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ORIGINAL RESEARCH

Overall survival and central nervous system activity of crizotinib in *ROS1*-rearranged lung cancer—final results of the EUCROSS trial

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Background: In 2019, we reported the first efficacy and safety analysis of EUCROSS, a phase II trial investigating crizotinib in *ROS1* fusion-positive lung cancer. At that time, overall survival (OS) was immature and the effect of crizotinib on intracranial disease control remained unclear. Here, we present the final analysis of OS, systemic and intracranial activity, and the impact of co-occurring aberrations.

Materials and methods: EUCROSS was a prospective, single-arm, phase II trial. The primary endpoint was best overall response rate (ORR) using RECIST 1.1. Secondary and exploratory endpoints were progression-free survival (PFS), OS, and efficacy in pre-defined subgroups.

Results: Median OS of the intention-to-treat population ($N = 34$) was 54.8 months [95% confidence interval (CI) 20.3 months-not reached (NR); median follow-up 81.4 months] and median all-cause PFS of the response-evaluable population ($N = 30$) was 19.4 months (95% CI 10.1-32.2 months). Time on treatment was significantly correlated with OS ($R = 0.82$; $P < 0.0001$). Patients with co-occurring *TP53* aberrations (28%) had a significantly shorter OS [hazard ratio (HR) 11; 95% CI 2.0-56.0; $P = 0.006$] and all-cause PFS (HR 4.2; 95% CI 1.2-15; $P = 0.025$). Patients with central nervous system (CNS) involvement at baseline ($N = 6$; 20%) had a numerically shorter median OS and all-cause PFS. Median intracranial PFS was 32.2 months (95% CI 23.7 months-NR) and the rate of isolated CNS progression was 24%.

Conclusions: Our final analysis proves the efficacy of crizotinib in *ROS1*-positive lung cancer, but also highlights the devastating impact of *TP53* mutations on survival and treatment efficacy. Additionally, our data show that CNS disease control is durable and the risk of CNS progression while on crizotinib treatment is low.

Key words: lung cancer, ROS1, crizotinib, overall survival, EUCROSS

INTRODUCTION

Rearrangements involving the proto-oncogene tyrosine-protein kinase ROS gene (*ROS1*) are rare oncogenic events in non-small-cell lung cancer (NSCLC) and define a subset of patients in whom therapy with the ROS1 tyrosine kinase inhibitors (TKIs) crizotinib and entrectinib is the standard of care in many countries in the first line of treatment.[1,2]

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However, not all patients benefit from ROS1 inhibition to the same extent.[3-5]

Several analyses have shown that co-occurring genetic aberration have an impact on the efficacy of targeted treatments and survival of cancer patients. Most notably, mutations that impair TP53 activity have been associated with shorter survival in retrospective analyses of lung cancer patients whose tumors harbor epidermal growth factor receptor (EGFR) mutations or anaplastic lymphoma kinase (ALK) fusions.[6,7] However, data on the impact in *ROS1* fusion-positive patients are limited, and more data are needed to assess the relevance of *TP53* mutations in these patients.

Similarly, central nervous system (CNS) metastases may be associated with shorter survival in *ALK* and *ROS1* rearrangement-positive NSCLC, and CNS progression is common in these patients.[8-11] Since the CNS penetration of crizotinib is low, its effect on CNS control is a matter of debate.[12] Therefore, a better understanding of the CNS activity of crizotinib is necessary to guide treatment decisions. This is even more important since new drugs such as lorlatinib or repotrectinib seem to be highly effective in CNS disease.[13,14]

In 2019, we reported a median progression-free survival (PFS) of 20.0 months for *ROS1* fusion-positive patients treated with crizotinib in the EUCROSS trial.[5] These data were in concordance with those of three other trials that investigated crizotinib in this population.[3,4,15] However, the median overall survival (OS) was not reached at that time in EUCROSS. We now report an updated analysis of the OS, systemic and CNS-specific activity, and the impact of co-occurring genetic aberrations of the EUCROSS patients.

MATERIALS AND METHODS

Study design and procedures

EUCROSS is an international, single-arm phase II trial conducted by the Lung Cancer Group Cologne and the Spanish Lung Cancer Group. A detailed description of the study design has been published previously.[5] Among others, the following key eligibility criteria were applied for patient enrollment: 18 years of age or older, locally advanced or metastatic NSCLC, *ROS1* fusion confirmed by central Zyto-Light SPEC dual color break-apart FISH (ZytoVision, Bremerhaven, Germany), ROS1 TKI-naïve, Eastern Cooperative Oncology Group performance status of 0 to 2, and measurable disease according to the RECIST version 1.1.[16] Patients with solid CNS metastases were eligible if they were asymptomatic and did not receive increasing doses of steroids. Patients received crizotinib at an initial dose of 250 mg twice daily until disease progression or unacceptable toxicity, among others. Owing to the market approval of crizotinib, treatment within the study was terminated in July 2018 and all ongoing patients received crizotinib outside of the trial.

Computed tomography scans were scheduled every 6 weeks for the first 6 months, every 8 weeks for the following 6 months, and every 12 weeks thereafter. Magnetic resonance imaging (MRI) of the brain was mandatory at baseline. Only patients with CNS metastases were followed up by MRI, in line with the aforementioned schedule. The other patients received brain MRIs at the investigators' discretion and according to the local practice. All efficacy assessments were carried out locally until the end of treatment within the study. Radiologic scans were additionally collected for an independent radiologic review (IRR) until the median PFS was reached (January 2017).

