Original Study

RELAY, Ramucirumab Plus Erlotinib (RAM+ERL) in Untreated Metastatic *EGFR*-Mutant NSCLC (*EGFR*+ NSCLC): Association Between *TP53* Status and Clinical Outcome

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ABSTRACT

Mutant TP53 is a negative prognostic factor in patients with EGFR+NSCLC. This exploratory analysis investigated the association between TP53 status and clinical outcome in RELAY. The findings indicated that RAM+ERL exhibited benefit compared with PBO+ERL, independent of TP53 status. The RELAY regimen is an efficacious first-line treatment option for all patients with EGFR+NSCLC, with or without TP53 mutation.

Background: Ramucirumab plus erlotinib (RAM+ERL) demonstrated superior progression-free survival (PFS) in RELAY, a randomised Phase III trial in patients with untreated, metastatic, EGFR-mutated, non–small-cell lung cancer (*EGFR*+ NSCLC). Here, we present the relationship between *TP53* status and outcomes in RELAY. **Materials and Methods:** Patients received oral ERL plus intravenous RAM (10 mg/kg IV) or placebo (PBO+ERL) every 2 weeks. Plasma was assessed by Guardant 360 next-generation sequencing and patients with any gene alteration detected at baseline were included in this exploratory analysis. Endpoints included PFS, overall response rate (ORR), disease control rate (DCR), DoR, overall survival (OS), safety, and biomarker analysis. The association between *TP53* status and outcomes was evaluated. **Results:** Mutated *TP53* was detected in 165 (42.7%; 74 RAM+ERL, 91 PBO+ERL) patients, wild-type *TP53* in 221 (57.3%; 118 RAM+ERL, 103 PBO+ERL) patients. Patient and disease characteristics and concurrent gene alterations were comparable between those with mutant and wildtype *TP53*. Independent of treatment, *TP53* mutations, most notably on exon 8, were associated with worse clinical outcomes. In all patients, RAM+ERL improved PFS. While ORR and DCR were comparable across all patients, DoR was superior with RAM+ERL. There were no clinically meaningful differences in the safety profiles between those with baseline *TP53* mutation and wild-type. **Conclusion:** This analysis indicates that while *TP53* mutations are a negative prognostic marker in *EGFR*+ NSCLC, the addition of a VEGF inhibitor improves outcomes in those with mutant *TP53*. RAM+ERL is an efficacious first-line treatment option for patients with *EGFR*+ NSCLC, independent of *TP53* status.

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Abbreviations: CI, confidence intervals; ctDNA, circulating tumor DNA; DBD, DNA-binding domain; DCR, disease control rate; DoR, duration of response; EGFR+ NSCLC, EGFR-mutated, non–small-cell lung cancer; EGFR, epidermal growth factor receptor; ITT, intent-to-treat; NGS, next-generation sequencing; NSCLC, non–small-cell lung cancer; ORR, overall response rate; OS, overall survival; PBO+ERL, Placebo plus erlotinib; PFS, progression-free survival; RAM+ERL, ramucirumab plus erlotinib; TKIs, tyrosine kinase inhibitors; VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor 2.

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Introduction

Non–small-cell lung cancers (NSCLCs) comprise a widely heterogenous group of tumors.[1] Due to the wide range of mutational profiles and complex pathogenesis of the disease, NSCLC has proven difficult to treat. One commonly mutated gene associated with the adenocarcinoma subtype of NSCLC is epidermal growth factor receptor (*EGFR*). Aberrations in the EGFR protein can lead to constitutive activation of various pathways involved in cell proliferation and survival, ultimately promoting oncogenesis.[2,3]

The introduction of EGFR tyrosine kinase inhibitors (TKIs) changed the treatment landscape for patients with *EGFR*-mutant (*EGFR*+) NSCLC. First-generation (erlotinib, gefitinib, and icotinib) and second-generation (afatinib and dacomitinib) TKIs have consistently demonstrated improved clinical outcomes in comparison to standard first-line platinum-based chemotherapy in these patients.[4,5] However, despite the potent anticancer activity exerted by these agents, resistance inevitably occurs. In approximately 50% of patients, resistance is caused by a secondary point mutation (T790M) in the *EGFR* gene which structurally prevents the binding of first- and second-generation TKIs.[6] Third generation TKI, osimertinib, further enhanced treatment by overcoming the limitation of T790M mutation-mediated resistance in patients treated with first- and second-generation TKIs. Still, acquired resistance to osimertinib inevitably develops, with no dominant pathway for follow-up therapeutic options.[4] Hence, novel treatment strategies that delay or prevent TKI resistance and further enhance efficacy in EGFR+NSCLC would be beneficial.

Concurrent gene alterations can impact clinical outcome and contribute to impaired efficacy of EGFR-TKI monotherapy.[7] *TP53* is one such gene demonstrated to have a negative impact on the survival outcomes of patients with EGFR+NSCLC treated with TKIs.[8,9] In EGFR+NSCLC, TP53 mutations are the most prevalent concurrent mutations with an incidence of approximately 50%, and are highly correlated with smoking.[8,10,11] The *TP53* gene is comprised of 11 exons which code for a transactivation domain, the DNA-binding domain (DBD), and the C-terminal domain. Exons 5 to 8 of the *TP53* gene encode the DBD, which mediates the transcriptional activity of the p53 tumor suppressor protein. The DBD is the region responsible for recognizing the promoter sequence of genes involved in DNA repair, apoptosis, and cell-cycle regulation.[12] In addition to its central role in response to cellular stress, there is growing evidence that the anticancer effects of p53 include the inhibition of angiogenesis through the regulation of proangiogenic factors such as vascular endothelial growth factor (VEGF)A and VEGF receptor 2 (VEGFR2), and HIFα under hypoxic conditions. Wild-type p53 also increases the transcription of antiangiogenic factors, COL4A and thrombospondin.[13-15] Thus, mutations in the DBD, particularly in exon 8, can lead to loss of these regulatory functions and promote uncontrolled cellular proliferation.[12,16] Mutations in the non-DBD exons have also been correlated with worse outcomes.[17] There are a wide range of *TP53* alterations which can produce a variety of oncogenic effects on the p53 protein.[10,18-20] These mutations could be classified according to mutation status, mutation number, mutation site, allele frequency, degree of disruption in protein structure or function, and protein expression.[21]

Results from several studies indicate that mutant *TP53* is a negative prognostic factor and that *EGFR*+ NSCLC patients with concurrent *TP53* mutations, most notably in exon 8, generally have more aggressive disease, increased rates of resistance to EGFR-TKIs and shorter survival.[9,12,16] A retrospective analysis of patients with *EGFR*+NSCLC evaluated tumor mutation profiles and correlated co-mutation with response to TKIs. The study demonstrated a median progression-free survival (PFS) of 7 months in those with concurrent *TP53* mutations compared with 15 months in patients wild-type *TP53*.[22] Similarly, a single institution retrospective analysis of patients with *EGFR*+NSCLC reported a significantly inferior median overall survival in patients harboring mutant *TP53* compared to those with wild-type *TP53* (33.3 months vs. 53.5 months, respectively).[23] *TP53* mutations may therefore identify a subgroup of patients with more aggressive disease that derive less benefit from EGFR-TKI monotherapy, including osimertinib.[24]

There is currently no approved targeted therapeutic for mutated p53 protein, though there is evidence indicating that *TP53*-mutant tumors respond favorably to VEGF pathway inhibitors. In a study involving 500 patients with refractory or progressive solid tumors, Wheler et al[25] evaluated the association between TP53 mutations and clinical outcomes with VEGF/VEGFR inhibitor therapy. Indeed, *TP53* mutations were associated with a positive therapeutic effect, leading the authors to conclude that *TP53* mutations predict sensitivity to antiangiogenics in a clinical setting. Moreover, *TP53* has been demonstrated to serve as a molecular determinant of response to anti-VEGF therapy across a variety of tumor types including carcinomas and sarcomas.[25-28] As studies have indicated the importance of the p53-VEGF pathway in angiogenesis,[13,15] it is possible that this may be one of the underling biological processes influencing the benefit observed with *TP53* mutant tumors. According to a body of literature, *TP53* mutations and elevated VEGF signaling may predict worse outcomes and identify patients that could benefit from VEGF inhibition.[29,30]

The VEGF pathway is a complementary target of EGFR inhibition,[31] as both the EGF and VEGF pathways share common downstream signaling and can function exclusively of one another to drive tumorigenesis.[32] Accordingly, recent clinical studies have implemented a dual inhibitory approach in an effort to overcome therapeutic resistance in *EGFR*+NSCLC.[33-35] The RELAY trial, a randomized, double-blind, placebo-controlled, Phase III trial assessed the efficacy and safety of combining erlotinib with ramucirumab, a recombinant human IgG1 monoclonal antibody receptor antagonist designed to block the ligand-binding site of VEGFR-2, as first-line treatment in metastatic EGFR+NSCLC. PFS was significantly longer in the ramucirumab plus erlotinib group than in the placebo plus erlotinib group (hazard ratio [HR], 0.591; 95% confidence interval [CI], 0.46-0.76; $P < .0001$; 19·4 vs. 12·4 months), and safety was consistent with the safety profiles of the individual compounds in advanced lung cancer.[36] The reported superior PFS and acceptable safety profile demonstrated the benefit of simultaneously inhibiting the VEGF and EGFR pathways, and led to worldwide regulatory approval and inclusion of the regimen in treatment guidelines.[37-39]

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Though it is known that *TP53* is implicated in angiogenesis, and mutations in the gene are associated with reduced responsiveness to EGFR-TKIs in patients with *EGFR*+NSCLC, there is a lack of published literature on the impact of *TP53* mutations on dual EGF/VEGF pathway inhibition in this indication. In this exploratory analysis, we examined the potential association between *TP53* status and efficacy and safety in patients with untreated metastatic *EGFR*+ NSCLC who received ramucirumab plus erlotinib in the Phase III RELAY trial.[36] In addition, the association between different *TP53* mutation sites (exon 8 vs. other) and outcome of treatment were assessed.

