ORIGINAL ARTICLE

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Efficacy of Lorlatinib in Treatment-Naive Patients With *ALK*-Positive Advanced NSCLC in Relation to *EML4::ALK* Variant Type and *ALK* With or Without *TP53* Mutations

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Received 25 April 2023; revised 27 July 2023; accepted 28 July 2023
Available online - 3 August 2023

ABSTRACT

Introduction: Lorlatinib, a third-generation ALK tyrosine kinase inhibitor, improved outcomes compared with crizotinib in patients with previously untreated *ALK*-positive advanced NSCLC in the phase 3 CROWN study. Here, we investigated response correlates using plasma circulating tumor DNA (ctDNA) and tumor tissue profiling.

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Drs. Bearz and Martini contributed equally as the first authorship.

Disclosure: Dr. Bearz has served as a consultant or on advisory boards for Amgen, AstraZeneca, Bristol Myers Squibb, Janssen, Merck, Novartis, Pfizer, Roche/Genentech, Sanofi, and Takeda. Dr. Martini, Dr. Shepard, Ms. Dall'O', Ms. Polli, and Dr. Thurm are employed by and own stocks in Pfizer. Dr. Jassem reports receiving speaker fees from AstraZeneca, Pfizer, and Roche; having advisory roles with AstraZeneca, Bristol Myers Squibb, Merck Sharp & Dohme, and Pfizer; and receiving travel support from Pfizer and Roche. Dr. Chang has received honoraria from AstraZeneca, Bristol Myers Squibb, Boehringer Ingelheim, Eli Lilly, Merck, Novartis, Pfizer, and Roche. Dr. Shaw is employed by and owns stock in Novartis. Dr. Zalcman reports receiving grant support from Inventiva, Roche-France, and Takeda; personal fees from AstraZeneca, Bristol Myers Squibb, Da Volterra, Merck Sharp & Dohme, and Pfizer; and nonfinancial support from AbbVie and Inventiva. Dr. Garcia Campelo has served as a consultant or on advisory boards for AstraZeneca, Bristol Myers Squibb, Janssen, Eli Lilly, Merck Sharp & Dohme, Novartis, Pfizer, Roche, Sanofi, and Takeda; served on speakers' bureau for AstraZeneca, Bristol Myers Squibb, Janssen, Eli Lilly, Merck Sharp & Dohme, Novartis, Pfizer, Roche, Sanofi, and Takeda; and received research funding from Bristol Myers Squibb. Dr. Penkov has received research funding from AbbVie, AstraZeneca, GlaxoSmithKline, Janssen, H3 Biomedicine, Merck, Novartis, Pfizer, Regeneron, Roche/Genentech, Sanofi, and Takeda. Dr. Hayashi has received grants and personal fees from AstraZeneca KK, Chugai Pharmaceutical, Boehringer Ingelheim Japan, Ono Pharmaceutical, and Bristol Myers Squibb; and personal fees from Eli Lilly Japan KK, Kyorin Pharmaceutical, Merck Biopharma, Merck Sharp & Dohme KK, Novartis Pharmaceuticals KK, Pfizer Japan Inc., Shanghai HaiHe Biopharma, and Taiho Pharmaceutical. Dr. Solomon has served as a consultant or on advisory boards for Amgen, AstraZeneca, Bristol Myers Squibb, Janssen, Merck, Novartis, Pfizer, Roche/Genentech, Sanofi, and Takeda. Dr. Kim declares no conflict of interest.

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ISSN: 1556-0864

https://doi.org/10.1016/j.jtho.2023.07.023

Methods: *ALK* fusions and *ALK* with or without *TP53* mutations were assessed by next-generation sequencing. End points included objective response rate (ORR), duration of response, and progression-free survival (PFS) by blinded independent central review on the basis of *EML4::ALK* variants and *ALK* with or without *TP53* or other mutation status.

Results: *ALK* fusions were detected in the ctDNA of 62 patients in the lorlatinib arm and 64 patients in the crizotinib arm. ORRs were numerically higher with lorlatinib versus crizotinib for *EML4::ALK* variant 1 (v1; 80.0% versus 50.0%) and variant 2 (v2; 85.7% versus 50.0%) but were similar between the arms for variant 3 (v3; 72.2% versus 73.9%). Median PFS in the lorlatinib arm was not reached for *EML4::ALK* v1 and v2 and was 33.3 months for v3; in the crizotinib arm, median PFS was 7.4 months, not reached, and 5.5 months, respectively. ORRs and PFS were improved with lorlatinib versus crizotinib regardless of *TP53* mutation status and in patients harboring preexisting bypass pathway resistance alterations. In the lorlatinib arm, PFS was lower in patients who had a co-occurring *TP53* mutation. Results from ctDNA analysis were similar to those observed with tumor tissue samples.

Conclusions: Patients with untreated *ALK*-positive advanced NSCLC derived greater clinical benefits, with higher ORRs and potentially longer PFS, when treated with lorlatinib compared with crizotinib, independent of *EML4::ALK* variant or *ALK* mutations, *TP53* mutations, or bypass resistance alterations.

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

Introduction

Lorlatinib is a third-generation *ALK* inhibitor designed to cross the blood-brain barrier to achieve high potency through exposure in the central nervous system and has the broadest coverage of *ALK* resistance mutations among *ALK* tyrosine kinase inhibitors (TKIs) identified to date.[1] In a phase 1/2 study (NCT01970865), lorlatinib was found to have clinical antitumor activity after the failure of previous ALK TKIs.[2,3] In the phase 3 CROWN study (NCT03052608) in patients with previously untreated *ALK*-positive advanced NSCLC, those who received lorlatinib had considerably longer progression-free survival (PFS), reduced time to intracranial progression, and higher frequency of intracranial response compared with those who received crizotinib.[4]

ALK-positive NSCLC is characterized by a chromosomal rearrangement resulting in expression of constitutively active *ALK* fusion proteins. The most common *ALK* fusion partner is *EML4*. More than 15 *EML4::ALK* fusion variants have been identified, the most common being variants 1 (v1, 37% of the cases), 2 (v2, 12% of the cases), and 3a/b (v3, 42% of the cases).[5,6] Retrospective analyses have suggested that the expression of particular *EML4::ALK* variants may affect the degree of clinical benefit experienced by patients in response to ALK TKIs and potentially influence the development of specific secondary *ALK* resistance mutations.[5,7] In addition, *EML4::ALK* v1 and v3 had differential sensitivity to six ALK TKIs.[8]

In addition to *ALK* fusion subtype, co-occurring mutations might influence sensitivity to the ALK TKIs similar to that reported in other molecularly driven tumors, such as *EGFR*-mutated NSCLC.[9,10] In particular, *TP53* mutations, co-occurring in approximately 25% of patients with *ALK*-positive advanced NSCLC, may be a marker of poor prognosis.[11,12] Other concomitant mutations have been linked to ALK TKI resistance, especially in the context of acquired resistance, including *EGFR*, *KRAS*, or *BRAF* mutations,[13] and *MET* amplification.[14] Nevertheless, little is known about the intrinsic effects of these de novo potential resistance alterations in patients with treatment-naive, *ALK*-positive, advanced NSCLC.

