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Safety and Efficacy of Crizotinib in Patients With Advanced or Metastatic *ROS1*-Rearranged Lung Cancer (EUCROSS): A European Phase II Clinical Trial



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ABSTRACT

Introduction: *ROS1* rearrangements are found in 1% of lung cancer patients. Therapeutic efficacy of crizotinib in this subset has been shown in early phase trials in the United States and East Asia. Here we present data on efficacy and safety of a prospective phase II trial evaluating crizotinib in European *ROS1*-positive patients (EUCROSS).

Patients and Methods: The trial was a multicenter, single-arm phase II trial (ClinicalTrials.gov identifier: NCT02183870). Key eligibility criteria included patients who were 18 years of age or older with advanced/metastatic lung cancer and centrally confirmed *ROS1*-rearranged lung cancer (fluorescence-in situ hybridization). Treatment included 250 mg crizotinib twice daily. The primary endpoint was investigator-assessed objective response rate (ORR) (Response Evaluation Criteria in Solid Tumors, version 1.1). Key secondary endpoints were progression-free survival (PFS), overall survival, efficacy by independent radiologic review, safety, health-related quality of life, and molecular characterization of tumor tissue.

Results: Thirty-four patients received treatment. Four patients were excluded from efficacy analysis. Investigator ORR was 70% (95% confidence interval [CI]: 51–85; 21 of 30 patients) and median PFS was 20.0 months (95% CI: 10.1–not reached). Two patients with *ROS1* wild-type sequences assessed by DNA sequencing had progression as best response. *CD74-ROS1*-positive patients had a trend towards a higher ORR and longer median PFS. *TP53*-co-mutant patients had a significantly shorter median PFS than wild-type patients (7.0 months, 95% CI: 1.7–20.0 versus 24.1 months, 95% CI: 10.1–not reached; $p = 0.022$). Treatment-related adverse events were documented in 33 of 34 patients (97%).

Conclusions: Crizotinib is highly effective and safe in patients with *ROS1*-rearranged lung cancer. *ROS1-TP53*-co-aberrant patients had a significantly worse outcome compared to *TP53* wild-type patients.

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Keywords: Lung cancer; Targeted treatment; *ROS1*; Crizotinib; *TP53*

Introduction

The discovery of an increasing number of targetable driver aberrations in NSCLC has boosted the development of targeted therapies in molecularly defined subgroups.¹ *ROS1* encodes a receptor tyrosine kinase closely related to ALK receptor tyrosine kinase (ALK).^{2,3} Oncogenic rearrangements of *ROS1* are found in approximately 1% of NSCLC patients and involve numerous other genes on different chromosomes, most commonly *CD74*.^{2–9}

Studies revealed distinct characteristics of patients with *ROS1*-rearranged NSCLC such as association with non- or light-smoking history and young age.^{6,7,9–11}

Crizotinib (Xalkori, Pfizer Inc., New York, New York) is an oral tyrosine kinase inhibitor with high affinity to ALK and *ROS1*.¹² It has been approved for treatment of *ALK*- and *ROS1*-rearranged NSCLC by the European Medicines Agency and the US Food and Drug Administration.^{13–15} A US phase I trial and an East Asian phase II trial in patients with advanced *ROS1*-positive NSCLC showed an overall response rate (ORR) of approximately

70%, median progression-free survival (PFS) times between 15.9 months and 19.2 months and low toxicity.^{7,16} Other drugs, such as the ALK/ROS1 inhibitors ceritinib, lorlatinib, or entrectinib also showed high response rates in patients with *ROS1*-positive NSCLC.¹⁷⁻¹⁹ However, in terms of tolerability and efficacy, these drugs do not seem to be superior to crizotinib. So far, no prospective trial results have been published on crizotinib in European patients.

We therefore initiated the EUCROSS trial, a European phase II trial investigating crizotinib in *ROS1*-rearranged NSCLC. Here we present data on treatment efficacy and safety.

Patients and Methods

Patients and Eligibility Criteria

Patients 18 years of age or older with locally advanced or metastatic histologically confirmed NSCLC and *ROS1* rearrangement in local testing were allowed to enter screening after written informed consent, independent of the number of prior therapies. *ROS1* rearrangements were confirmed centrally by dual-color break-apart fluorescence in situ hybridization (FISH) before treatment initiation. Additional key eligibility criteria included an Eastern Cooperative Oncology Group performance status of 0 to 2, at least one measurable lesion according to the Response Evaluation Criteria for Solid Tumors (RECIST version 1.1), no prior ALK/ROS1 tyrosine kinase inhibitor treatment, and adequate hematologic and organ functions.²⁰ Patients with brain metastases before enrollment were excluded if symptomatic and/or increasing doses of steroids were applied.