All patients who received at least one dose of crizotinib defined the intention-to-treat population (ITT), and those who met all eligibility criteria and had an adequate baseline efficacy assessment defined the response-evaluable population.

Adverse events (AEs) were collected from all patients who received at least one dose of crizotinib. AEs were assessed according to the Medical Dictionary for Regulatory Activities (MedDRA).

Central targeted hybrid-capture-based massively parallel DNA sequencing covering 39 genes was carried out if feasible to assess molecular determinants of response and progression using pre-treatment samples (NEOplus, NEO New Oncology AG, Cologne, Germany).[17] Target genes: *ALK, ARAF, ATM, ATR, BRAF, BRCA1, BRCA2, CDK4, CDKN2A, CDKN2B, CTNNB1, DDR2, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, HRAS, IDH1, IDH2, KEAP1, KIT, KRAS, MAP2K1, MDM2, MET, MTOR, NFE2L2, NRAS, PDGFRA, PIK3CA, PTEN, RB1, RET, ROS1, STK11, TP53, TSC1, TSC2*. Cell-free blood DNA sequencing was carried out using a targeted next-generation sequencing (NGS) assay at disease progression (NEOliquid, NEO New Oncology AG, Cologne, Germany).[17]

The trial was registered at ClinicalTrials.gov with the identifier NCT02183870 and at the European Union Drug Regulating Authorities Clinical Trials (EudraCT) registry with the identifier 2013-002737-38.

A summary of timelines, data cut-offs, and milestones can be found in Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2024.102237.

Statistical analyses were carried out on SAS version 9.4 and R version 4.2.2.

Time-to-event analyses

All time-to-event analyses were carried out using the Kaplan—Meier estimator. Time-to-event endpoints were defined as the time interval from the first day of treatment within the trial to the observed event or censoring.

After the end of the study (January 2020), OS follow-up (FU) was carried out within the follow-up registry of the Spanish Lung Cancer Group and the national Network Genomic Medicine (nNGM). All patients gave their written informed consent.

Analysis of CNS efficacy

Imaging data, including brain scans, were prospectively collected for IRR until January 2017. Brain MRIs were reviewed centrally according to RECIST 1.1 to assess the intracranial overall response rate (icORR) and intracranial

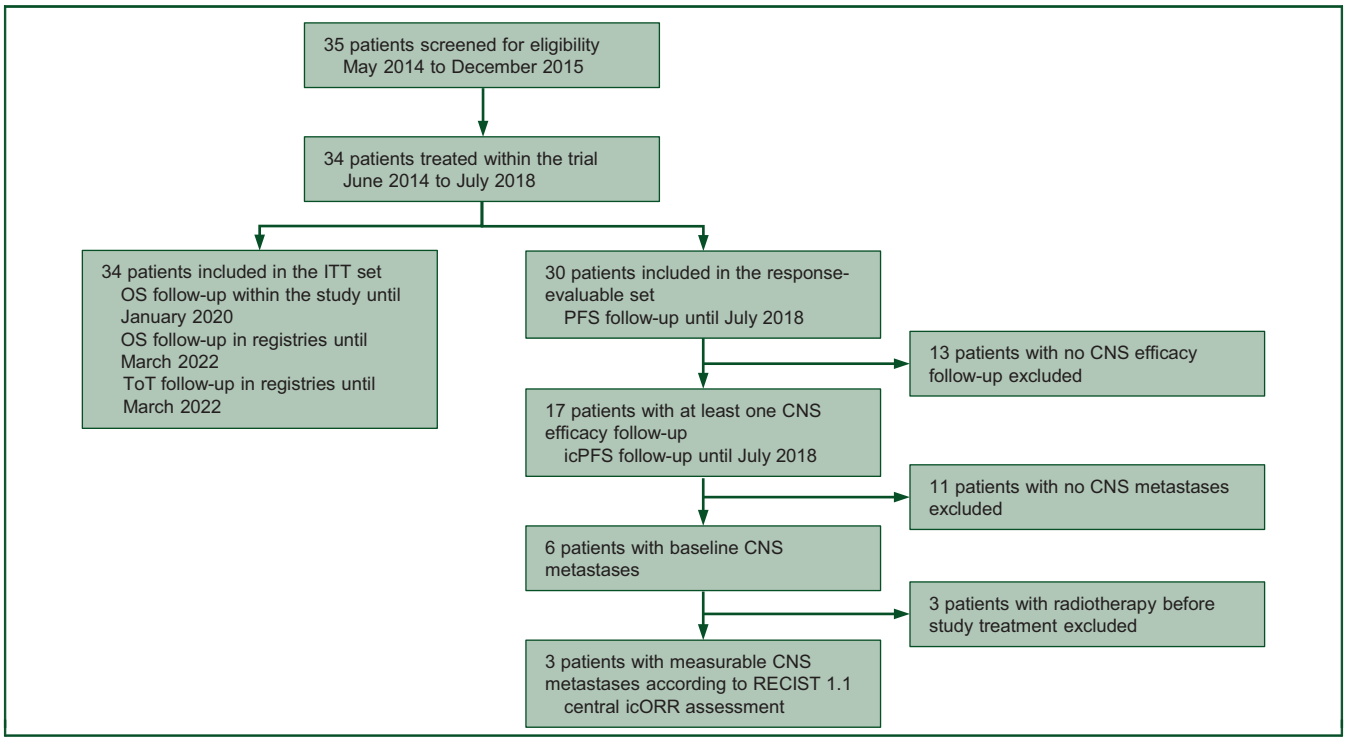

Figure 1. Analysis set flow chart.
CNS, central nervous system; icPFS, intracranial progression-free survival; ITT, intention-to-treat; OS, overall survival; PFS, progression-free survival.

progression-free survival (icPFS). IcPFS events were defined as intracranial progression, including new lesions and non-target progression, or death.

