF at week 4 was associated with a reduction in tumor size per RECIST on the first on-study tumor assessment (wk 8) in both groups, for both *ALK*-specific variants with a median tumor size change of −40.1% in the lorlatinib arm and −41.3% in the crizotinib arm and similarly for any somatic variants. In the lorlatinib arm at week 4, no patient had a mean VAF increase associated with *ALK* variants, and only 2 patients in the crizotinib arm had

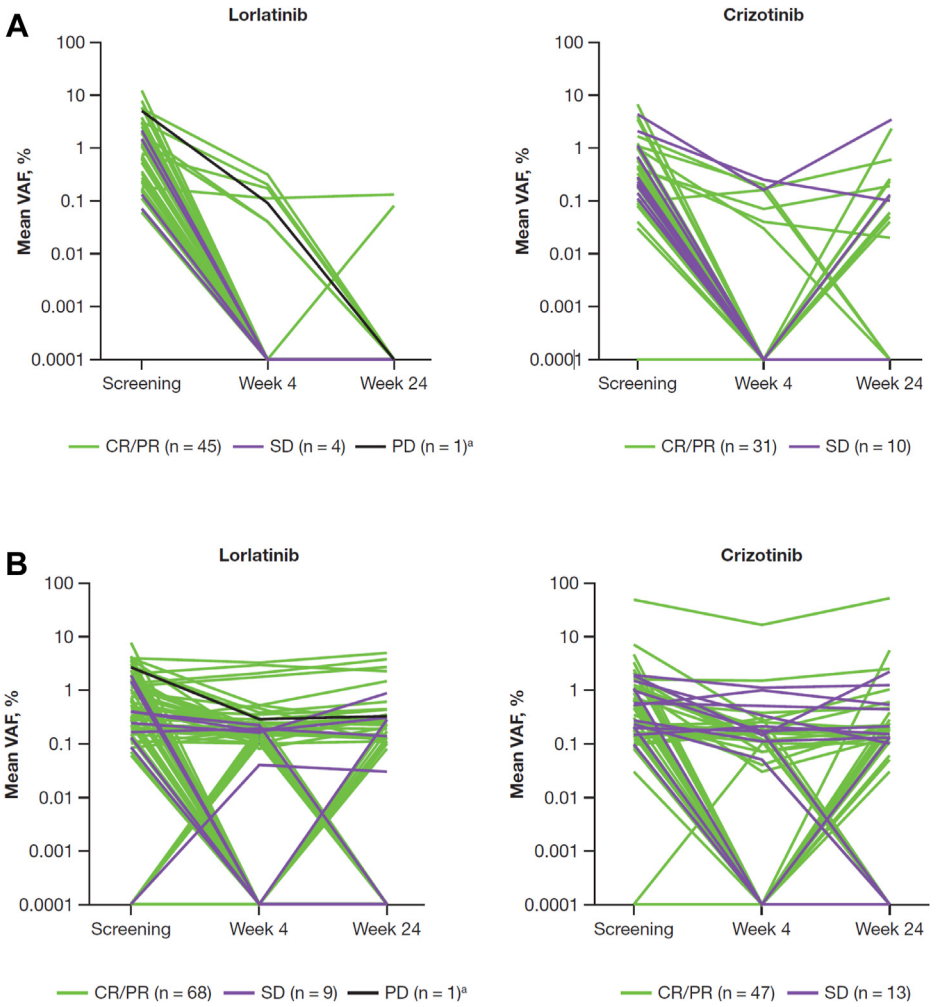
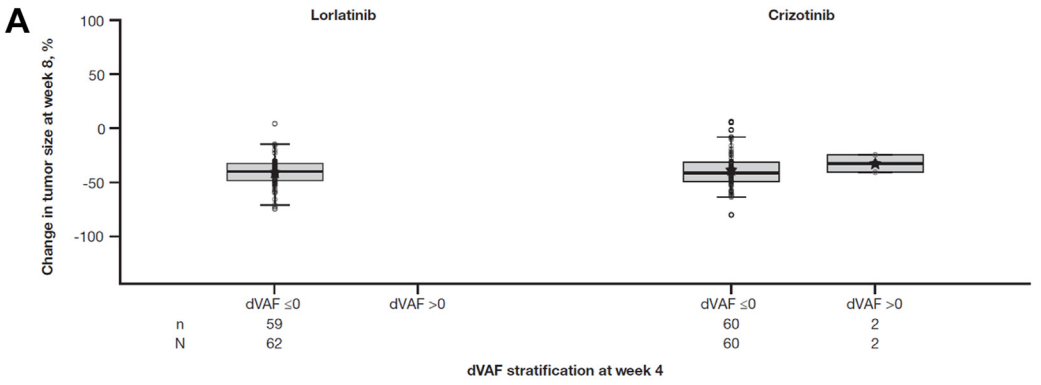
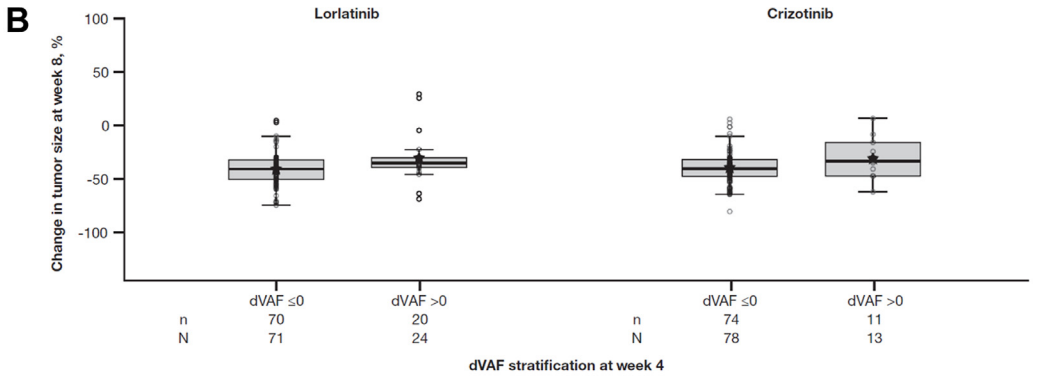
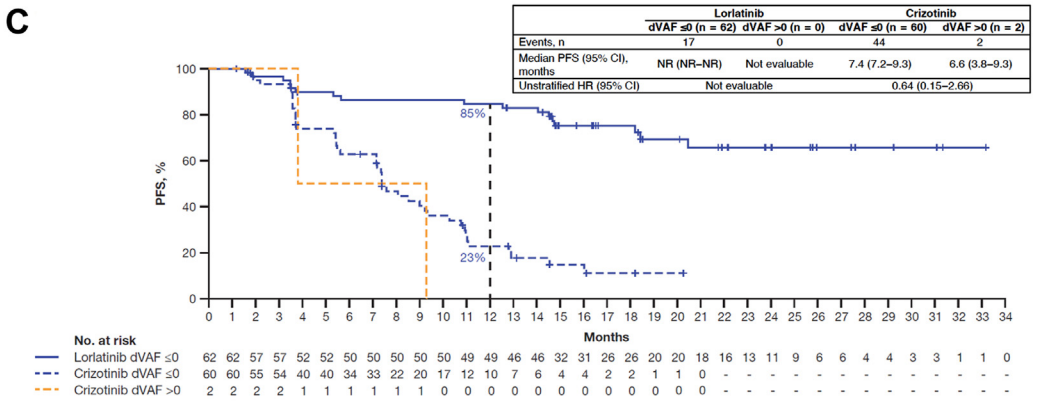