Here, we report a prospectively defined analysis of lorlatinib efficacy in the CROWN study in which molecular profiling of circulating tumor DNA (ctDNA) and tumor tissue was used to investigate the effects of *EML4::ALK* variant subtype, concomitant *TP53* mutations, and other potential concomitant resistance mutations on clinical outcomes in previously untreated patients with *ALK*-positive NSCLC.

Materials and Methods

Study Design

The global, randomized, phase 3 CROWN trial enrolled 296 patients with advanced *ALK*-positive NSCLC, of whom 291 received treatment. The study design, objectives, and eligibility criteria have been previously published.[4] Briefly, patients were randomly assigned 1:1 to receive either oral lorlatinib 100 mg daily or oral crizotinib 250 mg twice daily in 28-day cycles. Blood specimens for ctDNA profiling were collected at baseline, day 1 of cycle 2 (1 mo), day 1 of cycle 7 (6 mo), and end of treatment or patient withdrawal. Of note, 20 patients enrolled in the People's Republic of China were not included in these analyses as their biospecimens had not been received at the time of this analysis.

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

Molecular Profiling

Molecular profiling including both plasma ctDNA and tumor tissue was performed as described previously.[15] Briefly, tumor tissue was first profiled with a custom targeted *ALK* domain mutation detection next-generation sequencing (NGS) assay[15]; leftover tumor tissue was further analyzed using an NGS panel (Guardant360 TissueNext panel version 2.13; bioinformatics pipeline version 3.11; Guardant Health, Inc., Redwood City, CA). ctDNA from plasma collected at screening was analyzed with a validated, commercially available 74-gene ctDNA NGS assay (Guardant360 panel version 2.11; bioinformatics pipeline version 3.5.3; Guardant Health, Inc., Redwood City, CA).[16] Concordance assessments of plasma genotyping for *ALK* fusion detection versus tumor immunohistochemistry (IHC, Ventana D5F3 *ALK* IHC test) for *ALK* fusion expression and plasma-based *ALK* fusion variant versus tumor NGS were conducted retrospectively. We further explored the impact of pathogenic or likely pathogenic *TP53* mutations, as defined in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar), on clinical outcomes on the basis of ctDNA profiling.

End Points

Clinical efficacy parameters were described previously[4] (Supplementary Appendix).

Statistical Analysis

Objective response rates (ORRs), point estimates (percentage), and 95% confidence intervals (CIs) were calculated using the Clopper-Pearson exact method for binomial proportions. For the estimation of duration of response (DOR) and PFS, the Kaplan-Meier method was used, with 95% CIs based on the Brookmeyer and Crowley method. Hazard ratio estimates and associated 95% CIs were obtained using the Cox proportional hazards model. Wilson score method was used to assess agreement between tumor tissue and peripheral blood ctDNA biomarker results for ALK rearrangements. The data cutoff date was September 20, 2021.

Results

Analysis Population

Plasma samples collected at screening were available from 134 patients in the lorlatinib arm and 129 patients in the crizotinib arm (Fig. 1*A*). Because 90% of patients (263 of 291) from the treated population were included, baseline patient characteristics were similar to the overall study population and well balanced between the treatment arms; most had Eastern Cooperative Oncology Group performance status of 0 or 1, and approximately one-quarter of the patients in each treatment arm had brain metastases.[4]

Prevalence of ALK Rearrangements, EML4::ALK Variants, and ALK Mutations at Baseline

ctDNA was detected in most plasma samples collected at baseline, except in those from 32 patients (23.9%) in the lorlatinib arm and 25 patients (19.4%) in the crizotinib arm. Overall, *ALK* fusions were detected in the ctDNA of 62 patients in the lorlatinib arm and 64 in the crizotinib arm (Fig. 1*A*). In the lorlatinib arm, 20 patients had *EML4::ALK* v1, seven had v2, 18 had v3, 15 had other variants, and two had other *ALK* fusions. In the crizotinib arm, 26 patients had v1, two had v2, 23 had v3, nine had other variants, and four had other *ALK* fusions.

In total, 20 *ALK* mutations and one deletion were found at baseline in the ctDNA of 12 patients (five in the lorlatinib arm and seven in the crizotinib arm) and included variants of unknown significance and known *ALK* resistance mutations (Supplementary Table 1). Most patients (nine of 12; 75%) harbored one mutation, but one patient in the lorlatinib arm and two patients in the crizotinib arm harbored more than or equal to three mutations. Three patients harbored more than or equal to one mutation and *EML4::ALK* v1, none had v2, two patients had v3, and two patients had other *EML4::ALK* variants; five patients harbored *ALK* mutations without detectable *ALK* fusions. Despite a small sample size, *ALK* mutations did not seem to segregate preferentially with any variant subtype; thus, there was no evidence indicating that the presence of *ALK* mutations might influence the *EML4::ALK* variant analysis.

Tumor tissue samples were available from a total of 240 patients (82%): 125 in the lorlatinib arm and 115 in the crizotinib arm had available tumor tissue for *ALK* domain-specific NGS-targeted panel analysis; 114 samples (91.2%) in the lorlatinib arm and 104 (90.4%) in the crizotinib arm were successfully assessed for *ALK* domain mutations. Only one patient in the crizotinib arm harbored an *ALK* mutation (variant of unknown significance, R1120Q) at baseline. Leftover tumor tissue samples were available from 148 (50.9%) patients: 79 samples in the lorlatinib arm and 69 in the crizotinib arm were further analyzed using the broad NGS panel; no *ALK*