RESULTS

OS and systemic efficacy

Between June 2014 and December 2015, 34 patients were enrolled in the trial (ITT). Thirty patients were included in the response-evaluable population (Figure 1). The baseline patient demographics are outlined in the supplement (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2024.102237).

The median FU for OS of the ITT was 81.4 months [95% confidence interval (CI) 78.7-87.2 months]. At the data cut-off for OS, 17 (50%) patients died, and the median OS of the ITT was 54.8 months [95% CI 20.3 months-not reached (NR); Figure 2; Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2024.102237].

Median time-on-treatment (ToT) with crizotinib, including the period of treatment after the end of the trial, was 22.8 months (95% CI 15.9-63.7 months; median FU 81.4 months; 95% CI 78.7-87.2 months; Figure 2). OS significantly correlated with ToT ($R = 0.82$; $P < 0.0001$; Figure 2).

With 41.1 months (95% CI 34.9 months-NR), the median FU for investigator-assessed PFS was shorter than the median FU for OS or ToT, due to the earlier data cut-off (Figure 1; Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2024.102237). The median investigator-assessed PFS was 19.4 months (95% CI 10.1-32.2 months; Figure 2). The IRR PFS has been published previously.[5] Within the FU period for the investigator-assessed PFS, 10 out of 20 patients (50%) who experienced progression continued crizotinib treatment due to ongoing clinical benefit.

The ORR was unchanged with longer FU and was 70% (95% CI 50.6% to 85.3%; $N = 21$) in the response-evaluable population (Figure 2; Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2024.102237).

Impact of co-occurring aberrations and patterns of resistance

To determine molecular markers of response and progression, targeted DNA NGS was carried out in 20 patients with sufficient tumor tissue. *ROS1* fusions were confirmed in 18 samples. Two samples tested negative for *ROS1* fusions using direct DNA sequencing. *CD74::ROS1* fusions were most commonly detected ($N = 9$; 50%), followed by *EZR::ROS1* and *SLC34A2::ROS1* ($N = 3$; 16.7% each; Figure 3). The specific type of *ROS1* fusion had no effect on OS or PFS (data not shown). In total, 61% ($N = 11$ of 18) of the samples sequenced positive for *ROS1* fusions, and 55% ($N = 11$ of 20) of all samples that were sequenced harbored co-occurring genetic aberrations (Figure 3). Most notably, 27.8% ($N = 5$ of 18) of the *ROS1* fusion-positive samples were also positive for *TP53* mutations. Four of these were predicted to be deleterious (i.e. truncating or affecting splicing) and one resulted in a missense amino acid exchange.

OS in the patients sequenced positive for *ROS1* fusions was similar to the OS in those not amenable to sequencing (HR 0.98; 95% CI 0.38-2.5; $P = 0.96$). Patients with *TP53*

