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ORIGINAL RESEARCH

Ramucirumab plus erlotinib versus placebo plus erlotinib in previously untreated *EGFR*-mutated metastatic non-small-cell lung cancer (RELAY): exploratory analysis of next-generation sequencing results

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Background: Ramucirumab plus erlotinib (RAM + ERL) demonstrated superior progression-free survival (PFS) over placebo + ERL (PBO + ERL) in the phase III RELAY study of patients with epidermal growth factor receptor (EGFR)-mutated metastatic non-small-cell lung cancer (EGFR+ mNSCLC; NCT02411448). Next-generation sequencing (NGS) was used to identify clinically relevant alterations in circulating tumor DNA (ctDNA) and explore their impact on treatment outcomes.

Patients and methods: Eligible patients with EGFR+ mNSCLC were randomized 1 : 1 to ERL (150 mg/day) plus RAM (10 mg/kg)/PBO every 2 weeks. Liquid biopsies were to be prospectively collected at baseline, cycle 4 (C4), and postdiscontinuation follow-up. *EGFR* and co-occurring/treatment-emergent (TE) genomic alterations in ctDNA were analyzed using Guardant360 NGS platform.

Results: In those with valid baseline samples, detectable activating *EGFR* alterations in ctDNA (a*EGFR*+) were associated with shorter PFS [a*EGFR*+: 12.7 months (n = 255) versus a*EGFR*−: 22.0 months (n = 131); hazard ratio (HR) = 1.87, 95% confidence interval (CI) 1.42-2.51]. Irrespective of detectable/undetectable baseline a*EGFR*, RAM + ERL was associated with longer PFS versus PBO + ERL [a*EGFR*+: median PFS (mPFS) = 15.2 versus 11.1 months, HR = 0.63, 95% CI 0.46-0.85; a*EGFR*−: mPFS = 22.1 versus 19.2 months, HR = 0.80, 95% CI 0.49-1.30]. Baseline alterations co-occurring with a*EGFR* were identified in 69 genes, most commonly *TP53* (43%), *EGFR* (other than a*EGFR*; 25%), and *PIK3CA* (10%). PFS was longer in RAM + ERL, irrespective of baseline co-occurring alterations. Clearance of baseline a*EGFR* by C4 was associated with longer PFS (mPFS = 14.1 versus 7.0 months, HR = 0.481, 95% CI 0.33-0.71). RAM + ERL improved PFS outcomes, irrespective of a*EGFR* mutation clearance. TE gene alterations were most commonly in *EGFR* [T790M (29%), other (19%)] and *TP53* (16%).

Conclusions: Baseline a*EGFR* alterations in ctDNA were associated with shorter mPFS. RAM + ERL was associated with improved PFS outcomes, irrespective of detectable/undetectable a*EGFR*, co-occurring baseline alterations, or a*EGFR*+ clearance by C4. a*EGFR*+ clearance by C4 was associated with improved PFS outcomes. Monitoring co-occurring alterations and a*EGFR*+ clearance may provide insights into mechanisms of EGFR tyrosine kinase inhibitor resistance and the patients who may benefit from intensified treatment schedules.

Key words: NSCLC, EGFR, ramucirumab, erlotinib, next-generation sequencing, biomarkers

INTRODUCTION

Non-small-cell lung cancer (NSCLC) accounts for ∼85% of primary lung cancers worldwide. Most patients present with advanced or metastatic NSCLC (mNSCLC) at diagnosis.[1-3] Epidermal growth factor receptor (*EGFR*) mutations are important drivers of NSCLC, occurring in ∼40% of Asian and 10%-20% of white patients with NSCLC.[4]

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Activating *EGFR* (a*EGFR*) mutations, either an exon 19 deletion (ex19del) or a leucine to arginine substitution in exon 21 (ex21.L858R), account for the majority of *EGFR* mutations in patients with mNSCLC.[3,5,6]

a*EGFR*-mutated (a*EGFR*+) NSCLC is associated with sensitivity to EGFR tyrosine kinase inhibitors (EGFR-TKIs).[6,7] National Comprehensive Cancer Network (NCCN) guidelines for *EGFR*+ NSCLC recommend EGFR-TKI monotherapy or combined with ramucirumab (RAM) or bevacizumab (BEV).[8] *EGFR*+ NSCLC is a heterogenous disease with a high prevalence of co-occurring gene alterations,[9-11] which have been identified as a negative prognostic factor associated with poorer treatment outcomes with EGFR-TKI.[9,11]

Despite an initial response to EGFR-TKIs, most patients with *EGFR* mutations develop resistance within 1-2 years.[12,13] Factors contributing to progression and relapse include treatment-emergent (TE) gene alterations that confer resistance. Approximately 50% of patients whose disease progresses on a first- or second-generation EGFR-TKI acquire the *EGFR* T790M resistance mutation.[14-17] *EGFR* T790M confers sensitivity to third-generation TKI, osimertinib (OSI),[18] highlighting the need to better understand TE gene alterations. To identify emerging, potentially targetable, resistance mutations, a rebiopsy at progression is recommended.[19] Repeat biopsies, however, pose challenges and provide limited understanding of tumor heterogeneity and resistance mechanisms.[20] Use of circulating tumor-derived cell-free DNA detected (ctDNA) may help to overcome such issues. ctDNA analysis is established for dynamic monitoring of TE gene alterations[20-23] and clearance of *EGFR* mutations in ctDNA in response to first-line TKI treatment is reported to predict better treatment outcomes.[20,21,24-27]

The RELAY study assessed the safety and efficacy of RAM, a human vascular endothelial growth factor receptor 2 (VEGFR2) antagonist, combined with erlotinib (ERL), an EGFR TKI, in patients with untreated *EGFR*+ mNSCLC. RAM + ERL demonstrated superior clinical outcomes compared with placebo plus ERL (PBO + ERL).[28]

Next-generation sequencing (NGS) technology was used to identify clinically relevant genomic alterations in ctDNA from patients in RELAY treated with RAM + ERL or PBO + ERL. We report NGS results on alterations co-occurring with a*EGFR* at baseline, a*EGFR* mutation clearance during treatment, TE alterations, and associated clinical outcomes aiming to identify predictors of response and potential mechanisms of resistance.

PATIENTS AND METHODS

Study design

The RELAY study design and eligibility criteria are published elsewhere.[28] In brief, RELAY is an ongoing, global, phase III study of RAM + ERL or PBO + ERL in patients with previously untreated, *EGFR*+ mNSCLC.[28] Eligible patients had stage IV mNSCLC with an a*EGFR* mutation (ex19del/ex21.L858R) by local laboratory testing; Eastern Cooperative Oncology Group Performance Status (ECOG PS) 0-1; no central nervous system (CNS) metastasis nor T790M mutation at study entry. Randomization was stratified by *EGFR* mutation (ex19del/ex21.L858R), local *EGFR* testing method [Therascreen (Qiagen, Holden, Germany)/Cobas (Roche, Risch-Rotkreuz, Switzerland) versus other PCR/sequencing-based methods], sex (male/female), and region (East Asia/other). Randomized patients (1 : 1) received intravenous RAM (10 mg/kg) or matching PBO every 2 weeks combined with daily ERL (150 mg oral). Study treatment continued until radiographic progression as assessed by the investigator according to RECIST version 1.1, unacceptable toxicity, withdrawal of consent, noncompliance, or investigator decision. The study adhered to the Declaration of Helsinki, International Conference on Harmonization Guidelines for Good Clinical Practice, and applicable local regulations. All patients provided written informed consent. RELAY is registered at ClinicalTrials.gov (NCT02411448).

Assessments

Tumor assessments were conducted at baseline, then every 6 weeks for 72 weeks, followed by every 12 weeks thereafter until progression or unacceptable toxicity, and at the 30-day follow-up visit. Adverse events were assessed every cycle and graded as previously described.[28] Liquid biopsies were prospectively collected at baseline, cycle 4 (C4), and postprogression, and analyzed with Guardant360 NGS platform (Guardant Health, Redwood City, CA),[29] which evaluates 73 cancer-related genes.

Study endpoints and analysis populations

The primary endpoint was progression-free survival (PFS). Secondary endpoints included objective response rate, duration of response, overall survival (OS), safety, and tolerability. Prespecified NGS biomarker analyses were exploratory.

