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ORIGINAL REPORT

Pooled Analysis of the Prognostic and Predictive Effects of *TP53* Comutation Status Combined With *KRAS* or *EGFR* Mutation in Early-Stage Resected Non–Small-Cell Lung Cancer in Four Trials of Adjuvant Chemotherapy

Frances A. Shepherd, Benjamin Lacas, Gwénaël Le Teuff, Pierre Hainaut, Pasi A. Jänne, Jean-Pierre Pignon, Thierry Le Chevalier, Lesley Seymour, Jean-Yves Douillard, Stephen Graziano, Elizabeth Brambilla, Robert Pirker, Martin Filipits, Robert Kratzke, Jean-Charles Soria, and Ming-Sound Tsao, on behalf of the LACE-Bio Collaborative Group

Author affiliations and support information (if applicable) appear at the end of this article.

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Corresponding author: Frances A. Shepherd, MD, FRCPC, Department of Medical Oncology and Hematology, Princess Margaret Cancer Centre, Room 5-103, 610 University Ave, Toronto, ON, Canada, M5G 2M9; e-mail: frances.shepherd@uhn.ca.

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ABSTRACT

Purpose

Our previous work evaluated individual prognostic and predictive roles of *TP53*, *KRAS*, and *EGFR* in non–small-cell lung cancer (NSCLC). In this analysis, we explore the prognostic and predictive roles of *TP53/KRAS* and *TP53/EGFR* comutations in randomized trials of adjuvant chemotherapy versus observation.

Patients and Methods

Mutation analyses (wild-type [WT] and mutant) for *TP53*, *KRAS*, and *EGFR* were determined in blinded fashion in multiple laboratories. Primary and secondary end points of pooled analysis were overall survival and disease-free survival. We evaluated the role of *TP53/KRAS* comutation in all patients and in the adenocarcinoma subgroup as well as the *TP53/EGFR* comutation in adenocarcinoma only through a multivariable Cox proportional hazards model stratified by trial.

Results

Of 3,533 patients with NSCLC, 1,181 (557 deaths) and 404 (170 deaths) were used for *TP53/KRAS* and *TP53/EGFR* analyses. For *TP53/KRAS* mutation status, no prognostic effect was observed ($P = .61$), whereas a borderline predictive effect ($P = .04$) was observed with a deleterious effect of chemotherapy with *TP53/KRAS* comutations versus WT/WT (hazard ratio, 2.49 [95% CI, 1.10 to 5.64]; $P = .03$). *TP53/EGFR* comutation in adenocarcinoma was neither prognostic ($P = .83$), nor significantly predictive ($P = .86$). Similar results were observed for both groups for disease-free survival.

Conclusion

We could identify no prognostic effect of the *KRAS* or *EGFR* driver and *TP53* tumor suppressor comutation. Our observation of a potential negative predictive effect of *TP53/KRAS* comutation requires validation.

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INTRODUCTION

Lung cancer is one of the most molecularly complex cancers, second only to malignant melanoma[1-3]; however, many of these genetic changes may not have functional importance, and it seems that a much smaller number of true driver mutations—with prognostic or predictive value—characterize this malignancy. *KRAS*, a member of the *RAS* family, was one of the first oncogenes to be identified in non–small-cell lung cancer (NSCLC).[4-6] *KRAS* mutations occur most frequently in codons 12 and 13 and are usually found in nonsquamous histology and in cancers of smokers. Slebos et al[7] were among the first to suggest that *KRAS* was prognostic of a poorer outcome. Since then, several meta-analyses have reported similar results, although there has been considerable variability among studies with respect to the magnitude of prognostic effect.[8,9] In vitro experiments have suggested that *RAS* mutations might be associated with resistance to treatment[10]; however, clinical studies could not always confirm these preclinical effects.[11]

ASSOCIATED CONTENT

Appendix
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Mutations in the tumor suppressor gene, *TP53*, which encodes the p53 protein, are frequent in all subtypes of NSCLC, with reported mutation rates of approximately 39% to 46% in adenocarcinomas, 81% in squamous cell carcinomas, and 68% in large cell carcinomas.[12,13] *TP53* plays multiple roles in the prevention and suppression of abnormal cell growth through cell-cycle arrest, apoptosis, senescence, or control of metabolism and DNA repair. However, data on its prognostic or predictive effect in NSCLC are limited and inconclusive.[14-16]

Sensitizing epidermal growth factor receptor (EGFR) gene mutations were first reported in 2004[17,18] and, over the last decade, have become the most important molecular tool for treatment selection for advanced NSCLC.[19] They occur almost exclusively in adenocarcinoma and non-neuroendocrine large cell carcinoma, significantly more frequently in patients who have never smoked, and they confer exquisite sensitivity to EGFR tyrosine kinase inhibitors.

Multiple driver mutations are less common in NSCLC, and their prognostic and predictive significance has not been well studied. In an attempt to clarify the impact of multiple *TP53*, *KRAS*, and *EGFR* mutations—the three most frequent driver oncogenes in NSCLC—and their interaction on survival benefit from platinum-based adjuvant chemotherapy (ACT), we performed this pooled analysis of four randomized trials of ACT or observation (OBS). We hypothesized that comutation of a driver oncogene and a tumor suppressor gene might be prognostic of poorer outcome in untreated patients and potentially predictive of lesser benefit from ACT.

PATIENTS AND METHODS

Clinical Trials

This study used the LACE (Lung Adjuvant Cisplatin Evaluation)[20] database of 3,533 patients from three LACE cisplatin-based ACT trials—JBR.10[21,22] IALT,[23,24] and ANITA[25]—and CALGB-9633, which used carboplatin-based ACT.[26] Scientists, clinicians, and statisticians from these trials represent the LACE-Bio Collaborative Group.

Mutation Analyses

Mutations were analyzed by direct sequencing of PCR products of DNA that was extracted from formalin-fixed, paraffin-embedded sections. All personnel who conducted mutation analyses were blinded to study arm and outcome. All mutation analyses were performed in one laboratory for JBR.10 (Tsao) and IALT (Hainaut). For ANITA, *KRAS* and *TP53* were analyzed in the Tsao and Hainaut laboratories, respectively. For CALGB-9633, *KRAS* and *EGFR* were analyzed in the Jänne laboratory and *TP53* in

Table 1. Patient and Tumor Characteristics for Patients With Double Wild-Type, *KRAS*, or *TP53* Mutated and Double-Mutant Tumors

Characteristic	Double Wild-Type (n = 570)	$KRAS_{MUT}$ (n = 186)	$TP53_{MUT}$ (n = 376)	Double Mutant (n = 49)	Total (N = 1,181)
Age, years					
≤ 50	87 (15.3)	39 (21.0)	61 (16.2)	13 (26.5)	200 (16.9)
51-60	177 (31.1)	66 (35.5)	139 (37.0)	22 (44.9)	404 (34.2)
> 60	306 (53.7)	81 (43.5)	176 (46.8)	14 (28.6)	577 (48.9)
Sex					
Male	434 (76.1)	117 (62.9)	304 (80.9)	33 (67.3)	888 (75.2)
Female	136 (23.9)	69 (37.1)	72 (19.1)	16 (32.7)	293 (24.8)
T stage*					
T1	69 (12.1)	26 (14.0)	34 (9.0)	3 (6.1)	132 (11.2)
T2	439 (77.0)	154 (82.8)	270 (71.8)	44 (89.8)	907 (76.8)
T3/4	60 (10.5)	6 (3.2)	71 (18.9)	2 (4.1)	139 (11.8)
Unknown	2 (0.4)	0 (—)	1 (0.3)	0 (—)	3 (0.3)
N stage*					
N0	300 (52.6)	119 (64.0)	184 (48.9)	28 (57.1)	631 (53.4)
N1	195 (34.2)	55 (29.6)	125 (33.2)	14 (28.6)	389 (32.9)
N2	73 (12.8)	11 (5.9)	65 (17.3)	7 (14.3)	156 (13.2)
Unknown	2 (0.4)	1 (0.5)	2 (0.5)	0 (—)	5 (0.4)
Stage*					
I	270 (47.4)	117 (62.9)	156 (41.5)	26 (53.1)	569 (48.2)
II	212 (37.2)	57 (30.6)	134 (35.6)	16 (32.7)	419 (35.5)
III	86 (15.1)	12 (6.5)	85 (22.6)	7 (14.3)	190 (16.1)
Unknown	2 (0.4)	0 (—)	1 (0.3)	0 (—)	3 (0.3)
WHO performance status					
0	301 (52.8)	101 (54.3)	191 (50.8)	26 (53.1)	619 (52.4)
≥ 1	268 (47.0)	84 (45.2)	183 (48.7)	23 (46.9)	558 (47.2)
Missing	1 (0.2)	1 (0.5)	2 (0.5)	0 (—)	4 (0.3)
Histology					
Squamous	276 (48.4)	19 (10.2)	206 (54.8)	7 (14.3)	508 (43.0)
Adenocarcinoma	218 (38.2)	140 (75.3)	110 (29.3)	29 (59.2)	497 (42.1)
Other NSCLC	76 (13.3)	27 (14.5)	60 (16.0)	13 (26.5)	176 (14.9)
Treatment					
Observation	302 (53.0)	88 (47.3)	173 (46.0)	24 (49.0)	587 (49.7)
Adjuvant chemotherapy	268 (47.0)	98 (52.7)	203 (54.0)	25 (51.0)	594 (50.3)

NOTE. Data are given as No. (%).
Abbreviations: NSCLC, non–small-cell lung cancer; $KRAS_{MUT}$, KRAS mutant; $TP53_{MUT}$, TP53 mutant.
*Using 6th edition TNM staging classification.

the Hainaut laboratory. For technical reasons, *EGFR* test results are not available for the ANITA trial. Mutations are defined as wild-type (WT) or mutant (MUT), without further subtyping. *EGFR* mutation refers only to sensitizing mutations in exons 19 and 21. Full laboratory methods have been published previously.[11,15,27]

Statistical Methods

Overall survival (OS)—the primary end point—was defined as time from random assignment to death from any cause or last follow-up in surviving patients. Disease-free survival (DFS) was defined as time from random assignment to recurrence or death from any cause or last follow-up in surviving patients. We evaluated the prognostic and predictive value of *TP53/KRAS* comutation status and *TP53/EGFR* comutation status. For the latter, analysis was restricted to adenocarcinoma as *EGFR* mutation is uncommon in other histologic subtypes. Mutation status from these combinations was grouped into four classes (WT/WT, WT/MUT, MUT/WT, and MUT/MUT). Hazard ratios (HRs) and their CIs were estimated via a multivariable Cox proportional hazards model stratified by trial including the following core variables: treatment (ACT or OBS), sex, age (< 55, 55 to 64, ≥ 65 years), tumor stage (T1, T2, and T3/4), nodal stage (N0, N1, and N2), and histology (squamous, adenocarcinoma, or other), with minor modifications according to the type of combination (Appendix, online only). The predictive value of mutation status was assessed by a treatment-mutation status interaction. Prognostic analyses were performed in OBS patients. We assessed heterogeneity of prognostic and predictive values of mutation status across trials and according to histology (Appendix). Preplanned subgroup analyses were performed that assessed the prognostic effect of *TP53/KRAS* combination in adenocarcinoma, and *KRAS* (*EGFR*, respectively) in *EGFR* (*KRAS*, respectively) WT adenocarcinoma. Statistical significance was set at $P < .01$. Survival curves were based on Kaplan-Meier methods and presented with unadjusted HRs from the Cox model and *P* values using log rank statistic. Follow-up was estimated for both arms with the Schemper method.[28] Statistical analyses were performed by using SAS (SAS/STAT User's Guide, Version 9.3; SAS Institute, Cary, NC). All *P* values are two sided.