Study Design and Treatment

EUCROSS is an open-label, single-arm, multicenter, Fleming's single-stage phase II trial investigating crizotinib in *ROS1*-positive NSCLC patients at 20 sites in Germany, Spain, and Switzerland (Supplementary Table S1). The trial was coordinated by the Lung Cancer Group Cologne (University of Cologne) and the Spanish Lung Cancer Group. Patients were treated with initial doses of 250 mg crizotinib twice daily within 28-day cycles until disease progression, death, withdrawal of the informed consent, or unacceptable toxicity. Dose modifications and treatment interruptions were performed if clinically indicated or as prespecified in the protocol. Treatment beyond progression was allowed if patients derived ongoing clinical benefit from treatment continuation. As per amendment in 2016, efficacy assessments by computed tomography and/or magnetic resonance imaging were scheduled every 6 weeks for the first 6 months, every 8 weeks for the next 6 months, and every 12 weeks afterwards. Brain scans were mandated

at baseline and during follow-up if metastases were present at baseline or if new metastases were suspected. Clinical response status was evaluated locally for individual decision-making and endpoint analyses. A blinded independent radiologic review (IRR) was performed for selected efficacy endpoints. At baseline and throughout the treatment, patient-reported outcomes (PROs) of health-related quality of life (HRQoL) were collected using the European Organisation for Research and Treatment of Cancer QLQ-C30 (version 3) and lung cancer-specific QLQ-LC13 questionnaires.

After withdrawal from treatment, patients were followed quarterly for overall survival (OS).

The trial was registered at the US National Institutes of Health trial registry (NCT02183870) and was approved by the responsible institutional review boards or ethics committees. The trial was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines.

Outcomes

The primary endpoint was the efficacy of crizotinib in *ROS1*-rearranged NSCLC patients, measured as the ORR at the time of data cutoff for this report by local assessment. ORR was defined as the percentage of confirmed partial responses (PR) and complete responses (CR) according to RECIST (version 1.1). Patients were included in the efficacy analysis if an adequate baseline tumor assessment was performed, eligibility criteria were fulfilled, and at least 1 dose of crizotinib was administered. The intention-to-treat population (ITT) included all patients who received at least 1 dose of crizotinib.

Secondary efficacy endpoints were disease control rate (DCR; percentage of confirmed CR, PR, and stable disease), PFS, duration of response, and OS. PFS was defined as the time from first day of treatment until radiologic progression or death. OS was calculated from treatment initiation until death. Patients who did not meet these criteria were censored at the date of the last examination. In addition, efficacy endpoints were calculated based on the results of the IRR as well as separately for the defined subgroups. Safety and tolerability were assessed in the ITT population by grading of collected adverse events (AEs) and serious AEs according to Common Terminology Criteria for Adverse Events, version 4.0, as well as treatment-related therapy interruptions, and dose reductions. PROs were assessed as described above.

Molecular Analyses

ROS1 status was assessed centrally by experienced pathologists (Targos Molecular Pathology GmbH, Kassel, Germany) using validated ZytoLight SPEC dual color break-apart FISH (ZytoVision, Bremerhaven, Germany).

Criteria for *ROS1* positivity included that there were 20 of 100 cells with break-apart signals and/or isolated green signals.¹¹

The commercial hybrid-capture-based DNA sequencing panel NEOplus was used to test 72 cancer-related genes, to validate *ROS1* rearrangements, and to identify *ROS1* fusion partners (Supplementary Table S2) (NEO New Oncology AG, Cologne, Germany).²¹

Statistical Considerations and Analyses

For sample size calculation based on ORR according to Fleming's single-stage design, the following assumptions were prespecified: alpha 0.05, power 92%, lower proportion for rejection 20%, and a higher proportion for acceptance 45%, resulting in a sample size of 30 patients. The minimum number of objective responses to indicate effective treatment was 11 among the first 30 response-evaluable patients.

Confidence intervals (CIs) (level 95%) were calculated for all endpoint analyses if applicable. Time-to-event data (PFS, OS, and duration of response) were summarized by the Kaplan-Meier estimator. Statistical significance for differences in time-to-event endpoints between different strata was calculated using the log rank test and for differences in proportions using Fisher's exact test.

AEs were described by the frequency of patients exhibiting a specific event and by grade. AEs were summarized according to the Medical Dictionary for Regulatory Activities preferred term.

Scores collected within the HRQoL questionnaires were tested using a repeat measures model for linear time trends and summarized by mean and standard-error per time-point (2-cycle intervals until cycle 24 and aggregated as one timepoint thereafter) and analyzed for time trends using a repeat measures model. To account for the increasing proportions of missing data in later time intervals, we used various multiple imputation methods (fully conditional specification or monotone regression with or without prior transformation of the original scores to linearize the 0–100 scale). Scores at timepoints later than cycle 18 were not used due to the high proportion of missing values (there was complete data for less than 20 of the 34 patients). For multiple imputations, 10 imputed values of each missing value were generated using all previous available values of that score as predictors.²²

All analyses were conducted with the use of SAS version 9.3 (SAS Institute Inc., Cary, North Carolina).