Material and Methods

Study Design

As previously reported,[36] RELAY is a randomized, double-blind, Placebo-controlled, Phase III trial examining the efficacy of ramucirumab (RAM) (10 mg/kg intravenously) every 2 weeks plus erlotinib (ERL) (150 mg/day orally) in patients with untreated metastatic NSCLC with *EGFR* exon 19 deletion (ex19del) mutations or *EGFR* exon 21L858R (L858R) mutations.[36] Patients with known central nervous system metastases or T790M mutation were excluded from the trial. The primary endpoint of RELAY was PFS.[36] Secondary endpoints included overall response rate (ORR), disease control rate (DCR), duration of response (DoR), overall survival (OS), and safety. Exploratory endpoints included biomarker analysis. Tumor assessments were conducted using RECIST v1.1 and adverse events (AEs) were assessed at every cycle and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (v4.0). The protocol and amendments were approved by the ethics committees of all participating centers and all patients provided written informed consent before study entry. The trial was conducted according to the Declaration of Helsinki, the International Conference on Harmonization guidelines for good clinical practice, and applicable local regulations. The trial is registered at ClinicalTrials.gov (identifier: NCT02411448).

Biomarker Detection and Analysis Populations

Plasma samples were collected prior to the first dose of study drug, on day 1 of cycle 4, and at the 30-day poststudy treatment discontinuation follow up. Guardant 360 next-generation sequencing (NGS) (Guardant Health, Redwood City, CA) was used to screen circulating tumor DNA (ctDNA) for baseline and treatment-emergent gene alteration profiles. Germline mutations were excluded from the analysis.

For the analysis of baseline mutation profiles, NGS analyses were conducted in patients of the intent-to-treat (ITT) population from whom a valid baseline result (passed NGS testing QC) with at least one alteration was obtained. NGS analyses of postprogression follow-up alteration profiles were performed in patients who had disease progression by the poststudy treatment discontinuation visit and had at least one detectable alteration by NGS at baseline and at poststudy treatment discontinuation. Only genes detected were reported.

Classification of TP53 *Mutations*

The United States National Cancer Institute TP53 Database (https://tp53.isb-cgc.org/) was used to interpret *TP53* variants. *TP53* mutations were classified according to their predicted functional impact based on protein 3D structure and variant type using EffectGroup3.[40] The Sorting Intolerant From Tolerant algorithm, SIFT, was also applied on default settings to predict whether individual mutations affected protein function. Summaries were performed at the alteration level and did not represent individual patients.

Statistical Analyses

This exploratory post hoc analysis investigated the association between *TP53* status and clinical outcomes. Importantly, RELAY was not powered for analysis of *TP53* subgroups. Relationships between *TP53* status (mutant vs. wild-type; *TP53* exon 8 vs. nonexon 8) and clinical time-to-event outcomes were explored using an unadjusted Cox proportional hazards model comparing treatment within TP53 status subgroups. Corresponding hazard ratios (HR) and 95% confidence intervals (CI) were estimated and reported from this unadjusted Cox proportional hazards interaction model. The Kaplan-Meier method was used to plot time-to-event data and to provide summary statistics. Response rate CIs for overall response rate (ORR) and disease control rate (DCR) were calculated using the Wilson method. Descriptive summary statistics were used for safety measures and gene alteration frequencies within treatment and *TP53* subgroups. Statistical analyses were performed using SAS version 9.3 or higher or R Statistical Software version 3.4.4 or higher.

Results

Patient and Disease Characteristics

In RELAY, 449 patients (intention-to-treat [ITT] population) were randomized (Supplementary Figure 1). Of those, a total of 386 patients (86%) had a valid ctDNA baseline sample with at least one gene alteration detectable by NGS and were included in this analysis. Safety analyses were performed in all 386 patients as each received at least 1 dose of study drug. Patients were divided into subgroups according to *TP53* status, as shown in Table 1. 165 (42.7%) patients harbored a concurrent *TP53* mutation, and 221 (57.3%) patients had wild-type *TP53*. Of the 165 patients with *TP53* mutant tumors, 74 (44.8%) received RAM+ERL and 91 (55.2%) received PBO+ERL. Patients with *TP53* wild-type tumors were observed at similar rates in the RAM+ERL and PBO+ERL arms, as 118 patients (53.4%) and 103 (46.6%) patients in the wild-type subgroup received RAM+ERL and PBO+ERL respectively. At the time of data cutoff on 23 January, 2019, fewer patients with *TP53*-mutant tumors were still on study treatment (n = 22, 13.3%) in comparison to those with *TP53* wild-type (n = 68, 30.8%). Of those still on study treatment, more patients received RAM+ERL (TP53-mutant 21.6%; TP53-wild-type 32.2%) compared to PBO+ERL (TP53-mutant 6.6%, TP53 wild-type 29.1%)

The rates of patients of each race and *EGFR* exon 19 and exon21 mutations were comparable across the mutant and wild-type *TP53* subgroups (Table 1). Though the frequencies are not depicted,

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Table 1 Baseline Patient and Disease Characteristics

	n (%)	*TP53* Wild-Type (N = 221)	*TP53* Mutant (N = 165)	*TP53* Exon 8 (N = 41)	*TP53* Nonexon 8 (N = 124)
Sex	Female	147 (66.5)	94 (57.0)	22 (53.7)	72 (58.1)
Age	<65	94 (42.5)	92 (55.8)	19 (46.3)	73 (58.9)
Race	Asian	182 (82.4)	131 (79.4)	35 (85.4)	96 (77.4)
	White	38 (17.2)	33 (20.0)	6 (14.6)	27 (21.8)
Smoking history[a]	Ever	63 (28.5)	57 (34.5)	17 (41.5)	40 (32.3)
	Never	137 (62.0)	93 (56.4)	21 (51.2)	72 (58.1)
ECOG PS	0	120 (54.3)	80 (48.5)	23 (56.1)	57 (46.0)
***EGFR* mt type**	Ex19del	117 (52.9)	87 (52.7)	22 (53.7)	65 (52.4)
	L858R	102 (46.2)	78 (47.3)	19 (46.3)	59 (47.6)
CNS metastases at progression	No	215 (97.3)	161 (97.6)	39 (95.1)	122 (98.4)
	Yes	6 (2.7)	4 (2.4)	2 (4.9)	2 (1.6)
Liver metastases at progression	No	219 (99.1)	155 (93.9)	41 (100.0)	114 (91.9)
	Yes	2 (0.9)	10 (6.1)	0 (0.0)	10 (8.1)

CNS = central nervous system; ECOG PS = Eastern Cooperative Oncology Group Performance Score; ex19del = *EGFR* exon 19 deletion mutation; L858R = *EGFR* exon 21L858R; mutations mt = mutation; N = number of patients; n = number of patients in a sample; PBO+ERL, placebo plus erlotinib; RAM+ERL = ramucirumab plus erlotinib.
[a] Percentages may not total 100 due to the unknown status of some patients.

the values in Table 1 indicate that the rate of *TP53* mutations was similar in Asian and White patients and was comparable by *EGFR* mutation type. Interestingly, patients who were ever smokers were observed to have marginally higher rates of *TP53* alterations at baseline compared to never smokers (47.5% vs 40.4% respectively) (Supplementary Table 1). Baseline characteristics were not completely balanced between those with *TP53* mutant and wild-type tumors, though the majority of differences were ≤10%. The only parameter with a difference greater than 10% was age, as of the patients with *TP53*-mutant tumors, 55.8% were younger than 65, versus 42.5% of patients with wild-type *TP53*. Liver metastases at progression were more common in the *TP53* mutant subgroup, while those with *TP53* wildtype tumors had a greater proportion of patients with ECOG PS 0 (Table 1).

Approximately a quarter of the patients (24.8%) with a concurrent *TP53* mutation at baseline had a mutation in exon 8, and the remaining 124 (75.2%) had nonexon 8 mutations. Though the frequencies are not depicted, the values in Table 1 indicate that rate of exon 8 mutations was similar in Asian and White patients and was comparable by *EGFR* mutation type (Table 1).

Patient and disease characteristics were not completely balanced by treatment arm, though the majority of differences were ≤5%, as depicted in Supplementary Table 2.

Baseline Genetic Profiles

Among the 165 patients with concurrent *TP53* mutations present at baseline, there were 8 commonly affected exons. Supplementary Table 3 shows the distribution and percentages of all *TP53* exon mutations detected at baseline. The most frequent *TP53* mutations were in exon 5 (26.7%; n = 44), followed by exon 8 (24.8%; n = 41) and exon 7 (24.2%; n = 40). Twenty-four (14.5%) and 17 (10.3%) patients harbored exon 6 and exon 4 mutations, respectively. Eighteen (10.9%) patients had mutations detected across exons 9, 10, and 11.