Figure 2. Mean VAF dynamics and association with tumor response at weeks 4 and 24 (ctDNA population with measurable lesions and assessments at screening, week 4, and week 24) in patients with (*A*) *ALK* fusions and/or variants[a] and (*B*) any somatic variants.[b] [a]Treatment with lorlatinib was associated with a rapid reduction in mean VAF of *ALK* fusions and/or variants at 4 weeks, with 39 of 45 patients (86.7%) with a best overall response (BOR) of CR/PR achieving clearance and the remaining six (13.3%) experiencing some decrease in mean VAF; between weeks 4 and 24, five more patients (11.1%) achieved clearance, whereas two (4.4%) had an increase in mean VAF. In the crizotinib arm, 25 of 31 patients (80.6%) with CR/PR achieved clearance, five (16.1%) experienced some decrease in mean VAF, and one (3.2%) experienced an increase in mean VAF at week 4. Between weeks 4 and 24, 18 patients (58.1%) continued to have clearance of the *ALK* alterations, another three (9.7%) achieved clearance by week 24, and one (3.2%) continued to have a decrease in mean VAF without clearance; however, nine patients (29.0%) who had a decrease or clearance of *ALK* alteration at week 4 had an increase in mean VAF at week 24. [b]In the lorlatinib arm, 29 of 68 patients (42.6%) with CR/PR achieved clearance at week 4, 26 (38.2%) had some decrease in mean VAF, and 13 (19.1%) had an increase in mean VAF. By week 24, another 13 patients (19.1%) achieved clearance, whereas 20 (29.4%) who had experienced a mean VAF decrease or clearance by week 4 saw an increase at week 24. In the crizotinib arm, 24 of 47 patients (51.1%) with CR/PR achieved clearance at week 4, whereas 16 (34.0%) had some decrease and seven (14.9%) had an increase in mean VAF. By week 24, another eight patients (17.0%) achieved clearance, whereas 24 (51.1%) who had experienced a mean VAF decrease or clearance by week 4 saw an increase in mean VAF at week 24. [c]One lorlatinib-treated patient had PD as their best response, assessed by BICR but not the investigator, and so continued to receive lorlatinib treatment. BICR, blinded independent central review; CR, complete response; ctDNA, circulating tumor DNA; PD, progressive disease; PR, partial response; SD, stable disease; VAF, variant allele fraction.

such an increase (Fig. 3*A*). Therefore, mean VAF association with tumor response at week 8 for *ALK* fusions and/or variants could not be evaluated in the lorlatinib arm. Nevertheless, patients with a decrease in mean VAF at week 4 had a confirmed ORR of 75.8% in the lorlatinib arm and 53.3% in the crizotinib arm (Table 1). With all detected somatic variants included, patients with a decrease in mean VAF at week 4 had a confirmed ORR of 77.5% in the lorlatinib arm and 55.1% in the crizotinib arm. Patients who had an increase in mean VAF at week 4 (dVAF >0) still derived benefit, with a median tumor size change of -35.3% in the lorlatinib arm and -33.5%