Results

Patient Population

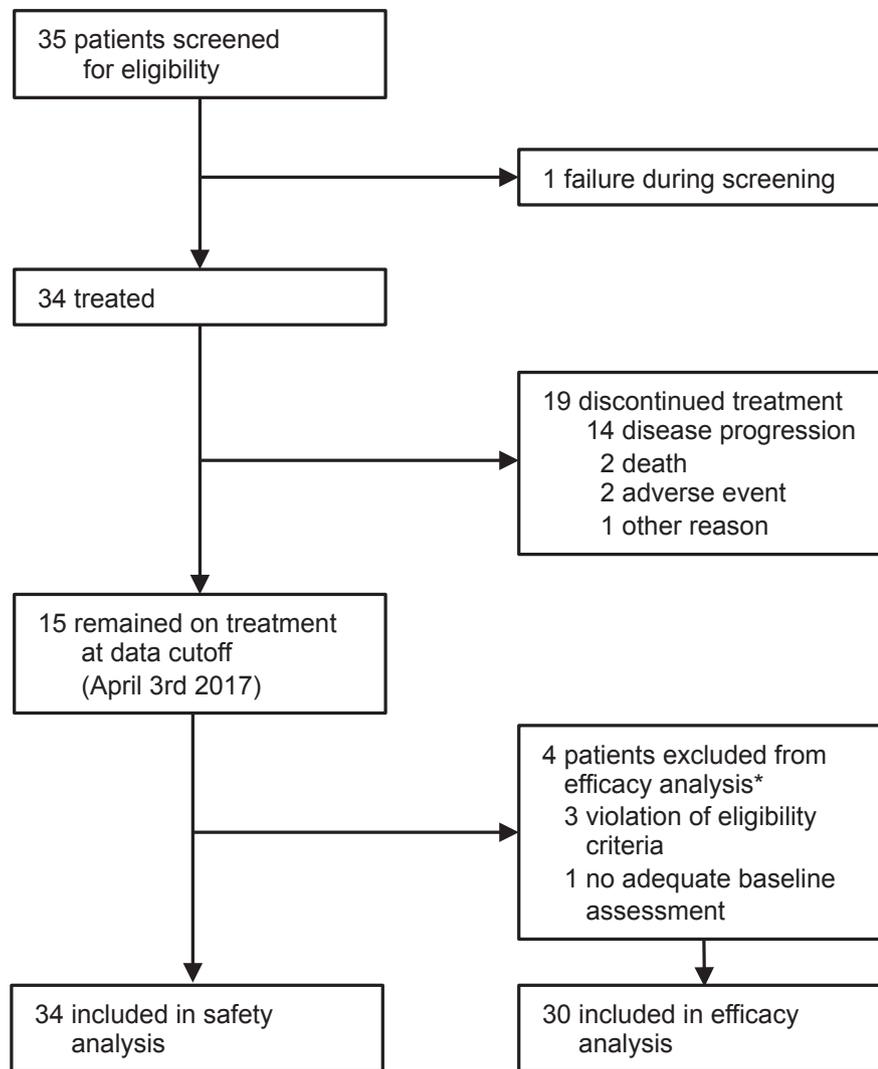
Between June 2014 and December 2015, 35 NSCLC patients with locally confirmed *ROS1* rearrangement

were screened for participation in the trial (Figure 1). Thirty-four patients received treatment with crizotinib. In 4 of these, major protocol violations, including ineligibility in 3 and inadequate baseline tumor assessment in 1 occurred (Supplementary Table S3). Because of ethical considerations, in particular missing availability of crizotinib outside of the trial and lack of alternative treatment options, these patients were allowed to start treatment within EUCROSS and were included into the ITT and safety population (N = 34). The efficacy population was constituted of the other 30 patients. At the time of data cutoff, 19 patients had stopped treatment (56%) and 15 patients continued therapy within the trial (44%). The clinical and epidemiologic characteristics of all patients are summarized in Table 1. In the efficacy population (n = 30), 16 (53%) patients were treatment-naïve or had one prior treatment. Fourteen (47%) had received two or more lines of systemic therapy. Median follow-up at data cutoff was 20.6 months.