Table 2 depicts the genetic profile at baseline of the 386 patients included in the analysis. The types of concurrent genetic alterations were comparable between *TP53* mutant and wild-type tumors and the majority of differences in incidence were less than 5%, and all were below 10% (Table 2). The most common concurrent gene alterations in the *TP53* mutation subgroup were in *PIK3CA* (15.8%; n = 26), *CDK6* (10.3%; n = 17), and *BRAF* (9.7%; n = 16), which were observed at a higher frequency than in the *TP53* wild-type subgroup (5.9%, 1.4%, and 3.6%, respectively). Overall, concurrent gene alterations were found at a higher incidence in *TP53* mutant tumors, with the exception of *KRAS, mTOR, NF1*, and *CTNNB1*, which were observed at respective rates of 3.0%, 2.4%, 6.7%, and 4.2% in *TP53* mutant tumors, and respective rates of 4.1%, 3.6%, 8.6%, and 4.5% in the *TP53* wild-type subgroup. Of those that presented with exon 8 mutations at baseline, 12.2% (n = 5), 12.2% (n = 5), and 9.8% (n = 4) had additional mutations in *MET, NF1*, and *SMAD4* respectively (Table 2). These alterations occurred at a higher frequency in comparison to those harboring *TP53* nonexon 8 mutations at baseline. Notably however, differences observed between the groups were all less than 10%.

Approximately one fifth (20.6%) of patients with mutant *TP53* and 13.6% of patients with wild-type *TP53* had no other concurrent somatic alteration besides *EGFR* (Supplementary Table 4). Conversely, at least one additional concurrent alteration (not *EGFR* or *TP53*) was detected in 74.6% of patients with mutant *TP53* and 30.2% of patients with wild-type *TP53* (Supplementary Table 4).

Concurrent *EGFR, TP53* and *RB1* alterations were more frequently found in those with *TP53* exon 8 mutations in comparison to the *TP53*-mutant and *TP53* nonexon 8 subgroups, although differences were small. Patients with *EGFR*/*RB1*/*TP53*-mutant

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Table 2 Concurrent Baseline Gene Alterations According to *TP53* Status

n(%)	*TP53* Wild-Type (N = 221)	*TP53* Mutant (N = 165)	*TP53* Exon 8 (N = 41)	*TP53* nonexon 8 (N = 124)
APC	14 (6.3)	13 (7.9)	2 (4.9)	11 (8.9)
BRAF	8 (3.6)	16 (9.7)	5 (12.2)	11 (8.9)
BRCA1	5 (2.3)	12 (7.3)	4 (9.8)	8 (6.5)
CCND1	2 (0.9)	3 (1.8)	1 (2.4)	2 (1.6)
CDK4	4 (1.8)	3 (1.8)	1 (2.4)	2 (1.6)
CDK6	3 (1.4)	17 (10.3)	6 (14.6)	11 (8.9)
CTNNB1	10 (4.5)	7 (4.2)	1 (2.4)	6 (4.8)
ERBB2	3 (1.4)	11 (6.7)	2 (4.9)	9 (7.3)
KRAS	9 (4.1)	5 (3.0)	2 (4.9)	3 (2.4)
MET	8 (3.6)	12 (7.3)	5 (12.2)	7 (5.6)
MTOR	8 (3.6)	4 (2.4)	1 (2.4)	3 (2.4)
NF1	19 (8.6)	11 (6.7)	5 (12.2)	6 (4.8)
PIK3CA	13 (5.9)	26 (15.8)	6 (14.6)	20 (16.1)
PTEN	2 (0.9)	4 (2.4)	1 (2.4)	3 (2.4)
RB1	5 (2.3)	8 (4.8)	3 (7.3)	5 (4.0)
SMAD4	5 (2.3)	8 (4.8)	4 (9.8)	4 (3.2)

N = number of patients; n = number of patients in a sample.

NSCLC represented 4.8% (n = 8), 7.3% (n = 3), and 4.0% (n = 5) of the *TP53*-mutant, *TP53* exon 8, and *TP53* nonexon 8 subgroups, respectively.

As shown in Supplementary Table 5, the percentage of concurrent gene alterations were not completely balanced by treatment arm, though the majority of differences were less than 5%. Gene alterations with a difference of ≥10% were observed in patients with TP53 exon 8 mutations. These included, *ERBB2, NF1, SMAD4*, and *KRAS* which were more frequent in the RAM+ERL arm, and *PIK3CA* and *RB1*, which were more prevalent in the PBO+ERL arm.

There was no evidence of a significant association between *TP53* status and clearance of activating *EGFR* alterations (a*EGFR*) in ctDNA by cycle 4 (Supplementary Table 6). Of the 78 patients in the *TP53* wild-type subgroup with a*EGFR* detected in their plasma, 84.6% cleared a*EGFR* by cycle 4, while 76.0% of those with mutant TP53 cleared a*EGFR* by cycle 4.

TP53 *Analysis*

Variant classification based on protein 3D structure and variant type (EffectGroup3) categorized the detected *TP53* mutations as missense in DNA-binding loops (n = 87), other missense (n = 40), in-frame deletions or insertions (n = 5), frameshift, splice site, and nonsense (n = 53), and not classified (n = 8) (Supplementary Table 7). Utilizing the SIFT algorithm, 124 mutations were classified as damaging, 4 as tolerated mutations, and 65 were not classified.

Progression-Free Survival

Irrespective of treatment, patients with a concurrent *TP53* mutation had a shorter PFS in comparison to patients with TP53 wild-type tumors (12.25 vs. 19.35 months, respectively; HR 1.867; 95% CI, 1.448-2.407) (Supplementary Figure 2A). In patients with *TP53* mutant tumors, RAM+ERL demonstrated superior PFS compared with PBO+ERL, with a median PFS of 15.2 months and 10.6 months, respectively (HR 0.54; 95% CI, 0.37- 0.79) (Figure 1A). A similar trend was observed among patients with *TP53* wild-type tumors, with a median PFS of 20.8 months for RAM+ERL versus 15.7 months for PBO+ERL (HR 0.79; 95% CI 0.55-1.12). Patients carrying *TP53* exon 8 mutations had a shorter median PFS than those with nonexon 8 mutations (Supplementary Figure 2B), however both TP53 exon and nonexon 8 benefitted from treatment with RAM+ERL (HR 0.628 and 0.491, respectively) (Figure 1B).

Analysis was also conducted on the impact of RAM+ERL on PFS in different subpopulations by *TP53* status. The presence of baseline *TP53* alterations were associated with shorter PFS in comparison to wild-type *TP53* in the East Asian population (Supplementary Figure 3A). However, RAM+ERL increased PFS compared with PBO+ERL irrespective of *TP53* mutation status. In the North American/European subpopulation of RELAY, RAM+ERL demonstrated a superior median PFS compared with PBO+ERL in patients with mutant *TP53* (19.35 vs. 7.88 months, respectively [HR 0.20, 95% CI, 0.08-0.45]), while there was a lack of treatment benefit in those with wild-type *TP53* (Supplementary Figure 3B). PFS was also assessed by *EGFR* ex19del mutations and EGFR L858R mutations. The effect of a baseline *TP53* mutation is consistent regardless of EGFR activating mutation, as in patients with concurrent *TP53* mutations at baseline, RAM+ERL demonstrated a superior PFS compared with PBO+ERL in both patients with *EGFR* ex19del mutations (17.97 vs. 9.86 months, respectively; HR 0.50, 95%; CI, 0.29-0.85) and *EGFR* L858R mutations (14.65 vs. 10.84 months, respectively; HR 0.56, 95%; CI, 0.34-0.95) (Figure 2A). In *TP53* wild-type patients, there was a trend toward increased PFS benefit from RAM+ERL for the L858R subgroup, and no PFS benefit was observed from RAM+ERL in the ex19del subgroup (Figure 2B). Increased PFS benefit was observed among

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Figure 1 Kaplan-Meier estimates of median progression-free survival by (A) mutant or wild-type *TP53*, and (B) TP53 exon 8 mutations or nonexon 8 mutations. CI = confidence intervals; HR = hazard ratio; PBO+ERL = placebo plus erlotinib; RAM+ERL = ramucirumab plus erlotinib.