Efficacy analyses were carried out in the population of randomized patients [intent-to-treat (ITT) population].

NGS analyses were carried out on the following populations (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2023.101580) to identify genomic alterations in ctDNA. The translational research (TR) population consisted of patients who had a valid ctDNA sample (passed NGS testing QC) and who had one or more detectable baseline genetic alteration. ctDNA was analyzed during treatment (C4, day 1) to identify clearance of alterations in ctDNA detectable by NGS and postprogression to identify clearance or emergence of gene alterations.

To examine TE alterations following progression, the TR population was further subdivided into two populations of patients who had disease progression prior to poststudy treatment discontinuation visit: TE population 1 (TEpop1) consisted of patients who had one or more detectable genetic alterations by NGS at both baseline and postprogression; TE population 2 (TEpop2) consisted of patients who specifically had a detectable a*EGFR* alteration at baseline and postprogression. Although population selection criteria did not restrict alteration type (to germline/somatic),

all analyses of Guardant360 (Guardant Health) NGS results were restricted to somatic mutations.[30-32]

Statistical analyses

RELAY was not powered for subgroup analyses and no adjustments were made for testing multiple comparisons; therefore results of subgroup analyses should be interpreted with caution, including comparisons within Guardant360 NGS TR populations.

Hazard ratios (HRs) and 95% confidence intervals (CIs) for time-to-event outcome endpoints were estimated with unstratified Cox proportional hazards regression modeling. 95% Wilson CIs were generated for the proportion of patients with TE alterations. Relationships between somatic gene alterations and treatment arms on PFS were explored using an unstratified Cox proportional hazards model including main effects for treatment and mutation status in addition to an interaction term, mutation status: treatment. Kaplan−Meier estimation was used to calculate median survival times and plot time-to-event outcomes. The likelihood ratio G-test for independence was used to compare baseline characteristics between analysis populations and gene alteration frequencies across treatment arms for baseline and TE gene alterations. Fisher's exact test was used to compare clearance of ctDNA a*EGFR* rates over the course of treatment between treatment arms. Descriptive summary statistics were used for summaries of safety outcomes.

RESULTS

Patient population

In RELAY, 449 patients were randomized (1 : 1) between January 2016 and February 2018 to receive RAM + ERL (n = 224) or PBO + ERL (n = 225; ITT population; Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2023.101580). The safety population (n = 446) received one or more study dose. At primary data cut-off (23 January 2019), 29% (n = 64) of patients in the RAM + ERL arm and 19% (n = 43) in the PBO + ERL arm were still on treatment.

A valid baseline ctDNA sample, with one or more gene alterations detectable by NGS (TR population), was obtained from 86% of patients (n = 386; RAM + ERL = 192, PBO + ERL = 194). As expected, baseline characteristics were similar between the ITT and TR populations (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2023.101580). The RAM + ERL safety profile was consistent with that of the individual agents.[28] Similarly, the safety profile of the TR population was consistent with the overall RELAY safety population (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2023.101580).

In the ITT population, patients in the RAM + ERL arm demonstrated a significantly lower risk of progression or death (stratified HR = 0.59, 95% CI 0.46-0.76; unstratified HR = 0.64, 95% CI 0.50-0.81).[28] In addition, a superior median PFS (mPFS) was observed in RAM + ERL (19.4 months) compared with PBO + ERL (12.4 months). In the TR population, mPFS was consistent with the overall RELAY results of 19.4 and 12.5 months in the RAM + ERL and PBO + ERL arms, respectively (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2023.101580).

Baseline detection of aEGFR mutations in ctDNA associates with shorter PFS

All patients had a documented a*EGFR* mutation (ex19del/ex21.L858R) by local testing (a prerequisite of enrollment in RELAY). Of those with one or more baseline gene alterations, an a*EGFR* mutation was detected by NGS (ctDNA a*EGFR*+) in 66.1% of patients [n = 255 (RAM + ERL = 122, PBO + ERL = 133)].

Overall, patients with a*EGFR* positivity detectable by NGS in baseline ctDNA had a shorter mPFS (12.7 months) than those whose a*EGFR* alteration was not detected (ctDNA *EGFR*−; 22.0 months; HR = 1.91, 95% CI 1.44-2.55; Figure 1A). Patients who were ctDNA a*EGFR*+ at baseline in RAM + ERL had a longer mPFS (15.2 months) than those in PBO + ERL (11.1 months; HR = 0.63, 95% CI 0.46-0.85). Patients who were ctDNA *EGFR*− at baseline in the RAM + ERL arm (22.1 months) had a more modestly numerically increased mPFS compared with those in PBO + ERL (19.4 months; HR = 0.80, 95% CI 0.49-1.30; Figure 1B).

Baseline alterations co-occurring with aEGFR mutations

Examination of other genes in the Guardant panel at baseline allowed us to determine co-occurring somatic gene alterations. Of those with a detectable a*EGFR* mutation in baseline ctDNA, 88.2% (n = 225; RAM + ERL = 107, PBO + ERL = 118) had a detectable co-occurring gene alteration at baseline. Such alterations were identified in 69 genes, most frequently in *TP53* (n = 165, 42.7%), *EGFR* other than ex19del/ex21.L858R (*EGFR* other; n = 97, 25.1%), and *PIK3CA* (n = 39, 10.1%; Figure 2A and B). Other genetic alterations observed in >5% patients were *NF1* (n = 30, 7.8%), *APC* (n = 27, 7.0%), *BRAF* (n = 24, 6.2%), *CDK6* (n = 20, 5.2%), and *MET* (n = 20, 5.2%; Figure 2A).

At baseline, 42 '*EGFR* other' alterations were identified (40 single-nucleotide variants, one gene amplification, and one splice-acceptor variant). Of the 40 single-nucleotide variants identified, 16 were in the tyrosine kinase domain.

Overall, the presence of any detectable baseline co-occurring alteration was associated with worse PFS compared with those without (12.5 versus 19.4 months; HR = 1.94, 95% CI 1.19-3.14; Figure 2C). This was similarly true for the most frequently identified co-occurring alterations (Figure 2E, G, and I).

Irrespective of whether a baseline co-occurring alteration was detected (RAM + ERL 15.2 versus PBO + ERL 10.8 months; HR = 0.62, 95% CI 0.45-0.85) or not (RAM + ERL 28.1 versus PBO + ERL 16.2; HR = 0.61, 95% CI 0.24-1.52), patients benefitted from combined RAM + ERL. Given the similar benefit observed in both groups, the interaction between detection of co-occurring alterations and

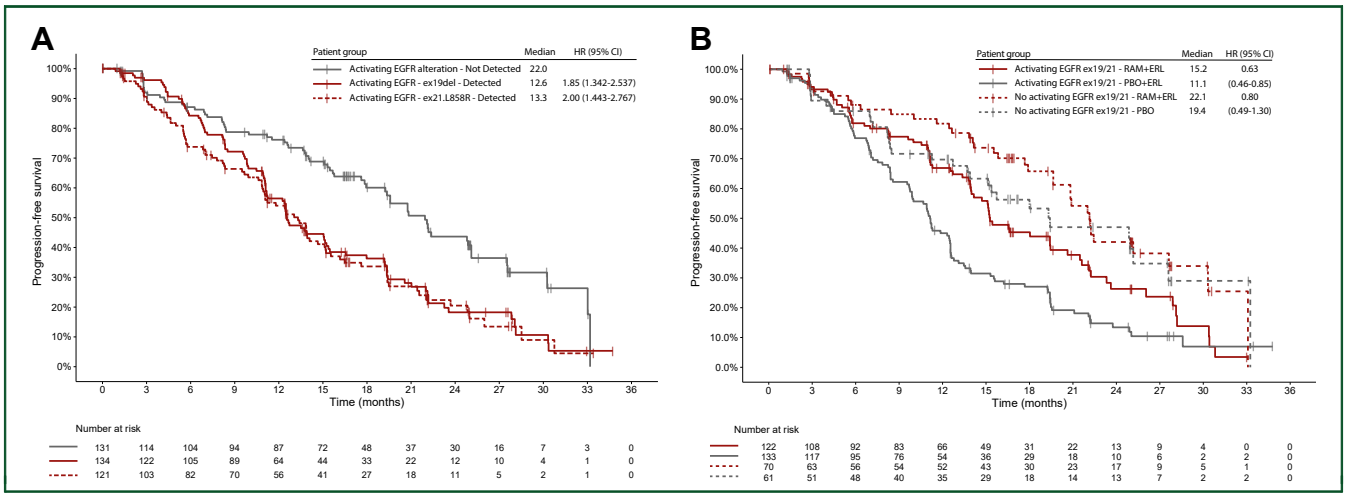


Figure 1. Kaplan–Meier curves of PFS among patients in the TR population with detectable or undetectable activating *EGFR* mutations in baseline ctDNA. Kaplan–Meier curves for PFS of patients grouped by (A) detection of activating *EGFR* alteration in baseline ctDNA and (B) further grouped by treatment arm. The TR population included patients with a valid baseline sample and one or more detectable genomic alterations in any gene.
CI, confidence interval; ctDNA, circulating tumor DNA; *EGFR*, epidermal growth factor receptor; HR, hazard ratio; PBO + ERL, placebo plus erlotinib; PFS, progression-free survival; RAM + ERL, ramucirumab plus erlotinib; TR, translational research.