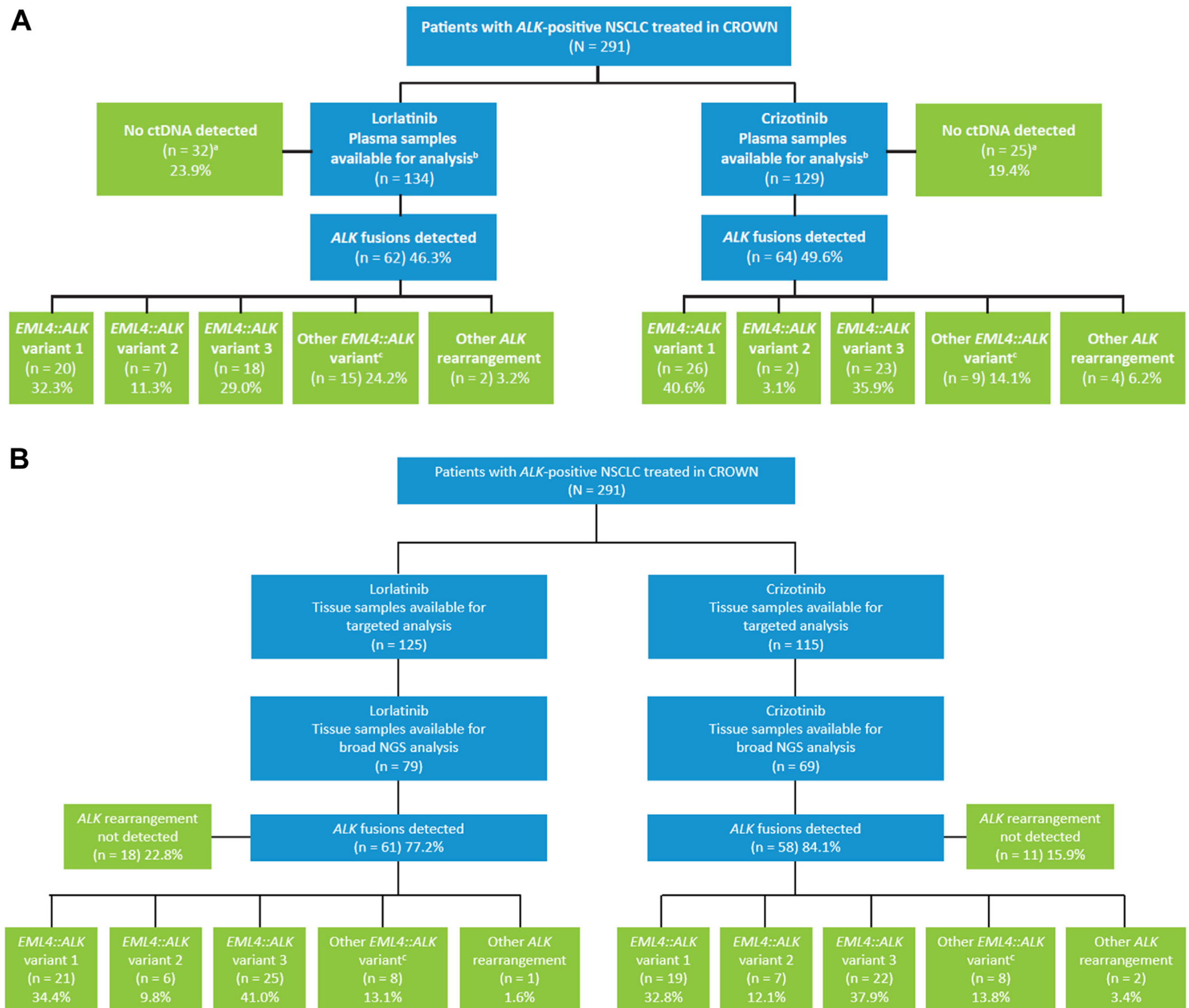

Figure 1. Samples obtained from plasma ctDNA and tumor tissue. (*A*) *EML4::ALK* fusion variants identified in plasma ctDNA at screening. (*B*) *EML4::ALK* fusion variants identified in tumor tissue. [a]Additional five patients in the lorlatinib arm and four patients in the crizotinib arm had samples that failed analysis, were uninformative, or were not analyzed. [b]Plasma samples prospectively collected for ctDNA analysis at baseline; patients enrolled in the People's Republic of China (N = 20) not included in this analysis. [c]Other variants are 4, 5, 7, and 8 combined. ctDNA, circulating tumor DNA.

mutations were reported in this subgroup analysis (Fig. 1*B*).

Agreement Between ALK Gene Rearrangements Detected in ctDNA and Tumor Tissue at Baseline

A subgroup analysis was performed to evaluate the agreement between ctDNA and tumor tissue-based analysis of *ALK* gene rearrangements by IHC. All patients enrolled in the CROWN study were identified as *ALK* positive using the Ventana D5F3 ALK IHC assay on tumor tissue. A total of 250 patients (125 in the lorlatinib arm and 125 in the crizotinib arm) had assessable *ALK* gene rearrangement status by blood-based Guardant360 Health test (Supplementary Table 2). *ALK* gene rearrangements were detected in 60 patients (48.0% [95% CI: 38.7%–56.7%]) in the lorlatinib arm and 64 patients (51.2% [95% CI: 42.5%–60.6%]) in the crizotinib arm.

Next, we evaluated the concordance between the ctDNA and tumor tissue *EML4::ALK* variant subtypes using the broad NGS panel assay. *ALK* fusions were detected in 119 (80.4%) of 148 patients (Fig. 1*B* and Supplementary Fig. 1). The overall agreement between the tumor tissue and the ctDNA for *ALK* fusion was 96.7% (95% CI: 88.6%–99.1%); more specifically, for the *EML4::ALK* variant subtypes, the agreement ranged from 94.7% (95% CI: 75.4%–99.1%) for *EML4::ALK* v1

Table 1. Efficacy by *EML4::ALK* Fusion Variant and Other *ALK* Rearrangements at Baseline

	Lorlatinib				Crizotinib			
EML4::ALK Variant	n	ORR, % (95% CI)	Median DOR, mo (95% CI)	Median PFS, mo (95% CI)	n	ORR, % (95% CI)	Median DOR, mo (95% CI)	Median PFS, mo (95% CI)
Plasma ctDNA								
1	20	80.0 (56.3-94.3)	NR (NR-NR)	NR (NR-NR)	26	50.0 (29.9-70.1)	5.7 (5.6-NR)	7.4 (5.5-9.3)
2	7	85.7 (42.1-99.6)	NR (15.9-NR)	NR (32.9-NR)	2	50.0 (1.3-98.7)	NR (NR-NR)	NR (3.7-NR)
3	18	72.2 (46.5-90.3)	NR (12.8-NR)	33.3 (14.7-NR)	23	73.9 (51.6-89.8)	6.5 (3.7-9.2)	5.5 (5.3-9.2)
Other[a]	15	86.7 (59.5-98.3)	31.3 (16.4-NR)	33.1 (12.6-NR)	9	66.7 (29.9-92.5)	9.3 (5.6-NR)	11.0 (7.2-16.0)
Other *ALK* fusion	2	100.0 (15.8-100.0)	12.9 (NR-NR)	14.3 (14.1-14.5)	4	25.0 (0.6-80.6)	NR (NR-NR)	10.8 (3.5-19.8)
Tumor tissue								
1	21	85.7 (63.7-97.0)	NR (NR-NR)	NR (25.8-NR)	19	47.4 (24.4-71.1)	NR (18.4-NR)	12.9 (7.4-NR)
2	6	100.0 (54.1-100.0)	NR (31.1-NR)	NR (32.9-NR)	7	57.1 (18.4-90.1)	18.2 (9.2-27.3)	29.0 (11.0-NR)
3	25	80.0 (59.3-93.2)	NR (NR-NR)	NR (NR-NR)	22	68.2 (45.1-86.1)	7.8 (4.6-12.9)	8.1 (5.3-12.8)
Other[a]	8	75.0 (34.9-96.8)	NR (10.8-NR)	NR (12.6-NR)	8	62.5 (24.5-91.5)	10.1 (3.8-NR)	11.0 (5.6-16.0)
Other *ALK* fusion	1	100.0 (2.5-100.0)	NR (NR-NR)	NR (NR-NR)	2	50.0 (1.3-98.7)	NR (NR-NR)	10.8 (NR-NR)
ALK rearrangement not detected	18	61.1 (35.7-82.7)	NR (29.4-NR)	31.1 (2.5-NR)	11	54.5 (23.4-83.3)	12.9 (8.7-NR)	14.8 (7.6-27.4)

[a]Variants 4, 5, 7, and 8 (combined).
CI, confidence interval; ctDNA, circulating tumor DNA; DOR, duration of response; NR, not reached; ORR, objective response rate; PFS, progression-free survival.

to 100.0% for v2 and other *ALK* fusions (Supplementary Table 3).