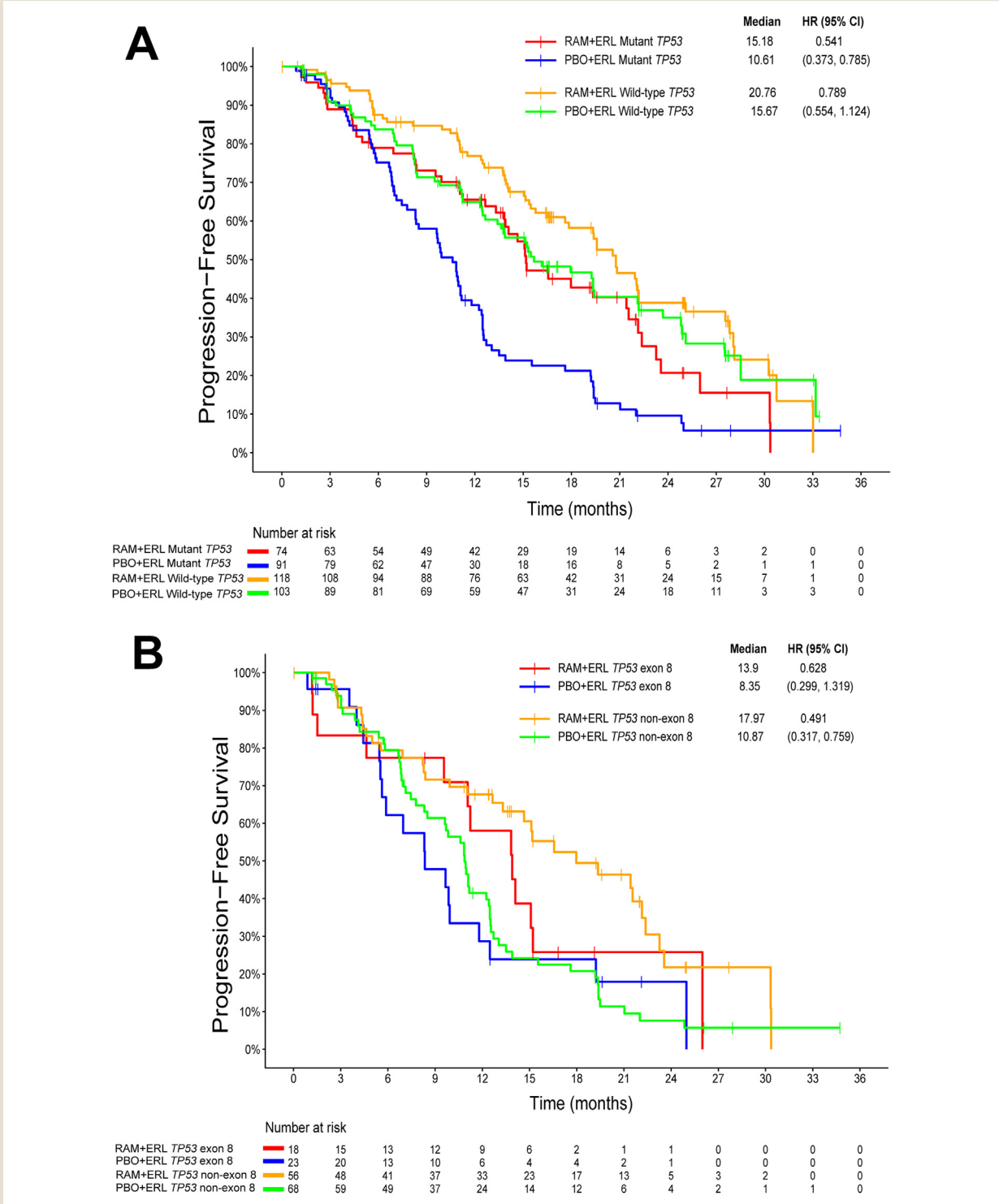

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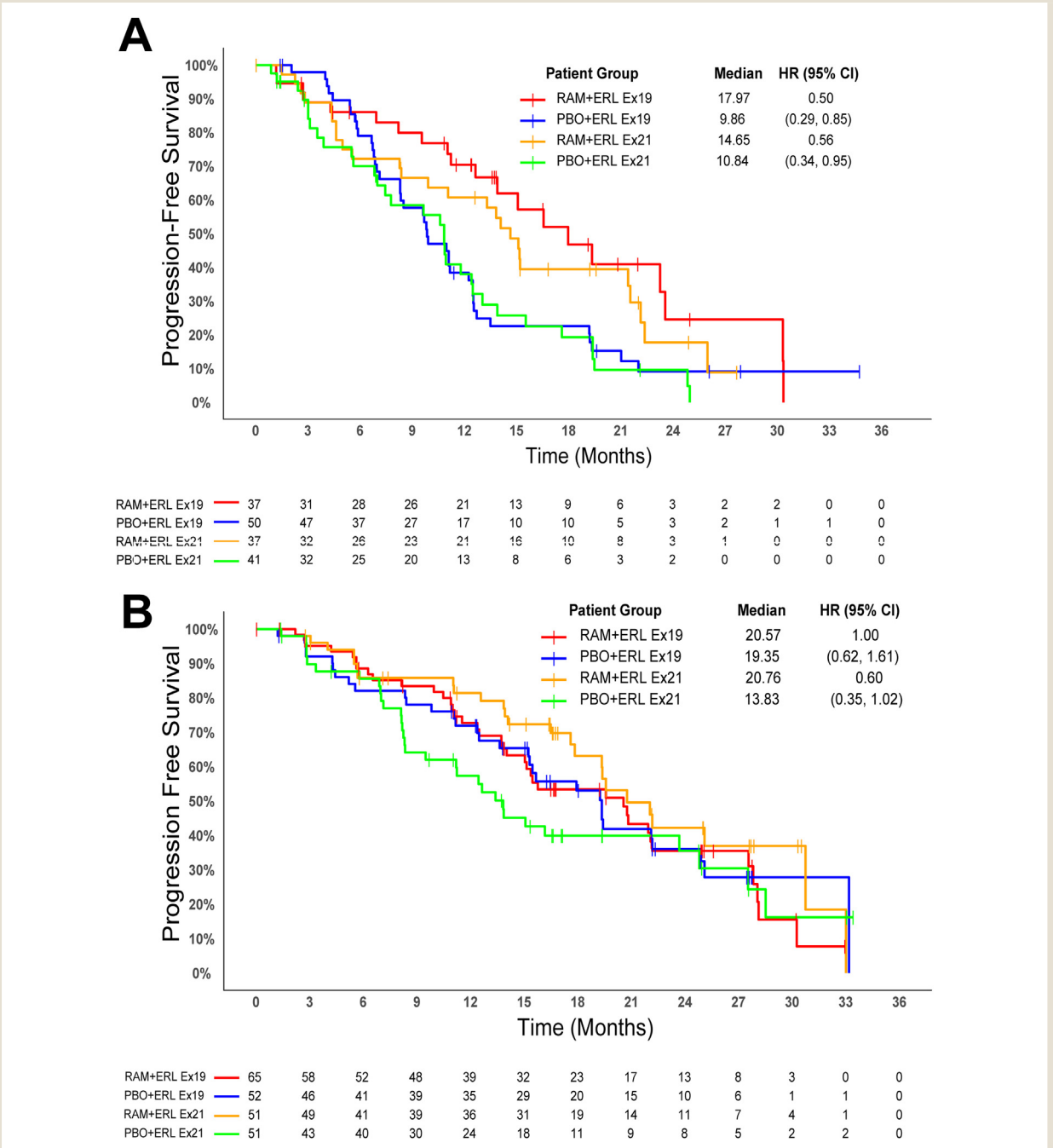
Figure 2 Kaplan-Meier curves of progression-free survival by baseline activating EGFR mutations in (A) patients with TP53 mutant tumors, and (B) TP53 wild-type tumors at baseline. CI = confidence intervals; HR = hazard ratio; PBO+ERL = placebo plus erlotinib; RAM+ERL = ramucirumab plus erlotinib; Ex19 = EGFR exon 19 deletion; Ex21 = EGFR exon 21 L858R mutation.



ever smokers compared with never smokers, with the biggest treatment effect observed in ever smokers who had a *TP53* mutation at baseline (9.82 vs. 15.11 months; HR 0.44; 95% CI, 0.21-0.92; PBO+ERL vs RAM+ERL, respectively) (Supplementary Figure 4).

Overall Response and Disease Control Rates

TP53 mutant and *TP53* wild-type tumors had similar ORRs and DCRs, though ORR was observed to be approximately 5% higher in those receiving RAM+ERL compared with PBO+ERL, regardless of *TP53* status (Table 3). A best response of progressive

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Table 3 Overall Response and Disease Control Rates According to *TP53* Status

	TP53 Wild-Type		*TP53* Mutant		*TP53* Exon 8		*TP53* Nonexon 8	
	RAM+ERL N = 118	PBO+ERL N = 103	RAM+ERL N = 74	PBO+ERL N = 91	RAM+ERL N = 18	PBO+ERL N = 23	RAM+ERL N = 56	PBO+ERL N = 68
CR, n (%)	3 (2.5)	1 (1.0)	0 (0)	1 (1.1)	0 (0)	0 (0)	0 (0)	1 (1.5)
PR, n (%)	89 (75.4)	75 (72.8)	61 (82.4)	69 (75.8)	14 (77.8)	17 (73.9)	47 (83.9)	52 (76.5)
SD, n (%)	22 (18.6)	24 (23.3)	9 (12.2)	16 (17.6)	1 (5.6)	3 (13.0)	8 (14.3)	13 (19.1)
PD, n (%)	0 (0)	2 (1.9)	3 (4.1)	2 (2.2)	3 (16.7)	1 (4.4)	0 (0)	1 (1.5)
NE, n (%)	4 (3.4)	1 (1.0)	1 (1.4)	3 (3.3)	0 (0)	2 (8.7)	1 (1.8)	1 (1.5)
ORR (95% CI)	78.0 (69.7, 84.5)	73.8 (64.6, 81.3)	82.4 (72.2, 89.4)	76.9 (67.3, 84.4)	77.8 (54.8, 91.0)	73.9 (53.5, 87.5)	83.9 (72.2, 91.3)	77.9 (66.7, 86.2)
DCR (95% CI)	96.6 (91.6, 98.7)	97.1 (91.8, 99.0)	94.6 (86.9, 97.9)	94.5 (87.8, 97.6)	83.3 (60.8, 94.2)	87.0 (67.9, 95.5)	98.2 (90.6, 99.7)	97.1 (89.9, 99.2)

CI = confidence intervals; DCR = disease control rate; ORR = overall response rate; N = number of patients; n = number of patients in a sample; PBO+ERL = placebo plus erlotinib; RAM+ERL = ramucirumab plus erlotinib.

disease (PD) was below 5% in both patients with mutant and wild-type *TP53* independent of treatment. Notably however, patients with tumors harboring *TP53* mutations on exon 8 who received RAM+ERL had the highest rate of PD (16.7%) (Table 3).