RESULTS

Patients

Of 3,533 patients who were randomly assigned (ANITA, n = 840; JBR.10, n = 482; IALT, n = 1,867; CALGB-9633, n = 344), 1,534 patients were eligible for *TP53/KRAS* combination analysis and 435 for *TP53/EGFR* combination analysis (Appendix Fig. A1, online only).

TP53/KRAS *and* TP53/EGFR *Combinations*

TP53/KRAS analysis included only patients for whom both *TP53* and *KRAS* status were available (n = 1,181). Baseline demographics for patients with and without *TP53/KRAS* status known are shown in Appendix Table A1 (online only). Compared with patients with unknown status, there were more women

Table 2. Patient and Tumor Characteristics for Patients With Double Wild-Type, *EGFR*, or *TP53* Mutated and Double-Mutant in Adenocarcinoma

Characteristic	Double Wild-Type (n = 260)	$EGFR_{MUT}$ (n = 31)	$TP53_{MUT}$ (n = 95)	Double Mutant (n = 18)	Total (N = 404)
Age, years					
≤ 50	51 (19.6)	7 (22.6)	26 (27.4)	3 (16.7)	87 (21.5)
51-60	91 (35.0)	9 (29.0)	35 (36.8)	7 (38.9)	142 (35.1)
> 60	118 (45.4)	15 (48.4)	34 (35.8)	8 (44.4)	175 (43.3)
Sex					
Male	166 (63.8)	8 (25.8)	61 (64.2)	12 (66.7)	247 (61.1)
Female	94 (36.2)	23 (74.2)	34 (35.8)	6 (33.3)	157 (38.9)
T stage*					
T1	45 (17.3)	4 (12.9)	11 (11.6)	4 (22.2)	64 (15.8)
T2	206 (79.2)	27 (87.1)	78 (82.1)	12 (66.7)	323 (80.0)
T3/4	7 (0.7)	0 (—)	6 (6.3)	2 (11.1)	15 (3.7)
Missing/unknown	2 (0.8)	0 (—)	0 (—)	0 (—)	2 (0.5)
N stage*					
N0	160 (61.5)	16 (51.6)	66 (69.5)	9 (50.0)	251 (62.1)
N1	78 (30.0)	12 (38.7)	21 (22.1)	6 (33.3)	117 (29.0)
N2	19 (7.3)	3 (9.7)	8 (8.4)	3 (16.7)	33 (8.2)
Missing/unknown	3 (1.2)	0 (—)	0 (—)	0 (—)	3 (0.7)
Stage*					
I	158 (60.8)	16 (51.6)	64 (67.4)	7 (38.9)	245 (60.6)
II	81 (31.2)	12 (38.7)	21 (22.1)	8 (44.4)	122 (30.2)
III	19 (7.3)	3 (9.7)	10 (10.5)	3 (16.7)	35 (8.7)
Missing/unknown	2 (0.8)	0 (—)	0 (—)	0 (—)	2 (0.5)
WHO performance status					
0	155 (59.6)	16 (51.6)	49 (51.6)	9 (50.0)	229 (56.7)
≥ 1	104 (40.0)	15 (48.4)	45 (47.4)	9 (50.0)	173 (42.8)
Missing/unknown	1 (0.4)	0 (—)	1 (1.1)	0 (—)	2 (0.5)
Treatment					
Observation	133 (51.2)	21 (67.7)	50 (52.6)	9 (50.0)	213 (52.7)
Adjuvant chemotherapy	127 (48.9)	10 (32.3)	45 (47.4)	9 (50.0)	191 (47.3)

NOTE. Data are given as No. (%).
Abbreviations: $TP53_{MUT}$, *TP53* mutant; $EGFR_{MUT}$, *EGFR* mutant.
*Using 6th edition TNM staging classification.

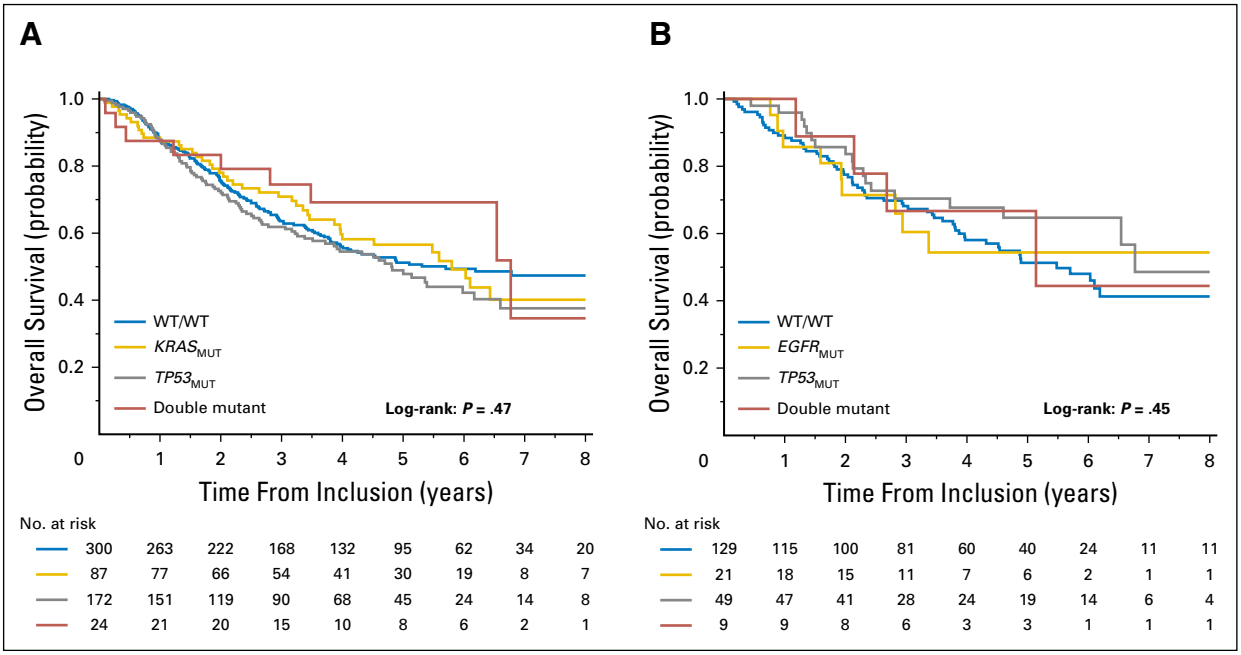

Fig 1. Overall survival. Unadjusted Kaplan-Meier curves of the prognostic effect in the observation arm. (A) *TP53/KRAS* combination. (B) *TP53/EGFR* combination. MUT, mutant; WT, wild type.

($P = .02$) with known status and more T2 tumors ($P = .003$)—stage was not significantly different between the two groups ($P = .10$). Among patients with known status, 888 (75%) were male and 569 (48%) were stage I, with mostly squamous cell carcinoma (43%) and adenocarcinoma (42%). Mutation distribution was as follows: 570 (48%) with no mutation, 186 (16%) with *KRAS* mutation, 376 (32%) with *TP53* mutation, and 49 (4%) with *TP53* and *KRAS* comutations (Table 1). Median follow-up was 5.5 years (range, 0.1 to 11.3 years) and 557 patients died.

TP53/EGFR analysis included only patients with adenocarcinoma with both *TP53* and *EGFR* status available (n = 404). Baseline demographics for patients with and without known *TP53/EGFR* status are shown in Appendix Table A2 (online only). There were no significant differences between groups. Among patients with known status, 247 (61%) were male and 245 (61%) were stage I. Mutation distribution was as follows: 260 (64%) with no mutation, 31 (8%) with *EGFR* mutation, 95 (24%) with *TP53* mutation, and only 18 (4%) with *TP53* and *EGFR* comutations (Table 2). Median follow-up was 5.5 years (range, 0.2 to 11.1 years) and 170 patients died.

Prognostic Effect of TP53 *(WT and MUT) Combined With* KRAS *(WT and MUT)*

In the OBS arm, there was no significant difference in OS on the basis of mutation status (Fig 1A, Appendix Table A3, online only; multivariable Cox model, $P = .61$). No heterogeneity across trials ($P = .66$) or histology ($P = .97$) was observed.

Table 3. Predictive Effect of *TP53* and *KRAS* Combination on Overall Survival (n = 1,176)

TP53 and *KRAS* Combination	ACT (No. of deaths/No. of patients)	OBS (No. of deaths/No. of patients)	HR ACT *v* OBS [95% CI]
Double WT (n = 568)	109/268	142/300	0.81 [0.63 to 1.04] (HR_1); $P = .09$
$KRAS_{MUT}$ (n = 185)	41/98	42/87	0.71 [0.45 to 1.10] (HR_2); $P = .13$
HR $KRAS_{MUT}$ *v* double WT [95% CI]	1.11 [0.76 to 1.61]; $P = .58$	1.26 [0.88 to 1.79]; $P = .21$	0.88 [0.53 to 1.46]*; $P = .63$
$TP53_{MUT}$ (n = 374)	108/202	89/172	0.99 [0.75 to 1.32] (HR_3); $P = .97$
HR $TP53_{MUT}$ *v* double WT [95% CI]	1.33 [1.01 to 1.74]; $P = .04$	1.08 [0.82 to 1.41]; $P = .60$	1.23 [0.84 to 1.80]*; $P = .28$
Double mutant (n = 49)	17/25	9/24	2.49 [1.10 to 5.64] (HR_4); $P = .03$
HR double mutant *v* double WT [95% CI]	2.77 [1.63 to 4.69]; $P = .0002$	0.90 [0.45 to 1.77]; $P = .75$	3.09 [1.31 to 7.27]*; $P = .01$
Test for interaction ($HR_1 = HR_2 = HR_3 = HR_4$): $P = .04$†			

NOTE. Five patients were excluded because of missing values for some covariates. Heterogeneity across trials: $P = .73$; heterogeneity across histologic subtypes: $P = .85$.

Abbreviations: ACT, adjuvant chemotherapy; HR, hazard ratio; $KRAS_{MUT}$, *KRAS* mutant; OBS, observation; $TP53_{MUT}$, *TP53* mutant; WT, wild-type.

*HR of interaction between treatment and two mutation categories (double WT and $KRAS_{MUT}$, double WT and $TP53_{MUT}$, double WT and double mutant). This HR of interaction and the corresponding test compare the treatment effect (ACT *v* OBS) across two mutation categories.

†Equivalent to testing the heterogeneity between the four HRs.

Results were similar for DFS (Appendix Table A4 and Appendix Fig A2A, online only).