Efficacy, Subgroup Analyses and Molecular Analyses

ORR was 70% (95% CI: 51%–85%; n = 21 of 30 patients) and DCR was 90% (95% CI: 74%–98%; n = 27 of 30 patients) according to local assessment (Table 2, Fig. 1A). ORR and DCR determined by IRR were 73% (95% CI: 54%–88%; n = 22 of 30 patients) and 83% (95% CI: 65%–94%; n = 25 of 30 patients) (Table 2), respectively. At data cutoff, 16 of 30 patients (53%) of the efficacy population showed progressive disease (PD) according to local assessment or had died. Fifteen (50%) patients had PD according to IRR or had died. Median PFS and duration of response were 20.0 months (95% CI: 10.1–not reached [NR]) and 19.0 months (95% CI: 9.1–NR), respectively, as assessed locally and 20.0 months (95% CI: 9.6–NR) and 19.0 months (95% CI: 8.3–NR), respectively, as assessed by IRR (Table 2, Fig. 1C). Median OS (95% CI: 17.1–NR) was not met at data cutoff, but survival rates at 12 months and 24 months were 83% (95% CI: 69%–97%) and 63% (95% CI: 42–84), respectively (Fig. 1D, Supplementary Table S4). Efficacy and OS in the ITT population were similar (Supplementary Table S5 and S6).

Tumor tissue of 20 (67%) patients from the efficacy population was available for central DNA sequencing. *ROS1* rearrangements were confirmed in 18 samples (90%), with *CD74* (n = 9, 50%) being the most frequent fusion partner, followed by *ezrin (EZR)* (n = 3, 17%), *SCL34A2* (n = 3, 17%), and *PTP4A1*, *SCD4*, and *TMP3* (n = 1, 6% each) (Supplementary Figure 1). Two patients who were classified *ROS1*-rearranged based on extra-green FISH signals did not display rearrangements by DNA sequencing and had PD as best response. In the



*Due to ethical considerations these patients were allowed to start or continue treatment within the EUCROSS trial despite protocol violations prior to the start of treatment.

Figure 1. Trial flow chart.

patients with confirmed *ROS1* rearrangement by sequencing, investigator-assessed ORR was 89% (95% CI: 65%–99%; $n = 16$ of 18 patients) (Table 2). ORR calculated based on IRR was similar (Table 2). Patients with *CD74-ROS1* fusions had a nonsignificantly higher ORR and longer PFS than patients with other *ROS1* fusions (Fig. 2, Supplementary Figure 1, Supplementary Figure 2).

Co-occurring genetic aberrations were found in 11 of 18 samples (61%). Mutations in *TP53* were most frequent (28%; 5 of 18 samples) (Supplementary Figure 3). No aberrations in *EGFR*, *KRAS*, *BRAF*, *ALK*, or *MET* were found. Patients without co-occurring genetic aberrations

had a nonsignificantly longer median PFS as compared to those with at least one co-aberration (24.1 months, 95% CI: 2.2–NR versus 11.5 months, 95% CI: 6.9–20.0; $p = 0.175$) (Supplementary Figure 3). Median PFS was significantly shorter in patients with *TP53*-mutant sequences as compared to wild-type sequences (7.0 months, 95% CI: 6.8–7.2 versus 24.1 months, 95% CI: 1.0–47.2) (hazard ratio: 3.89; 95% CI: 1.12–13.6; $p = 0.022$) (Supplementary Table 7).

The status of brain metastases at baseline was recorded in 29 (97%) patients in the efficacy population. Stable brain metastases were present in six (21%). Although displaying a similar ORR and DCR, patients

Table 1. Baseline Patient Characteristics and Demographics of the ITT Population

Characteristics	ITT Population (N = 34)
Median age, years (range)	56 (33-84)
Sex	
Female	19 (56)
Male	15 (44)
Ethnicity	
Caucasian	31 (91)
Asian	2 (6)
Other	1 (3)
ECOG performance status at study entry	
0	12 (35)
1	20 (59)
2	2 (6)
Smoking status	
Never-smoker	23 (68)
Ex-smoker	11 (32)
Number of systemic antineoplastic regimens	
0	7 (21)
1	12 (35)
2	5 (15)
>2	10 (29)
Histologic subtype	
Adenocarcinoma	31 (91)
Adenosquamous carcinoma	3 (9)
UICC stage at time of study entry	
IV	34 (100)
Brain metastases at study entry	
No	26 (76)
Yes	7 (21)
Unknown	1 (3)
ROS1 fusion type by sequencing	
No sequencing performed	14 (41)
Sequencing performed	20 (59)
No ROS1 fusion	2 (11.0)
CD74-ROS1	9 (5.0)
SLC34A2-ROS1	3 (16.7)
PTP4A1/EZR-ROS1	1 (5.6)
EZR-ROS1	3 (16.7)
SDC4-ROS1	1 (5.6)
TPM3-ROS1	1 (5.6)

Values are n (%) unless otherwise stated.

ECOG, Eastern Cooperative Oncology Group; ITT, intent to treat; UICC, Union for International Cancer Control.

with brain metastases had a shorter median PFS than patients without brain metastases at 9.4 months (95% CI: 1.7–NR) versus 20.0 months (95% CI: 10.1–NR) (hazard ratio: 1.53; 95% CI, 0.488–4.77; $p = 0.464$) (Supplementary Table 8 and 9).