Duration of Response

DoR favored the RAM+ERL arm versus the PBO+ERL arm in both patients with *TP53* mutant and wild-type tumors (Figure 3). Patients with *TP53* mutation treated with RAM+ERL were associated with a shorter median DoR relative to patients with wild-type *TP53* (15.2 [95% CI, 12.520.3] vs. 18.2 [95% CI, 14.120.6] months) (Figure 3A). Among patients with *TP53*-mutant tumors in the RAM+ERL arm, those carrying exon 8 mutations exhibited a shorter median DoR than those with nonexon 8 mutations (13.3 [95% CI, 8.2-NR] vs. 18.0 [11.1-20.5] months, respectively)(Figure 3B).

Treatment Emergent Gene Alterations

Treatment-emergent gene alterations at 30-day follow-up after disease progression are displayed in Table 4. There was a slight increase in the number of patients who developed genetic alterations among those with *TP53*-mutant tumors (57 of 84) versus those with wildtype *TP53* (52 of 84), though the difference was not significant ($P = .419$). The total number of emergent alterations were increased, and the number of unique mutations were decreased among those with *TP53* mutation compared to those with *TP53* wild-type (data not shown). *EGFR* T790M was the most likely mutation to develop postprogression. *EGFR* T790M mutation rates were increased in patients with *TP53* mutant tumors compared to those with *TP53* wild-type (37% for *TP53*-mutant tumors, 20% for wild-type *TP53* overall), and similar across both treatment arms. Among patients with wild-type *TP53* at baseline, the most likely alterations to emerge postprogression were *TP53* (27.0%) in the RAM+ERL arm, and *EGFR* (non-T790M variants) (23.4%) in the PBO+ERL arm. Of the patients in the *TP53* mutant and wild-type subgroups with postprogression *TP53* detected at the 30-day follow-up, newly emergent *TP53* alterations were detected as early as 4 cycles, independent of treatment (Supplementary Figure 5).

Five (3%) patients harbored concurrent *EGFR, TP53*, and *RB1* alterations at disease progression (3 in the RAM+ERL arm; 2 in the PBO+ERL arm). A single patient (0.6%) patient in the RAM+ERL arm was triple emergent for *EGFR, TP53*, and *RB1* alterations at progression (data not shown).

Postdiscontinuation Therapy

Among patients with a *TP53* mutation, 44 (59.5%) in the RAM+ERL arm and 75 (82.4%) in the PBO+ERL arm received any postdiscontinuation therapy (Supplementary Table 8). In those with wild-type *TP53*, postdiscontinuation therapy was administered to 64 (54.2%) and 60 (58.3%) patients in the RAM+ERL and PBO+ERL arms, respectively. For both *TP53* groups, EGFR-TKIs, predominantly erlotinib, and osimertinib, were the most frequent postdiscontinuation therapy, followed by chemotherapy. Of those with *TP53* mutant tumors, EGFR-TKIs were used more frequently in patients treated with PBO+ERL (65.9%) than RAM+ERL (50.0%). In patients with wild-type *TP53*, EGFR-TKIs were used at similar rates in both treatment arms. Osimertinib was used more frequently as any subsequent therapy in patients with *TP53* mutant tumors treated with PBO+ERL (35.2%) compared with RAM+ERL (25.7%). For patients with wild-type *TP53*, osimertinib was received by 22.9% of those in the RAM+ERL arm and 16.5% of those in the PBO+ERL. These findings should be interpreted with caution as osimertinib use may be dictated by the presence of T790M mutation. Chemotherapy was administered more frequently in patients with *TP53* mutations treated with PBO+ERL (45.1%) than RAM+ERL (25.7%). Similarly, in patients with wild-type *TP53*, chemotherapy was more commonly used in the PBO+ERL (31.1%) arm than the RAM+ERL (19.5%) arm.

Independent of *TP53* status, EGFR-TKIs were the most common first subsequent therapy. *TP53* status did not appear to impact the rate at which chemotherapy was administered as first subsequent therapy. In both patients with *TP53* mutant and wild-type tumors, chemotherapy was used as first subsequent therapy at slightly higher rates in patients treated PBO+ERL than RAM+ERL.

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Figure 3 Kaplan-Meier estimates of median duration of response by (A) mutant or wild-type *TP53*, and (B) *TP53* exon 8 mutations or nonexon 8 mutations. CI = confidence intervals; HR = hazard ratio; PBO+ERL = placebo plus erlotinib; RAM+ERL = ramucirumab plus erlotinib.

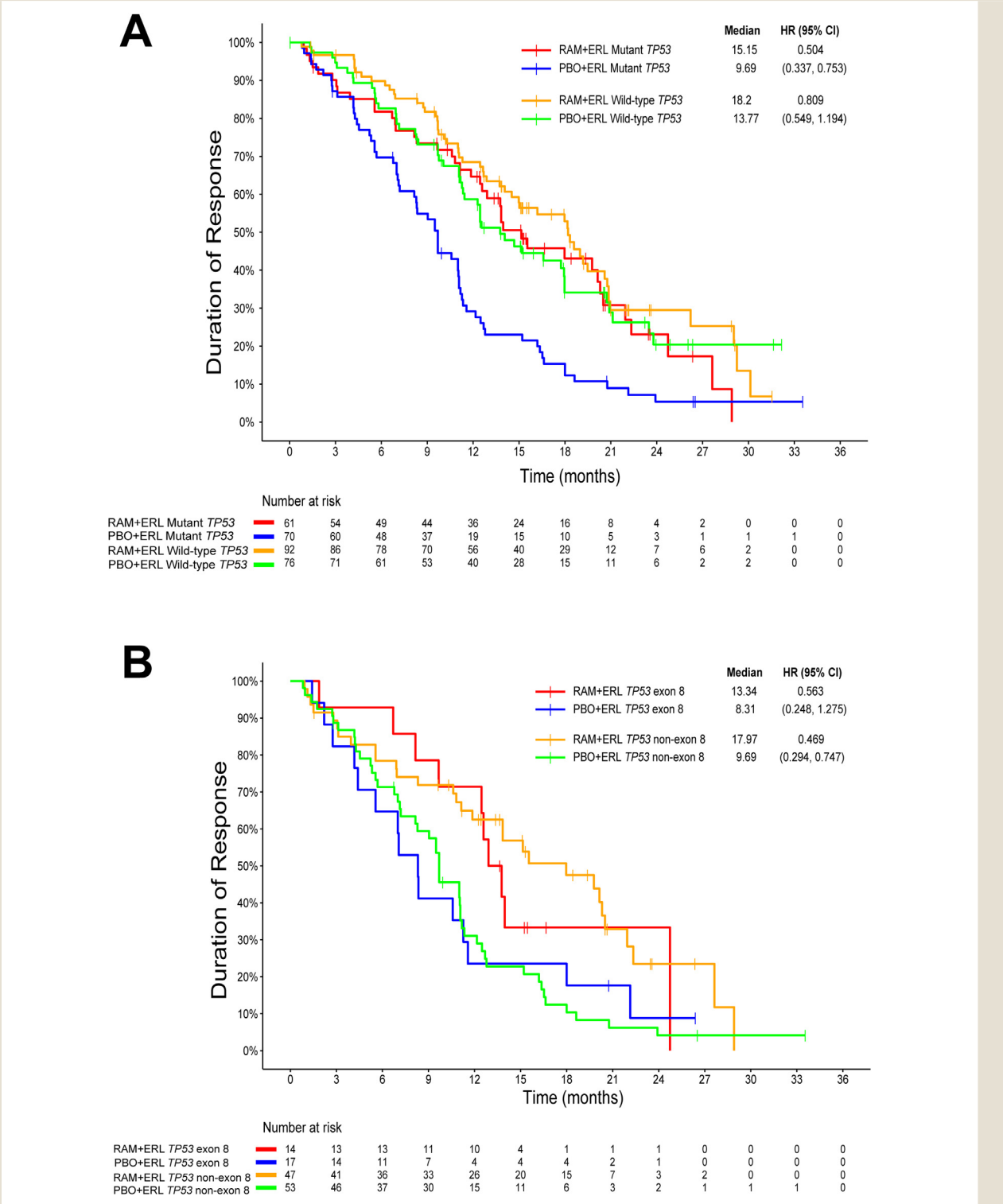

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Table 4 Treatment Emergent Gene Alterations After Disease Progression

n(%)	TP53 Wild-Type		TP53 Mutant		TP53 Exon 8		TP53 Nonexon 8	
	RAM+ERL N = 37	PBO+ERL N = 47	RAM+ERL N = 30	PBO+ERL N = 54	RAM+ERL N = 11	PBO+ERL N = 13	RAM+ERL N = 19	PBO+ERL N = 41
Any	52 (61.9)		57 (67.9)		15 (62.5)		42 (70.0)	
NF1	2 (5.4)	1 (2.1)	4 (13.3)	1 (1.9)	1 (9.1)	1 (7.7)	3 (15.8)	0 (0.0)
PIK3CA	1 (2.7)	3 (6.4)	0 (0.0)	4 (7.4)	0 (0.0)	1 (7.7)	0 (0.0)	3 (7.3)
MET	3 (8.1)	2 (4.3)	1 (3.3)	5 (9.3)	1 (9.1)	2 (15.4)	0 (0.0)	3 (7.3)
FGFR2	2 (5.4)	0 (0.0)	1 (3.3)	3 (5.6)	0 (0.0)	1 (7.7)	1 (5.3)	2 (4.9)
KIT	2 (5.4)	0 (0.0)	1 (3.3)	1 (1.9)	0 (0.0)	0 (0.0)	1 (5.3)	1 (2.4)
TP53	10 (27.0)	7 (14.9)	4 (13.3)	6 (11.1)	2 (18.2)	1 (7.7)	2 (10.5)	5 (12.2)
***EGFR* other**[a]	7 (18.9)	11 (23.4)	4 (13.3)	10 (18.5)	2 (18.2)	2 (15.4)	2 (10.5)	8 (19.5)
***EGFR* T790M**	7 (18.9)	10 (21.3)	10 (33.3)	21 (38.9)	4 (36.4)	3 (23.1)	6 (31.6)	18 (43.9)
KRAS	4 (10.8)	0 (0.0)	2 (6.7)	3 (5.6)	0 (0.0)	1 (7.7)	2 (10.5)	2 (4.9)
RB1	1 (2.7)	1 (2.1)	1 (3.3)	1 (1.9)	1 (9.1)	0 (0.0)	0 (0.0)	1 (2.4)
NONE	13 (35.1)	19 (40.4)	6 (20.0)	21 (38.9)	2 (18.2)	7 (53.8)	4 (21.1)	14 (34.1)

A cut-off frequency of ≥5% was used.
N = number of patients; n = number of patients in a sample; PBO+ERL = placebo plus erlotinib; RAM+ERL = ramucirumab plus erlotinib.
[a] EGFR other includes all non-T790M gene alterations.