Predictive Effect of TP53 *(WT and MUT) Combined With* KRAS *(WT and MUT)*

As shown in Table 3 and Figure 2, there was no significant difference in OS benefit from chemotherapy when patients with *KRAS* mutation (HR, 0.71 [95% CI, 0.45 to 1.10]; $P = .13$) were compared with those with double WT tumors (HR, 0.81 [95% CI, 0.63 to 1.04]; $P = .09$; interaction test, $P = .63$). Patients with *TP53* mutant tumors did not seem to benefit from chemotherapy (HR, 0.99 [95% CI, 0.75 to 1.32]; $P = .97$). In contrast, patients with double-mutant tumors had significantly worse OS with chemotherapy (HR, 2.49 [95% CI, 1.10 to 5.64]; $P = .03$) compared with patients with double WT tumors (interaction test, $P = .01$). This latter result led to a chemotherapy effect on OS that was marginally heterogeneous across mutation status ($P = .04$). This marginal predictive effect was homogeneous across trials ($P = .73$) and histology ($P = .85$). For DFS, the same results were observed with an interaction ($P = .04$) as a result of a marginal effect of ACT in the *KRAS* mutant group (HR, 0.62 [95% CI, 0.41 to 0.92]; $P = .02$; Appendix Table A5 and Appendix Fig A3, online only).

Prognostic Effect of TP53 *(WT and MUT) Combined With* EGFR *(WT and MUT) in Adenocarcinoma*

Trials that were included in this analysis include only IALT, JBR.10, and CALGB and are limited to patients with adenocarcinoma as few *EGFR* results were available for other histologies. In the OBS arm, there was no significant difference in OS on the basis of mutation status (Appendix Table A6, online only, and Fig 1B),

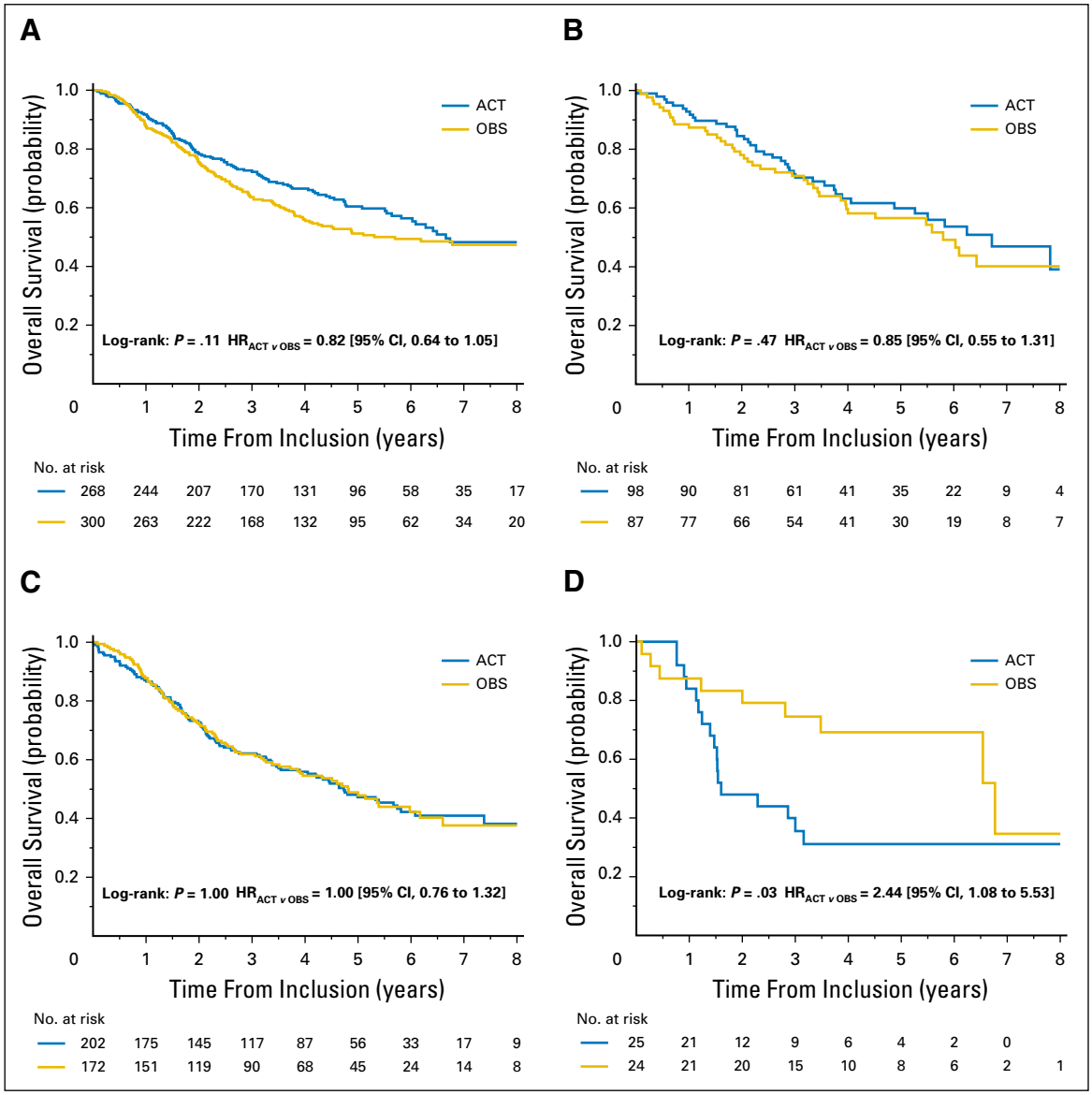

Fig 2. Overall survival. Unadjusted Kaplan-Meier curves of treatment effect according to *TP53* and *KRAS* mutation status. (A) Double wild type. (B) *TP53* wild-type, *KRAS* mutant. (C) *TP53* mutant, *KRAS* wild type. (D) Double mutant. ACT, adjuvant chemotherapy; OBS, observation.

Table 4. Predictive Effect of *TP53* and *EGFR* Combination on Overall Survival in Adenocarcinoma (n = 399)

TP53 and *EGFR* Combination	ACT (No. of deaths/No. of patients)	OBS (No. of deaths/No. of patients)	HR ACT *v* OBS [95% CI]
Double WT (n = 256)	47/127	64/129	0.67 [0.46 to 0.99] (HR_1); P = .05
$EGFR_{MUT}$ (n = 31)	4/10	9/21	0.67 [0.20 to 2.21] (HR_2); P = .51
HR $EGFR_{MUT}$ *v* double WT [95% CI]	1.15 [0.40 to 3.28]; P = .79	1.15 [0.57 to 2.35]; P = .70	1.00 [0.29 to 3.49]*; P = 1.00
$TP53_{MUT}$ (n = 94)	19/45	20/49	0.89 [0.47 to 1.69] (HR_3); P = .72
HR $TP53_{MUT}$ *v* double WT [95% CI]	1.13 [0.65 to 1.97]; P = .66	0.86 [0.51 to 1.44]; P = .57	1.32 [0.62 to 2.78*; P = .47
Double mutant (n = 18)	3/9	4/9	1.06 [0.23 to 4.94] (HR_4); P = .94
HR double mutant *v* double WT [95% CI]	1.10 [0.33 to 3.69]; P = .87	0.70 [0.25 to 1.95]; P = .50	1.57 [0.32 to 7.70]*; P = .58
Test for interaction (HR_1 = HR_2 = HR_3 = HR_4): P = .86			

NOTE. Five patients were excluded because of missing values for some covariates. Heterogeneity across trials: P = .86.
Abbreviations: ACT, adjuvant chemotherapy; $EGFR_{MUT}$, *EGFR* mutant; HR, hazard ratio; OBS, observation; $TP53_{MUT}$, *TP53* mutant; WT, wild-type.
*HR of interaction between treatment and two mutation categories (double WT and $EGFR_{MUT}$, double WT and $TP53_{MUT}$, double WT and double mutant). This HR of interaction and the corresponding test compare the treatment effect (chemotherapy *v* observation) across two mutation categories.

EGFR, TP53, or double mutation compared with double WT subgroup (multivariable Cox proportional hazards model, P = .83). No heterogeneity across trials (P = .38) was observed. Results were similar for DFS (Appendix Table A7 and Appendix Fig A2B, online only).

Predictive Effect of TP53 *(WT and MUT) Combined With* EGFR *(WT and MUT) in Adenocarcinoma*

As shown in Table 4 and Figure 3, there was no significant difference in OS benefit from chemotherapy when patients with *EGFR* mutation (HR, 0.67 [95% CI, 0.20 to 2.21]; P = .51) were compared with those with double WT tumors (HR, 0.67 [95% CI, 0.46 to 0.99]; P = 0.05; interaction P = 1.00). Patients with *TP53* mutant tumors did not seem to benefit from chemotherapy (HR, 0.89 [95% CI, 0.47 to 1.69]; P = .72), nor did patients with double-mutant tumors (HR, 1.06 [95% CI, 0.23 to 4.94]; P = 0.94). Overall, chemotherapy effect on OS was homogeneous across mutation status (P = .86). For DFS, there was a beneficial effect of ACT in double WT patients (HR, 0.63 [95% CI, 0.45 to 0.90]; P = .01) but significant interaction was not observed (P = .74; Appendix Table A8 and Appendix Fig A4, online only).

Adenocarcinoma Subgroup Analyses

Because *EGFR* and *KRAS* mutations occur mainly in adenocarcinoma, this subgroup was analyzed separately. Patient selection is presented in Appendix Fig A5 (online only).

TP53 *(WT and MUT) Combined With* KRAS *(WT and MUT)*

The prognostic effect of *TP53* and *KRAS* comutation was assessed in 377 patients with adenocarcinoma—257 patients and 120 deaths in the OBS arm—and no survival advantage was observed for any mutation subgroup (data not shown).

KRAS *in* EGFR *WT*

The prognostic effect of *KRAS* mutation was assessed further in the subgroup of *EGFR* WT adenocarcinoma—207 patients and 99 deaths in the OBS arm—and no OS difference was observed for patients with *KRAS* mutations (HR, 0.97 [95% CI, 0.62 to 1.53]; P = .91), and no heterogeneity among trials (P = .12).

EGFR *in* KRAS *WT*

The prognostic effect of *EGFR* mutation was assessed further in the subgroup of *KRAS* WT adenocarcinoma—160 patients and 77 deaths in the OBS arm—and no OS advantage was observed for patients with *EGFR* mutations (HR, 1.18 [95% CI, 0.66 to 2.12]; P = 0.57), and no heterogeneity among trials (P = .91).

DISCUSSION

In this study, we elected to focus on three oncogenes—*KRAS*, *TP53*, and *EGFR*—because they are the most frequently mutated in NSCLC and, importantly, the most frequently comutated genes.[1-3]

We previously reported that *KRAS* mutation was not a significant prognostic marker in resected early-stage NSCLC, whether analyzed collectively or by mutation subtype.[11] More recently, we have reported similar results for *TP53*.[16] In advanced disease, *EGFR* mutation is frequently associated with longer survival, although inconsistent results have been reported from surgical series.[29,30] *EGFR* mutation occurs rarely in NSCLC subtypes other than adenocarcinoma. When we limited our analyses to adenocarcinoma, we could identify no prognostic effect for *EGFR* mutation even when patients with *KRAS*-mutated tumors were removed from the analysis (MUT *v* WT HR, 1.18 [95% CI, 0.66 to 2.12]; P = .57).