The number of prior treatment lines had no detectable influence on ORR, DCR, PFS, and OS (Supplementary Table 8 and 9).

At data cutoff for this report, tumor tissue ($n = 2$) or cell-free tumor DNA ($n = 2$) collected at progression was available of four patients. Matched hybrid-capture–based DNA sequencing revealed the emergence of co-occurring aberrations known to cause treatment resistance in the

two tissue samples (Supplementary Table 10). One patient acquired an *ROS1* p.L2026M substitution as well as a *TP53* p.P278H missense mutation. The other patient acquired the known resistance mutation *PIK3CA* p.E545K. No confirmed mechanism of resistance was detected in the two plasma samples. In one, no *ROS1*-rearrangement could be detected.

Safety and Toxicity

All patients who received at least 1 dose of crizotinib ($N = 34$) were evaluable for toxicity and safety analysis. Treatment interruptions were necessary in 17 (50%) patients. Dose reduction to crizotinib 200 mg twice daily was necessary in 16 (47.1%) patients and to crizotinib 250 mg every day in two (5.9%). Dose reductions were performed due to bradycardia (11.8%; $n = 4$; all grade 2), edema (8.8%; $n = 3$; grade 1 or 2), neutropenia (8.8%; $n = 3$; all grade 3), and vomiting (5.9%; $n = 2$; grades 2 and 3) among other reasons (Supplementary Table 11). In six patients, dose reductions were performed due to grade 1 or 2 events and to the discretion of the investigators. In 10 patients (29.4%), dose reductions were in accordance with the recommendations in the trial protocol. Three patients discontinued treatment for other reasons than progression (8.8%). AEs were recorded in all but one patient (33 of 34 patients [97%]) (Supplementary Table 12). Thirty-three patients (97%) had AEs of any grade that were suspected to be treatment related (Table 3). Eight treatment-related AEs in five patients (14.7%) fulfilled seriousness criteria (Supplementary Table 13). One of these — pulmonary embolism — resulted in the patient's death. Grades 1 and 2 treatment-related AEs were documented in 32 (94%) and 22 (65%) patients. Grade 3 events were recorded in eight (24%) patients. The most common treatment-related AEs ($\geq 10\%$) are summarized in Table 3. One of the most frequent treatment-related AEs was sinus bradycardia (47%; $n = 16$). The recorded mean heart rate decreased by 19 beats/min (84.5 beats/min to 65.5 beats/min) during treatment and recovered after treatment termination (82.8 beats/min) (Supplementary Table 14).

Disease-Related Quality of Life and PROs

PROs of HRQoL were assessed using QLQ-C30 and QLQ-LC13. Global health status showed increasing mean values from baseline throughout treatment (Supplementary Figure 4). Similarly, the mean values of all other QLQ-C30 components scores, with the exception of cognitive functioning, increased steadily over time. However, in multiple imputation analyses the time trends were not statistically significant (Supplementary Table 15). Concerning the QLQ-LC13 symptom scores,

Table 2. Overview of Response and PF assessment of the Response-Evaluable (n = 30) and the DNA Sequencing-Positive Population (n = 18)

	Local Radiologic Assessment		Independent Radiologic Assessment	
	n (%) ^a	95% CI	n (%) ^a	95% CI
Response-evaluable population (n = 30)				
Objective response rate	21 (70.0)	50.6-85.3	22 (73.3)	54.1-87.7
Complete response	0 (0.0)		0 (0.0)	
Partial response	21 (70.0)		22 (73.3)	
Disease control rate	27 (90.0)	73.5-97.9	25 (83.3)	65.3-94.4
Stable disease	6 (20.0)		3 (10.0)	
Non-CR/non-PD	0 (0.0)		2 (6.7)	
Progressive disease	2 (6.7)		2 (6.7)	
NE	1 (3.3)		1 (3.3)	
PFS				
Median PFS (months)	20.0	10.1-NR	20.0	9.6-NR
Events censored	14 (47)		15 (50)	
PFS at 12 months (%)	56.5	38.7-74.3	56.7	38.9-74.4
PFS at 24 months (%)	45.6	25.6-65.6	45.8	25.8-65.8
Sequencing-positive population (n = 18)				
Objective response rate	16 (88.9)	65.3-98.6	15 (83.3)	58.6-96.4
Complete response	0 (0.0)		0 (0.0)	
Partial response	16 (88.9)		15 (83.3)	
Disease control rate	17 (94.4)	72.7-99.9	16 (88.9)	65.3-98.6
Stable disease	1 (5.6)		1 (5.6)	
Non-CR/non-PD*	0 (0.0)		1 (5.6)	
Progressive disease	0 (0.0)		0 (0.0)	
NE	1 (5.6)		1 (5.6)	
PFS				
Median PFS (months)	16.8	9.6-NR	16.8	7.4-NR
Events censored	7 (38.9)		8 (44.4)	
PFS at 12 months (%)	55.0	31.8-78.2	55.6	30.5-74.5
PFS at 24 months (%)	38.5	13.0-64.0	38.9	15.1-62.4

^aValues are expressed as n (%) unless otherwise stated.