A
Lorlatinib
Crizotinib
Change in tumor size at week 8, %
100
50
0
-50
-100
dVAF ≤0
dVAF >0
dVAF ≤0
dVAF >0
n 59 60 2
N 62 60 2
dVAF stratification at week 4


B
Lorlatinib
Crizotinib
Change in tumor size at week 8, %
100
50
0
-50
-100
dVAF ≤0
dVAF >0
dVAF ≤0
dVAF >0
n 70 20 n 74 11
N 71 24 N 78 13
dVAF stratification at week 4


C
Lorlatinib
Crizotinib
dVAF ≤0 (n = 62) dVAF >0 (n = 0) dVAF ≤0 (n = 60) dVAF >0 (n = 2)
Events, n 17 0 44 2
Median PFS (95% CI), months NR (NR–NR) Not evaluable 7.4 (7.2–9.3) 6.6 (3.8–9.3)
Unstratified HR (95% CI) Not evaluable 0.64 (0.15–2.66)
PFS, %
100
80
60
40
20
0
85%
23%
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
Months
No. at risk
Lorlatinib dVAF ≤0 62 62 57 57 52 52 50 50 50 50 50 49 49 46 46 32 31 26 26 20 20 18 16 13 11 9 6 6 4 4 3 3 1 1 0
Crizotinib dVAF ≤0 60 60 55 54 40 40 34 33 22 20 17 12 10 7 6 4 4 2 2 1 1 0 - - - - - - - - - - - - -
Crizotinib dVAF >0 2 2 2 2 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 - - - - - - - - - - - -

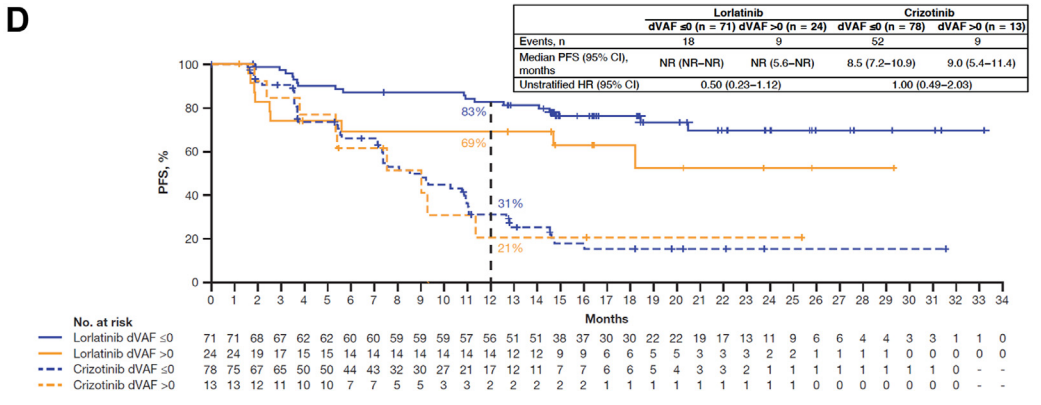

D
Lorlatinib
Crizotinib
dVAF ≤0 (n = 71) dVAF >0 (n = 24) dVAF ≤0 (n = 78) dVAF >0 (n = 13)
Events, n 18 9 52 9
Median PFS (95% CI), months NR (NR–NR) NR (5.6–NR) 8.5 (7.2–10.9) 9.0 (5.4–11.4)
Unstratified HR (95% CI) 0.50 (0.23–1.12) 1.00 (0.49–2.03)
PFS, %
100
80
60
40
20
0
83%
69%
31%
21%
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
Months
No. at risk
Lorlatinib dVAF ≤0 71 71 68 67 62 62 60 60 59 59 57 56 51 51 38 37 30 30 22 22 19 17 13 11 9 6 6 4 4 3 3 1 1 0
Lorlatinib dVAF >0 24 24 19 17 15 15 14 14 14 14 14 14 12 12 9 9 6 6 5 5 3 3 3 2 2 1 1 1 1 0 0 0 0 0
Crizotinib dVAF ≤0 78 75 67 65 50 50 44 43 32 30 27 21 17 12 11 7 7 6 6 5 4 3 3 2 1 1 1 1 1 1 1 1 0 - -
Crizotinib dVAF >0 13 13 12 11 10 10 7 7 5 5 3 3 2 2 2 2 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 - -

Table 1. Summary of Objective Response by RECIST Based on BICR at Week 8 by dVAF Stratification at Week 4

ALK Fusion and/or Mutation	Lorlatinib (n = 62)		Crizotinib (n = 62)	
dVAF	≤0	>0	≤0	>0
Patients, n (%)	62 (100)	0	60 (96.8)	2 (3.2)
Objective response rate, n (%)				
Confirmed CR/PR	47 (75.8)	0	32 (53.3)	1 (50.0)
Unconfirmed CR/PR	7 (11.3)	0	14 (23.3)	0
SD	6 (9.7)	0	12 (20.0)	1 (50.0)
PD	2 (3.2)	0	2 (3.3)	0
Any somatic mutation	**Lorlatinib (n = 95)**		**Crizotinib (n = 91)**	
dVAF	≤0	>0	≤0	>0
Patients, n (%)	71 (74.7)	24 (25.3)[a]	78 (85.7)[a]	13 (14.3)[a]
Objective response rate, n (%)				
Confirmed CR/PR	55 (77.5)	14 (58.3)	43 (55.1)	7 (53.8)
Unconfirmed CR/PR	7 (9.9)	3 (12.5)	15 (19.2)	0
SD	8 (11.3)	3 (12.5)	12 (15.4)	4 (30.8)
PD	1 (1.4)	3 (12.5)	5 (6.4)	1 (7.7)
IND	0	0	1 (1.3)	0