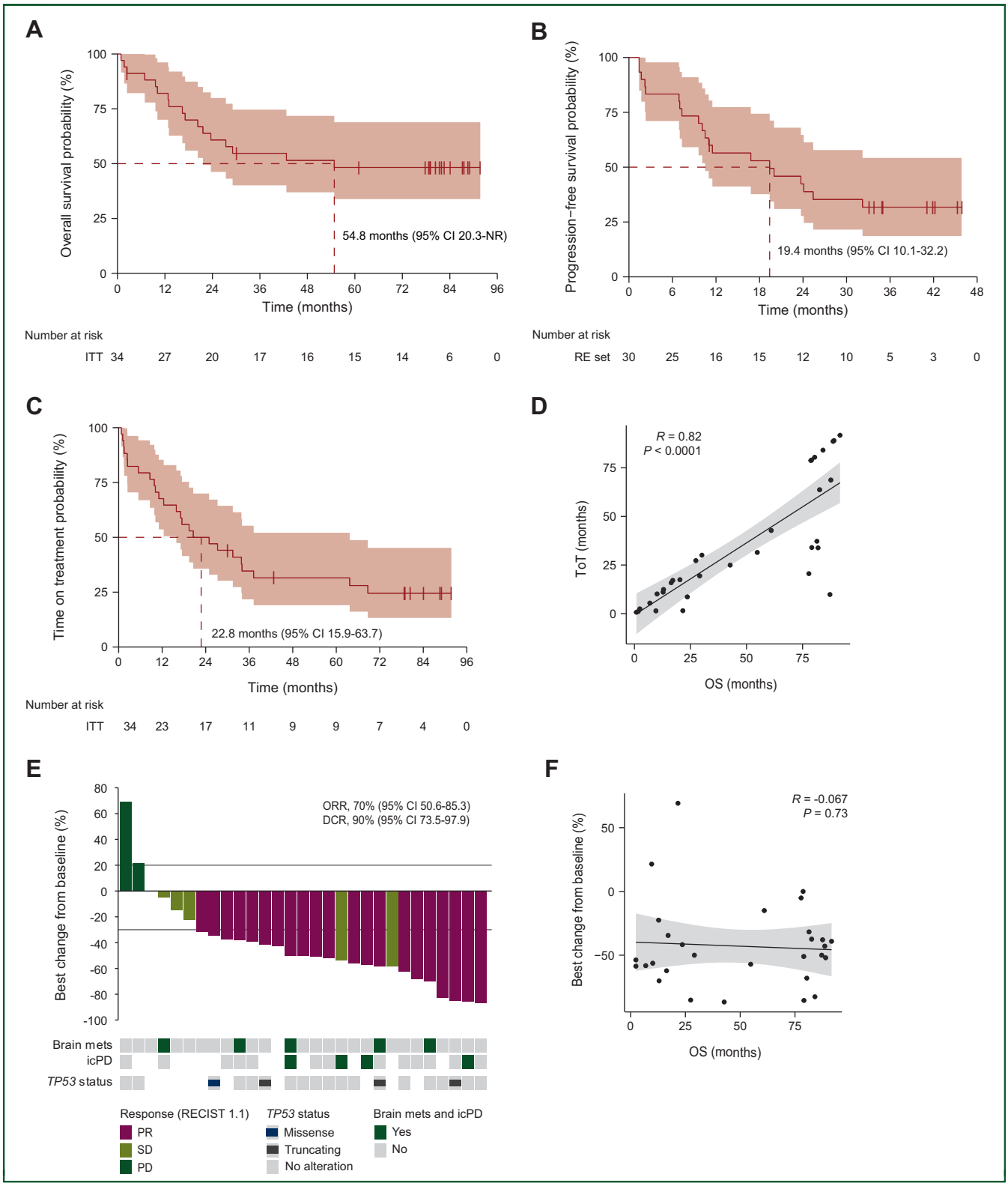

Figure 2. Survival outcomes and treatment efficacy.
(A) OS of the ITT ($N = 34$), (B) all-cause PFS of the response-evaluable population, and (C) ToT of the ITT including time on treatment outside of the trial. (D) Correlation between ToT and OS. (E) Waterfall plot of best change from baseline according to RECIST 1.1 of the response-evaluable population ($N = 30$) and (F) correlation between best change from baseline and OS.
CI, confidence interval; DCR, disease control rate; icPD, intracranial progressive disease; ITT, intention-to-treat; NR, not reached; ORR, overall response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; RE, response-evaluable; SD, stable disease; ToT, time-on-treatment.