CI, confidence interval; CR, complete response; PD, progressive disease; PFS, progression-free survival; PR, partial response; NE, not evaluable; NR, not reached.

only coughing, dyspnea, and chest pain showed a trend to lower mean scores over time. However, only in the case of coughing was this trend significant in the multiple imputation analysis ($p = 0.0027$ to 0.042) (Supplementary Figure 5, Supplementary Table 16).

Discussion

To our knowledge, this is the first prospective phase II trial of crizotinib in European patients. Comparable to the results of the US PROFILE 1001 phase I trial and the phase II trial in Eastern Asian patients, we observed an ORR of 70% based on local assessment and 73% based on IRR.^{7,16} Similarly, a median PFS of 20.0 months was in the same range as in the two trials. In contrast, a retrospective analysis of crizotinib in European *ROS1*-positive patients (EUROS1) revealed a markedly shorter median PFS of only 9.1 months.¹⁰ We believe that the differences in PFS may be explained in part by the heterogeneous selection of patients in the EUROS1 study including the lack of a central validation of the *ROS1*

status. Additionally, retrospective analyses of PFS may be biased by previous treatment decisions made by the investigators. No differences were detected for efficacy between patients who received crizotinib as first- or second-line treatment and patients who received treatment in later lines. Thus, crizotinib seemed to be equally efficacious in heavily pretreated patients. Similarly, the presence of brain metastases at baseline did not have a negative impact on ORR. However, median PFS was markedly shorter in these patients as compared to those without brain metastases at 9.4 months in patients with brain metastases versus 20.0 months in patients without brain metastases. Because patient numbers were low and statistical significance was not met, these results must be interpreted with caution. Remarkably, Wu et al.¹⁶ reported almost identical ORR and median PFS results depending on the status of brain metastases — 73.9% and 10.2 months in patients with brain metastases versus 71.2% and 18.8 months in patients without brain metastases.