[a]One patient with a dVAF of more than 0 in both treatment arms and two patients with a dVAF less than or equal to 0 in the crizotinib arm did not have a tumor assessment at week 8.
BICR, blinded independent central review; CR, complete response; dVAF, change in variant allele frequency; IND, indeterminant; PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.

in the crizotinib arm. Of these patients, 58.3% in the lorlatinib arm and 53.8% in the crizotinib arm experienced a confirmed ORR (Fig. 3*B* and Table 1).

Evaluation of Absolute ctDNA

In patients with *ALK* fusions and/or variants, the HR of PFS for dVAF less than or equal to 0 versus more than 0 could not be evaluated in the lorlatinib arm because no patient in this group had a mean VAF increase. The HR of PFS for a dVAF less than or equal to 0 (n = 60) versus a dVAF of more than 0 (n = 2) was 0.64 (95% CI: 0.15–2.66) in the crizotinib arm (Fig. 3*C*). A reduction in dVAF (≤0) was associated with a potentially longer PFS in the lorlatinib arm compared with the crizotinib arm (HR = 0.15; 95% CI: 0.08–0.28). With regard to all detected somatic variants, a reduction in dVAF (≤0) was associated with a potentially longer PFS in the lorlatinib arm compared with the crizotinib arm (HR = 0.18; 95% CI: 0.10–0.31). An increase in dVAF (>0) was also associated with a potentially longer PFS in the lorlatinib versus the crizotinib arm (HR = 0.46, 95% CI: 0.18–1.18). The HR for a dVAF less than or equal to 0 versus more than 0 in the lorlatinib arm was 0.50 (95% CI: 0.23–1.12; Fig. 3*D*). A similar association was not observed in the crizotinib arm (dVAF ≤0 versus >0 HR = 1.00, 95% CI: 0.49–2.03). The association observed for the lorlatinib group was not retained when using the dVAF derived from the ctDNA assessment at week 24 (HR = 1.13, 95% CI: 0.32–3.93) (Supplementary Table 3). This was also true for the crizotinib group (HR = 1.58, 95% Cl: 0.68–3.67).

Evaluation of Relative ctDNA

We next applied a molecular response approach to explore whether the association between molecular response, on the basis of ctDNA dynamics at week 4, and RECIST response would be more informative. In patients treated with lorlatinib, deeper molecular response values at week 4 (i.e., a greater reduction in VAF from

Figure 3. dVAF association with tumor response and PFS (ctDNA population with assessments at screening and week 4). (*A*) Tumor size change at week 8 for *ALK* fusions and/or variants; (*B*) tumor size change at week 8 for any somatic variants; (*C*) PFS for *ALK* fusions and/or variants; (*D*) PFS for any somatic variants. Circles in (*A*) and (*B*) represent individual patient values, and stars represent the mean. Tumor size (mm) corresponds to sum of the dimension of all measurable lesions (using the longest diameter for no-nodal lesions and the short axis for nodal lesions). Patients with no measurable lesions at baseline or with week 8 tumor measurement outside the time window (d 56 + 15 days) are excluded (n = 3 for *ALK* fusions or variants and n = 5 for any somatic variants in the lorlatinib arm, and n = 0 for *ALK* fusions or variants and n = 6 for any somatic variants in the crizotinib arm). CI, confidence interval; ctDNA, circulating tumor DNA; dVAF, change in variant allele frequency; HR, hazard ratio; N, total number in the assessable population; n, number with tumor burden data available; NR, not reached; PFS, progression-free survival.