Table 5 Overview of Safety Profile According to Baseline *TP53* Status

N (%)	*TP53* Wild-Type		*TP53* Mutant	
	RAM+ERL N = 118	PBO+ERL N = 103	RAM+ERL N = 74	PBO+ERL N = 91
Patients with at least 1 TEAE, any Grade	118 (100.0)	103 (100.0)	74 (100.0)	91 (100.0)
Patients with at least 1 TEAE, Grade ≥3	86 (72.9)	53 (51.5)	55 (74.3)	54 (59.3)
Patients with at least 1 SAE	41 (34.7)	19 (18.4)	17 (23.0)	23 (25.3)
Patients who discontinued study treatment due to an AE	16 (13.6)	10 (9.7)	9 (12.2)	14 (15.4)
Patients who discontinued study treatment due to an SAE	7 (5.9)	4 (3.9)	1 (1.4)	5 (5.5)
Deaths on study treatment due to AE	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)

AE = adverse events; N = number of patients; n = number of patients in a sample; PBO+ERL = placebo plus erlotinib; RAM+ERL = ramucirumab plus erlotinib; TEAE = treatment-emergent adverse events; SAE = serious adverse events.

Safety

An overview of safety profile according to baseline *TP53* status is presented in Table 5. All patients reported at least one treatment-emergent adverse event (TEAE). There were no clinically meaningful differences in the safety profiles between those with baseline *TP53* mutation and wild-type. The rate of grade 3 or higher TEAEs was increased in the RAM+ERL arm, irrespective of *TP53* status. Patients with *TP53* wild-type treated with RAM+ERL had a higher incidence of SAEs (34.7%) compared to other patients in the analysis population. In the RAM+ERL arm, hypertension was the most common grade ≥3 AE observed in both patients with TP53 mutant (25.7%) and wild-type (22.0%) tumors (Supplementary Table 9). Dermatitis acneiform was the second most frequent grade ≥3 AE in the RAM+ERL arm, with a rate of ~16% in the *TP53* mutant and wild-type subgroups. Study treatment discontinuation rates due to AEs were comparable between *TP53* mutant and wild-type tumors in both the RAM+ERL arm (12.2% vs. 13.6%, respectively) and the PBO+ERL arm (15.4% vs. 9.7%, respectively). One death on study treatment due to an AE (interstitial lung disease) occurred in a patient with *TP53* wild-type treated with RAM+ ERL.

Discussion

In the current exploratory analysis, we examined the effect of *TP53* status, and specific exons, on clinical outcomes using data from the RELAY trial. The incidence rate of concurrent baseline *TP53* mutations observed was consistent with rates previously reported in patients with *EGFR*+metastatic NSCLC.[9] Our findings indicated that dual inhibition of the EGF and VEGF pathways with RAM+ERL exhibited benefit compared with PBO+ERL, independent of *TP53* status. Overall, safety profiles were similar between the treatment arms and were generally consistent with the ITT population of RELAY. In addition, the data further confirmed that the presence of mutant *TP53* at baseline was a negative prognostic indicator. However, while concurrent *TP53* mutations appear to carry a poorer prognosis, clinical outcomes indicated a trend for greater RAM+ERL benefit in those with mutant *TP53*. This analysis may inform future research efforts, particularly of combined EGFR an VEGF inhibition. Our findings are consistent with those reported by Zhao et al in the Phase III ACTIVE study, which explored the concept of EGFR and VEGF inhibition using gefitinib plus apatinib in treatment-naive

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advanced *EGFR*+NSCLC.[28] In comparison to gefitinib plus placebo, combined treatment demonstrated superior PFS in the ITT (10.2 vs. 13.7 months, respectively). Post hoc analyses of trial data showed that patients harbouring a *TP53* mutation benefitted most from the treatment combination (PFS HR 0.56), while those with *TP53* wild-type tumors received little benefit from the combination (PFS HR 0.92). The link between *TP53* mutations and overexpression of VEGF may offer a biological explanation for why better outcomes was observed in patients with *EGFR*+ *TP53*-mutant tumors with dual EGFR/VEGF pathway inhibition.[13,14] This association between *TP53* and VEGF has been observed across different tumor types and plausibly represents an underlying biological process. Indeed, our findings are aligned with multiple studies that indicate *TP53*-mutant tumors may benefit most from VEGF inhibition.[25-28]

The presence of *TP53* mutations in exon 8 were associated with inferior PFS and DoR compared to those with nonexon 8 mutation. Mutations in exon 8 impact the DBD of p53 and can lead to loss of regulatory functions. Evidence also suggests that mutations in exon 8 may be involved in the primary resistance mechanism to EGFR-TKIs, possibly explaining the association with inferior outcomes in this study.[12] Despite their association with poor prognosis, those with *TP53* exon 8 mutations demonstrated improved clinical outcomes in RELAY, a finding also noted in the ACTIVE trial.[28]

In RELAY, both East Asian patients with *TP53* mutant and wild-type tumors benefitted from RAM+ERL, while a difference in the median PFS of approximately 1-year in favor of RAM+ERL was observed in the North American/European population with *TP53* mutations (HR 0.20; 95% CI, 0.08-0.45). No PFS benefit was evident in the North American/European population with wild-type *TP53*. However, these findings are limited by the small sample sizes of the subgroups. Patients with a concurrent *TP53* mutation at baseline, treated with RAM+ERL, had an improved outcome compared to PBO+ERL regardless of ex19del or L858R mutation status, whereas no treatment benefit was observed with RAM+ERL in wild-type *TP53* and the ex19del mutation subgroup. These findings suggest that RAM+ERL may not be a better first-line treatment option than PBO+ERL in *TP53* wild-type patients with ex19del mutations. *TP53* mutations were detected at similar rates in the *EGFR* ex19del and L858R subgroups, indicating that while L858R mutations are associated with poorer prognosis, the subgroup was not enriched for *TP53* mutations.[9]

Interestingly, a treatment interaction analysis indicated that for PFS, the benefit of combination treatment was greater in ever-smokers than never-smokers. This is consistent with the findings of the BOOSTER and BEVERLEY trials, wherein dual inhibition of EGF and VEGF was evaluated in patients with *EGFR*+NSCLC.[41,42] In our analysis, the biggest treatment effect was observed in ever-smokers who had a *TP53* mutation at baseline. While it remains unclear whether *TP53* mutations were the underlying reason for the greater treatment effect observed in smokers, smoking and *TP53* alterations at baseline were associated with a poor prognosis, as although RAM+ERL had a greater treatment effect compared to PBO+ERL in this subpopulation, PFS was worse compared to never smokers and *TP53* wild-type tumors.

ORR and DCR did not differ by *TP53* status or between treatment arms and were generally consistent with rates previously reported for RELAY,[36] however, prolonged DoR was observed in RAM+ERL treated patients, reflecting the extension in PFS. Patients with *TP53* exon 8 mutations present at baseline had approximately 10% lower DCR in both treatment arms compared with the rest of the analysis population. Notably, patients with *TP53* exon 8 mutations in the PBO+ERL arm had a 6-month PFS-rate of approximately 60% despite the high percentage of patients who achieved tumor responses, indicating that approximately 40% of patients did not show sufficient clinical benefit and may be resistant to single agent EGFR-TKI treatment. For those with *TP53* exon 8 mutations in the RAM+ERL arm, the 6-month PFS rate was 77%, suggesting that the addition of a VEGF inhibitor may overcome primary resistance.