treatment arms was not significant ($P = 0.963$; Figure 2D). This benefit was also observed for the most frequently identified co-occurring alterations (Figure 2F, H, and J).

EGFR *ctDNA clearance*

To investigate the prevalence and impact of ctDNA a*EGFR* clearance during treatment, the a*EGFR* mutation status of patients and associated PFS was examined.

Of the 255 patients who were ctDNA a*EGFR*+ at baseline, 207 patients (RAM + ERL = 102, PBO + ERL = 105) had a valid ctDNA sample with one or more alterations detected at C4. Of those, a*EGFR* mutations were undetectable in 79.2% ($n = 164$) of patients and detectable in 20.8% ($n = 43$) by C4 (Figure 3A). mPFS was longer ($n = 164$; 14.1 months) in those whose a*EGFR* mutation cleared by C4 compared with those with a detectable a*EGFR* mutation ($n = 43$; 7.0 months; HR = 0.48, 95% CI 0.33-0.71; Figure 3B). Addition of RAM to ERL improved PFS outcomes, irrespective of a*EGFR* mutation clearance (Figure 3C).

Of the 120 patients (RAM + ERL = 44, PBO + ERL = 76) who were ctDNA a*EGFR*+ at baseline and had a valid postprogression ctDNA sample, 20.0% ($n = 24$) had undetectable a*EGFR* mutations postprogression and 80.0% ($n = 96$) had a detectable a*EGFR* mutation.

Of the 104 patients (RAM + ERL = 40, PBO + ERL = 64) who were ctDNA a*EGFR*+ at baseline who had valid NGS samples at all three timepoints (baseline, C4, and postprogression), 25.0% ($n = 26$) were ctDNA a*EGFR*+ at C4, 80.7% ($n = 84$) were ctDNA a*EGFR*+ at progression, and 38.5% ($n = 40$) had a detectable T790M mutation, demonstrating the usefulness of serial monitoring of liquid biopsies in shedding tumors (Supplementary Figure S3A and B, available at https://doi.org/10.1016/j.esmoop.2023.101580).

Among the 40 patients (RAM + ERL = 18, PBO + ERL = 22) who were ctDNA a*EGFR*− at baseline and had valid NGS samples available at all three timepoints, 100% ($n = 40$) were persistently negative at C4 and 72.5% ($n = 29$) were persistently negative postprogression (Supplementary Figure S3C and D, available at https://doi.org/10.1016/j.esmoop.2023.101580).

TE gene alterations and clinical outcomes

To investigate TE gene alterations—absent at baseline but present postprogression—two prespecified analysis populations were used: TEpop1 [patients with a valid ctDNA sample and ≥1 alteration detectable by NGS at baseline and postprogression ($n = 168$)] and TEpop2 [patients with a valid ctDNA sample and specifically an a*EGFR* mutation detectable by NGS at baseline and postprogression ($n = 96$)].

In general, baseline characteristics were similar between ITT and TE populations; however, patients in both TE analysis populations were more likely to have an ECOG PS of 1 and were more likely to be <65 years old compared with the ITT population (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2023.101580).

TE gene alterations were detected in the ctDNA of 64.9% ($n = 109$) patients in TEpop1 and 72.9% ($n = 70$) patients in TEpop2. Overall, the distribution of TE gene alterations was similar between RAM + ERL and PBO + ERL arms (Table 1).

The most common TE gene alterations were *EGFR T790M* (TEpop1 = 28.6%; TEpop2 = 46.9%), *EGFR other* (excluding T790M; TEpop1 = 19.0%; TEpop2 = 13.5%), *TP53* (TEpop1 = 16.1%; TEpop2 = 15.6%), *NF1* (TEpop1 = 4.8%; TEpop2 = 6.3%), and *APC* (TEpop1 = 4.8%; TEpop2 = 5.2%). T790M emergence was not significantly different between treatment arms (Table 1); there was no noticeable difference in T790M emergence between those with either baseline a*EGFR* mutation subtype, although T790M emergence was slightly more common among those with detectable baseline co-occurring *TP53* alterations than those without (Supplementary Table S3, available at

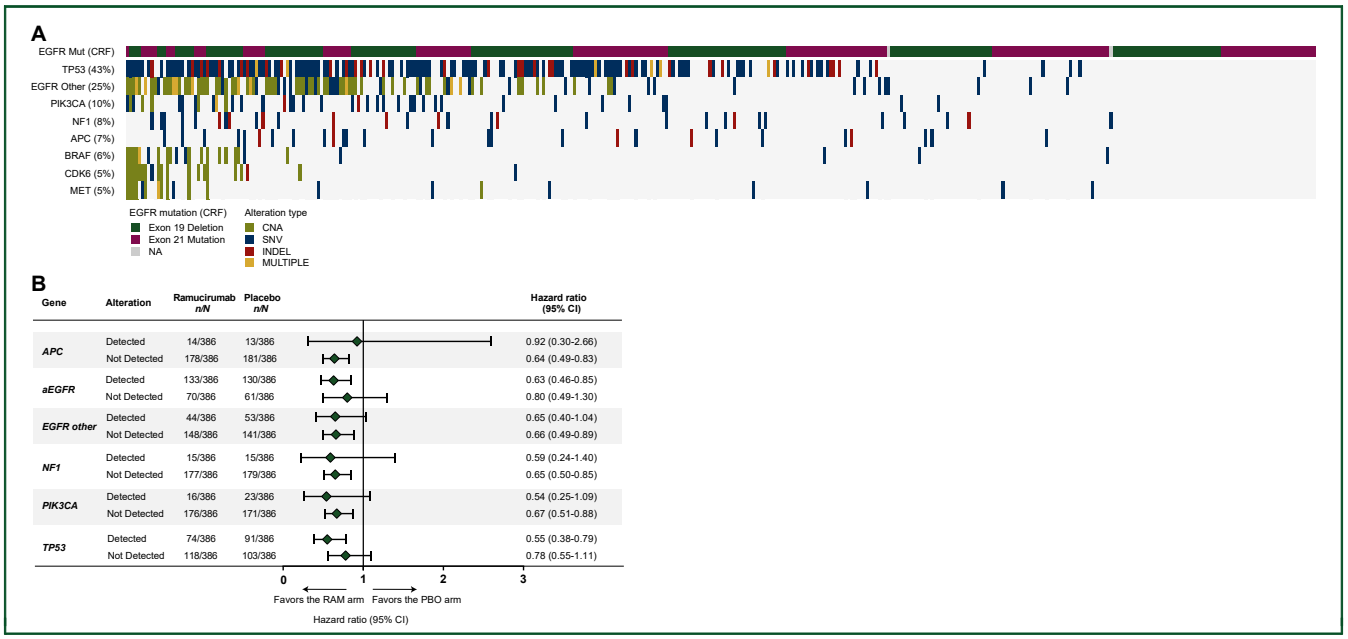


Figure 2. Baseline alterations co-occurring with activating *EGFR* alterations and associated clinical outcomes. (A) Heatmap of genomic alterations co-occurring with *EGFR* activating alterations. (B) Forest plot representing the hazard ratio associated with co-occurring gene alterations at baseline detected by NGS. Kaplan—Meier plots representing mPFS associated with the (C) presence or absence of any detectable baseline co-occurring alterations and (D) presence/absence of baseline co-occurring alterations within each treatment arm. Statistical analysis of the interaction between baseline co-occurring gene alteration and treatment arm is represented by the interaction *P* value calculated using the log-likelihood ratio test (D). Kaplan—Meier plots representing mPFS associated with the presence or absence of co-occurring (E) *TP53*, (G) *EGFR other*, and (I) *PIK3CA* alterations and by treatment arm (F, H, and J).
CI, confidence interval; CNA, copy number alteration; *EGFR*, epidermal growth factor receptor; HR, hazard ratio; INDEL, insertion/deletion; mPFS, median progression-free survival; Mut, mutation; NA, not applicable; NGS, next-generation sequencing; PBO + ERL, placebo plus erlotinib; PFS, progression-free survival; RAM + ERL, ramucirumab plus erlotinib; SNV, single-nucleotide variant.

https://doi.org/10.1016/j.esmoop.2023.101580). TE *NF1* alterations may be numerically higher in RAM + ERL in both TE analysis populations, while TE *APC* and *GNAS* alterations may be numerically higher in the RAM + ERL arm of TEpop2.