Treatment Effect of Lorlatinib Versus Crizotinib by EML4::ALK Fusion Variant Subtypes or ALK Mutation Status at Baseline

On the basis of ctDNA, ORRs were numerically higher in the lorlatinib arm versus the crizotinib arm for *EML4::ALK* v1 (80.0% [95% CI: 56.3%–94.3%] versus 50.0% [95% CI: 29.9%–70.1%]), v2 (85.7% [95% CI: 42.1%–99.6%] versus 50.0% [95% CI: 1.3%–98.7%]), and other *EML4::ALK* variants (86.7% [95% CI: 59.5%–98.3%] versus 66.7% [95% CI: 29.9%–92.5%]), and for fusions of *ALK* with another gene (e.g., *KIF5B*, *TFG*) (100% [95% CI: 15.8%–100.0%] versus 25.0% [95% CI: 0.6%–80.6%]). For *EML4::ALK* v3, ORRs were similar between the lorlatinib and crizotinib arms (72.2% [95% CI: 46.5%–90.3%] versus 73.9% [95% CI: 51.6%–89.8%]) (Table 1). Median DORs (mDORs) were not reached (NR) for *EML4::ALK* v1, v2, and v3 in the lorlatinib arm and were 5.7 months, NR, and 6.5 months in the crizotinib arm, respectively. Median PFS (mPFS) in the lorlatinib arm was NR for *EML4::ALK* v1 and v2 and was 33.3 months for v3; in the crizotinib arm, mPFS was 7.4 months, NR, and 5.5 months, respectively. The rate of progression or death was lower with lorlatinib versus crizotinib, with hazard ratios (HRs) of 0.15 (95% CI: 0.048–0.449) and 0.15 (95% CI: 0.048–0.453) for v1 and v3, respectively (Fig. 2*A*). Similarly, on the basis of the broad tumor tissue profiling, ORRs were numerically higher in the lorlatinib arm versus the crizotinib arm; mPFS was NR for either v1 or v3 in the lorlatinib arm and was 12.9 and 8.1 months in the crizotinib arm, respectively. Although the number of patients with *EML4::ALK* v2 was small, the mPFS was NR in the lorlatinib arm and was 29.0 months in the crizotinib arm. The rate of progression or death was lower with lorlatinib versus crizotinib regardless of the variant type, with an HR of 0.40 (95% CI: 0.156–1.052) for v1 and an even greater effect for v3, with an HR of 0.14 (95% CI: 0.049–0.415) (Fig. 2*B*). In patients with tissue samples in which *ALK* rearrangement was not detected, mPFS was 31.1 months (95% CI: 2.5–NR) for lorlatinib and 14.8 months (95% CI: 7.6–27.4) for crizotinib (Table 1).

Although numbers were small, *ALK* mutations could be detected at baseline in ctDNA of 12 patients and only in one patient's archived tumor tissue; however, they did not seem to affect outcomes for ORR, DOR, or PFS in either treatment arm. In patients harboring *ALK* mutations at baseline in ctDNA, mDOR and mPFS were NR in the lorlatinib arm and 6.5 and 9.0 months in the