With the introduction of next-generation sequencing techniques, it is now recognized that NSCLC is a molecularly complex cancer[1-3]; however, most molecular changes are of little or unknown functional significance. Somatic driver mutations occur less frequently, with multiple driver mutations reported in approximately 3% to 44% of tumors studied, depending on platform used.[31,32]

TP53 mutation occurs in almost all small-cell lung cancers and is the most frequent driver mutation in NSCLC, occurring in approximately 50% of this malignancy.[1-3] Our *TP53* mutation rate in adenocarcinoma seems to be somewhat lower than that reported in some series but is consistent with a recent study from the Clinical Lung Cancer Genome Project that found *TP53* mutations in 26.9% of cases,[33] which is virtually identical to our result. The first multiplex platforms that were designed to study specific driver mutations did not include *TP53* because of the multiplicity and

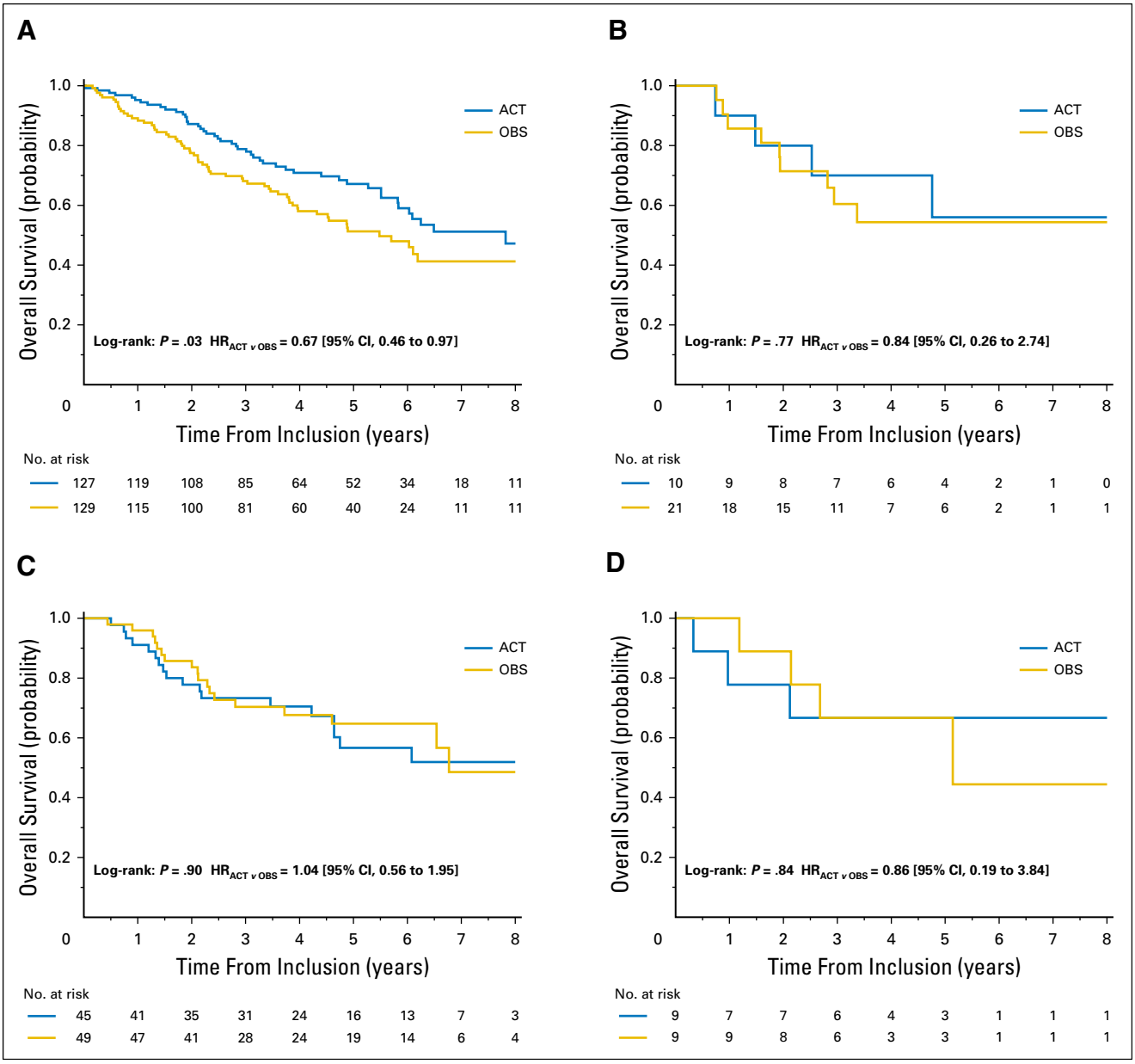

Fig 3. Overall survival. Unadjusted Kaplan-Meier curves of treatment effect according to *EGFR* and *TP53* mutation status, in adenocarcinoma. (A) Double wild type. (B) *TP53* wild-type, *EGFR* mutant. (C) *TP53* mutant, *EGFR* wild type. (D) Double mutant. ACT, adjuvant chemotherapy; OBS, observation.

complexity of genetic changes in this gene; therefore, early publications that have reported on mutation analyses in NSCLC undoubtedly underestimated the frequency of dual mutations. Kris et al[31] reported only 3% of patients with dual mutations when up to 10 mutations were assessed. In contrast, Jao et al[32] found dual mutations in 44% of tumors from a study by using next-generation sequencing platforms that included *TP53*. Furthermore, in studies that have included *TP53* testing, this mutation is the most frequent comutation in all subtypes of NSCLC.[1-3]

With more than 1,000 patients, our study is the first and the largest, to our knowledge, to report on the prognostic and predictive effects of *TP53* mutation in combination either with *KRAS* mutation or with *EGFR* mutation. Furthermore, our study examines a homogeneous group of surgically staged patients with complete follow-up of more than 5 years' duration. After accounting for other prognostic variables in the OBS arm, we could demonstrate no prognostic effect of dual *TP53/KRAS* mutation compared with the WT/WT cohort or with cohorts with only one mutation. Results were similar when examining the adenocarcinoma subset that accounted for almost 70% of *KRAS* mutations. Because *EGFR* mutations, as expected, were virtually limited to adenocarcinoma, our analyses of the *TP53/EGFR* combination were limited to this NSCLC subgroup. Once again, we could demonstrate no prognostic effects of dual mutation. Similar results were reported recently by Kosaka et al,[34] who identified no significant prognostic effect of *TP53, KRAS*, or *EGFR* mutations on multivariable analysis of 397 surgically resected adenocarcinomas. They did not report on the outcomes of patients with dual-mutant

tumors. Cortot et al[35] also reported a high frequency of dual *EGFR* or *KRAS* mutations with alterations in the p53/arf[14] pathway, and emphasized the importance of further investigation to understand the effect of mutations in tumor suppressor genes in association with other driver mutations. Most recently, Reily et al[36] reported a nonsignificant trend to shorter survival in 92 patients with co-occurring *KRAS/TP53* mutated tumors compared with 147 with *KRAS* alone (HR, 1.25; $P = .396$). They also found that alterations of *STK11, KEAP1*, or *NF2E2L* with *KRAS* mutation were associated with significantly shorter survival on univariable analysis.

Dual mutation status, however, does seem to be predictive and to influence the survival benefit that is derived from ACT. There were trends toward a beneficial effect from ACT in patients with WT/WT tumors and even in those with *KRAS*-mutated tumors. In contrast, patients with *TP53*-mutated tumors seemed to derive no benefit from ACT (HR, 0.99), and those with double-mutant tumors who were treated with ACT had significantly worse OS (HR, 2.49; $P = .03$; interaction $P = .01$). These trends also were observed in our analyses of *TP53/EGFR* in adenocarcinoma, although significant differences could not be demonstrated in this smaller subset. Patients with WT/WT tumors and those with *EGFR* mutant tumors seemed to benefit from ACT (HR, 0.67 for both groups), whereas there was no apparent benefit from ACT in those with double MUT *TP53/EGFR* tumors (HR, 1.06).

There are preclinical data that suggest that the silencing of p53 in A549 cell lines—that carry a *KRAS* mutation—enhances resistance of cells to cisplatin and paclitaxel, and that intact p53 is required for chemotherapy-induced apoptosis.[37] Our findings are consistent with this hypothesis; however, we could not find any preclinical work that examined the effect of dual *TP53/EGFR* mutation with respect to response to chemotherapy.

Our work, to our knowledge, is the first to examine the negative predictive effect of dual mutations on survival benefit from ACT. Tomasini et al[38] reported on a small group of patients who were treated with platinum-based chemotherapy for all stages of NSCLC who had undergone molecular profiling, including *TP53* analysis. They observed a negative effect that was similar to ours in the adjuvant setting. Among 218 patients, 28 had tumors with dual *TP53/KRAS* mutations. Whereas there was no difference when comparing survival outcomes among the four groups of WT/WT, MUT/MUT, or single mutation, patients with dual *TP53/KRAS* mutations had significantly shorter overall survival than those with WT/WT tumors (HR, 2.06 [95% CI, 1.09 to 3.88] $P = .02$). Interaction could not be demonstrated as all patients received chemotherapy and there was no untreated control arm.

Dual *TP53/EGFR* mutations have been reported to occur frequently by other groups,[39-42] and in one small study[40] were associated with lower response to gefitinib and shorter survival. Labbe et al[39] examined the effect of dual *TP53/EGFR* mutation in 103 patients with *EGFR* exons 19 or 21 mutations who received EGFR tyrosine kinase inhibitors for advanced disease. There was no difference in response rate for patients with comutated tumors, but progression-free survival was significantly shorter for patients who were treated with EGFR tyrosine kinase inhibitors (HR, 1.82; $P = .039$). Similar results were reported by Yu et al,[40] who reported an even greater negative effect of dual mutation (HR, 2.7; $P = .017$).

This LACE-Bio report is the first to suggest that *TP53/EGFR* mutations also may have a negative effect on the benefit from chemotherapy. Patients with WT/WT adenocarcinoma seemed to benefit from platinum-based ACT, whereas those with *TP53* or dual mutations did not. The mechanistic basis for this observation remains unclear. As these analyses were limited to the adenocarcinoma subset, our results lack statistical power and so they should be interpreted with caution and must be validated in other studies.