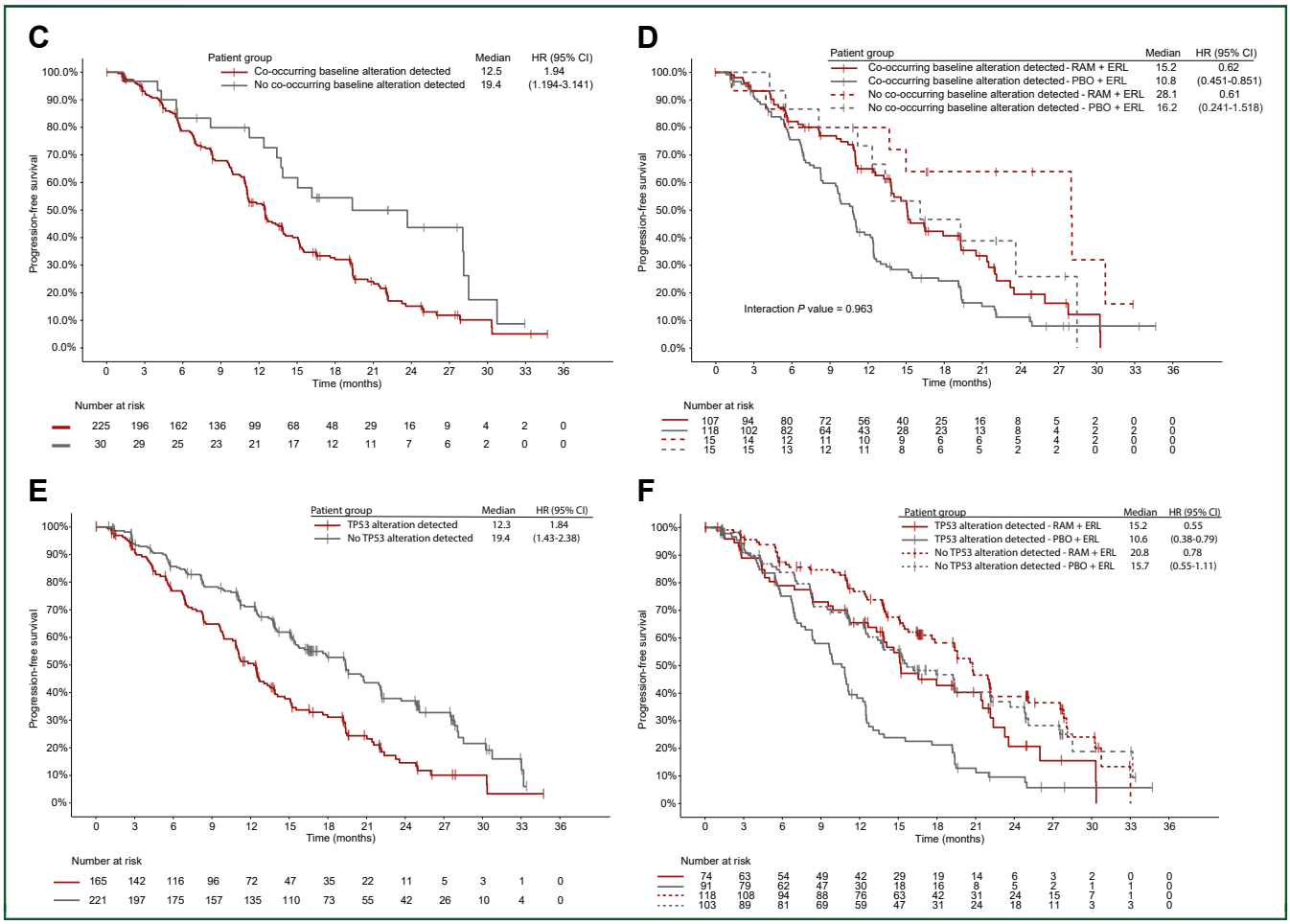
DISCUSSION

The phase III RELAY study demonstrated superior PFS in patients with previously untreated a*EGFR*+ mNSCLC without CNS metastasis when treated with RAM + ERL compared with PBO + ERL.[28] To further understand the clinical benefit observed in response to RAM + ERL and to gain greater understanding of resistance mechanisms, we carried out exploratory analyses using NGS to assess a*EGFR* mutation dynamics and genomic alterations co-occurring with a*EGFR* in the ctDNA of patients from RELAY. The impact of identified alterations on treatment outcomes was also explored.

NGS allows for simultaneous analysis of multiple genes to identify genomic alterations in ctDNA that may play a role in efficacy and clinical outcomes.[33] The ESMO Precision Medicine Working Group recommends using NGS analysis of ctDNA in treatment-naïve patients with lung cancer when tissue genotyping may be delayed or invasive and as a complementary/alternative to tissue NGS for biomarker evaluation.[34] NGS analysis of ctDNA is well established for detection of resistance mutation, *EGFR* T790M, in a*EGFR*+ NSCLC and other mechanisms in oncogene-addicted NSCLC (e.g. *ALK*- or *ROS1*-positive disease).[34]

In this exploratory analysis, an a*EGFR* mutation was detectable in ctDNA of 66% of patients. According to the RELAY inclusion criteria, patients were required to have an a*EGFR* mutation by local laboratory tissue testing. Detection rates of *EGFR* mutations in ctDNA vary according to the method used.[35] In RELAY, *EGFR* mutations in ctDNA were assessed using Guardant360, which shows a 100% positive agreement with an externally validated plasma-based NGS assay for ex21.L858R, 96.8% for ex19del, and 95.0% for *EGFR* T790M.[36] Similarly, healthy donor samples pre-screened by an externally validated orthogonal method show 100% average negative agreement for *EGFR* ex19del, L858R, T790M, and ex20 insertions (97.4% across the panel).[36] Variances in concordance rates, between tissue and plasma samples, have similarly been reported elsewhere, for patients with NSCLC,[37,38] possibly explained by reports that plasma ctDNA concentrations correlate with radiographic tumor volume[39-41] and, in mNSCLC, are reportedly affected by anatomic location and genomic subtype.[37,38]

In RELAY, undetectable a*EGFR* mutation at baseline was associated with a longer mPFS (a*EGFR*− *22*.0 versus a*EGFR*+ *12*.7 months), indicating that detection of a*EGFR* mutations in baseline ctDNA is a negative prognostic factor, as reported elsewhere.[42,43] In both first-line treatment arms of the FLAURA study, comparing first- with third-generation *EGFR*-TKIs in patients with a*EGFR*+ mNSCLC, detectable a*EGFR* mutations in baseline ctDNA were associated with worse PFS (a*EGFR*+ *15*.0 versus a*EGFR*− 23.5 months).[43] This correlation may be due to the higher tumor burden