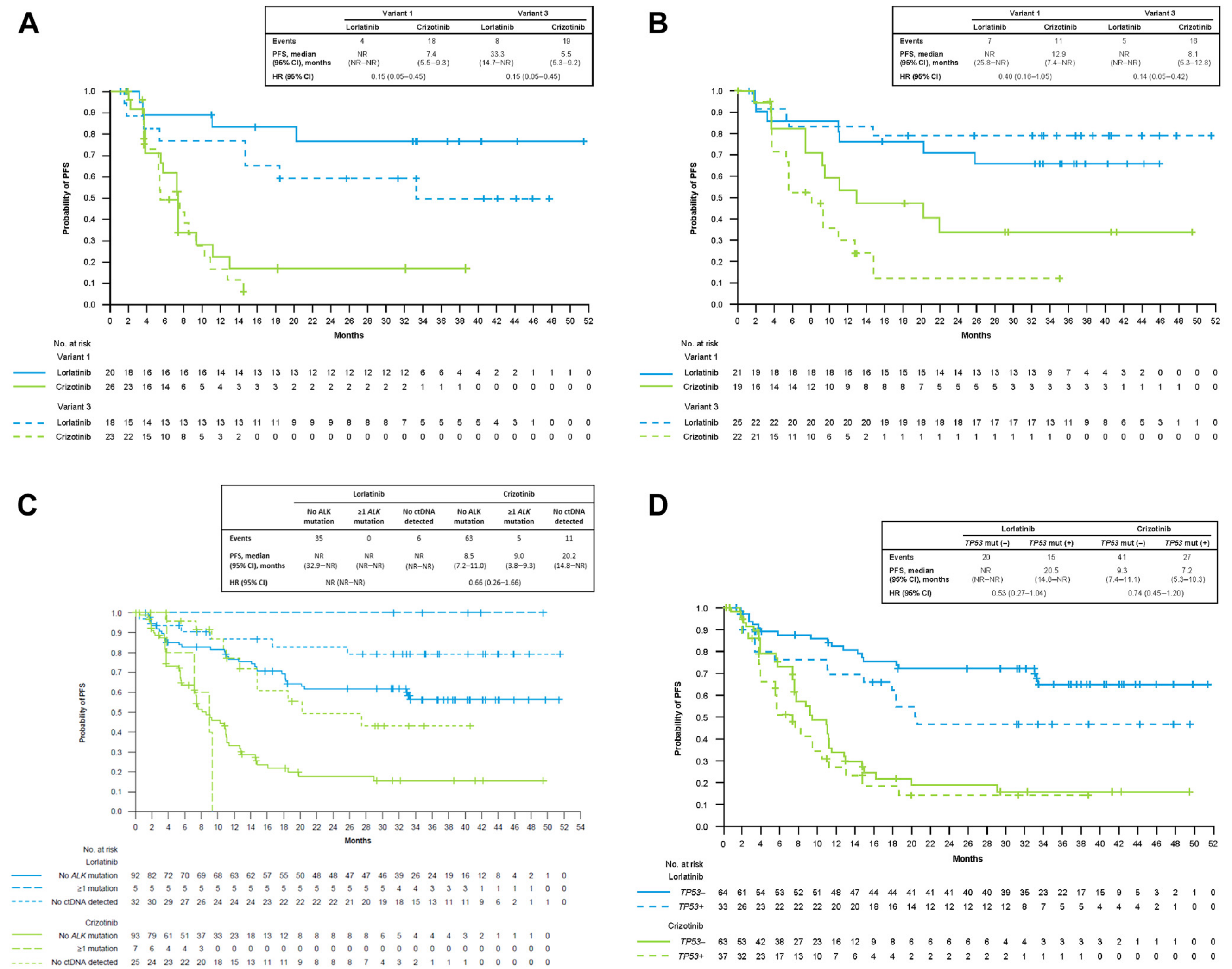

Figure 2. Kaplan-Meier plot of PFS on the basis of BICR assessment. (*A*) Presence of *EML4::ALK* variants 1 and 3 at baseline, by treatment, on the basis of plasma ctDNA analysis. (*B*) Presence of *EML4::ALK* variants 1 and 3 at baseline, by treatment, on the basis of tumor tissue analysis. (*C*) No *ALK* mutation versus more than or equal to one plasma *ALK* mutation at baseline, on the basis of plasma ctDNA. (*D*) According to *TP53* mutation status, based on plasma ctDNA. *TP53* mutations only included known pathogenic or likely pathogenic as per the ClinVar or COSMIC databases. (*E*) According to *TP53* mutation status, on the basis of tumor tissue analysis. (*F*) According to preexisting potential bypass pathway alteration status in the lorlatinib and crizotinib arms, on the basis of plasma ctDNA analysis. (*G*) According to preexisting potential bypass pathway alteration status in the lorlatinib and crizotinib arms, on the basis of tumor tissue analysis. BICR, blinded independent central review; CI, confidence interval; ctDNA, circulating tumor DNA; HR, hazard ratio; NR, not reached; PFS, progression-free survival.

crizotinib arm, respectively (Supplementary Table 4). Of note, the absence of detectable ctDNA at baseline was associated with more favorable ORR and longer DOR and PFS in the crizotinib arm compared with no *ALK* mutation and more than or equal to one *ALK* mutation detected at baseline. The presence or absence of ctDNA did not seem to influence lorlatinib efficacy (Fig. 2*C*).

Effect of TP53 Mutation Status at Baseline on Efficacy of Lorlatinib

In patients with detectable ctDNA at baseline, *TP53* mutations were the most frequent co-alterations, found in 82 of 197 patients (41.6%, Table 2). In general, ORRs were numerically higher in the *TP53* mutation–negative groups (lorlatinib 78.6% [95% CI: 65.6%–88.4%]; crizotinib 61.0% [95% CI: 47.4%–73.5%]) than in *TP53* mutation–positive groups (lorlatinib 70.7% [95% CI: 54.5%–83.9%]; crizotinib 56.1% [95% CI: 39.7%–71.5%]) (Table 2). In the *TP53*-positive and -negative subgroups, mDOR was NR in both subgroups in the lorlatinib arm and 7.4 and 9.5 months, respectively, in the crizotinib arm. Similarly, mPFS was NR in both subgroups in the lorlatinib arm and 7.2 and 10.8 months, respectively, in the crizotinib arm. ORRs were numerically higher and PFS was longer with

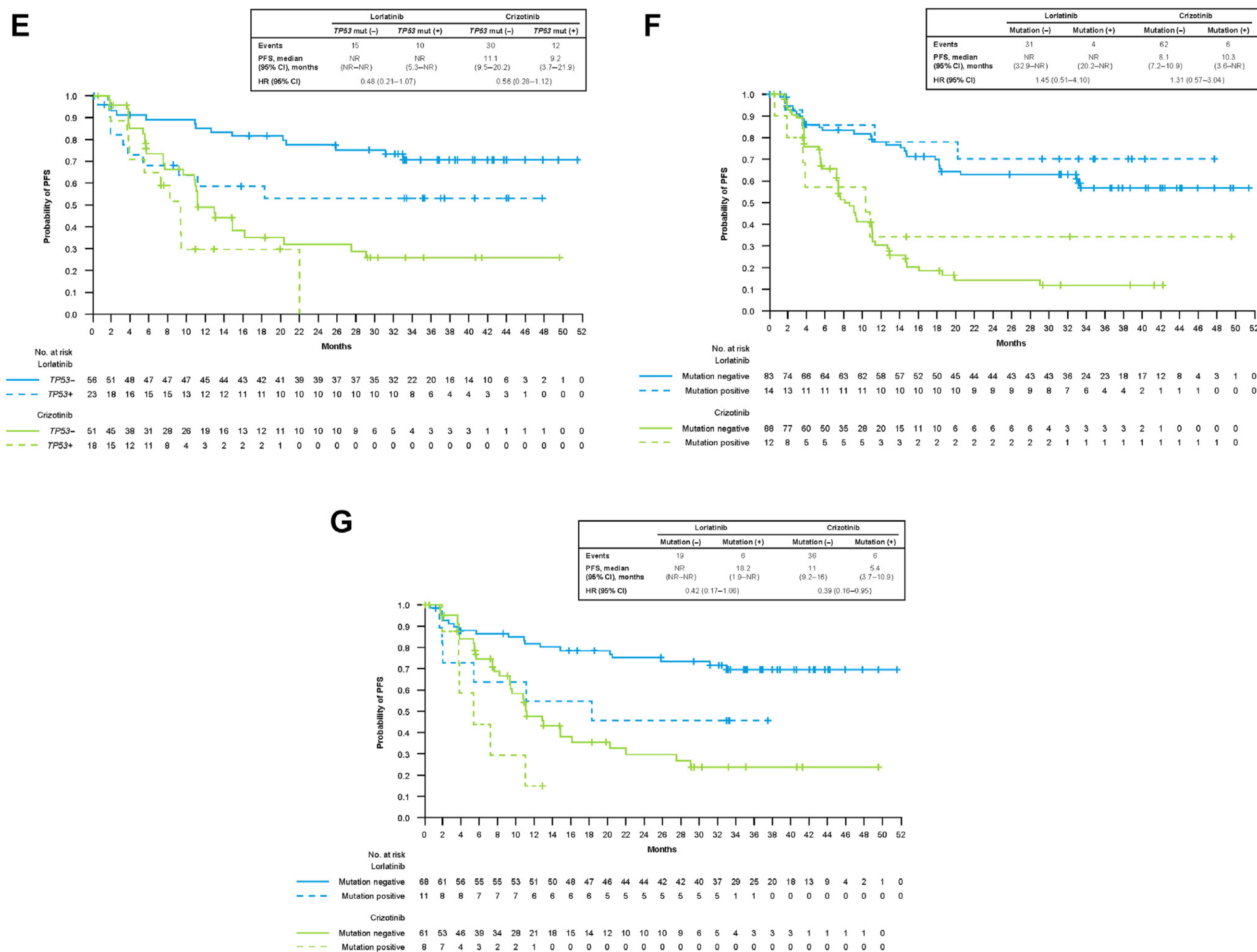

Figure 2. continued

lorlatinib versus crizotinib regardless of *TP53* mutation status (Fig. 2*D*).