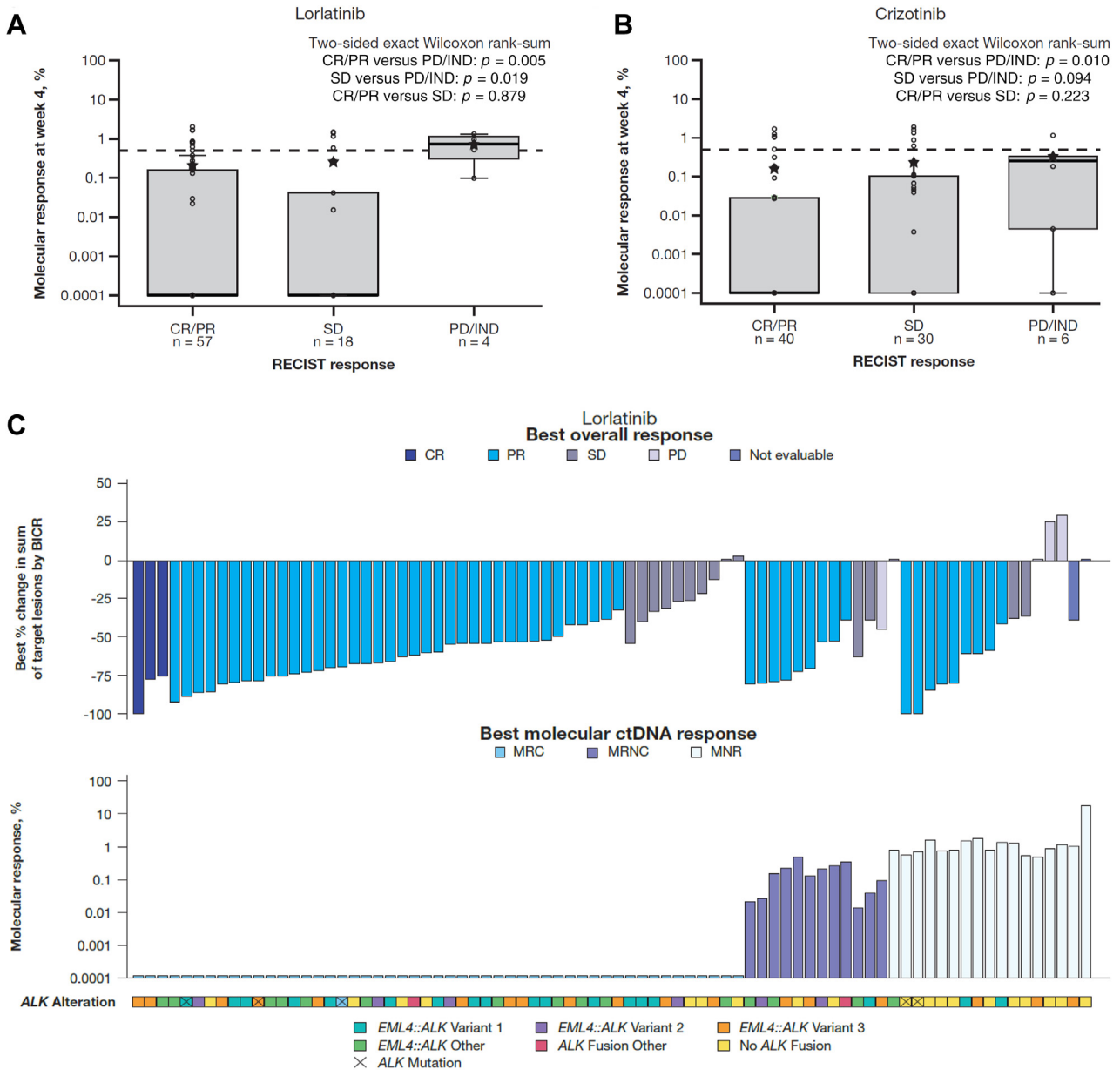


Figure 4. Rank-sum association between molecular response at week 4 and RECIST response at week 8 (lorlatinib [*A*]; crizotinib [*B*]), best tumor response to treatment and ctDNA molecular response (lorlatinib [*C*]; crizotinib [*D*]), and progression-free survival (lorlatinib [*E*]; crizotinib [*F*]). Circles in (*A*) and (*B*) represent individual patient values, and stars represent mean. Box plots represent median and 25%/75% quartiles, with whiskers to the last point within 1.5× the IQR. Waterfall plots of best overall response as determined by BICR ranked by molecular response at week 4 using a cutoff of 50%; *ALK* alterations (*EML4*::*ALK* fusion variants 1, 2, 3, other, *ALK* fusion other, and *ALK* mutation) on the basis of ctDNA profiling. Kaplan-Meier progression-free survival curves used the molecular response cutoff of 50% (ctDNA population). CI, confidence interval; CR, complete response; ctDNA, circulating tumor DNA; HR, hazard ratio; IND, indeterminant; IQR, interquartile range; MNR, molecular nonresponder; MRC, molecular responder cleared; MRNC, molecular responder not cleared; NCTD, no ctDNA detected; NR, not reached; PD, progressive disease; PFS, progression-free survival; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.

baseline) were observed in patients who had a CR/PR or SD versus progressive disease (PD) (CR/PR versus PD, $p = 0.005$; SD versus PD, $p = 0.019$), but there was no difference between CR/PR versus SD ($p = 0.88$) (Fig. 4*A* and *C*); the MRC category captured all the patients experiencing a BOR of CR and none of the PD group, and although the MNR category captured most of the patients (three of four) with a BOR of PD, it still included nine patients achieving a BOR of PR, including some with a deep response (Fig. 4*C*). For the crizotinib-treated patients, the molecular response values were only deeper in patients with a CR/PR versus PD ($p = 0.010$) (Fig. 4*B*);