In summary, *TP53* comutation with *KRAS* or *EGFR* is not a significant prognostic marker in patients with resected NSCLC. Dual *TP53/KRAS* mutation seems to be predictive of shortened survival in patients who are treated with platinum-based ACT, but the statistically significant result must be interpreted with caution in view of the small sample size, and validation studies are needed in both the adjuvant and advanced disease settings. Preclinical models are also warranted and may clarify the potential interaction of dual mutation and chemotherapy. Similar, although nonsignificant, trends toward a lack of survival benefit from ACT in patients with dual *TP53/EGFR* mutations as well as preclinical studies support our observation that tumor suppressor gene mutations combined with other genetic mutations may be associated with relative resistance to chemotherapy. With recent improvements in technology, next-generation sequencing is now cost effective and should be considered for all newly diagnosed patients with NSCLC to assess the mutational profile of each cancer. Although not a therapeutic target at this time, routine inclusion of *TP53* mutation testing may clarify the role that this tumor suppressor gene plays, both alone and in combination with other driver mutations in lung cancer, and determine whether there is a negative interaction with treatment.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Frances A. Shepherd, Gwénaël Le Teuff, Pierre Hainaut, Jean-Pierre Pignon, Thierry Le Chevalier, Lesley Seymour, Jean-Charles Soria, Ming-Sound Tsao
Administrative support: Jean-Pierre Pignon, Lesley Seymour
Provision of study materials or patients: Frances A. Shepherd, Thierry Le Chevalier, Lesley Seymour, Jean-Yves Douillard, Stephen Graziano, Robert Pirker, Martin Filipits, Robert Kratzke, Jean-Charles Soria
Collection and assembly of data: Benjamin Lacas, Gwénaël Le Teuff, Pierre Hainaut, Pasi A. Jänne, Jean-Pierre Pignon, Thierry Le Chevalier, Lesley Seymour, Stephen Graziano, Elizabeth Brambilla, Robert Pirker, Martin Filipits, Ming-Sound Tsao
Data analysis and interpretation: Benjamin Lacas, Gwénaël Le Teuff, Pierre Hainaut, Pasi A. Jänne, Jean-Pierre Pignon, Thierry Le Chevalier, Lesley Seymour, Jean-Yves Douillard, Elizabeth Brambilla, Robert Pirker, Martin Filipits, Robert Kratzke, Jean-Charles Soria, Ming-Sound Tsao
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

REFERENCES

1. Cancer Genome Atlas Research Network: Comprehensive molecular profiling of lung adenocarcinoma. Nature 511:543-550, 2014 [Erratum: Nature 514:262, 2014]
2. Cancer Genome Atlas Research Network: Comprehensive genomic characterization of squamous cell lung cancers. Nature 489:519-525, 2012 [Erratum: Nature 491:288, 2012]
3. Lawrence MS, Stojanov P, Polak P, et al: Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 499:214-218, 2013
4. Harvey JJ: An unidentified virus which causes the rapid production of tumours in mice. Nature 204: 1104-1105, 1964
5. Rodenhuis S, van de Wetering ML, Mooi WJ, et al: Mutational activation of the K-ras oncogene. A possible pathogenetic factor in adenocarcinoma of the lung. N Engl J Med 317:929-935, 1987
6. Prior IA, Lewis PD, Mattos C: A comprehensive survey of Ras mutations in cancer. Cancer Res 72:2457-2467, 2012
7. Slebos RJC, Kibbelaar RE, Dalesio O, et al: K-ras oncogene activation as a prognostic marker in adenocarcinoma of the lung. N Engl J Med 323: 561-565, 1990
8. Huncharek M, Muscat J, Geschwind JF: K-ras oncogene mutation as a prognostic marker in non-small cell lung cancer: A combined analysis of 881 cases. Carcinogenesis 20:1507-1510, 1999
9. Mascaux C, Iannino N, Martin B, et al: The role of RAS oncogene in survival of patients with lung cancer: A systematic review of the literature with meta-analysis. Br J Cancer 92:131-139, 2005
10. Rodenhuis S, Boerrigter L, Top B, et al: Mutational activation of the K-*ras* oncogene and the effect of chemotherapy in advanced adenocarcinoma of the lung: A prospective study. J Clin Oncol 15: 285-291, 1997
11. Shepherd FA, Domerg C, Hainaut P, et al: Pooled analysis of the prognostic and predictive effects of KRAS mutation status and KRAS mutation subtype in early-stage resected non-small-cell lung cancer in four trials of adjuvant chemotherapy. J Clin Oncol 31:2173-2181, 2013
12. Kato S, Han SY, Liu W, et al: Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. Proc Natl Acad Sci USA 100:8424-8429, 2003
13. Huang CL, Taki T, Adachi M, et al: Mutations of p53 and K-ras genes as prognostic factors for non-small cell lung cancer. Int J Oncol 12:553-563, 1998
14. Scoccianti C, Vesin A, Martel G, et al: Prognostic value of TP53, KRAS and EGFR mutations in nonsmall cell lung cancer: The EUELC cohort. Eur Respir J 40:177-184, 2012
15. Ma X, Rousseau V, Sun H, et al: Significance of TP53 mutations as predictive markers of adjuvant cisplatin-based chemotherapy in completely resected non-small-cell lung cancer. Mol Oncol 8:555-564, 2014
16. Ma X, Le Teuff G, Lacas B, et al: Prognostic and predictive effect of TP53 mutations in non-small cell lung cancer patients from adjuvant cisplatin-based therapy randomized trials: A LACE-Bio pooled analysis. J Thorac Oncol 11:850-861, 2016
17. Paez JG, Jänne PA, Lee JC, et al: EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. Science 304:1497-1500, 2004
18. Lynch TJ, Bell DW, Sordella R, et al: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 350:2129-2139, 2004
19. Russo A, Franchina T, Ricciardi GR, et al: A decade of EGFR inhibition in EGFR-mutated non small cell lung cancer (NSCLC): Old successes and future perspectives. Oncotarget 6:26814-26825, 2015
20. Pignon J-P, Tribodet H, Scagliotti GV, et al: Lung adjuvant cisplatin evaluation: A pooled analysis by the LACE Collaborative Group. J Clin Oncol 26: 3552-3559, 2008
21. Winton T, Livingston R, Johnson D, et al: Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. N Engl J Med 352: 2589-2597, 2005
22. Butts CA, Ding K, Seymour L, et al: Randomized phase III trial of vinorelbine plus cisplatin compared with observation in completely resected stage IB and II non-small-cell lung cancer: Updated survival analysis of JBR-10. J Clin Oncol 28:29-34, 2010
23. Arriagada R, Bergman B, Dunant A, et al: Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. N Engl J Med 350:351-360, 2004
24. Arriagada R, Dunant A, Pignon JP, et al: Long-term results of the International Adjuvant Lung Cancer Trial evaluating adjuvant cisplatin-based chemotherapy in resected lung cancer. J Clin Oncol 28:35-42, 2010
25. Douillard JY, Rosell R, De Lena M, et al: Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB-IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): A randomised controlled trial. Lancet Oncol 7:719-727, 2006
26. Strauss GM, Herndon JE II, Maddaus MA, et al: Adjuvant paclitaxel plus carboplatin compared with observation in stage IB non-small-cell lung cancer: CALGB 9633 with the Cancer and Leukemia Group B, Radiation Therapy Oncology Group, and North Central Cancer Treatment Group Study Groups. J Clin Oncol 26:5043-5051, 2008
27. Jänne PA, Wang X, Socinski M.A, et al: Randomized phase II trial of erlotinib alone or with carboplatin and paclitaxel in patients who were never or light former smokers with advanced lung adenocarcinoma: CALGB 30406 trial. J Clin Oncol 30: 2063-2069, 2012
28. Schemper M, Smith TL: A note on quantifying follow-up in studies of failure time. Control Clin Trials 17:343-346, 1996
29. Fang S, Wang Z: *EGFR* mutations as a prognostic and predictive marker in non-small-cell lung cancer. Drug Des Devel Ther 8:1595-1611, 2014
30. Zhang Z, Wang T, Zhang J, et al: Prognostic value of epidermal growth factor receptor mutations in resected non-small cell lung cancer: a systemic review with meta-analysis. PLoS One 9:e106053.0, 2014
31. Kris MG, Johnson BE, Berry LD, et al: Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. JAMA 311:1998-2006, 2014
32. Jao K., Tomasini P., Kamel-Reid S., et al: Prognostic effect of single versus multiple somatic mutations in non-small cell lung cancer (NSCLC). J Clin Oncol 33, 2015 (abstr 7521)
33. Clinical Lung Cancer Genome Project (CLCGP); Network Genomic Medicine (NGM): A genomics-based classification of human lung tumors. Sci Transl Med 5:209ra153, 2013
34. Kosaka T, Yatabe Y, Onozato R, et al: Prognostic implication of EGFR, KRAS, and TP53 gene mutations in a large cohort of Japanese patients with surgically treated lung adenocarcinoma. J Thorac Oncol 4:22-29, 2009
35. Cortot AB, Younes M, Martel-Planche G, et al: Mutation of TP53 and alteration of p14(arf) expression in EGFR- and KRAS-mutated lung adenocarcinomas. Clin Lung Cancer 15:124-130, 2014
36. Reily GJ, Jordan E, Kim HR, et al: Association of outcomes and co-occurring genomic alterations in patients with *KRAS*-mutant non-small cell lung cancer. J Clin Oncol 34, 2016 (abstr 9019)
37. Yang L, Zhou Y, Li Y, et al: Mutations of p53 and KRAS activate NF-κB to promote chemoresistance and tumorigenesis via dysregulation of cell cycle and suppression of apoptosis in lung cancer cells. Cancer Lett 357:520-526, 2015
38. Tomasini P, Jao K, Kamel-Reid S, et al: Predictive value of co-existing KRAS and TP53 mutations on response to chemotherapy in non-small cell lung cancer (NSCLC). J Clin Oncol 33, 2015 (abstr 11060)
39. Labbe C, Korpanty G, Tomasini P, et al: Prognostic and predictive effects of *p53* mutation in patients with *EGFR*-mutated non-small cell lung cancer (NSCLC). J Clin Oncol 34, 2016 (abstr 11585)
40. Yu HA, Jordan E, Ni A, et al: Concurrent genetic alterations identified by next-generation sequencing in untreated, metastatic EGFR-mutant lung cancers. J Clin Oncol 34, 2016 (abstr 9053)
41. Lim SM, Kim HR, Cho EK, et al: Targeted sequencing identifies genetic alterations that confer primary resistance to EGFR tyrosine kinase inhibitor (Korean Lung Cancer Consortium). Oncotarget 7: 36311-36320, 2016
42. Bria E, Pilotto S, Amato E, et al: Molecular heterogeneity assessment by next-generation sequencing and response to gefitinib of EGFR mutant advanced lung adenocarcinoma. Oncotarget 6: 12783-12795, 2015

Affiliations

Frances A. Shepherd and **Ming-Sound Tsao**, Princess Margaret Cancer Centre; University of Toronto, Toronto; **Lesley Seymour**, Queen's University, Kingston, Ontario, Canada; **Benjamin Lacas, Gwénaël Le Teuff, Jean-Pierre Pignon, Thierry Le Chevalier,** and **Jean-Charles Soria**, Institut Gustave-Roussy; **Benjamin Lacas, Gwénaël Le Teuff, Jean-Pierre Pignon, Thierry Le Chevalier,** and **Jean-Charles Soria**, University Paris XI, Paris; **Pierre Hainaut**, International Agency for Research on Cancer, Lyon; **Jean-Yves Douillard**, R Gauducheau, St Herblain; **Elizabeth Brambilla**, Inserm U823, Institut Albert Bonniot, Département de Pathologie CHU, Albert Michallon University Joseph Fourrier, Grenoble, France; **Pasi A. Jänne**, Dana-Farber Cancer Institute, Boston, MA; **Stephen**

Graziano, State University of New York Upstate Medical University, Syracuse, NY; **Robert Kratzke**, University of Minnesota Medical School, Minneapolis, MN; and **Robert Pirker** and **Martin Filipits**, Medical University of Vienna, Vienna, Austria.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Pooled Analysis of the Prognostic and Predictive Effects of *TP53* Comutation Status Combined With *KRAS* or *EGFR* Mutation in Early-Stage Resected Non–Small-Cell Lung Cancer in Four Trials of Adjuvant Chemotherapy

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Frances A. Shepherd
No relationship to disclose