Although the numbers were smaller for patients included in the broad tumor tissue profiling, results were similar to those observed in ctDNA. The frequency of *TP53* mutations was lower in the tumor tissue (29.1% in the lorlatinib arm and 26.1% in the crizotinib arm) than in ctDNA (42.3% in the lorlatinib arm and 41.0% in the crizotinib arm), probably due to the detection of clonal hematopoiesis or potential emergence of new *TP53* mutations from the time of diagnosis. It is also possible that some of the tissue samples analyzed were of low tumor cellularity and *TP53* mutations were not detected. Nevertheless, the impact of the *TP53* mutation status on ORR, DOR, and PFS remained similar (Table 2 and Fig. 2*E*). Furthermore, the differences observed between *TP53*-positive and -negative subgroups were also conserved within the treatment arms for *EML4::ALK* v1 or v3 (Supplementary Table 5).

We further explored the impact of pathogenic or likely pathogenic *TP53* mutations on clinical outcomes on the basis of ctDNA profiling. Using these restrictions, pathogenic *TP53* mutations were detected in 70 of 197 patients (35.5%); however, outcomes were similar to those observed with all *TP53* mutations (Supplementary Table 6). Finally, we also investigated whether a detectable *ALK* fusion co-occurring with a *TP53* mutation could identify a subgroup of patients less likely to benefit from treatment with lorlatinib or crizotinib. Results in each subgroup were consistent with those in the overall population, with longer DOR and PFS with lorlatinib versus crizotinib, regardless of *TP53* mutation status (Table 3). In the lorlatinib arm, mPFS was 20.2 months in patients who had an *ALK* fusion co-occurring with a *TP53* mutation and NR in other subgroups.

Efficacy by Potential Preexisting Bypass Pathway Resistance Alterations at Baseline

First, we explored the impact of canonical alteration in the MAPK-PI3K pathway on the basis of ctDNA profiling. A total of nine patients (two in the lorlatinib

Table 2. Efficacy by *TP53* Mutation Status for Patients With Detectable ctDNA at Baseline

Plasma ctDNA	Lorlatinib (n = 97)		Crizotinib (n = 100)	
Mutation status[a]	*TP53* positive	*TP53* negative	*TP53* positive	*TP53* negative
n (%)	41 (42.3)	56 (57.7)	41 (41.0)	59 (59.0)
ORR, % (95% CI)	70.7 (54.5-83.9)	78.6 (65.6-88.4)	56.1 (39.7-71.5)	61.0 (47.4-73.5)
Median DOR, mo (95% CI)	NR (18.3-NR)	NR (NR-NR)	7.4 (5.6-16.6)	9.5 (7.4-12.9)
Median PFS, mo (95% CI)	NR (18.2-NR)	NR (NR-NR)	7.2 (5.4-9.3)	10.8 (7.4-11.4)
HR[b] for PFS (95% CI)				
Mutation negative vs. mutation positive		0.65 (0.333-1.261)		0.71 (0.437-1.151)
Lorlatinib vs. crizotinib	0.28 (0.149-0.534)	0.24 (0.133-0.428)		
Tumor tissue	**Lorlatinib (n = 79)**		**Crizotinib (n = 69)**	
Mutation status[a]	*TP53* positive	*TP53* negative	*TP53* positive	*TP53* negative
n (%)	23 (29.1)	56 (70.9)	18 (26.1)	51 (73.9)
ORR, % (95% CI)	69.6 (47.1-86.8)	82.1 (69.6-91.1)	55.6 (30.8-78.5)	58.8 (44.2-72.4)
Median DOR, mo (95% CI)	NR (NR-NR)	NR (NR-NR)	7.8 (6.2-20.0)	12.9 (9.2-NR)
Median PFS, mo (95% CI)	NR (5.3-NR)	NR (NR-NR)	9.2 (3.7-21.9)	11.1 (9.5-20.2)
HR[b] for PFS (95% CI)				
Mutation negative vs. mutation positive		0.48 (0.214-1.065)		0.56 (0.282-1.117)
Lorlatinib vs. crizotinib	0.48 (0.202-1.151)	0.25 (0.131-0.469)		

Note: Based on blinded independent central review by Kaplan-Meier estimates, based on Brookmeyer and Crowley method.
[a]*TP53* mutations included both known pathogenic or likely pathogenic variants and variants of unknown significance per the ClinVar database.
[b]Unstratified hazard ratio based on Cox proportional hazards model. HR <1.00 favors test (vs. reference).
CI, confidence interval; ctDNA, circulating tumor DNA; DOR, duration of response; HR, hazard ratio; NR, not reached; ORR, objective response rate; PFS, progression-free survival.

arm and seven in the crizotinib arm) harbored a detectable *BRAF* (V600E or V600Dup), *KRAS* (G12S, G12C, or G12D), or *PIK3CA* (K111E, E545K, or E542K) mutation, and/or an amplification of these genes. The number of patients with such alterations was too small to derive a meaningful hypothesis (Supplementary Table 7).

We further broadened our investigation to include additional known acquired mechanisms of resistance to *ALK* TKIs, such as alterations in the receptor tyrosine kinase family of receptors (e.g., *EGFR*, *ERBB2*, *KIT*, *MET* mutation or amplification), or alterations in the cell cycle regulation (e.g., *CDK4*, *CDK6*, *CCND1*, *CCND2*, *CCNE1* amplification, or *CDKN2A* or *RB1* deleterious mutations). Of note, 17 patients had detectable alterations in *EGFR* or *ERBB2* in their baseline ctDNA, mainly amplification (Supplementary Fig. 1).

After the pooling of all these potential bypass mechanisms, a total of 28 patients had detectable alterations linked to potential resistance bypass mechanisms (15 in the lorlatinib arm and 13 in the crizotinib arm) on the basis of ctDNA profiling. The ORR was numerically lower for patients harboring such alterations compared with those who did not when treated with crizotinib (30.8% [95% CI: 9.1%–61.4%] versus 63.2% [95% CI: 52.2%–73.3%]) but not with lorlatinib (Table 4). The mutation status did not seem to influence PFS, with HRs of 1.45 (95% CI: 0.511–4.103) and 1.12 (95% CI: 0.511–2.460) with lorlatinib and crizotinib, respectively (Fig. 2*F*).

The rate of progression or death was lower with lorlatinib versus crizotinib regardless of the presence (HR = 0.28 [95% CI: 0.088–0.991]) or absence (HR = 0.26 [95% CI: 0.162–0.403]) of potential resistance alterations. These observations were confirmed in the tumor tissue profiling set (Table 4 and Fig. 2*G*). Of note, one patient treated with lorlatinib with an ongoing partial response in tumor lesions at the data cutoff date was found to have a *KRAS* G12V mutation and the presence of *ALK* fusion in the tumor tissue but had no ctDNA detected at baseline.