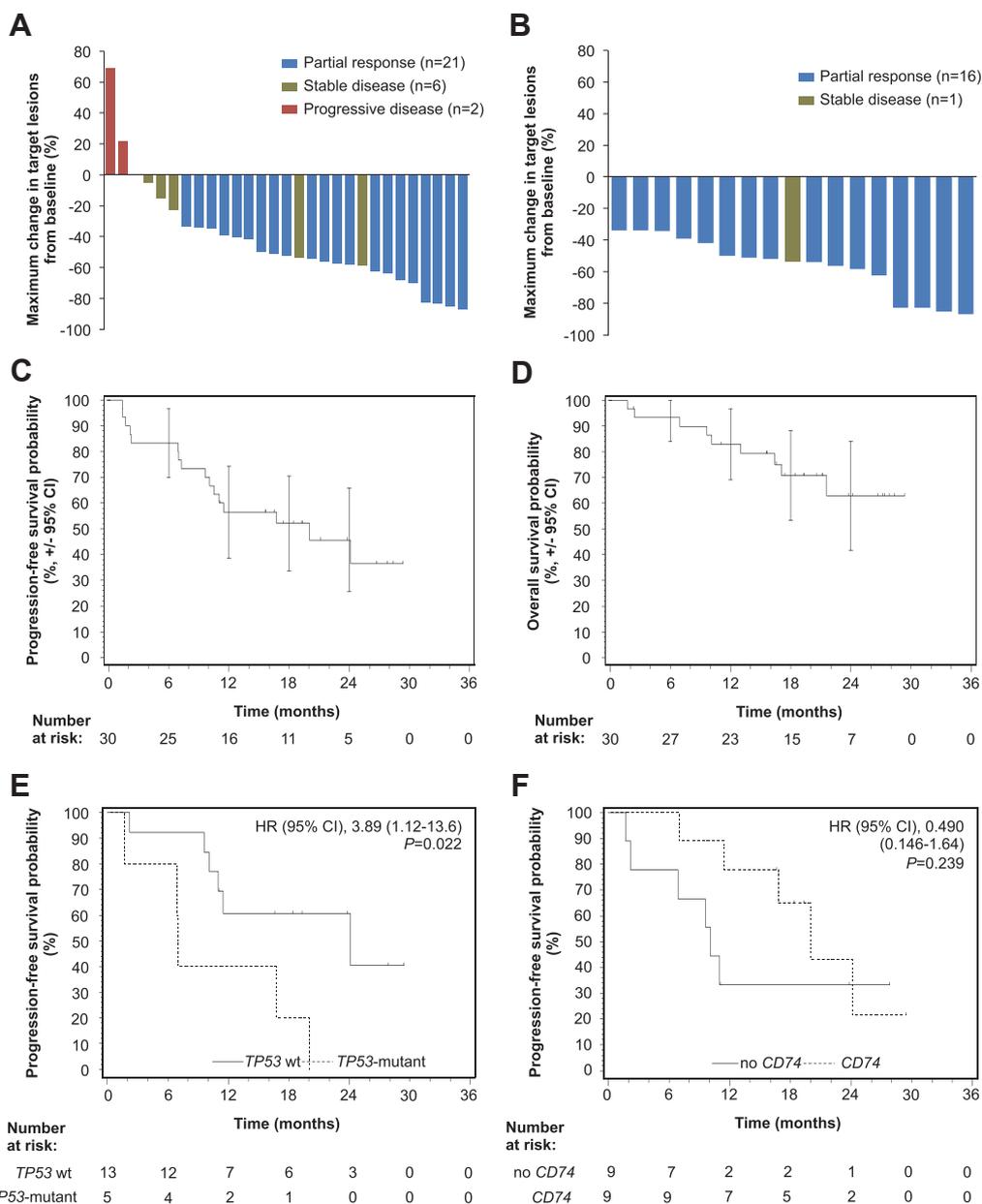


Figure 2. (A) Maximum change in target lesions from baseline (%) as assessed by the investigators in the efficacy population (n = 30). The maximum change in target lesions according to Response Evaluation Criteria in Solid Tumors 1.1 was assessed in 29 patients. One patient was not evaluable due to early death and (B) in patients evaluable for response and *ROS1* fusion-positive as determined by DNA sequencing (n = 18). Each bar represents one patient. (C) Progression-free survival and (D) overall survival as assessed by the investigators in all patients evaluable for efficacy (n = 30). (E) Progression-free survival stratified by *TP53* status and (F) *ROS1* fusion type (n = 18). CI, confidence interval; HR, hazard ratio.

Two of 20 samples that were DNA sequenced were *ROS1*-negative and, interestingly, these were obtained of the 2 only patients exhibiting progressive disease as best response. FISH-positivity in these patients was based on an atypical rearrangement pattern of extra-green signals. In the phase I study of Shaw et al.⁷ a similar case was described and the patient experienced PD at first staging. In the subgroup of FISH- and sequencing-positive patients, the ORR of 89% was numerically higher than in

the efficacy population. Rearrangements of *ROS1* with *CD74* were most frequent and the distribution of fusion types was similar to that reported in previous studies.²⁻⁹ Comparing the efficacy of crizotinib grouped by rearrangement type, we found that patients with *CD74-ROS1* had a higher ORR and longer median PFS than patients with other fusion types. However, this effect was not statistically significant. Efficacy of crizotinib was independent of *ROS1* translocation type in the PROFILE 1001

Table 3. Summary of Treatment-Related Adverse Events With a Frequency of 10% or Greater or That Resulted in Death (N = 34)

AE	Grade ^a					
	Any	1	2	3	4	5
Any related AE	33 (97)	32 (94)	22 (65)	8 (24)	0.0 (0)	1 (3)
In ≥10% of patients						
Visual disturbances	22 (65)	22 (65)	—	—	—	—
Diarrhea	19 (56)	14 (41)	5 (15)	—	—	—
Edema	17 (50)	11 (32)	6 (18)	—	—	—
Bradycardia	16 (47)	11 (32)	5 (15)	—	—	—
Nausea	14 (41)	8 (24)	5 (15)	1 (3)	—	—
ALT increased	12 (35)	10 (29)	1 (3)	1 (3)	—	—
Vomiting	11 (32)	6 (18)	4 (12)	1 (3)	—	—
Leukopenia/neutropenia	11 (32)	6 (18)	2 (6)	3 (9)	—	—
AST increased	9 (26)	9 (26)	—	—	—	—
Blood creatinine increased	7 (21)	6 (18)	1 (3)	—	—	—
Asthenia/fatigue	6 (18)	6 (18)	—	—	—	—
Constipation	5 (15)	3 (9)	2 (6)	—	—	—
Abdominal pain	5 (15)	5 (15)	—	—	—	—
Dizziness	5 (15)	3 (9)	2 (6)	—	—	—
Anemia	5 (15)	5 (15)	—	—	—	—
Dysgeusia	4 (12)	3 (9)	—	1 (3)	—	—
Blood AP increased	4 (12)	4 (12)	—	—	—	—
Resulted in death						
Pulmonary embolism	1 (3)	—	—	—	—	1 (3)

^aValues shown are n (%).