NGS screening after disease progression revealed that patients with *TP53* mutations at baseline have a higher proportion of acquired *EGFR* T790M mutation after progression compared to those with wild-type *TP53* at baseline. Emergent T790M may be indicative of involvement in acquired resistance to EGFR-TKIs and may be contributing to the poor prognosis associated with *TP53*. What is more, the rates of T790M mutation were similar between treatment groups after disease progression, suggesting that the addition of ramucirumab did not impact the frequency of erlotinib-associated T790M mutation. These findings indicate a potential opportunity to identify an optimal treatment sequence for these patients, as osimertinib demonstrates anticancer activity in tumors with T790M-positive mutation status.[43] Osimertinib was indeed a commonly administered postdiscontinuation therapy in both treatment arms. While published data suggest that *TP53* mutations, particularly in exon 8, reduce the efficacy of first-line osimertinib in EGFR+NSCLC,[24] this study demonstrated the benefit of combining RAM+ERL in all patients with *EGFR*+NSCLC, independent of *TP53* status. These data may suggest the potential of utilizing ramucirumab to improve outcomes with osimertinib in *TP53*+ *EGFR*+ NSCLC. The ongoing RAMOSE, TORG.1833, and WJOG14420L trials may provide insights into whether combining ramucirumab with osimertinib will further improve outcomes in EGFR+ NSCLC, and if specific subgroups, including those with mutant *TP53*, benefit more from the addition of a VEGF inhibitor.[44-46]

In *EGFR*+ NSCLC, concurrent *TP53* and *RB1* alterations characterize a subset of patients at increased risk for small cell transformation. Moreover, the transformation of tumor histology from NSCLC to SCLC is a known mechanism of acquired resistance to EGFR-TKIs in *EGFR*+NSCLC.[9,47,48] In this analysis, concurrent *RB1* mutations seemed most prevalent in those with *TP53* exon 8 mutations, possibly identifying this group as at increased risk of small cell transformation. However, it was not possible to evaluate this further as ancillary molecular testing was performed using liquid biopsy samples, while confirmatory diagnosis of SCLC requires histological examination.

The activity of p53 depends on the structural conformation of the protein. In its active form, p53 is a tetramer with a high affinity for DNA. Mutations in the protein may alter the conformation and inhibit DNA binding. Depending on the protein domain

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affected, mutations can confer new gain of function activities that enhance tumor progression. Most studies investigating the prognostic role of *TP53* status focus on discriminating patients with wild-type versus mutant tumors. Though our analysis demonstrates the clinical value of this distinction, evidence also indicates the potential benefit of classifying *TP53* mutations based on their functional effects on the p53 protein. Using the TP53 Database (https://tp53.isb-cgc.org/), a wide range of genetic variants were identified among those with *TP53*-mutant tumors in this analysis, including missense and nonsense mutations, in-frame deletions or insertions, and others that could not be classified. According to the SIFT algorithm, the functional impact could not be classified for the majority of variants. This indicates the difficulty in categorizing *TP53* mutations in a clinical setting. Due to the majority of clinical samples having a single *TP53* mutation detected, and the overall complexity of *TP53* gene and encoded p53 protein, there are significant challenges detecting and classifying these mutations according to their potential clinical impact. Of those identified using the SIFT algorithm, detected mutations were predicted as either damaging or tolerated. Notwithstanding, while the identified mutations were predicted to be detrimental, caution should be used when interpreting the predicted impact of detected *TP53* mutations. Due to the complex nature of *TP53* signaling, functional studies would be needed for verification.

As mutant *TP53* is implicated in many tumor types, there is significant interest and ongoing research to identify an effective therapeutic strategy to target the aberration. Although despite intensive efforts, no targeted agent has received approval for use in a clinical setting, indicating the complexity of treating patients with mutated *TP53*. To this end, the development of *TP53* reactivating compounds is an interesting advancement in the treatment of *TP53*-mutant tumors. Eprenetapopt is a small molecule with the ability to selectively bind mutant *TP53*, leading to thermodynamic stabilization of the molecule. The resulting functional conformation has been shown to induce apoptosis and increase oxidative stress in *TP53*-mutant tumor cells.[49,50] Though the agent is still in early clinical development stages, combining eprenetapopt and pembrolizumab has demonstrated safety, tolerability, and early signs of anticancer activity in multiple tumor types, including NSCLC.[51] Given the complexity of the numerous responses regulated by the p53 pathway and the high incidence of *TP53* mutations in NSCLC, this is an interesting and promising development for future therapeutic combinations in *TP53*-mutant NSCLC.

There were several limitations to this analysis. Firstly, while this study is a relevant contribution to the field, formal statistical tests were not performed in the *TP53* subgroups, owing to the small sample for some subgroups and the exploratory nature of the analyses. These factors should be taken into consideration when interpreting these findings from RELAY. Second, as the stratification at randomization was applied to the RELAY ITT population and not to each *TP53* subgroup, discrepancies in ECOG PS score and the proportion of patients under 65 may be contributing to the difference observed between treatment arms. Finally, molecular profiling was performed using only ctDNA with no NGS of companion biopsies at baseline and/or progression. In accordance with the study protocol and informed consent, tissue biopsies were collected at baseline and were utilized for confirmatory EGFR testing only. Less invasive liquid biopsy samples were utilized to evaluate ctDNA and characterize the tumor molecular profile. Thus, some of the detected baseline genetic alterations may not be derived from ctDNA, but may indicate clonal hematopoiesis of indeterminate potential.

In conclusion, this analysis confirms that *TP53* mutations are a negative prognostic marker in *EGFR*+ NSCLC and extends on other reports that the addition of a VEGF inhibitor improves outcomes in *TP53* mutant tumors. Ramucirumab plus erlotinib is an efficacious first-line treatment option for all patients with *EGFR*+ and *TP53* mutant NSCLC. In patients with wild-type TP53, no treatment benefit from the addition of ramucirumab to erlotinib was observed in the subgroup with EGFR ex19del mutation.

Clinical Practice Points

- Results from several studies indicate that mutant *TP53* is a negative prognostic factor and that *EGFR*+ NSCLC patients with concurrent *TP53* mutations, most notably in exon 8, generally have more aggressive disease, increased rates of resistance to EGFR-TKIs and shorter survival. *TP53* plays a central role in response to cellular stress, and there is growing evidence of its involvement in angiogenesis through the regulation of vascular endothelial growth factor (VEGF)A and VEGF receptor 2 (VEGFR2). Although *TP53* is implicated in angiogenesis, and mutations in the gene are associated with reduced responsiveness to EGFR-TKIs in patients with *EGFR*+ NSCLC, there is a paucity of literature on the impact of *TP53* mutations on dual EGF/VEGF pathway inhibition.
- Our data further confirmed that the presence of mutant *TP53* at baseline was a negative prognostic indicator. The findings indicated that dual EGF/VEGF pathway inhibition with RAM+ERL exhibited benefit compared with PBO+ERL, independent of *TP53* status. Clinical outcomes indicated a trend for greater RAM+ERL benefit in those with mutant *TP53*. Overall, safety profiles were similar between the treatment arms and were generally consistent with the ITT population of the RELAY trial.
- This exploratory analysis provides further knowledge on the impact of co-occurring *TP53* mutations in *EGFR*+ NSCLC and may inform future ramucirumab efforts in this setting.

Authors' Contributions

M. Nishio, L. Paz Ares, M. Reck, K. Nakagawa, E. Garon, S. Popat, and S. Novello acquired the data. M. Ceccarelli, H. Graham and C. Visseren-Grul analyzed and interpreted the data. M. Nishio, L. Paz Ares, M. Reck, K. Nakagawa, E. Garon, S. Popat, and S. Novello interpreted the data. M. Nishio, L. Paz Ares, M. Reck, K. Nakagawa, S. Popat, and S. Novello conceived of the idea. C. Visseren-Grul designed and drafted the manuscript. All authors revised the work critically for important intellectual content, made substantial contributions, give final approval for the work to be published, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Availability of Data and Material

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 US and EU 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.

Disclosure

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cllc.2023.02.010.

References

1. Zito Marino F, Bianco R, Accardo M, et al. Molecular heterogeneity in lung cancer: from mechanisms of origin to clinical implications. *Int J Med Sci*. 2019;16(7):981–989.
2. Bethune G, Bethune D, Ridgway N, Xu Z. Epidermal growth factor receptor (EGFR) in lung cancer: an overview and update. *J Thorac Dis*. 2010;2(1):48–51.
3. Passaro A, Malapelle U, Del Re M, et al. Understanding EGFR heterogeneity in lung cancer. *ESMO Open*. 2020;5(5) e000919-e.
4. Leonetti A, Sharma S, Minari R, Perego P, Giovannetti E, Tiseo M. Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. *British J Cancer*. 2019;121(9):725–737.
5. Shi YK, Wang L, Han BH, et al. First-line icotinib versus cisplatin/pemetrexed plus pemetrexed maintenance therapy for patients with advancedEGFR mutation–positive lung adenocarcinoma (CONVINCE): a phase 3, open-label, randomized study. *Ann Oncol*. 2017;28(10):2443–2450.