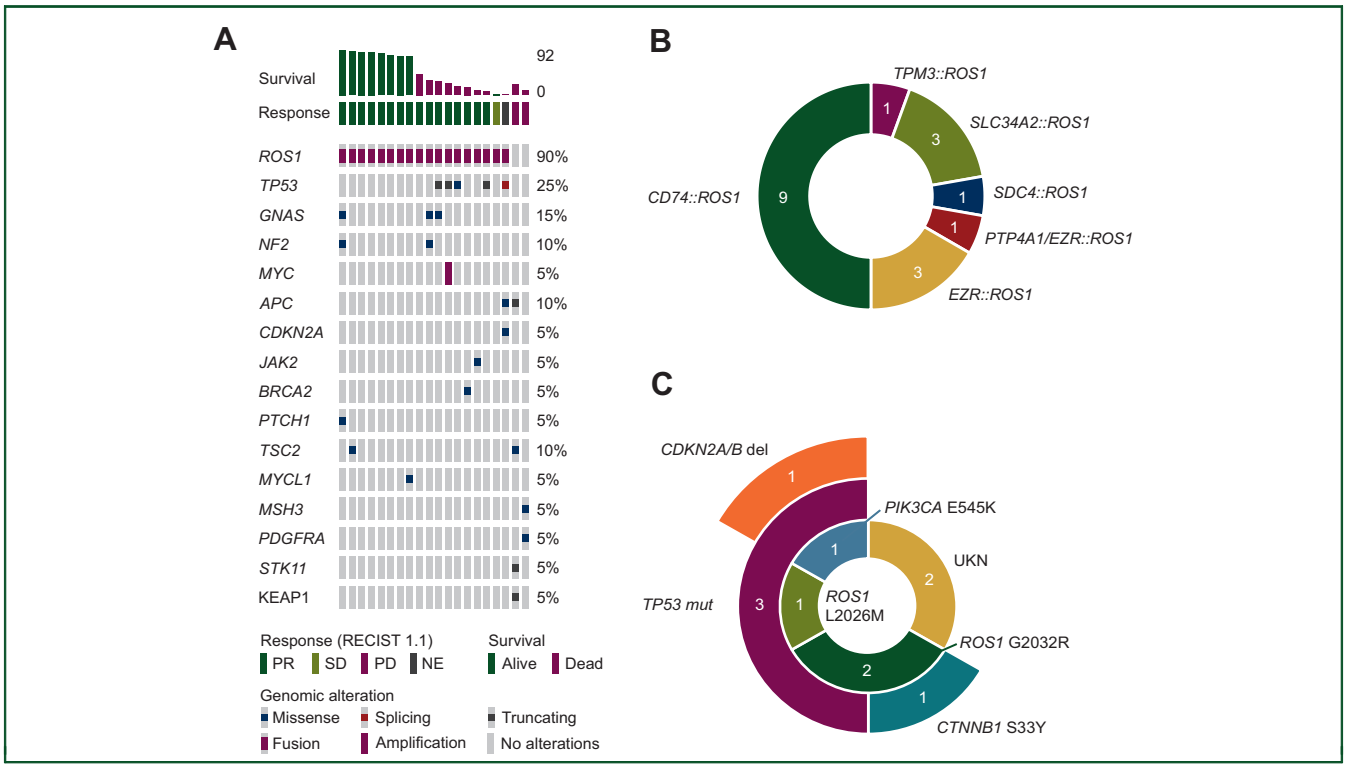

Figure 3. Genomic characteristics.
(A) Depiction of co-occurring genetic aberrations, OS, and RECIST-assessed response sorted by the duration of OS. (B) Distribution of ROS1 fusion type in the 18 *ROS1*-positive patients by DNA NGS. (C) Results of DNA NGS of blood or tissue samples collected at progression.
NE, not evaluable; NGS, next-generation sequencing; OS, overall survival; PD, progressive disease; PR, partial response; SD, stable disease.

alterations ($N = 5$; 28%) had a significantly shorter OS and PFS than patients without *TP53* alterations ($N = 13$; 72%; HR for OS, 11; 95% CI 2.0-56.0; $P = 0.006$ and HR for PFS, 4.2; 95% CI 1.2-15; $P = 0.025$; Figure 4). The median OS was 17.1 months (95% CI 1.7 months-NR) for patients with co-occurring *TP53* alterations and not reached (95% CI 16.4 months-NR) for those without *TP53* alterations. The median PFS was 7.0 months (95% CI 6.9 months-NR) for patients with *TP53* alterations and 32.3 months (95% CI 11.0 months-NR) for patients without *TP53* alterations. Other co-occurring aberrations were not sufficiently enriched to draw any additional statistically valid conclusions. However, we found that the patients with alterations in *BRCA2*, *MYC*, *APC*, *CDKN2A*, and *JAK2* had a shorter PFS and OS than the median (Supplementary Table S5, available at https://doi.org/10.1016/j.esmoop.2024.102237). Patients with alterations in *GNAS* and *NF2* had either a shorter or a longer PFS and OS than the median (Supplementary Table S5, available at https://doi.org/10.1016/j.esmoop.2024.102237). In the 18 patients with confirmed *ROS1* fusion by sequencing, the ORR was 88.9% (95% CI 65.3% to 98.9%; Figure 4; Supplementary Table S6, available at https://doi.org/10.1016/j.esmoop.2024.102237). The two patients who were tested negative for *ROS1* fusions by direct DNA sequencing had progressive disease (PD) at the first staging. Depth of response was not correlated with *TP53* mutation status (Figure 4).

Blood ($N = 3$) and tissue samples ($N = 5$) were collected from eight patients at progression to crizotinib. DNA NGS was not feasible in one blood and one tissue sample because of the low DNA quality of the samples. Potential mechanisms of resistance were identified in four patients, including the secondary *ROS1* mutations G2032R ($N = 2$) and L2026M ($N = 1$), as well as the *PIK3CA* substitution E545K ($N = 1$; Figure 3). In two samples, no mechanism of resistance could be detected.

CNS activity

The prevalence of brain metastases at baseline was 20% ($N = 6$) in the response-evaluable population and 21% ($N = 7$) in the ITT group (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2024.102237). Patients with baseline CNS disease had a numerically higher risk of all-cause disease progression (HR 1.8; 95% CI 0.65-5.0; $P = 0.257$) and death (HR 2.3; 95% CI 0.81-5.5; $P = 0.177$; Figure 5). The median OS of patients with brain metastases in the ITT was 13.0 months (95% CI 1.7 months-NR), while the median OS of patients without brain metastases was not reached (95% CI 21.6 months-NR). The median PFS of patients with brain metastases was 9.4 months (95% CI 6.9 months-NR) and that of patients without brain metastases was 23.7 months (95% CI 10.5 months-NR). Seventeen patients who had at least one MRI follow-up scan were