Finally, to further expand this analysis, data from both ctDNA and tumor tissue analyses were pooled for all these potential bypass mechanisms, and a total of 42 patients had detectable alterations linked to potential resistance bypass mechanisms (22 in the lorlatinib arm and 20 in the crizotinib arm). Although the ORRs were similar in the lorlatinib arm, they were numerically lower for patients harboring such alterations when treated with crizotinib (40.0% [95% CI: 19.1%–63.9%]) versus patients who did not harbor these alterations (65.1% [95% CI: 55.4%–74.0%]); the mutation status did not seem to considerably influence PFS, with HR of

Table 3. Efficacy by *ALK* Fusion and *TP53* Mutation Status in Patients With Detectable ctDNA at Baseline

Mutation Status[a]	Lorlatinib (n = 97)				Crizotinib (n = 100)			
ALK fusion	Negative		Positive		Negative		Positive	
TP53 mutation[b]	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
n (%)	19 (19.6)	16 (16.5)	37 (38.1)	25 (25.8)	24 (24.0)	12 (12.0)	35 (35.0)	29 (29.0)
ORR, % (95% CI)	63.2 (38.4-83.7)	68.8 (41.3-89.0)	86.5 (71.2-95.5)	72.0 (50.6-87.9)	58.3 (36.6-77.9)	58.3 (27.7-84.8)	62.9 (44.9-78.5)	55.2 (35.7-73.6)
Median DOR, mo (95% CI)	NR (NR-NR)	NR (NR-NR)	NR (NR-NR)	NR (15.9-NR)	12.9 (9.4-NR)	12.8 (5.6-NR)	7.4 (5.6-11.0)	6.2 (4.6-NR)
Median PFS, mo (95% CI)	NR (11.3-NR)	NR (10.8-NR)	NR (33.3-NR)	20.2 (14.8-NR)	11.4 (7.6-NR)	9.3 (2.4-18.5)	8.5 (7.4-10.9)	5.5 (5.3-9.2)
PFS at 12 mo, % (95% CI)	65.8 (39.1-83.0)	72.7 (42.5-88.8)	86.3 (70.2-94.1)	77.3 (53.7-89.8)	47.6 (25.7-66.7)	45.5 (12.6-74.1)	24.1 (10.0-41.5)	20.4 (7.3-38.2)

Note: Based on blinded independent central review by Kaplan-Meier estimates, based on Brookmeyer and Crowley method.
[a]Only patients with ctDNA detected.
[b]Including both known pathogenic or likely pathogenic variants and variants of unknown significance as per the ClinVar database.
CI, confidence interval; ctDNA, circulating tumor DNA; DOR, duration of response; NR, not reached; ORR, objective response rate; PFS, progression-free survival.

0.67 (95% CI: 0.324–1.404) for the lorlatinib arm but might have influenced the activity of crizotinib (HR = 0.51 [95% CI: 0.292–0.902]; Supplementary Table 8), which might emphasize that the main oncogenic driver in these tumors remains the *ALK* fusion.

Discussion

Targeted therapy for advanced *ALK*-positive NSCLC is a rapidly developing therapeutic area, with newer ALK TKIs approved for patients who develop resistance to first- or second-generation ALK inhibitors.[17,18] The third-generation TKI lorlatinib was found to have clinical antitumor activity as a second-line or first-line treatment in patients with *ALK*-positive NSCLC.[2–4,15,19]

In this analysis of data from the CROWN trial, we investigated the clinical efficacy of lorlatinib compared with the first-generation ALK TKI crizotinib in relation to *EML4::ALK* variant or *ALK* mutation status at baseline. Our analysis revealed that *EML4::ALK* variant or *ALK* mutation at baseline did not affect the clinical efficacy of lorlatinib. ORRs were numerically higher in the lorlatinib arm than in the crizotinib arm for most variants (except *EML4::ALK* v3 on the basis of ctDNA analysis). Median PFS was also numerically longer in the lorlatinib arm than in the crizotinib arm for most variants (except *EML4::ALK* v2 on the basis of ctDNA analysis). With 3 years of follow-up,[19] mPFS was NR for v1 and 33.3 months for v3 in the lorlatinib arm. This finding is potentially important because v1 and v3 are the most common fusion variants found among *ALK*-positive NSCLCs (43% and 40% of the cases).[5] In vitro, ALK TKI sensitivity was dependent on *EML4::ALK* variants, with v3 having lower sensitivity.[20] Results from a retrospective analysis indicate that v3 leads to an acceleration of metastatic spread and earlier progression after ALK TKI therapy.[21] ALK resistance mutations are more common in v3, specifically G1202R.[5] Given the potency of lorlatinib, it may be able to block the development of resistance mutations such as G1202R in patients harboring *EML4::ALK* v3. In the current study, patients with *EML4::ALK* v3 derived great benefit from lorlatinib versus crizotinib treatment, suggesting that the broader coverage and potency of lorlatinib may improve several efficacy measures for this specific variant compared with crizotinib. Although cross-trial comparisons should be used with caution, mPFS of 33.3 months with lorlatinib in patients harboring *EML4::ALK* v3 indicates favorable clinical activity of lorlatinib in this patient population. In the ALEX trial, on the basis of plasma analysis, mPFS with alectinib was 34.8 and 17.7 months for *EML4::ALK* v1 and v3, respectively.[22] In the ALTA-1 trial, on the basis of

Table 4. Efficacy by Potential Preexisting Bypass Pathway Alteration Status

Plasma ctDNA	Lorlatinib (n = 97)		Crizotinib (n = 100)	
Mutation status[a]	Positive	Negative	Positive	Negative
n (%)	15 (15.5)	82 (84.5)	13 (13.0)	87 (87.0)
ORR, % (95% CI)	73.3 (44.9-92.2)	75.6 (64.9-84.4)	30.8 (9.1-61.4)	63.2 (52.2-73.3)
Median DOR, mo (95% CI)	NR (NR-NR)	NR (NR-NR)	NR (8.5-NR)	9.2 (6.8-12.8)
Median PFS, mo (95% CI)	NR (20.2-NR)	NR (32.9-NR)	10.3 (2.2-NR)	8.5 (7.2-10.9)
HR[b] for PFS (95% CI)				
Mutation negative vs. mutation positive		1.45 (0.511-4.103)		1.12 (0.511-2.460)
Lorlatinib vs. crizotinib	0.28 (0.080-0.991)	0.26 (0.162-0.403)		
Tumor tissue	**Lorlatinib (n = 79)**		**Crizotinib (n = 69)**	
Mutation status	Positive	Negative	Positive	Negative
n (%)	11 (13.9)	68 (86.1)	8 (11.6)	61 (88.4)
ORR, % (95% CI)	72.7 (39.0-94.0)	79.4 (67.9-88.3)	62.5 (24.5-91.5)	57.4 (44.1-70.0)
Median DOR, mo (95% CI)	NR (9.4-NR)	NR (NR-NR)	7.4 (4.6-NR)	12.9 (8.7-27.3)
Median PFS, mo (95% CI)	18.2 (1.9-NR)	NR (NR-NR)	5.4 (3.7-10.9)	11.0 (9.2-16.0)
HR[b] for PFS (95% CI)				
Mutation negative vs. mutation positive		0.42 (0.169-1.061)		0.39 (0.159-0.947)
Lorlatinib vs. crizotinib	0.44 (0.129-1.492)	0.27 (0.153-0.482)		