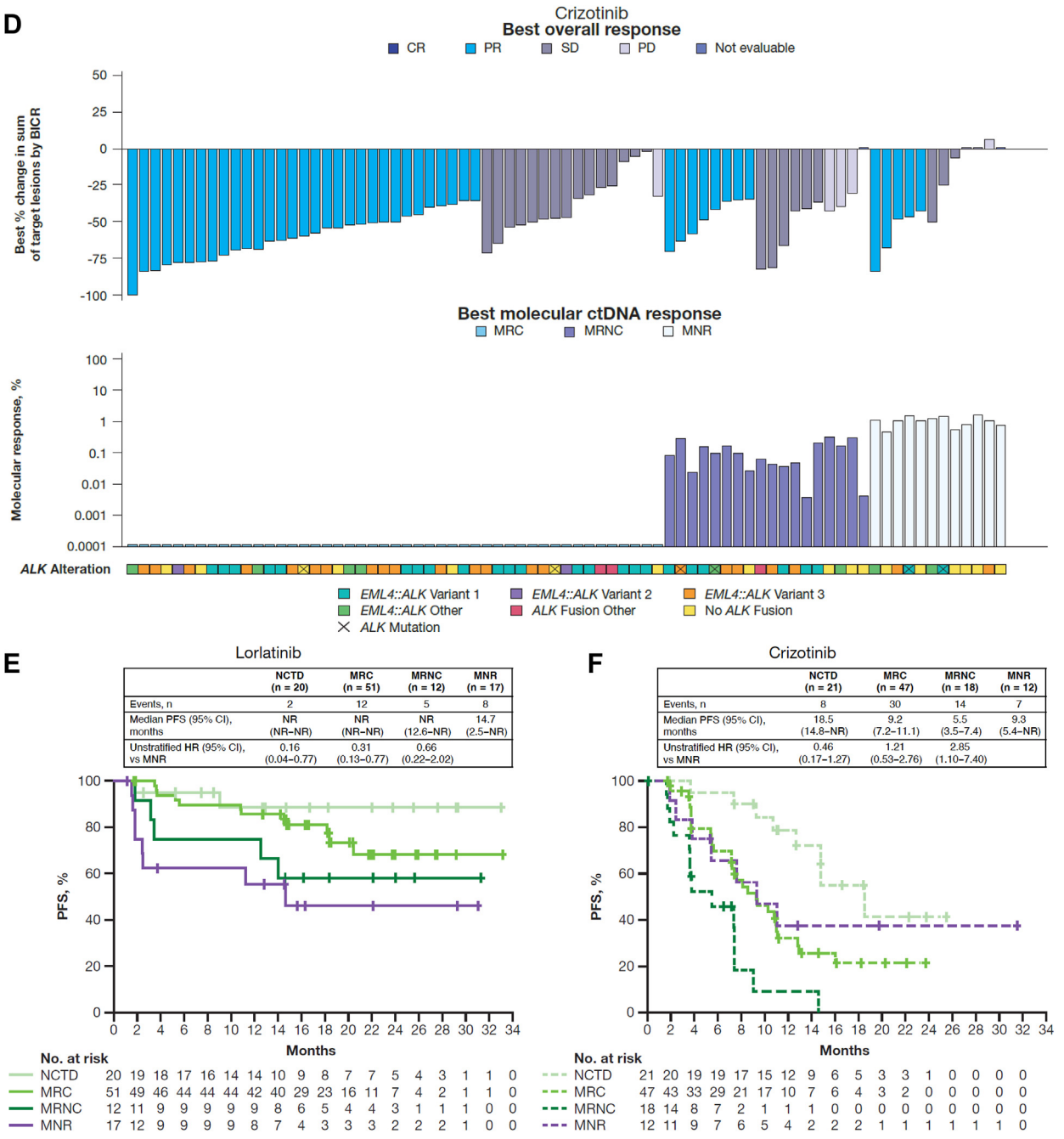


Figure 4. (*continued*).

the MRC category captured one patient with a BOR of PD, whereas the MRNC category captured most of the patients (three of five) with a BOR of PD, and the MNR category still included five patients achieving a BOR of PR (Fig. 4*D*). In addition, the type of *ALK* fusions, the *EML4::ALK* variant subtypes, and *ALK* mutations did not cluster preferentially in a specific ctDNA molecular response category.

On lorlatinib treatment, the NCTD group (assessed at wk 4) had potentially the longest PFS, followed by the MRC, MRNC, and MNR groups (Fig. 4*E*). The 12-month PFS was 89% (95% CI: 61%–97%), 86% (95% CI: 72%–93%), 75% (95% CI: 41%–91%), and 56% (95% CI: 29%–76%) for the NCTD, MRC, MRNC, and MNR groups, respectively. In contrast, in the crizotinib treatment arm, patients in the MRC and MNR groups had similar PFS, whereas patients in the MRNC group had the shortest PFS. Similar to the lorlatinib treatment group, patients in the NCTD group of the crizotinib arm had the longest PFS (Fig. 4*F*). The 12-month PFS was 79% (95% CI: 53%–92%), 32% (95% CI: 18%–47%), 9% (95% CI: 1%–33%), and 38% (95% CI: 12%–64%) for the NCTD,