Benjamin Lacas
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Gwénaël Le Teuff
No relationship to disclose

Pierre Hainaut
No relationship to disclose

Pasi A. Jänne
Stock or Other Ownership: Gatekeeper Pharmaceuticals
Consulting or Advisory Role: AstraZeneca, Boehringer Ingelheim, Pfizer, Chugai Pharma, Genentech, ARIAD Pharmaceuticals, Ignyta, Loxo, ACEA Biosciences
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Jean-Pierre Pignon
Research Funding: Bayer (Inst)

Thierry Le Chevalier
No relationship to disclose

Lesley Seymour
Stock or Other Ownership: AstraZeneca
Consulting or Advisory Role: Boehringer Ingelheim
Research Funding: Merck (Inst), AstraZeneca (Inst), Novartis (Inst), Innate Pharma (Inst)

Jean-Yves Douillard
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Research Funding: Merck Serono (Inst)

Stephen Graziano
No relationship to disclose

Elizabeth Brambilla
No relationship to disclose

Robert Pirker
Consulting or Advisory Role: Merck Sharp & Dohme, Synta
Speakers' Bureau: Boehringer Ingelheim, Eli Lilly, Roche, Pierre Fabre

Martin Filipits
Consulting or Advisory Role: AstraZeneca, Boehringer Ingelheim, Merck Sharp & Dohme, Eli Lilly, Ratiopharm, Novartis, Roche, Pfizer

Robert Kratzke
No relationship to disclose

Jean-Charles Soria
No relationship to disclose

Ming-Sound Tsao
Consulting or Advisory Role: AstraZeneca, Boehringer Ingelheim
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Appendix

Statistical Methods

To quantify the association between mutation status (both for *TP53*/*KRAS* and *TP53*/*EGFR*) and overall survival and disease-free survival, we first used the Cox proportional hazards regression model developed for *TP53* analysis.[16] This model (core model) was stratified by trial and included the following covariates: treatment (adjuvant chemotherapy [ACT] and observation [OBS]), sex, age ($<$ 55, 55 to 64, $>$ 64 years), tumor stage (T1, T2, T3/4), nodal stage (N0, N1, N2), and histology (squamous, adenocarcinoma, other). Histology was removed for *TP53*/*EGFR* analyses because they were limited to adenocarcinoma. We applied a process of several steps to develop a final model from this core model for both *TP53*/*KRAS* analysis and *TP53*/*EGFR* analysis. The first step was to study the correlation between *KRAS* and *EGFR*, respectively, and each covariate (age, sex, T stage, N stage, stage, histology only for *KRAS*, performance status, type of surgery, and lymphoid infiltration) via a univariable and multivariable logistic regression stratified by trial. Significant covariates ($P < .20$, univariable analysis) were included to a stratified logistic multivariable model. For *KRAS*, age, and histology remained statistically significant ($P < .05$). For *EGFR*, only sex remained statistically significant ($P < .05$). These covariates (age and histology for *KRAS* and sex for *EGFR*) were included in the core model. We added a composite marker (WT/WT, WT/MUT, MUT/WT, and MUT/MUT) that combined *TP53* and *KRAS* (model 1) and combined *TP53* and *EGFR* (model 2) to the core model. The next step was to check whether covariates not previously selected in each model may have an impact on the regression coefficients of the composite marker. If a variable caused a $\geq$ 20% change, the variable would be added to the model (Hosmer DW, et al: New York, NY, Wiley, 2008). For model 1, neither type of surgery, nor performance status changed the regression coefficients by more than 20%. For model 2, performance status was included in the model because it changed the regression coefficients by more than 20%. The last step was to check the proportional hazards assumption. This was performed by using the Schoenfeld residuals (survival R package), and no violation of this hypothesis was observed. Finally, model 1 included the following covariates: treatment (ACT and OBS), sex, age ($<$ 55, 55-64, $>$ 64 years), tumor stage (T1, T2, T3/4), nodal stage (N0, N1, N2), histology (squamous, adenocarcinoma, other), and composite marker *TP53/KRAS*. Model 2 included the following covariates: treatment (ACT and OBS), sex, age ($<$ 55, 55-64, $>$ 64 years), tumor stage (T1, T2, T3/4), nodal stage (N0, N1, N2), performance status (0, $\geq$ 1), and composite marker (*TP53/EGFR*). Univariable analyses showing relations between all analyzed covariates and overall survival are presented in Appendix Tables A9 and A10.

Heterogeneity of prognostic and predictive values of mutation status (*TP53/KRAS* and *TP53/EGFR*) across trials and according to histology was evaluated by introducing interaction terms in each model (two-order interaction for prognostic analyses and three-order interaction for predictive analyses).

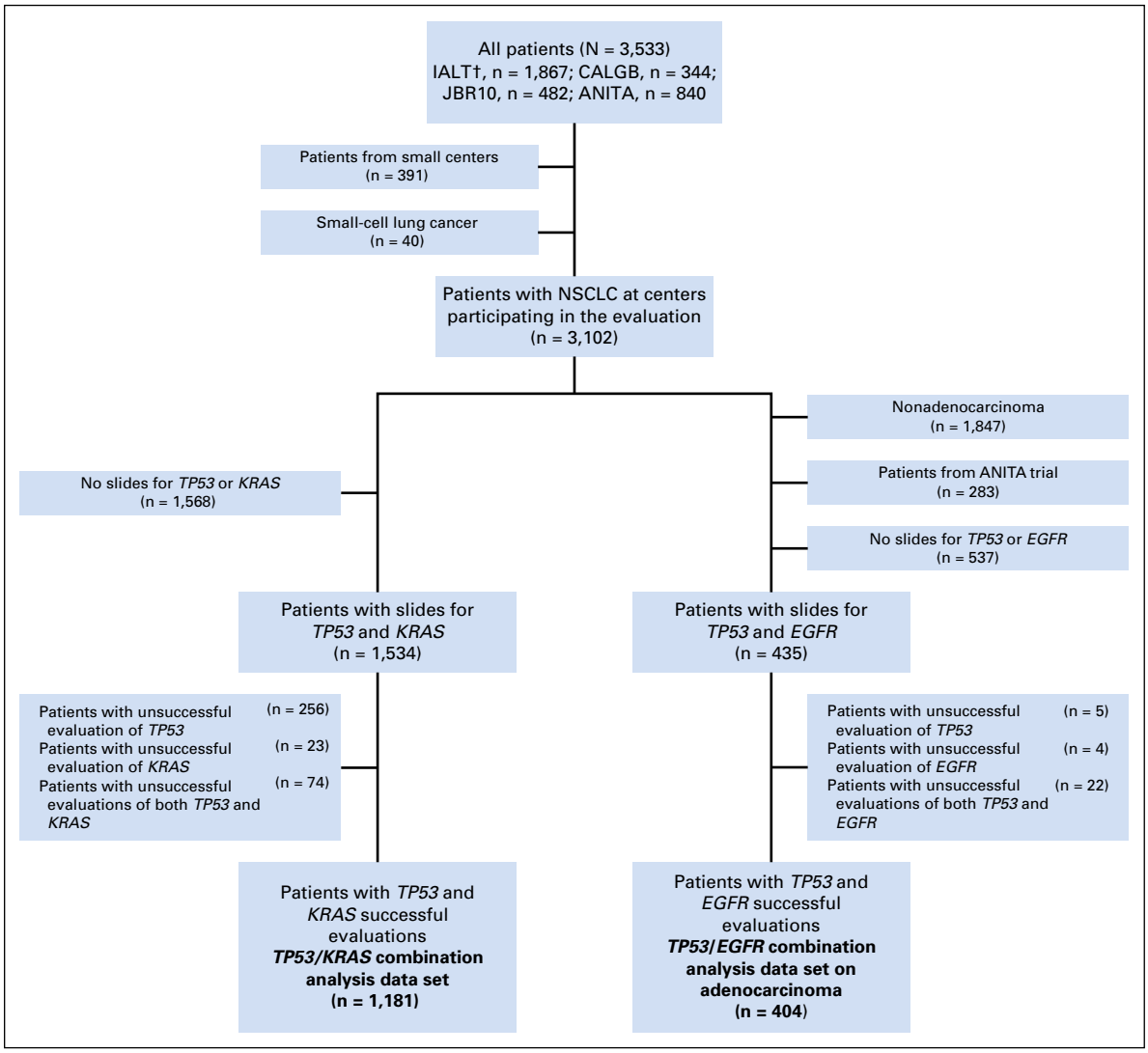

Fig A1. Flowchart of patients entered in the four trials and included in the (*TP53*, *KRAS*) and (*TP53*, *EGFR*) analyses. NSCLC, non–small-cell lung cancer.

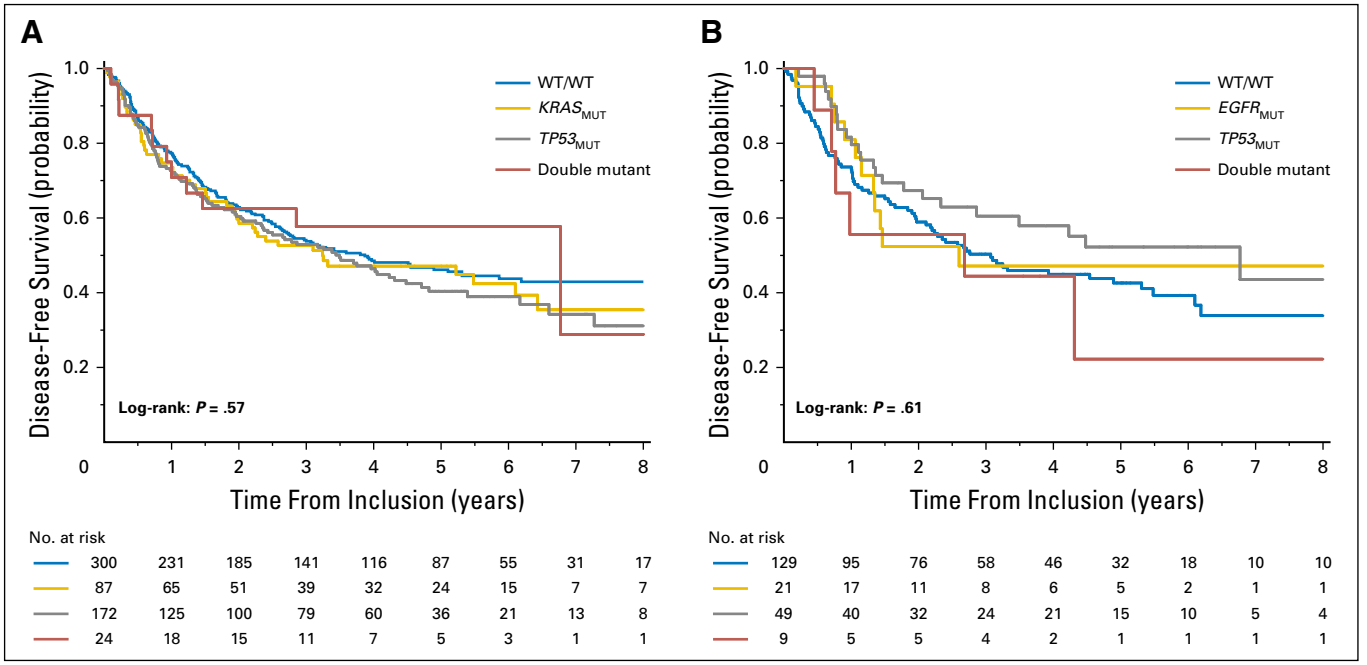

Fig A2. Disease-free survival. Unadjusted Kaplan-Meier curves of the prognostic effect in the observation arm. (A) *TP53/KRAS* combination. (B) *TP53/EGFR* combination. MUT, mutant; WT, wild type.