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in patients who are a*EGFR*+ at baseline, who may progress sooner, and could benefit from stricter follow-up and more aggressive treatment schedules. This highlights the relevance of liquid biopsies for monitoring genomic alterations during treatment and follow-up, which can capture the dynamic aspects of the disease.[44] Notably, patients in RELAY benefitted from combined RAM + ERL, independent of plasma a*EGFR* mutation status at baseline, although to a greater extent in the a*EGFR*+ group. Moreover, while subgroup analysis of FLAURA found a less substantial benefit in terms of PFS for patients with baseline *EGFR* ex21.L858R,[45] and no improvement in OS,[46] compared with those with the ex19del, this difference between a*EGFR* subtypes detectable by NGS at baseline was not observed in RELAY (Supplementary Figure S4, available at https://doi.org/10.1016/j.esmoop.2023.101580). A recent meta-analysis found that similar PFS benefits were observed in patients with baseline *EGFR* ex19del/ex21.L858R with an intensified treatment approach of first-line EGFR-TKI plus chemotherapy.[47] Recent studies indicate that *EGFR* variant allele frequency (VAF) may be associated with clinical outcomes among patients treated with TKIs in mNSCLC.[48,49] The relationship between specific *EGFR* VAF quantity and outcomes was not analyzed here, however future investigations of VAF may provide further clarity on treatment outcomes for those with *EGFR* alterations. The observed benefit of RAM + ERL in RELAY adds support to the importance of angiogenesis in EGFR-TKI resistance. The VEGF pathway is a key mediator of angiogenesis and VEGF expression is thought to increase in response to upregulated *EGFR* signaling in *EGFR*+ mNSCLC,[50,51] perhaps explaining the greater benefit observed in the a*EGFR*+ group.

Here, we report widespread genomic alterations co-occurring with a*EGFR* at baseline, consistent with previous reports that mNSCLC is a heterogenous disease, with a high prevalence of co-occurring gene alterations.[9-11] Existence of submolecular characteristics, including *EGFR* mutation subtypes and co-occurring alterations, may partially account for the significant variance in clinical efficacies observed in patients with a*EGFR*+ mNSCLC in response to EGFR-TKIs.[12,52-55] To date, concomitant genomic alterations that impact clinical outcomes in *EGFR*+ mNSCLC remain largely unknown, although a role for *TP53* mutations, present as a concurrent mutation in 30%-72% of patients, has been reported.[11] In RELAY, genomic alterations in *TP53* were the most common baseline co-occurring alteration (43%) followed by alterations in *EGFR* (other than a*EGFR* mutations) and *PIK3CA*. Presence of concomitant baseline alterations has previously been associated with poorer survival

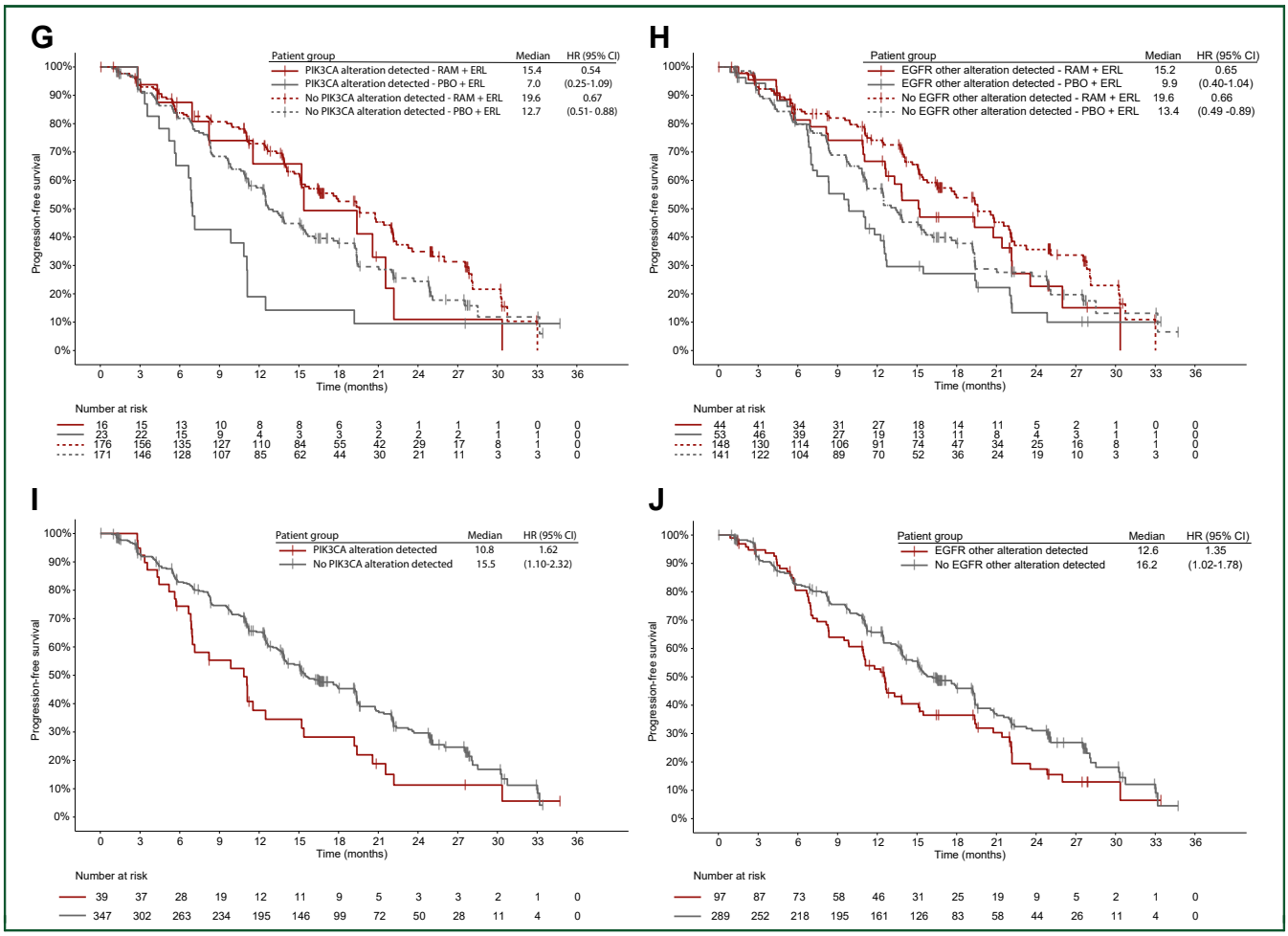

Figure 2. Continued.

outcomes,[56] although investigations into the impact of baseline alterations co-occurring specifically with a*EGFR* remain limited. In a study by Chen et al.,[57] baseline alterations co-occurring with a*EGFR* were compared between patients in short (≤6 months) and long (≥24 months) PFS groups. *TP53, EGFR* other, and *PIK3CA* alterations were common baseline co-occurring alterations and were numerically more prevalent in the short versus long PFS group. In RELAY, detectable baseline alterations in *TP53, EGFR* other, and *PIK3CA* were also the most prevalent alterations co-occurring with a*EGFR* and were associated with worse PFS. However, patients benefitted from combined RAM + ERL, regardless of baseline co-occurring alterations. Evidence suggests that p53 inhibits angiogenesis through the regulation of proangiogenic factors (e.g. VEGF and VEGFR2) and promotes transcription of anti-angiogenic factors (e.g. thrombospondin-1),[58-60] perhaps partially explaining the benefit observed with the addition of VEGFR2 antagonist, RAM, to ERL.