Note: Based on blinded independent central review by Kaplan-Meier estimates, based on Brookmeyer and Crowley method.
[a]Only patients with ctDNA detected.
[b]Unstratified hazard ratio based on Cox proportional hazards model. HR <1.00 favors test (vs. reference).
CI, confidence interval; ctDNA, circulating tumor DNA; DOR, duration of response; HR, hazard ratio; NR, not reached; ORR, objective response rate; PFS, progression-free survival.

plasma analysis, mPFS with brigatinib was 29.0 and 16.0 months, respectively.[23]

Evaluation of the ctDNA and tumor tissue to determine *EML4::ALK* variant subtypes revealed overall high agreement between the assays. This finding is important considering that determining variant status from ctDNA at baseline and during the course of treatment has the potential to guide treatment decisions and improve outcomes for patients.[6] Nevertheless, such hypotheses are mainly based on small data sets, and analyses require detectable ctDNA in plasma, which may not be feasible in patients with advanced NSCLC. In addition, NGS technology might not be as readily or broadly available in every country or clinical site as IHC, which remains one of the standard methods for *ALK* fusion detection. Finally, the IHC assay does not identify the fusion partner or the fusion variant subtype. The preferential use of lorlatinib instead of crizotinib as first line may overcome the absence of that information owing to its efficacy independent from the variant type.

The absence of detectable ctDNA at baseline suggested association with higher ORR and longer DOR and PFS than detected ctDNA in the crizotinib arm and represents a good prognostic group.[24] These results might be capturing a different biology of slow-growing tumors, which have a better prognosis and are less likely to "shed" ctDNA.[25] In addition, metastases may be located in tissue that is not highly vascularized.[26] Nevertheless, lorlatinib had similar efficacy despite the presence of ctDNA at baseline and its associated more aggressive tumor type, indicating that the treatment is effective even when the disease is widely metastatic with a high disease burden.

As expected, very few ALK TKI treatment-naive patients harbored *ALK* mutations. Yet, regardless of the presence of mutations, patients derived greater benefit from lorlatinib treatment. Co-existing missense or nonsense *TP53* mutations are predictive of poor outcomes in oncogene-driven NSCLC, including *EGFR* mutation-positive NSCLC and *ALK*-positive NSCLC.[11,12] The combination of *EML4::ALK* v3 and *TP53* co-mutation may be a predictor of especially poor outcomes.[6,27] We found that with crizotinib treatment, PFS was longer in the *TP53*-negative than *TP53*-positive group (mPFS = 10.8 mo versus 7.2 mo; HR = 0.71 [95% CI: 0.437–1.151]). In the lorlatinib arm, a similar observation was made, with mPFS being NR in both *TP53*-positive and -negative groups (HR = 0.65 [95% CI: 0.333–1.261]). Overall, patients whose tumor harbored a *TP53* mutation did worse than those who did not. Nevertheless, outcomes were improved in the lorlatinib group versus the crizotinib group

regardless of *TP53* status; these results were numerically superior to those achieved with alectinib or brigatinib. In a retrospective cohort of 90 patients with *ALK*-positive NSCLC who had been treated with alectinib, patients with co-occurring *TP53* mutation had substantially worse PFS than patients without *TP53* mutation (mPFS = 11.7 mo versus NR, respectively; HR = 0.33 [95% CI: 0.17–0.65])[28]; a similar observation was also reported in the ALEX trial.[29] In the ALTA-1L trial, in the brigatinib arm, mPFS was 18.0 months in patients with co-occurring *TP53* mutation and 24.0 months in those without *TP53* mutation.[23] As documented in many cancer settings,[30] overcoming *TP53* mutations remains an unmet need, and this is definitely the case for patients with *ALK*-positive NSCLC, in particular those harboring *EML4::ALK* v3.

A subanalysis restricted to alterations with known or likely pathogenic effects did not change the overall result and indicates that lorlatinib as first-line treatment is effective despite co-existing *TP53* mutations. Although subanalysis of the mutation location was not performed, these results are in contrast to NSCLC treated with immune checkpoint inhibitors, in which *TP53*-mutated status correlated with overall survival benefit of immunotherapy.[31]

This study has several limitations. Despite all enrolled patients being *ALK* positive on the basis of IHC test results, the detection rate of *ALK* fusions by the NGS assay used in the tumor tissue analysis was 80.4%. The disagreement rate between IHC and NGS could be explained, at least in part, by limitations in the NGS assay[32] and the IHC assay, as observed in other trials,[33,34] as there is no perfect method for gene fusion analysis. Although ctDNA analysis can provide meaningful information, the sensitivity of the assay may be limited, as revealed by the low rate of *ALK* fusion detection with potential false negatives affecting the ability to distinguish between the groups. Nevertheless, despite this issue, results indicate important predictive value.[35] In addition, the results were supported and confirmed with the broad tumor tissue NGS platform used in this study, with very high agreement observed in *EML4::ALK* variant subtype detection. Plasma ctDNA was collected during the screening, cycle 2 day 1, cycle 7 day 1, and at the end of the study, but tumor tissue was mainly derived from archived diagnostic specimens. Any potential changes since diagnosis may lead to incongruency between the two sample types. It is unknown whether the ctDNA results from this study are due to a broad modification in all tumor sites or to a single-disease site mutation. The relatively low number of cases in some subgroups (e.g., *EML4-ALK* v2) limits the potential for finding relevant group differences in multiple analyses. A larger sample size could have led to more robust results related to the subgroup differences in the current study.

In conclusion, the results of the baseline biomarker analyses further emphasize the superior efficacy of lorlatinib compared with crizotinib, with consistent activity across *EML4::ALK* fusion variant subtypes and in patients with and without the *TP53* co-mutation and in patients harboring preexisting bypass pathway resistance alterations. With 3 years of follow-up, these more mature data further support lorlatinib as a standard treatment option for treatment-naive *ALK*-positive NSCLC.

CRediT Authorship Contribution Statement

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Acknowledgments

The authors thank the participating patients and their families, investigators, subinvestigators, research nurses, study coordinators, and operations staff. Editorial and

medical writing support was provided by Meredith Rogers, MS, CMPP, and Marius Dettmer, PhD, of CMC AFFINITY, McCann Health Medical Communications, and Eleanor Porteous, MSc, of Clinical Thinking, Inc., and was funded by Pfizer.

This study was sponsored by Pfizer.

Data Availability Statement

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.2023.07.023.

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