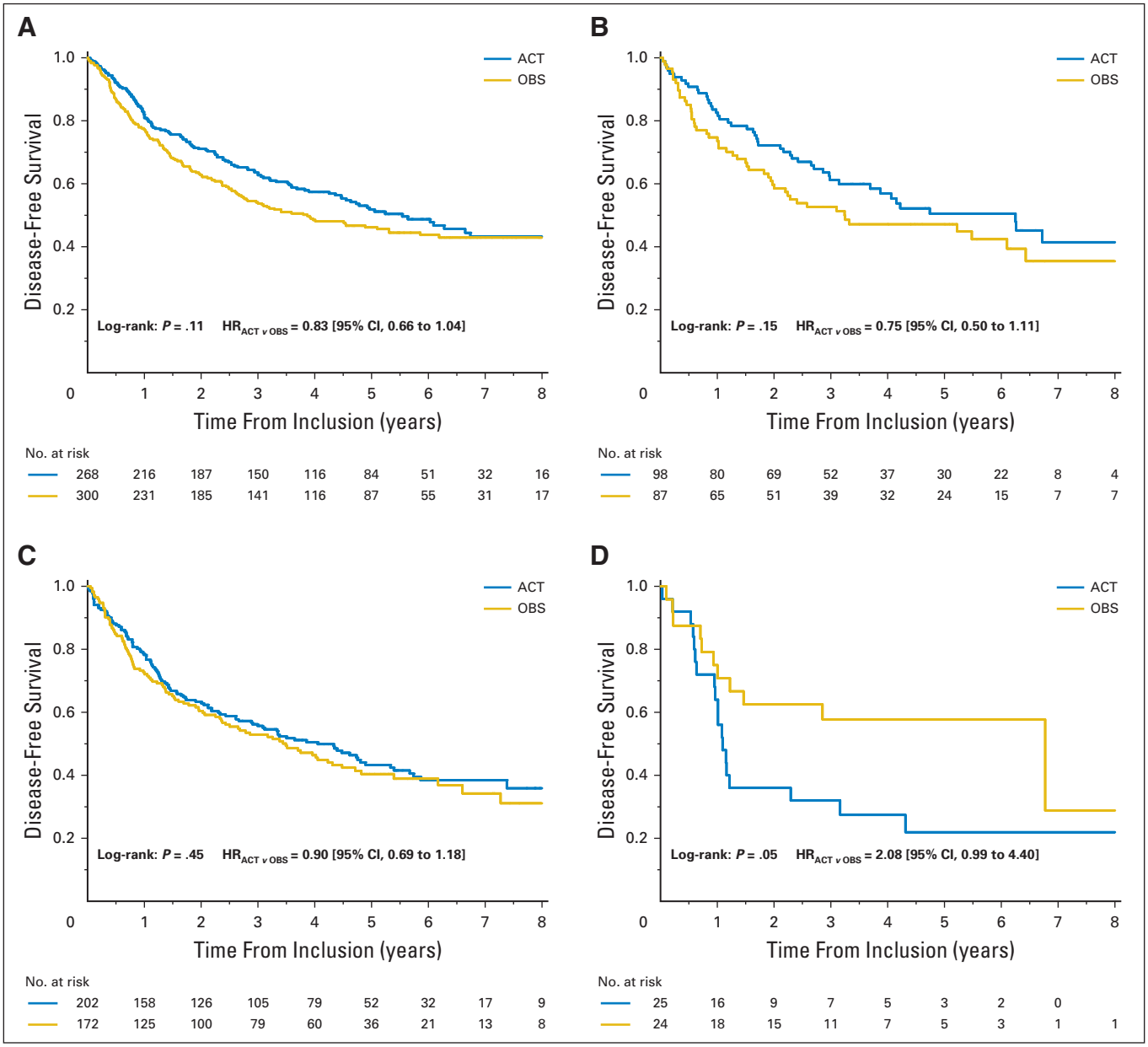

Fig A3. Disease-free survival - Unadjusted Kaplan-Meier curves of treatment effect according to *TP53* and *KRAS* mutation status. (A) Double wild type. (B) *TP53* wild-type, *KRAS* mutant. (C) *TP53* mutant, *KRAS* wild type. (D) Double mutant. ACT, adjuvant chemotherapy; OBS, observation.

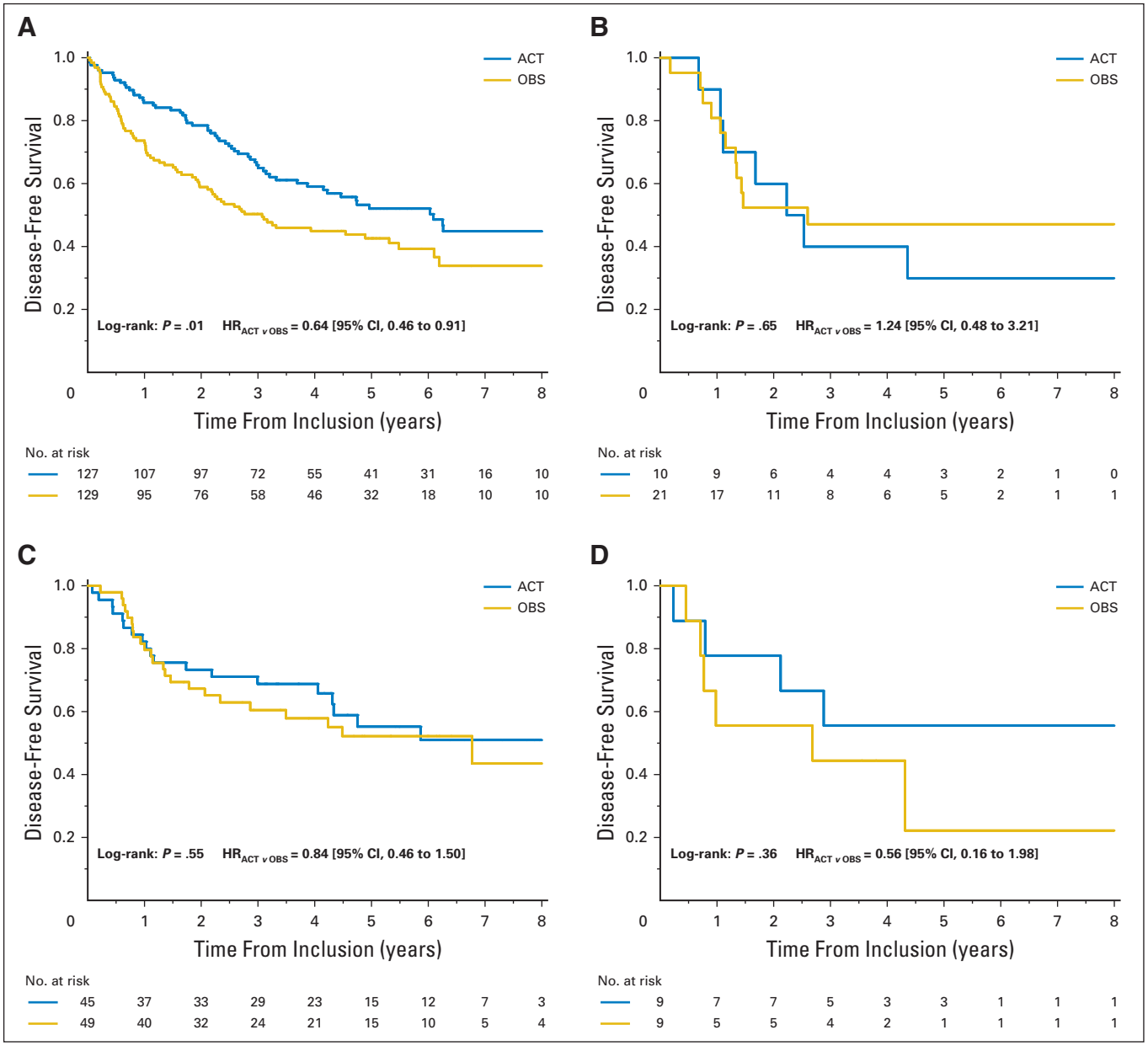

Fig A4. Disease-free survival - Unadjusted Kaplan-Meier curves of treatment effect according to *TP53* and *EGFR* mutation status. (A) Double wild type. (B) *TP53* wild-type, *EGFR* mutant. (C) *TP53* mutant, *EGFR* wild type. (D) Double mutant. ACT, adjuvant chemotherapy; OBS, observation.

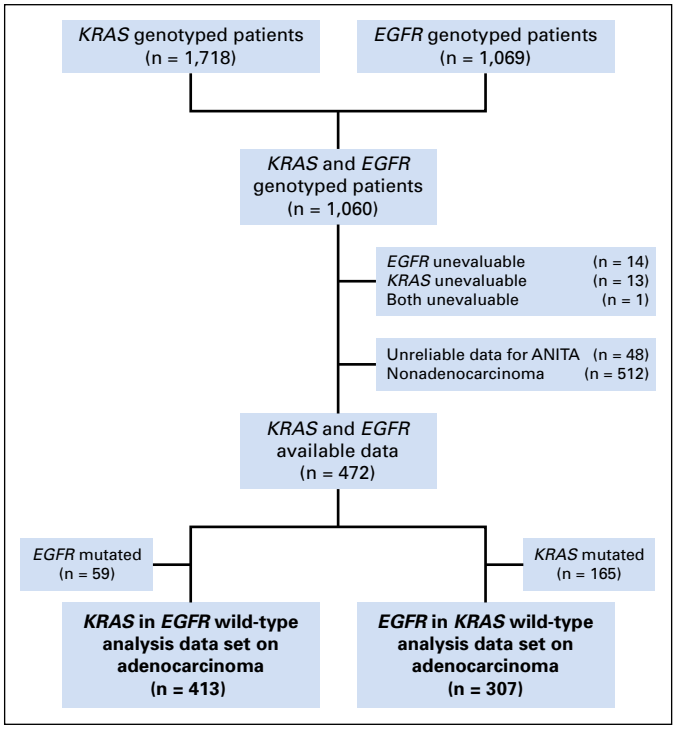

Fig A5. Subgroup analyses. Flowchart of patients with adenocarcinoma entered in three trials and with *KRAS* and *EGFR* available mutation status.