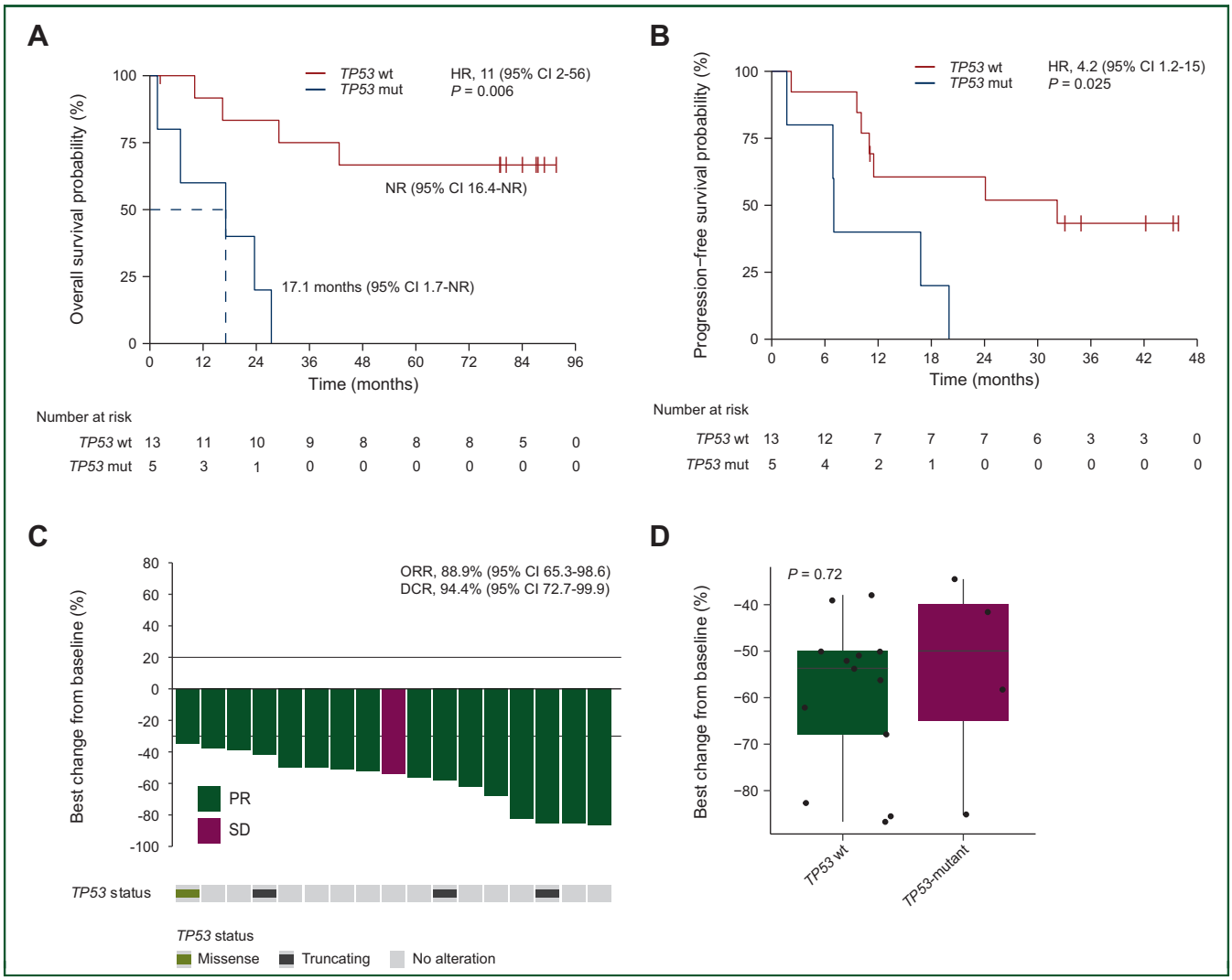

Figure 4. Survival outcomes and treatment efficacy by genomic subgroup.
(A) OS and (B) PFS stratified by *TP53* status in patients with *ROS1* fusion confirmed by DNA sequencing ($N = 18$). (C) Waterfall plot of radiographic response according to RECIST 1.1 in patients with *ROS1* fusion confirmed by DNA sequencing ($N = 18$) and (D) mean best response from baseline stratified by *TP53* mutation status.
CI, confidence interval; DCR, disease control rate; HR, hazard ratio; mut, mutant; NR, not reached; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PR, partial response; SD, stable disease; wt, wild-type.

assessed for icPFS analysis. Six (35%) of these patients had CNS progression. Median icPFS was 32.2 months (95% CI 23.7 months-NR) and notably longer than the 19.4 months of all-cause PFS (Figure 5). Within the group of patients followed for icPFS, more patients had all-cause PD ($N = 7$; 41%) than isolated intracranial progressive disease ($N = 4$; 24%; Figure 5).

Three patients with brain metastases had radiotherapy before the study treatment and were excluded from the icORR assessment (Figure 1). Three patients had CNS metastases that were evaluable as target lesions using RECIST 1.1. All three patients had stable disease (SD) as the best intracranial response.

Safety and tolerability

All patients who received at least one dose of crizotinib were included in the safety and tolerability analyses ($N = 34$; ITT). Thirty-three of 34 patients (97%) experienced at least one treatment-related AE of any grade. Thirty-two patients had at least one treatment-related grade 1 AE (94%), 24 had a treatment-related grade 2 AE (70%), 11 had a treatment-related grade 3 AE (32%), and one had a treatment-related grade 5 AE (3%). The latter case was a fatal pulmonary embolism that occurred shortly after the initiation of crizotinib treatment. The most common treatment-related AEs (>20% prevalence) were visual disturbances ($N = 23$; 68%), diarrhea ($N = 19$; 56%), edema ($N = 18$; 53%), bradycardia ($N = 16$; 47%), nausea ($N = 15$; 44%), increased alanine aminotransferase ($N = 12$; 35%), decreased leukocyte and neutrophil count ($N = 11$; 32%), vomiting ($N = 11$; 32%), increased aspartate aminotransferase ($N = 9$; 26%), and increased blood creatinine levels ($N = 9$; 26%) (Supplementary Table S7, available at https://doi.org/10.1016/j.esmoop.2024.102237). A line listing of all AEs is provided in Supplementary Table S8, available at