The PFS benefits associated with combining first-generation EGFR-TKIs and anti-VEGF treatment observed in RELAY, NEJ026, and other studies for first-line treatment of EGFR+ mNSCLC[28,61,62] have not been observed with third-generation TKI, OSI, plus anti-VEGF treatment to date. Phase II WJOG8715L and BOOSTER, comparing BEV + OSI with OSI alone for the second-line treatment of patients with EGFR+ mNSCLC[63,64] who had acquired *EGFR* T790M, revealed no significant prolongation of PFS or OS.[45,65,66] Similarly, no significant PFS improvement was observed in a randomized phase II trial, WJOG9717L, comparing first-line OSI with/without BEV, although the small sample size may have limited the ability to demonstrate significant differences between arms.[67] Comparisons with RELAY or NEJ026 cannot be extrapolated due to lack of first-line phase III data and differences in enrolled patients' characteristics, including CNS metastasis, smoking status, and comutation profile. Results of ongoing randomized phase II [OSI with/without RAM, US: NCT03909334; Japan: TORG1833[68] (registration number 184146)] and phase III studies (OSI with/without BEV, NCT04181060), may reveal whether these combinations could improve OSI efficacy in previously untreated patients with *EGFR*+ mNSCLC. Moreover, while investigation of the comutation profile of patients treated with OSI is limited, recent NGS analysis of ctDNA from FLAURA (first-line OSI treatment) reported TP53 as the most common baseline co-occurring genomic alteration,[69] similar to observations in RELAY. Patients with co-occurring baseline TP53 alterations benefitted from

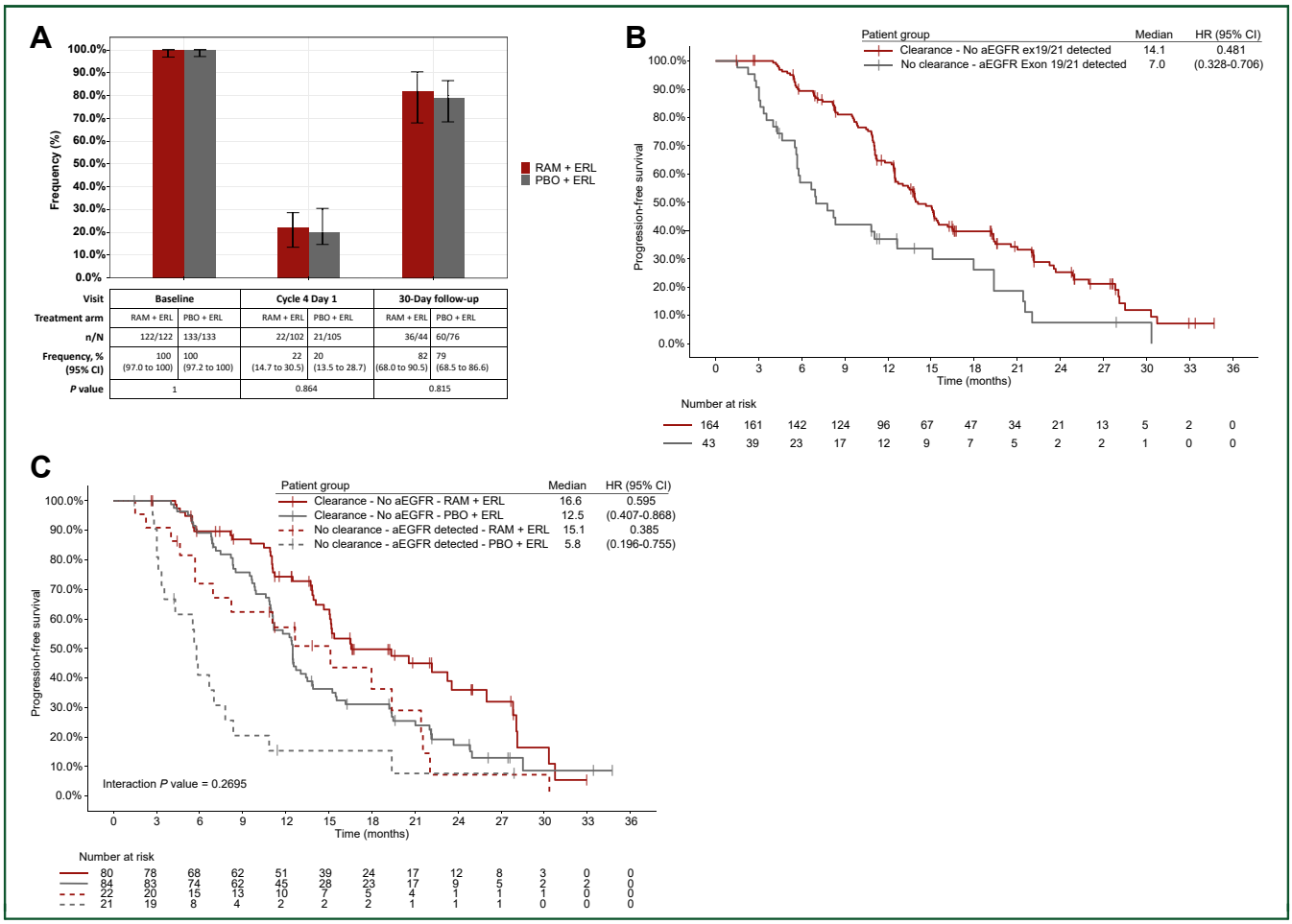

Figure 3. Clearance of activating *EGFR* mutations and clinical outcomes. (A) Bar graph representing detection of a*EGFR* mutations during treatment and post-progression in those with a detectable ctDNA activating alteration at baseline. Kaplan–Meier curves representing (B) mPFS associated with detection of a*EGFR* alterations during treatment and (C) mPFS associated with detection of aEGFR within each treatment arm. Statistical analysis of the interaction between aEGFR detection during treatment and treatment arm is represented by the interaction *P* value (C).
aEGFR, activating epidermal growth factor receptor; CI, confidence interval; ctDNA, circulating tumor DNA; *EGFR*, epidermal growth factor receptor; HR, hazard ratio; mPFS, median progression-free survival; PBO + ERL, placebo plus erlotinib; RAM + ERL, ramucirumab plus erlotinib.

combined anti-VEGFR–EGFR-TKI therapy, both in RELAY with RAM + ERL (Figure 2F and [70]) and in the phase III ACTIVE study of combined VEGFR2 TKI apatinib with gefitinib.[62] However, co-occurring TP53 alterations, especially within exon 8, reportedly reduce OSI efficacy,[71,72] and worsen prognosis in those with brain metastasis.[72] Further investigation of comutations, especially in treatment-naïve patients, may provide additional insights into the impact of combining anti-VEGF therapy with OSI and which patient subgroups it could benefit.

Clearance of detectable a*EGFR* alterations in ctDNA during treatment has been associated with improved PFS for an extensive range of therapies, including in response to first-line EGFR-TKIs[21,39,73] and in those receiving second-line treatment following acquired resistance to EGFR-TKIs.[74] Here, clearance of a*EGFR* mutations by C4 in patients who had detectable a*EGFR*+ at baseline was similarly associated with improved mPFS (a*EGFR*+ 14.1 months versus a*EGFR*− 7.0 months). These observations are similar to those of the NEJ026 trial, comparing BEV + ERL with ERL alone, where clearance was associated with improved PFS in both treatment groups (BEV + ERL: 15.5 months versus 6.0 months; ERL: 11.1 versus 4.3 months).[73] The benefit of combined BEV + ERL was most pronounced in those who were a*EGFR*+ at baseline and had clearance during treatment (a*EGFR*− at 6 weeks). Here, patients with and without a*EGFR* mutation clearance benefitted from combined RAM treatment, indicating that clearance of a*EGFR* mutations early in treatment is prognostic, as, irrespective of treatment, patients who do not clear their a*EGFR* during EGFR-TKI treatment have poorer PFS outcomes than those who do. The PACE-Lung study is currently exploring if patients treated with first-line OSI, without a*EGFR* mutation clearance in the ctDNA after 3-4 weeks, will benefit from a more intensified treatment schedule.

Currently, there are no standard criteria to define populations for analysis of TE genomic alterations. In line with populations used in the primary RELAY study,[28] two similar analysis populations, TEpop1 and TEpop2, are presented. The requirement for one or more detectable alterations ensured that the patient population had tumors that were shedding DNA, such that genomic alterations in the tumor

Table 1. Treatment-emergent gene alterations.