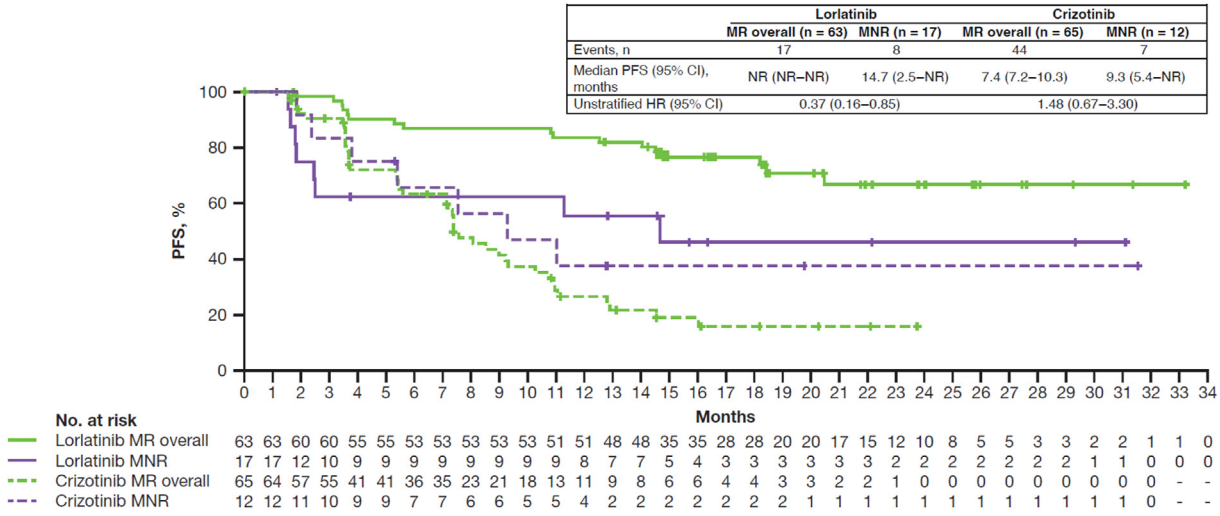


Figure 5. Kaplan-Meier plot of PFS based on BICR by molecular response at week 4 considering two-group stratification comparing molecular responders (MRC + MRNC) versus nonresponders (MNR). BICR, blinded independent central review; CI, confidence interval; HR, hazard ratio; MNR, molecular nonresponder; MR, molecular responder; NR, not reached; PFS, progression-free survival.

MRC, MRNC, and MNR groups, respectively. When comparing between treatment arms, patients treated with lorlatinib achieved potentially longer PFS than those treated with crizotinib in the NCTD group (HR = 0.24, 95% CI: 0.05–1.12), MRC group (HR = 0.18, 95% CI: 0.09–0.35), MRNC group (HR = 0.20, 95% CI: 0.07–0.63), or MNR group (HR = 0.86, 95% CI: 0.31–2.40). The association between PFS and molecular response observed for the lorlatinib group was not retained when using the molecular response derived from the ctDNA assessment at week 24 (Supplementary Fig. 2).

Finally, when simplifying the grouping and looking only at molecular responders (MRC + MRNC) versus nonresponders (MNRs), patients treated with lorlatinib who had a molecular response at week 4 (molecular responders) had longer PFS (median PFS, not reached [NR]; 95% CI: NR–NR) compared with the MNR group (14.7 mo, 95% CI: 2.5–NR) with an HR of 0.37 (95% CI: 0.16–0.85) (Fig. 5). Patients treated with crizotinib who had a molecular response at week 4 had similar PFS compared with patients without a molecular response (median PFS, 7.4 mo; 95% CI: 7.2–10.3 versus 9.3 mo; 95% CI: 5.4–NR, respectively; HR = 1.48, 95% CI: 0.67–3.30).

Discussion

The inclusion of ctDNA sample collection and analysis in large clinical trials is becoming more important as ctDNA emerges as a potential new biomarker to predict outcomes. Here, we report results from a prospectively planned analysis of early ctDNA dynamics from the phase 3 CROWN study. We evaluated two different methods to integrate the ctDNA data and its correlation with clinical efficacy to identify potential early biomarkers of response to lorlatinib or crizotinib in patients with treatment-naive, advanced, *ALK*-positive NSCLC.

In patients treated with lorlatinib, dVAF less than or equal to 0 was associated with tumor size reduction and longer PFS than dVAF of more than 0 in samples with *ALK* alterations and with any genomic alterations. Patients treated with lorlatinib who achieved a molecular response with or without clearance of ctDNA at week 4 had potentially longer PFS than those without a molecular response. Although no clear association was observed at week 24, it seemed that the week 4 time point was most informative in stratifying patients on the basis of early response or PFS outcome. This finding was not observed to the same extent in patients treated with crizotinib. Integrating the emergence of new variants on-treatment did not improve the performance of the model.

Thus, although early ctDNA dynamics, such as a dVAF less than or equal to 0 at 4 weeks, predicted better outcomes with lorlatinib treatment in patients with advanced *ALK*-positive NSCLC, they were inconclusive for crizotinib treatment. The differences in ctDNA dynamics between the two groups and their association or lack of association with treatment efficacy could be explained, at least in part, by the superior efficacy of lorlatinib as compared with crizotinib observed in the CROWN study. In particular, the superior efficacy of lorlatinib for central nervous system (CNS) lesions may play a role in the differences between the two groups.[5] In the CROWN study, lorlatinib reduced CNS progression versus crizotinib in patients with treatment-naive, advanced, *ALK*-positive NSCLC with or without brain

metastases at baseline.[22] The high rate of isolated intracranial disease progression under crizotinib has been linked to reduced CNS concentrations owing to poor penetration.[23,24]