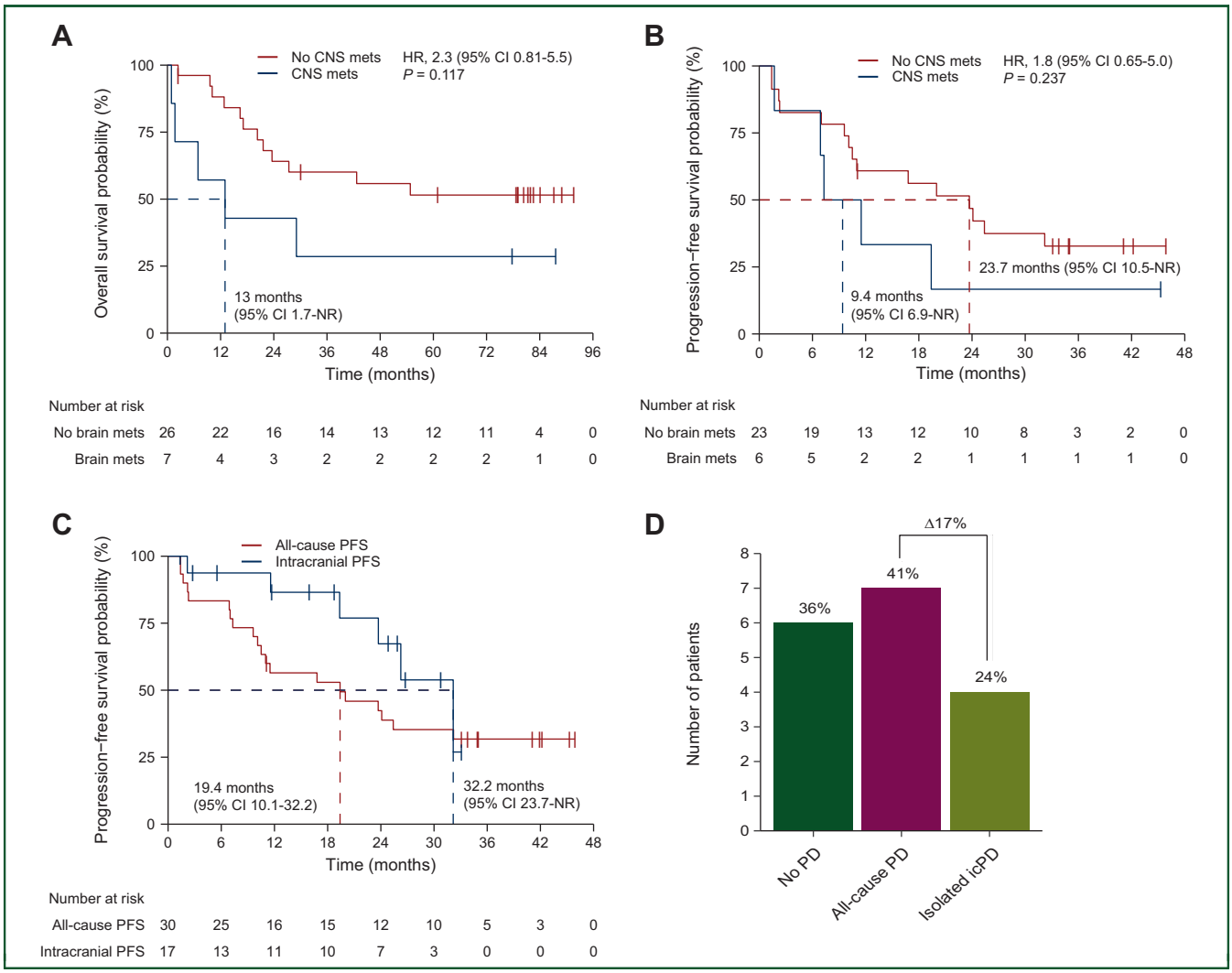

Figure 5. Intracranial survival outcomes and efficacy.
(A) OS of the ITT ($N = 34$) and (B) the response-evaluable set stratified by the status of baseline CNS disease. (C) All-cause PFS of the response-evaluable population (including CNS progression) and intracranial PFS of the patient followed for CNS progression. (D) Cumulative numbers and frequency of all-cause PD (including synchronous systemic and icPD) and isolated icPD.
CI, confidence interval; CNS, central nervous system; HR, hazard ratio; icPD, intracranial progressive disease; ITT, intention-to-treat; NR, not reached; OS, overall survival; PD, progressive disease; PFS, progression-free survival.

https://doi.org/10.1016/j.esmoop.2024.102237. In summary, the safety, tolerability, and AE profiles were in line with the published study data.[5] No new safety or toxicity signals were observed.