	TE population 1 (*N* = 168)		TE population 2 (*N* = 96)	
	RAM + ERL (*n* = 67)	PBO + ERL (*n* = 101)	RAM + ERL (*n* = 36)	PBO + ERL (*n* = 60)
Gene name, % (95% Wilson CI)				
EGFR	37.3 (26.7 to 49.3)	42.6 (33.4 to 52.3)	44.4 (29.5 to 60.4)	55.0 (42.5 to 66.9)
T790M	25.4 (16.5 to 36.9)	30.7 (22.5 to 40.3)	41.7 (27.1 to 57.8)	50.0 (37.7 to 62.3)
Others	16.4 (9.4 to 27.1)	20.8 (14.0 to 29.7)	5.6 (1.5 to 18.1)	18.3 (10.6 to 29.9)
TP53	20.9 (12.9 to 32.1)	12.9 (7.7 to 20.8)	22.2 (11.7 to 38.1)	11.7 (5.8 to 22.2)
NF1	9.0 (4.2 to 18.2)	2.0 (0.5 to 6.9)	13.9 (6.1 to 28.7)	1.7 (0.3 to 8.9)
APC	7.5 (3.2 to 16.3)	3.0 (1.0 to 8.4)	11.1 (4.4 to 25.3)	1.7 (0.3 to 8.9)
MET	6.0 (2.3 to 14.4)	6.9 (3.4 to 13.6)	8.3 (2.9 to 21.8)	8.3 (3.6 to 18.1)
KRAS	9.0 (4.2 to 18.2)	3.0 (1.0 to 8.4)	5.6 (1.5 to 18.1)	5.0 (1.7 to 13.7)
FGFR2	4.5 (1.5 to 12.4)	3.0 (1.0 to 8.4)	5.6 (1.5 to 18.1)	3.3 (0.9 to 11.4)
GNAS	3.0 (0.8 to 10.2)	—	5.6 (1.5 to 18.1)	—
BRAF	3.0 (0.8 to 10.2)	5.9 (2.8 to 12.4)	2.8 (0.5 to 14.2)	10.0 (4.7 to 20.1)
PIK3CA	1.5 (0.3 to 8.0)	6.9 (3.4 to 13.6)	2.8 (0.5 to 14.2)	6.7 (2.6 to 15.9)
BRCA2	3.0 (0.8 to 10.2)	4.0 (1.6 to 9.7)	—	5.0 (1.7 to 13.7)
ERBB2	3.0 (0.8 to 10.2)	4.0 (1.6 to 9.7)	2.8 (0.5 to 14.2)	5.0 (1.7 to 13.7)
AR	1.5 (0.3 to 8.0)	2.0 (0.5 to 6.9)	—	3.3 (0.9 to 11.4)
CDK6	1.5 (0.3 to 8.0)	2.0 (0.5 to 6.9)	2.8 (0.5 to 14.2)	3.3 (0.9 to 11.4)
RB1	3.0 (0.8 to 10.2)	2.0 (0.5 to 6.9)	2.8 (0.5 to 14.2)	1.7 (0.3 to 8.9)
CDK4	1.5 (0.3 to 8.0)	2.0 (0.5 to 6.9)	2.8 (0.5 to 14.2)	1.7 (0.3 to 8.9)
SMAD4	1.5 (0.3 to 8.0)	2.0 (0.5 to 6.9)	2.8 (0.5 to 14.2)	1.7 (0.3 to 8.9)
KIT	4.5 (1.5 to 12.4)	1.0 (0.2 to 5.4)	2.8 (0.5 to 14.2)	1.7 (0.3 to 8.9)
PTEN	3.0 (0.8 to 10.2)	2.0 (0.5 to 6.9)	2.8 (0.5 to 14.2)	3.3 (0.9 to 11.4)
ARID1A	3.0 (0.8 to 10.2)	3.0 (1.0 to 8.4)	2.8 (0.5 to 14.2)	1.7 (0.3 to 8.9)
FGFR1	1.5 (0.3 to 8.0)	1.0 (0.2 to 5.4)	2.8 (0.5 to 14.2)	—
MAP2K1	3.0 (0.8 to 10.2)	—	2.8 (0.5 to 14.2)	—
MAPK3	1.5 (0.3 to 8.0)	—	2.8 (0.5 to 14.2)	—
MTOR	—	1.0 (0.2 to 5.4)	—	1.7 (0.3 to 8.9)
MYC	1.5 (0.3 to 8.0)	2.0 (0.5 to 6.9)	2.8 (0.5 to 14.2)	—
RET	1.5 (0.3 to 8.0)	—	—	—
STK11	—	1.0 (0.2 to 5.4)	—	—

a*EGFR*, activating epidermal growth factor receptor; CI, confidence interval; *N*, total number of patients; *n*, number of patients per category; PBO + ERL, placebo plus erlotinib; RAM + ERL, ramucirumab plus erlotinib; TEpop1, TE population 1 (i.e. patients who had ≥1 detectable genetic alteration by NGS at both baseline and poststudy treatment discontinuation); TEpop2, TE population 2 (i.e. patients who had a detectable a*EGFR* alteration at both baseline and poststudy treatment discontinuation); TR, patients who had a valid baseline circulating tumor DNA sample with one or more detectable genetic alteration.

could be detected in the liquid biopsy sample. The types and frequencies of co-occurring baseline and TE alterations appeared similar between treatment arms, although sample sizes were limited. As progressive disease was required for the poststudy treatment discontinuation follow-up sample, fewer patients in RAM + ERL met this criterion, as progression was less frequent in those patients compared with the PBO + ERL group within the timeframe of the primary analysis dataset. Moreover, addition of RAM to ERL did not impact the profile of TE genomic alterations or treatment resistance. As with other genes, the sensitivity of T790M detection in plasma ctDNA is reportedly lower than the detection rate in tissue samples.[75,76] Accordingly, it should be noted that rates of T790M at progression may be higher than identified here by NGS, highlighting the need to confirm negative plasma-based results with tissue samples. The most abundant TE genomic alterations were identified in the *EGFR* (particularly the T790M resistance mutation) and *TP53* genes. No differences were identified between TEpop1 and TEpop2. Final NGS analysis at the time of OS maturity is expected to provide a clearer understanding of the impact of TE alterations.

Limitations of this study include that co-occurring and TE alterations were identified by NGS from liquid biopsies only. A considerable proportion of patients enrolled in RELAY did not have disease progression while on first-line treatment, therefore further follow-up is warranted. As with any analysis of blood liquid biopsies, it is possible that non-tumor mutations due to clonal hematopoiesis of indeterminate potential[77] can occur, however previous reports indicate that actionable NSCLC-driver mutations are not among the alterations commonly associated with clonal hematopoiesis of indeterminate potential.[78,79] Similar to other studies,[21,39,42,74] a*EGFR* clearance was examined at an early treatment timepoint; more frequent ctDNA assessments during treatment may provide a more complete picture. RELAY was not powered for subgroup analysis; further, no adjustments were made to control the type I error rate when conducting multiple testing procedures and biomarker analyses were exploratory. The inherent limitations of NGS should also be considered.

Conclusions

In RELAY, detection of a*EGFR* alterations, in ctDNA, was associated with poorer PFS outcomes. a*EGFR* clearance during treatment was associated with improved PFS outcomes. Moreover, RAM + ERL was associated with improved PFS outcomes, irrespective of detectable or undetectable a*EGFR*, co-occurring baseline alterations, or a*EGFR* clearance by C4. Monitoring co-occurring gene alterations and a*EGFR* clearance may provide insights into

mechanisms of EGFR-TKI resistance and which patients may benefit from more intensified treatment schedules. Further studies are warranted to demonstrate the potential clinical utility of serial ctDNA *EGFR* testing in NSCLC management.

ACKNOWLEDGEMENTS

Eli Lilly and Company thank the clinical trial participants and their caregivers, without whom this work would not have been possible. The authors acknowledge Michelle Carlsen, a full-time employee of Eli Lilly and Company for statistical expertise and support as well as Dwayne Byrne, PhD, a full-time employee of Eli Lilly and Company, for project management support and scientific communication expertise. National Comprehensive Cancer Network (NCCN) and NCCN are registered trademarks of NCCN.

FUNDING

Eli Lilly and Company sponsored this clinical trial (no grant number), provided study drugs, and participated in regulatory and ethics approval, safety monitoring, data collection, and statistical analyses.