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6. Suda K, Onozato R, Yatabe Y, Mitsudomi T. EGFR T790M mutation: a double role in lung cancer cell survival? *J Thorac Oncol*. 2009;4(1):1–4.
7. Guo Y, Song J, Wang Y, et al. Concurrent genetic alterations and other biomarkers predict treatment efficacy of EGFR-TKIs in EGFR-Mutant non-small cell lung cancer: a review. *Front Oncol*. 2020;10:2796.
8. Hou H, Qin K, Liang Y, et al. Concurrent TP53 mutations predict poor outcomes of EGFR-TKI treatments in Chinese patients with advanced NSCLC. *Cancer Manag Res*. 2019;11:5665–5675.
9. Qin K, Hou H, Liang Y, Zhang X. Prognostic value of TP53 concurrent mutations for EGFR- TKIs and ALK-TKIs based targeted therapy in advanced non-small cell lung cancer: a meta-analysis. *BMC Cancer*. 2020;20(1):328.
10. Mogi A, Kuwano H. TP53 mutations in nonsmall cell lung cancer. *J Biomed Biotechnol*. 2011;2011.
11. Greulich H. The genomics of lung adenocarcinoma: opportunities for targeted therapies. *Genes Cancer*. 2010;1(12):1200–1210.
12. Canale M, Petracci E, Delmonte A, et al. Impact of TP53 mutations on outcome in EGFR-mutated patients treated with first-line tyrosine kinase inhibitors. *Clin Cancer Res*. 2017;23(9):2195–2202.
13. Teodoro JG, Evans SK, Green MR. Inhibition of tumor angiogenesis by p53: a new role for the guardian of the genome. *J Mol Med (Berl)*. 2007;85(11):1175–1186.
14. Schwaederlé M, Lazar V, Validire P, et al. VEGF-A expression correlates with TP53 mutations in non-small cell lung cancer: implications for antiangiogenesis therapy. *Cancer Res*. 2015;75(7):1187–1190.
15. Assadian S, El-Assaad W, Wang XQ, et al. p53 inhibits angiogenesis by inducing the production of Arresten. *Cancer Res*. 2012;72(5):1270–1279.
16. Canale M, Petracci E, Delmonte A, et al. Concomitant tp53 mutation confers worse prognosis in EGFR-mutated non-small cell lung cancer patients treated with TKIs. *J Clin Med*. 2020;9(4):1047.
17. Li XM, Li WF, Lin JT, et al. Predictive and prognostic potential of TP53 in patients with advanced non-small-cell lung cancer treated with EGFR-TKI: analysis of a phase III randomized clinical trial (CTONG 0901). *Clin Lung Cancer*. 2021;22(2) 100-9.e3.
18. Molina-Vila MA, Bertran-Alamillo J, Gascó A, et al. Nondisruptive p53 mutations are associated with shorter survival in patients with advanced non-small cell lung cancer. *Clin Cancer Res*. 2014;20(17):4647–4659.
19. Lapke N, Lu Y-J, Liao C-T, et al. Missense mutations in the TP53 DNA-binding domain predict outcomes in patients with advanced oral cavity squamous cell carcinoma. *Oncotarget*. 2016;7(28):44194–44210.
20. Blandino G. Drugging the master regulator TP53 in cancer: mission possible? *J Clin Oncol*. 2021;39(14):1595–1597.
21. Poeta ML, Manola J, Goldwasser MA, et al. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N Engl J Med*. 2007;357(25):2552–2561.
22. VanderLaan PA, Rangachari D, Mockus SM, et al. Mutations in TP53, PIK3CA, PTEN and other genes in EGFR mutated lung cancers: correlation with clinical outcomes. *Lung Cancer*. 2017;106:17–21.
23. Aggarwal C, Davis CW, Mick R, et al. Influence of TP53 mutation on survival in patients with advanced EGFR-mutant non-small-cell lung cancer. *JCO Precis Oncol*. 2018;2018 PO.18.00107.
24. Chen L, Mu X, Wu H, Zhao Y. 405P - Association between TP53 mutations and efficacy of Osimertinib for brain metastasis from EGFR-mutant lung cancer. *Ann Oncol*. 2019;30:v149.
25. Wheler JJ, Janku F, Naing A, et al. TP53 alterations correlate with response to VEGF/VEGFR inhibitors: implications for targeted therapeutics. *Mol Cancer Ther*. 2016;15(10):2475–2485.
26. Koehler K, Liebner D, Chen JL. TP53 mutational status is predictive of pazopanib response in advanced sarcomas. *Ann Oncol*. 2016;27(3):539–543.
27. Graziano F, Fischer NW, Bagaloni I, Di, et al. TP53 mutation analysis in gastric cancer and clinical outcomes of patients with metastatic disease treated with ramucirumab/paclitaxel or standard chemotherapy. *Cancers (Basel)*. 2020;12(8):2049.
28. Zhao H, Yao W, Min X, et al. Apatinib plus gefitinib as first-line treatment in advanced EGFR-mutant NSCLC: the phase III ACTIVE study (CTONG1706). *J Thorac Oncol*. 2021;16(9):1533–1546.
29. Joshi H, Bhanot G, Børresen-Dale AL, Kristensen V. Potential tumorigenic programs associated with TP53 mutation status reveal role of VEGF pathway. *Br J Cancer*. 2012;107(10):1722–1728.
30. Li AM, Boichard A, Kurzrock R. Mutated TP53 is a marker of increased VEGF expression: analysis of 7,525 pan-cancer tissues. *Cancer Biol Ther*. 2020;21(1):95–100.
31. Watanabe H, Ichihara E, Kayatani H, et al. VEGFR2 blockade augments the effects of tyrosine kinase inhibitors by inhibiting angiogenesis and oncogenic signaling in oncogene-driven non-small-cell lung cancers. *Cancer Sci*. 2021;112(5):1853–1864.
32. Le X, Nilsson M, Goldman J, Reck M, Nakagawa K, Kato T, et al. Dual EGFR-vegf pathway inhibition: a promising strategy for patients with EGFR-mutant NSCLC. *J Thorac Oncol*. 2021;16(2):205–215.
33. Saito H, Fukuhara T, Furuya N, et al. Erlotinib plus bevacizumab versus erlotinib alone in patients with EGFR-positive advanced non-squamous non-small-cell lung cancer (NEJ026): interim analysis of an open-label, randomised, multicentre, phase 3 trial. *Lancet Oncol*. 2019;20(5):625–635.
34. Nishio M, Nishio K, Reck M, et al. RELAY+: Exploratory study of ramucirumab plus gefitinib in untreated patients (pts) with epidermal growth factor receptor (EGFR)-mutated metastatic non-small cell lung cancer (NSCLC). *J Clin Oncol*. 2020;38(15_suppl):9564.
35. Zhou Q, Xu CR, Cheng Y, et al. Bevacizumab plus erlotinib in Chinese patients with untreated, EGFR-mutated, advanced NSCLC (ARTEMIS-CTONG1509): a multicenter phase 3 study. *Cancer cell*. 2021;39(9) 1279-91.e3.
36. Nakagawa K, Garon EB, Seto T, et al. Ramucirumab plus erlotinib in patients with untreated, EGFR-mutated, advanced non-small-cell lung cancer (RELAY): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol*. 2019;20(12):1655–1669.
37. National Comprehensive Cancer Network. Non-small cell lung cancer, version 3.2022, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw*. 2022;20(5):497–530.
38. Planchard D, Popat S, Kerr K, et al. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2018;29(Suppl 4):iv192–iv237.
39. Akamatsu H, Ninomiya K, Kenmotsu H, et al. The Japanese Lung Cancer Society Guideline for non-small cell lung cancer, stage IV. *Int J Clin Oncol*. 2019;24(7):731–770.
40. Olivier M, Langerød A, Carrieri P, et al. The clinical value of somatic TP53 gene mutations in 1,794 patients with breast cancer. *Clin Cancer Res*. 2006;12(4):1157–1167.
41. Soo R, Han JY, Dimopoulou G, et al. A randomized phase II study of second–line osimertinib (Osi) and bevacizumab (Bev) versus Osi in advanced non-small-cell lung cancer (NSCLC) with epidermal growth factor receptor (EGFR) and T790M mutations (mt): results from the ETOP BOOSTER trial. *Ann Oncol*. 2021;32(7):942–944.
42. Piccirillo MC, Bonanno L, Garassino MCC, et al. 1207O Bevacizumab + erlotinib vs erlotinib alone as first-line treatment of pts with EGFR mutated advanced non squamous NSCLC: final analysis of the multicenter, randomized, phase III BEVERLY trial. *Ann Oncol*. 2021;32:S950.
43. Soria JC, Ohe Y, Vansteenkiste J, et al. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N Engl J Med*. 2018;378(2):113–125.
44. Saltos A, Baik C, Sanborn RE, et al. P76.62 RAMOSE: an open-label randomized phase II study of Osimertinib with or without ramucirumab in TKI-Naïve EGFR-mutant metastatic NSCLC. *J Thorac Oncol*. 2021;16(suppl 3):S614.
45. Nakahara Y, Kato T, Isomura R, et al. A multicenter, open label, randomized phase II study of osimertinib plus ramucirumab versus osimertinib alone as initial chemotherapy for EGFR mutation-positive non-squamous non-small cell lung cancer: TORG1833. *J Clin Oncol*. 2019;37(suppl 15) TPS9120-TPS.
46. Haratake N, Hayashi H, Shimokawa M, et al. Phase III clinical trial for the combination of erlotinib plus ramucirumab compared with osimertinib in previously untreated advanced or recurrent non–small cell lung cancer positive for the L858R mutation of EGFR: REVOL858R (WJOG14420L). *Clin Lung Cancer*. 2022;23(3):e257–e263.
47. Offin M, Chan JM, Tenet M, et al. Concurrent RB1 and TP53 alterations define a subset of EGFR-mutant lung cancers at risk for histologic transformation and inferior clinical outcomes. *J Thorac Oncol*. 2019;14(10):1784–1793.
48. Oser MG, Niederst MJ, Sequist LV, Engelman JA. Transformation from non-small-cell lung cancer to small-cell lung cancer: molecular drivers and cells of origin. *Lancet Oncol*. 2015;16(4):e165–ee72.
49. Sallman DA, DeZern AE, Garcia-Manero G, et al. Eprenetapopt (APR-246) and azacitidine in TP53-mutant myelodysplastic syndromes. *J Clin Oncol*. 2021;39(14):1584–1594.
50. Duffy MJ, Synnott NC, O'Grady S, Crown J. Targeting p53 for the treatment of cancer. *Semin Cancer Biol*. 2020;79:58–67.
51. Park H, Shapiro G, Gao X, et al. 516MO Phase I/II study of eprenetapopt (APR-246) in combination with pembrolizumab in patients with solid tumor malignancies. *Ann Oncol*. 2021;32:S586.

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