DISCUSSION

Our final OS analysis and updated PFS data confirm the efficacy of crizotinib in patients with *ROS1* fusion-positive NSCLC and our data are strikingly consistent with the data reported for the PROFILE 1001 study by Shaw and colleagues.[3] More precisely, medians for OS were 54.8 months in EUCROSS and 51.4 months in PROFILE 1001, and medians for PFS were 19.4 months and 19.3 months, respectively. Two other phase II trials, one in East Asia and one in Italy, reported similar survival rates, underlining the high level of evidence for the use of crizotinib in *ROS1*-positive NSCLC.[4,15,18]

Targeted treatments have improved OS in subgroups such as *ALK*- or *EGFR*-positive NSCLC, and median survival times of up to 50 months have been frequently reported in randomized clinical trials and retrospective studies.[2,8] However, the impact of crizotinib on the OS of *ROS1*-positive NSCLC remains unknown. Thus, we assessed whether the duration of crizotinib treatment had an impact on OS and showed that a longer ToT significantly correlated with a longer OS, suggesting that the inter-individual efficacy of crizotinib affects the prognosis of patients and that crizotinib treatment may prolong survival.

Next, we focused our analyses on factors that might have an impact on efficacy and survival rates such as the profile of co-occurring genomic aberrations and the status of CNS involvement.

TP53 mutations have been associated with worse outcomes in *ALK* fusion-positive and *EGFR*-mutant NSCLC.[6,7] We previously showed that patients with *ROS1*-positive

and *TP53*-mutant disease had significantly shorter PFS rates than those with wild-type *TP53* disease.[5] However, the effect of *TP53* mutations on the survival of patients remains unclear. With adequate OS FU, we now found that co-occurring alterations in *TP53* were associated with significantly shorter survival. This finding further supports the hypothesis that the impact of *TP53* on treatment outcomes may be universal and independent of the underlying driver oncogene. Due to the low number of patients, we were unable to assess the impact of other co-occurring mutations on the efficacy of crizotinib and the survival of patients. Larger datasets are required to further investigate this. The improvement of treatments for lung cancer patients with co-occurring *TP53* mutations or other aberrations is an unmet medical need, which requires to be specifically addressed in future clinical studies.

Crizotinib poorly penetrates the blood—brain—barrier, resulting in lower efficacy against brain metastases as compared to newer ROS1 or ALK inhibitors such as entrectinib, alectinib, lorlatinib, or repotrectinib.[11,13,19] Additionally, the CNS activity of next-generation ALK inhibitors dramatically improves survival times in *ALK*-positive NSCLC, partly due to the delay of CNS disease.[8,11] Data on the significance of brain metastases in treatment-naïve *ROS1*-positive NSCLC are conflicting. However, retrospective analyses determined a relatively low prevalence of brain metastases in *ROS1*-positive NSCLC, also compared to *ALK*-positive cases (e.g. 19.4% versus 39.1%), suggesting an overall lower risk of brain metastases in *ROS1*-positive lung cancer.[10,20,21] That in mind, the use of brain-penetrant inhibitors may not have the same benefit in *ROS1*-positive NSCLC as in *ALK*-positive lung cancer. In concordance with this, the all-cause PFS rates of CNS-penetrant entrectinib or lorlatinib and non-penetrant crizotinib were similar in different prospective studies.[1,4,14,19] In our analyses, the icPFS was longer than the all-cause PFS, and the rate of isolated CNS progression was lower than that of systemic progression. Objective response rates of CNS metastases in *ROS1* fusion-positive lung cancer patients vary significantly between different drugs, including entrectinib and lorlatinib. However, data for crizotinib are restricted to small samples, including the assessable three patients in our analysis who did not receive prior CNS radiation. Except for one case of complete response, patients in the Italian METROS study ($N = 4$) and our trial ($N = 3$) all had SD as best response.[15] Although this strongly argues in favor of a low efficacy of crizotinib on brain metastases, the data need to be interpreted with caution, due to the low patient numbers. With an intracranial ORR of 19%, the efficacy of entrectinib on brain metastases seems to be marginally higher.[19] Compared to this, lorlatinib yielded a promising CNS response rate of 64% in the same clinical setting.[14] All these, especially the similar all-cause PFS rates between *ROS1* inhibitors with different CNS activity, raise doubts about whether the use of brain-penetrant inhibitors is necessarily warranted in *ROS1* fusion-positive lung cancer. However, it remains to be elucidated whether newer inhibitors with higher CNS penetrance and more potent inhibition of *ROS1* have a greater impact on efficacy and survival. Taken together, more data are needed to assess the value of CNS-penetrant inhibitors in patients with *ROS1* fusion-positive disease.

In addition to these subgroup analyses, we carried out DNA NGS on samples collected from six patients at PD. Although the number of samples was limited, the resistance pattern identified was in concordance with the patterns published for crizotinib thus far and points out the need for the approval of next-generation *ROS1* inhibitors that target secondary *ROS1* mutations.[13]

In view of this work, some limitations must be taken into consideration. Firstly, the overall number of enrolled patients was calculated based on the primary endpoint of ORR. Thus, the results for time-to-event endpoints must be interpreted with caution, and the lack of significance in some analyses may be attributed to the small patient numbers. Secondly, as the treatment within the trial was terminated earlier owing to the market approval of crizotinib, no pre-specified treatment data were collected thereafter, and efficacy analyses were not carried out.

Taken together, our analyses confirm the high efficacy of crizotinib in *ROS1*-positive NSCLC and give new insights into the efficacy in patients with CNS metastases. Additionally, our data provide the first prospective evidence for the negative impact of *TP53* mutations on the prognosis of *ROS1*-positive NSCLC.

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DISCLOSURES

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