AE, adverse event; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase.

trial, but a recently published retrospective study showed a reduced efficacy of crizotinib in *CD74-ROS1*-positive patients.^{7,8} Thus, the true impact of the type of rearrangement on crizotinib efficacy remains unclear and must be evaluated in larger cohorts. However, it seems that DNA sequencing positivity may be more predictive for response to crizotinib treatment than FISH alone. We found co-occurring genetic aberrations in 61% of patients. These had a nonsignificantly shorter median PFS, which most probably was caused by a significantly shorter median PFS in *TP53*-mutant patients. However, these results must be interpreted with caution due to the low patient numbers. The impact of *TP53* on the OS of NSCLC patients with targetable aberrations was investigated retrospectively in several studies but has not been analyzed in *ROS1*-positive patients or prospectively for the efficacy of crizotinib.²³ Just recently, a retrospective study found a significantly shorter PFS and OS in *ALK*-positive patients with co-occurring *TP53* mutations treated with *ALK* inhibitors.²⁴ The negative impact of *TP53* mutations in these reports and in our study may be caused by a higher genomic instability of *TP53*-mutant tumors as shown for *ALK*-rearranged NSCLC.²⁵ Still, efficacy of crizotinib in this subgroup is high and patients should not be excluded from treatment.

Safety and toxicity profiles of crizotinib were similar to previous study reports in *ROS1*- and *ALK*-positive

NSCLC in most aspects.^{13-16,26} However, we observed a higher rate of dose reductions than in prior trials with crizotinib (15.7% to 21%).^{13-16,26} Most dose reductions were performed in accordance with the recommendations given in the trial protocol (29.4%; n = 10). But, dose reductions in six patients (17.7%) were based on the investigator's decision only. Another aspect is that recommendations for the management of bradycardia were strict in the EUCROSS protocol. Dose reductions in patients with grade 2 bradycardia were recommended if no other reason such as co-medication triggered bradycardia. We also suspect that the high experience of the investigators and the prior reports on efficacy of crizotinib have encouraged dose reductions. Interestingly, the prevalence of sinus bradycardia (heart rate <60 beats/min; 16 [47%]) reported in this study was higher than in other trials. Two studies retrospectively investigating the occurrence of bradycardia in several PROFILE trials found that 42% to 69% of patients had at least one episode of bradycardia.^{27,28} Therefore, we suspect that sinus bradycardia might have been underreported in prior trials.

Mean global HRQoL as well as several functioning scores improved throughout the treatment with crizotinib. Also, mean coughing, dyspnea and chest pain scores tended to improve over time. However, most scores did not improve significantly. Bias may be introduced due to

missing data, especially at later times. It is plausible to expect that unfavorable score values tend to be preferentially missing at later times; therefore, the mean values observed may tend to be biased towards more favorable values. This possible bias cannot be eliminated entirely by multiple imputation methods.

Resistance towards crizotinib treatment inevitably develops in ROS1-rearranged NSCLC as in other lung cancer entities.^{29,30} But, the molecular mechanisms underlying kinase inhibitor resistance in ROS1-positive NSCLC are not as well understood as in ALK-rearranged or EGFR-mutant lung cancer, where target-specific next-generation inhibitors have already been approved for first-line therapy.^{31,32} A large number of secondary resistance mutations in ROS1 have been characterized and the multikinase inhibitor cabozantinib and the next-generation ROS1/ALK inhibitors repotrectinib and lorlatinib seem to be effective against several of these mutations, including the ROS1 p.L2026M mutation.^{19,32,33} However, none of these inhibitors has been approved so far and crizotinib remains the first-line standard of care in ROS1-rearranged NSCLC. The analysis of plasma from two trial participants collected at progression revealed no molecular mechanism of resistance. In one of these patients, no ROS1 fusion could be detected, arguing for a lack of sensitivity of the cell-free tumor DNA analysis in this patient. However, the hybrid-capture-based sequencing of the tissue of two patients revealed the acquisition of a ROS1 p.L2026M as well as a TP53 p.P278H substitution mutation in one and a PIK3CA p.E545K substitution in another. Currently, PIK3CA inhibitors are under clinical investigation and new inhibitors exhibit a more favorable safety profile than the first generation of drugs.³⁴ The understanding of mechanisms of resistance to crizotinib may enable the successful treatment by next-generation inhibitors targeting secondary resistance mutations or by combinations of inhibitors aiming off-target aberrations.

Conclusions

In summary, EUCROSS confirmed the high efficacy, tolerability, and safety of crizotinib in ROS1-rearranged NSCLC patients. We additionally found that TP53 wild-type patients had a significantly longer median PFS than TP53-mutant patients. Although this trial reached the primary endpoint, new questions have emerged. These relate to the impact of different rearrangement types on crizotinib efficacy, the added diagnostic value of DNA sequencing compared to FISH, and the significance of brain metastases in ROS1-positive lung cancer. Studies with larger patient numbers must investigate on these questions.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at: <https://doi.org/10.1016/j.jtho.2019.03.020>.

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