It should also be noted that the differences in the predictive ability of early ctDNA dynamics could be due to differences in disease burden, including actual spread and location(s) of the disease and proximity of the metastatic cells to the bloodstream, between the lorlatinib and crizotinib arms. Lorlatinib has been found to exhibit great efficacy in patients with *ALK* resistance mutations previously treated with an ALK TKI.[25] Although rare in treatment-naive patients, the presence of baseline *ALK* mutations, likely subclonal, conferring primary resistance to crizotinib but not to lorlatinib in some patients cannot be ruled out.[5]

Interestingly, the group of patients with NCTD at baseline seemed to have better responses and longer PFS, suggesting a different tumor biology compared with patients with detectable ctDNA at baseline in both the lorlatinib and crizotinib arms. In addition, higher ctDNA levels at baseline were associated with greater tumor burden. Similar results were found in another analysis of the phase 3 CROWN study that looked at molecular correlates of response to lorlatinib or crizotinib in patients with treatment-naive, advanced, *ALK*-positive NSCLC, specifically focusing on patients with variants of the *ALK* gene.[26] In that analysis, crizotinib-treated patients with NCTD at baseline were found to have higher ORR, longer median duration of response, and longer median PFS, whereas lorlatinib was equally efficacious in those with and without detectable ctDNA at baseline.

An association between undetectable ctDNA at baseline and early in the course of treatment and better outcomes has been observed in a number of clinical trials of different types of cancers, including NSCLC, melanoma, and colorectal and breast cancer.[27–34] Patients with NCTD in the plasma may have smaller tumors and slower tumor growth, possibly as a result of a lower cellular turnaround rate. These tumors may also have reduced access to the bloodstream (i.e., are less angiogenic), which may hinder the shedding of their DNA and their access to nutrients, oxygen, and other growth-critical elements.[35,36] The present analysis indicates that this is also likely the case in patients with advanced *ALK*-positive NSCLC. Although patients with NCTD do not fit in any of the molecular response categories described here, this study provides important information that could help guide treatment decisions.

ctDNA is increasingly being used in clinical trials to both monitor response to treatment and predict outcomes, as it is easily accessible, convenient to obtain, and reproducible compared with tumor biopsy.[8–11,37] Similar to our findings, an increase in ctDNA was associated with worse outcomes in the phase 3 FLAURA trial of osimertinib versus gefitinib or erlotinib in patients with treatment-naive, advanced, *EGFR*-positive NSCLC.[10,11] ctDNA progression was found to precede or co-occur with PD in 66% of patients across the treatment arms.[38] Several limitations are associated with the use of ctDNA, which are also applicable to this study. ctDNA profiling by next-generation sequencing has relatively low sensitivity, and detection of gene rearrangements such as *ALK* fusions is particularly challenging, which along with its high cost could represent a barrier to its use as a screening tool in the near future.[39] Although, in the present study, sample collection was prespecified and analyses were performed at a central laboratory using a validated assay, the timing of collection and handling of samples, along with differences among the various assays commercially available (e.g., sensitivity, specificity, DNA input requirements, size of the gene panel), could affect inter- and intra-study concordance of results.[40] To assist with this endeavor, the Friends of Cancer Research, a nonprofit cancer research thinktank and advocacy organization, has partnered with a number of pharmaceutical companies, cancer centers, and the US Food and Drug Administration to develop the ctDNA to Monitor Treatment Response Project (ctMoniTR Project), which aims to harmonize the use of ctDNA in clinical trials.[41] This will enable clinical trial results to be better compared, which will ultimately facilitate the use of ctDNA directly in patient care. In this study, the gene panel used was relatively small, with 74 genes assessed. Although a larger gene panel would have greater ability to detect comutations, it could lead to reduced sensitivity to detect the genes of interest. Moreover, the panel used here is validated and clinically available, whereas broader panels are more suitable for research settings. The optimal sequencing platform therefore requires further investigation.

In conclusion, in this study, early ctDNA dynamics predicted better outcomes with lorlatinib, but not with crizotinib, in patients with advanced *ALK*-positive NSCLC. The superior efficacy of lorlatinib likely enables better disease control and potential clearing of *ALK* alteration clones that might be otherwise resistant to crizotinib treatment. This likely translates into molecular responses, favorable ORRs, and prolonged PFS in patients receiving lorlatinib. These results support the use of ctDNA to dynamically monitor and predict treatment response early during therapy (i.e., at 4 wk), with the potential to be expanded to clinical practice. Further studies, including some prospective studies, are ongoing to validate these findings and investigate whether early intervention on the basis of ctDNA monitoring improves outcomes.

CRediT Authorship Contribution Statement

Ross A. Soo: Conceptualization, Data curation, Investigation, Methodology, Writing—review and editing.

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Anna Maria Calella: Data curation, Writing—review and editing.

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Chao-Hua Chiu: Data curation, Investigation, Writing—review and editing.

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Hye Ryun Kim: Data curation, Investigation, Writing—review and editing.

Benjamin J. Solomon: Conceptualization, Data curation, Investigation, Methodology, Writing—review and editing.

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Data Availability

On request and subject to review, Pfizer will provide the data that support the findings of this study. Subject to certain criteria, conditions, and exceptions, Pfizer may also provide access to the related individual deidentified participant data. See https://www.pfizer.com/science/clinical-trials/trial-data-and-results for more information.

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.2023.05.021.

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