DISCLOSURE

EBG declares grants from ABL-Bio, AstraZeneca, Bristol-Myers Squibb, Daiichi-Sankyo, Dynavax Technologies, EMD Serono, Eli Lilly and Company, Genentech, Iovance Biotherapeutics, Merck, Mirati Therapeutics, Neon, and Novartis; consulting fees from AbbVie, ABL-Bio, AstraZeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Dracen Pharmaceuticals, EMD Serono, Eisai, Eli Lilly and Company, Gilead, GlaxoSmithKline, Ipsen, Merck, Natera, Novartis, Personalis, Regeneron, Sanofi, Shionogi, and Xilio; payment for expert testimony from UCLA relating to motif neoepitopes for cancer immunotherapy; leadership/fiduciary role with LUNGevity and Jonsson Comprehensive Cancer Center at UCLA. JVH declares advisory board/committee membership with Genentech, Mirati Therapeutics, Eli Lilly and Company, Janssen Pharmaceuticals, Boehringer-Ingelheim Pharmaceuticals, DAVA Oncology, Regeneron, Takeda Pharmaceuticals, BerGenBio, Jazz Pharmaceuticals, Curio Science, Immunocore, and Novartis; research support from AstraZeneca, Boehringer-Ingelheim, Spectrum, and Takeda; royalties and licensing fees from Spectrum. MR declares consulting fees from Amgen, AstraZeneca, Boehringer-Ingelheim, BeiGene, BMS, GSK, Mirati, Merck, MSD, Eli Lilly and Company, Novartis, Pfizer, Roche, Regeneron, and Sanofi; honoraria from Amgen, AstraZeneca, Boehringer-Ingelheim, BeiGene, BMS, GSK, Mirati, Merck, MSD, Eli Lilly and Company, Novartis, Pfizer, Roche, Regeneron, and Sanofi; support for meetings/travel from Amgen, AstraZeneca, Boehringer-Ingelheim, BeiGene, BMS, GSK, Mirati, Merck, MSD, Eli Lilly and Company, Novartis, Pfizer, Roche, Regeneron, and Sanofi; board participation for Daiichi and Sanofi. KN declares grants from Nippon Boehringer-Ingelheim, West Japan Oncology Group, Thoracic Oncology Research Group, North East Japan Study Group, Clinical Research Support Center Kyushu, Nichirei Biosciences Inc., Eli Lilly and Company, Hitachi, Sysmex, and Otsuka Pharmaceutical; consulting fees from SymBio Pharmaceuticals, Solasia Pharma, Eli Lilly and Company, and Otsuka Pharmaceutical; honoraria from Chugai, Pfizer, Eli Lilly and Company, MSD, Novartis Pharma, AstraZeneca, Amgen, Merck Biopharma, Roche Diagnostics, Yakult Honsha, Guardant Health, Takeda Pharmaceuticals, Boehringer-Ingelheim Japan, FUJIREBIO, Bristol-Myers Squibb, Merck Biopharma, Janssen Pharmaceutical Daiichi-Sankyo, and Ono Pharmaceutical. MN declares honoraria from Ono Pharmaceuticals, Chugai Pharmaceutical, Taiho Pharmaceutical, Bristol-Myers Squibb, Daiichi-Sankyo, Eli Lilly and Company, AstraZeneca, MSD, AbbVie, Takeda, Pfizer, Boehringer-Ingelheim, Novartis, Nippon Kayaku, Merck, and Janssen. SN declares grants from Eli Lilly and Company; consulting fees from BMS, Eli Lilly and Company, Takeda, Roche, Pfizer, AstraZeneca, BI, MSD, AbbVie, PharmaMar, and BeiGene; support for meetings and/or travel from Eli Lilly and Company, Thermo Fisher, and Takeda; participation on advisory boards for Guardant Health, GSK, Novocure, Amgen, AstraZeneca, Janssen, and Eli Lilly and Company; and declares leadership/fiduciary roles as President of WALCE (Women against Lung Cancer in Europe) Onlus. LPA declares grants from MSD, AstraZeneca, Pfizer, and BMS; consulting fees from Eli Lilly and Company, MSD, Roche, PharmaMar, Merck, AstraZeneca, Novartis, Servier, Amgen, Pfizer, Sanofi, Bayer, BMS, Mirati, GSK, Janssen, Takeda, and Daiichi-Sankyo; honoraria from AstraZeneca, Janssen, Merck, and Mirati; member of the board of directors of Altum Sequencing and Genomica; principal investigator for studies sponsored by Alkermes, Amgen, AstraZeneca, Bristol-Myers Squibb, Daiichi-Sankyo, IO Biotech, Janssen-Cilag, Eli Lilly and Company, MSD, Novartis, Pfizer, PharmaMar, Roche, Sanofi, Takeda, and Tesaro. SP declares consulting fees from Amgen, AstraZeneca, Bayer, BeiGene, Blueprint, BMS, Boehringer-Ingelheim, Daiichi-Sankyo, Guardant Health, Incyte, Janssen, Eli Lilly and Company, Merck Serono, MSD, Novartis, Roche, Takeda, Pfizer, Seattle Genetics, Turning Point Therapeutics, and EQRx; honoraria from AstraZeneca, Bayer, Guardant Health, Janssen, Merck Serono, Roche, Takeda, and Pfizer; payment for expert testimony from Roche and Merck Serono; support for attending meetings and/or travel from Janssen and Roche; unpaid leadership/fiduciary roles in British Thoracic Oncology Group, ALK Positive UK, Lung Cancer Europe, Ruth Strauss Foundation, Mesothelioma Applied Research Foundation, and ETOP-IBCSG Partners Foundation Board. SPA declares no conflicts of interest. HG was an employee and stockholder of Eli Lilly and Company during the time of study analysis and manuscript preparation; contributions to the manuscript were made as part of the roles at Eli Lilly and Company. BDB and CVG are full-time employees and minor shareholders of Eli Lilly and Company. KN declares grants to institution from AstraZeneca, MSD, Ono Pharmaceutical Co. Ltd., Nippon Boehringer Ingelheim Co. Ltd., Novartis, Pfizer Japan Inc., Bristol-Myers Squibb, Eli Lilly and Company, Chugai Pharmaceutical, Daiichi-Sankyo, Merck, Paraxel International Corp., PRA Health Sciences, EPS Corporation, Kissei Pharmaceutical Co. Ltd., Taiho Pharmaceutical Co. Ltd., PPD-SNBL K.K., SymBio

Pharmaceuticals Limited, IQVIA Services Japan K.K., Syneos Health Clinical K.K., Nippon Kayaku Co. Ltd., EP-CRSU Co. Ltd., Mebix, Janssen Pharmaceutical K.K., AbbVie Inc., Bayer Yakuhin Ltd., Eisai, Mochida Pharmaceutical Co. Ltd. Covance Japan Inc., Japan Clinical Research Operations, Takeda Pharmaceutical Co. Ltd., GlaxoSmithKline, Sanofi, Sysmex Corporation, Medical Research Support, Otsuka Pharmaceutical Co. Ltd., SRL Inc., Pfizer R&D Japan G.K., and Amgen Inc.; consulting fees from Eli Lilly and Company, KYORIN Pharmaceutical Co. Ltd., Ono Pharmaceutical Co. Ltd., and Pfizer Japan Inc.; honoraria from Ono Pharmaceutical Co. Ltd., Amgen Inc. Nippon Kayaku Co. Ltd., AstraZeneca K.K., Chugai Pharmaceutical Co. Ltd., Eli Lilly and Company, MSD, Pfizer Japan Inc., Nippon Boehringer-Ingelheim Co. Ltd., Taiho Pharmaceutical Co. Ltd., Bayer Yakuhin, CMIC ShiftZero K.K., Life Technologies Japan Ltd., Neo Communication, Roche Diagnostics K.K., AbbVie Inc, Merck Biopharma Co. Ltd., Kyowa Kirin Co. Ltd., Takeda Pharmaceutical Co. Ltd., 3H Clinical Trial Inc., Care Net Inc., Medical Review Co. Ltd., Medical Mobile Communications Co. Ltd., Yodosha Co. Ltd., Nikkei Business Publications Inc., Japan Clinical Research Operations, CMIC Co. Ltd., Novartis, TAIYO Pharma Co. Ltd., KYORIN Pharmaceutical Co. Ltd., and Bristol-Myers Squibb K.K.; patents planned, issued, or pending with Daiichi-Sankyo.

DATA SHARING

Lilly provides access to all individual participant data collected during the trial, after anonymization, with the exception of pharmacokinetic or genetic data. Data are available to request 6 months after the indication studied has been approved in the United States and European Union and after primary publication acceptance, whichever is later. No expiration date of data requests is currently set once data are made available. Access is provided after a proposal has been approved by an independent review committee identified for this purpose and after receipt of a signed data sharing agreement. Data and documents, including the study protocol, statistical analysis plan, clinical study report, blank or annotated case report forms, will be provided in a secure data sharing environment. For details on submitting a request, see the instructions provided at www.vivli.org.

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