Table A1. Patient and Tumor Characteristics for Patients With and Without *TP53*/*KRAS* Mutation Status Results

Characteristic	*TP53*/*KRAS* Unknown Status* (n = 1,921)	*TP53*/*KRAS* Known Status (n = 1,181)	*P*†
Age, years			.16
≤ 50	400 (20.8)	200 (16.9)	
51-60	656 (34.2)	404 (34.2)	
> 60	865 (45.0)	577 (48.9)	
Sex			.02
Male	1,526 (79.4)	888 (75.2)	
Female	391 (20.4)	293 (24.8)	
Missing/unknown	4 (0.2)	0 (—)	
T stage‡			.003
T1	253 (13.2)	132 (11.2)	
T2	1,359 (70.7)	907 (76.8)	
T3/4	299 (15.6)	139 (11.8)	
Missing/unknown	10 (0.5)	3 (0.3)	
N stage‡			.13
N0	987 (51.4)	631 (53.4)	
N1	533 (27.8)	389 (32.9)	
N2	390 (20.3)	156 (13.2)	
Missing/unknown	11 (0.6)	5 (0.4)	
Stage‡			.10
I	846 (44.0)	569 (48.2)	
II	590 (30.7)	419 (35.5)	
III	481 (25.0)	190 (16.1)	
Missing/unknown	4 (0.2)	3 (0.3)	
WHO performance status			.55
0	988 (51.4)	619 (52.4)	
≥ 1	920 (47.9)	558 (47.3)	
Missing/unknown	13 (0.7)	4 (0.3)	
Histology			.05
Squamous	970 (50.5)	508 (43.0)	
Adenocarcinoma	758 (39.5)	497 (42.1)	
Other NSCLC	185 (9.6)	176 (14.9)	
Missing/unknown	8 (0.4)	0 (—)	
Treatment arm			.96
Observation	964 (50.2)	587 (49.7)	
Adjuvant chemotherapy	957 (49.8)	594 (50.3)	

Abbreviation: NSCLC, non–small-cell lung cancer.
*Patients without known status include patients without tumor samples (n = 1,568) or with unsuccessful evaluation of *TP53* and/or *KRAS* (n = 353).
†χ^2 test from a logistic model stratified by trial. Patients with unknown values were excluded from the corresponding analyses.
‡Using 6th edition TNM staging classification.

Table A2. Patient and Tumor Characteristics for Patients With and Without *TP53*/*EGFR* Mutation Status Results* in Adenocarcinoma

Characteristic	*TP53*/*EGFR* Unknown Status* (n = 568)	*TP53*/*EGFR* Known Status (n = 404)	*P*†
Age, years			.72
≤ 50	132 (23.2)	87 (21.5)	
51-60	195 (34.3)	142 (35.2)	
> 60	241 (42.4)	175 (43.3)	
Sex			.39
Male	374 (65.9)	247 (61.1)	
Female	194 (34.2)	157 (38.9)	
T stage‡			.87
T1	114 (20.1)	64 (15.8)	
T2	389 (68.5)	323 (80.0)	
T3/4	64 (11.3)	15 (3.7)	
Missing/unknown	1 (0.2)	2 (0.5)	
N stage‡			.07
N0	330 (58.1)	251 (62.1)	
N1	143 (25.2)	117 (29.0)	
N2	94 (16.6)	33 (8.2)	
Missing/unknown	1 (0.2)	3 (0.7)	
Stage‡			.28
I	303 (53.4)	245 (60.6)	
II	151 (26.6)	122 (30.2)	
III	113 (19.9)	35 (8.7)	
Missing/unknown	1 (0.2)	2 (0.5)	
WHO performance status			.64
0	318 (56.0)	229 (56.7)	
≥ 1	249 (43.8)	173 (42.8)	
Missing/unknown	1 (0.2)	2 (0.5)	
Treatment arm			.25
Observation	272 (47.9)	213 (52.7)	
Adjuvant chemotherapy	296 (52.1)	191 (47.3)	

Abbreviation: NSCLC, non–small-cell lung cancer.
*Patients without known status include patients without tumor samples (n = 537) or with unsuccessful evaluation of *TP53* and/or *EGFR* (n = 31).
†χ^2 test from a logistic model stratified by trial. Patients with unknown values were excluded from the corresponding analyses.
‡Using 6th edition TNM staging classification.

Table A3. Prognostic Value of *TP53* and *KRAS* Combination on Overall Survival in the Observation Arm

TP53 × *KRAS*	No. of Deaths/No. of Patients	HR [95% CI]	*P*
$TP53_{WT}$ and $KRAS_{WT}$	142/300	Ref	—
$TP53_{WT}$ and $KRAS_{MUT}$	42/87	1.23 [0.85 to 1.78] (HR_1)	—
$TP53_{MUT}$ and $KRAS_{WT}$	89/172	1.06 [0.80 to 1.39] (HR_2)	—
$TP53_{MUT}$ and $KRAS_{MUT}$	9/24	0.83 [0.42 to 1.65] (HR_3)	—
Overall effect $HR_1 = 1$ and $HR_2 = 1$ and $HR_3 = 1$	—	—	.61
Interaction $HR_1 = HR_2 = HR_3$	—	—	.51

NOTE. Four patients were excluded because of missing values for some covariates. Heterogeneity across trials: *P* = .66; heterogeneity across histology: *P* = .97.
Abbreviations: HR, hazard ratio; $TP53_{MUT}$, *TP53* mutant; $KRAS_{MUT}$, *KRAS* mutant.

Table A4. Prognostic Value of *TP53* and *KRAS* Combination on Disease-Free Survival in the Observation Arm

TP53 × *KRAS*	No. of Events/No. of Patients	HR [95% CI]	*P*
$TP53_{WT}$ and $KRAS_{WT}$	161/300	Ref	—
$TP53_{WT}$ and $KRAS_{MUT}$	50/87	1.28 [0.91 to 1.79] (HR_1)	—
$TP53_{MUT}$ and $KRAS_{WT}$	102/172	1.06 [0.82 to 1.36] (HR_2)	—
$TP53_{MUT}$ and $KRAS_{MUT}$	11/24	0.91 [0.49 to 1.71] (HR_3)	—
Overall effect $HR_1 = 1$ and $HR_2 = 1$ and $HR_3 = 1$	—	—	.51
Interaction $HR_1 = HR_2 = HR_3$	—	—	.47

NOTE. Four patients were excluded because of missing values for some covariates. Heterogeneity across trials: $P = .41$; heterogeneity across histology: $P = .99$.
Abbreviations: HR, hazard ratio; $TP53_{MUT}$, *TP53* mutant; $KRAS_{MUT}$, *KRAS* mutant.

Table A5. Predictive Value of *TP53* and *KRAS* Combination on Disease-Free Survival (n = 1,176)**

TP53 and *KRAS* Combination	ACT Group (No. of events/No. of patients)	OBS Group (No. of events/No. of patients)	HR ACT *v* OBS [95% CI]
Double WT (n = 568)	129/268	161/300	0.82 [0.65 to 1.04] (HR_1); $P = .10$
$KRAS_{MUT}$ (n = 185)	47/98	50/87	0.62 [0.41 to 0.92] (HR_2); $P = .02$
$KRAS_{MUT}$ *v* double WT [95% CI]	1.00 [0.70 to 1.41]; $P = .98$	1.33 [0.96 to 1.85]; $P = .09$	0.75 [0.47 to 1.19]*; $P = .22$
$TP53_{MUT}$(n = 374)	116/202	102/172	0.91 [0.69 to 1.18] (HR_3); $P = .47$
$TP53_{MUT}$ *v* double WT [95% CI]	1.18 [0.92 to 1.53]; $P = .20$	1.07 [0.83 to 1.38]; $P = .58$	1.10 [0.77 to 1.57]*; $P = .60$
Double mutant (n = 49)	19/25	11/24	2.13 [1.01 to 4.51] (HR_4); $P = .05$
Double mutant *v* double WT [95% CI]	2.65 [1.61 to 4.34]; $P = .001$	1.02 [0.55 to 1.89]; $P = .95$	2.59 [1.18 to 5.68]*; $P = .02$
Test for interaction ($HR_1 = HR_2 = HR_3 = HR_4$): $P = .04$			

NOTE. Five patients were excluded because of missing values for some covariates. Heterogeneity across trials: $P = .87$; heterogeneity across histology: $P = .46$.
Abbreviations: ACT, adjuvant chemotherapy; HR, hazard ratio; $KRAS_{MUT}$, *KRAS* mutant; OBS, observation; $TP53_{MUT}$, *TP53* mutant; WT, wild-type.
*HR of interaction between treatment and two mutation categories (double WT and $KRAS_{MUT}$, double WT and $TP53_{MUT}$, double WT and double mutant). This HR of interaction and the corresponding test compare the treatment effect (chemotherapy *v* observation) across two mutation categories.

Table A6. Prognostic Value of *TP53* and *EGFR* Combination on Overall Survival in the Adenocarcinoma Observation Arm

TP53 × *EGFR*	No. of Deaths/No. of Patients	HR [95% CI]	*P*
$TP53_{WT}$ and $EGFR_{WT}$	64/129	Ref	—
$TP53_{WT}$ and $EGFR_{MUT}$	9/21	1.10 [0.53 to 2.28] (HR_1)	—
$TP53_{MUT}$ and $EGFR_{WT}$	20/49	0.85 [0.50 to 1.46] (HR_2)	—
$TP53_{MUT}$ and $EGFR_{MUT}$	4/9	0.69 [0.25 to 1.94] (HR_3)	—
Overall effect $HR_1 = 1$ and $HR_2 = 1$ and $HR_3 = 1$	—	—	.83
Interaction $HR_1 = HR_2 = HR_3$	—	—	.73

NOTE. Five patients were excluded because of missing values for some covariates. Heterogeneity across trials: $P = .38$.
Abbreviations: HR, hazard ratio; $EGFR_{MUT}$, *EGFR* mutant; $TP53_{MUT}$, *TP53* mutant.

Table A7. Prognostic Value of *TP53* and *EGFR* Combination on Disease-Free Survival in Adenocarcinoma and Observation Arm

TP53 × *EGFR*	No. of Events/No. of Patients	HR [95% CI]	*P*
$TP53_{WT}$ and $EGFR_{WT}$	77/129	Ref	—
$TP53_{WT}$ and $EGFR_{MUT}$	11/21	0.93 [0.49 to 1.79] (HR_1)	—
$TP53_{MUT}$ and $EGFR_{WT}$	25/49	0.75 [0.46 to 1.21] (HR_2)	—
$TP53_{MUT}$ and $EGFR_{MUT}$	6/9	0.92 [0.39 to 2.17] (HR_3)	—
Overall effect $HR_1 = 1$ and $HR_2 = 1$ and $HR_3 = 1$	—	—	.71
Interaction $HR_1 = HR_2 = HR_3$	—	—	.81

NOTE. Five patients were excluded because of missing values for some covariates. Heterogeneity across trials: $P = .78$.
Abbreviations: HR, hazard ratio; $EGFR_{MUT}$, *EGFR* mutant; $TP53_{MUT}$, *TP53* mutant.

Table A8. Predictive Value of *TP53* and *EGFR* Combination on Disease-Free Survival in Adenocarcinoma (n = 399)**

TP53 and *EGFR* Combination	ACT (No. of events/No. of patients)	OBS (No. of events/No. of patients)	HR ACT *v* OBS [95% CI]
Double WT (n = 256)	59/127	77/129	0.63 [0.45 to 0.90] (HR_1); $P = .01$
$EGFR_{MUT}$ (n = 31)	7/10	11/21	1.09 [0.42 to 2.85] (HR_2); $P = .85$
$EGFR_{MUT}$ *v* double WT [95% CI]	1.71 [0.76 to 3.84]; $P = .19$	0.99 [0.52 to 1.88]; $P = .98$	1.73 [0.62 to 4.79]*; $P = .29$
$TP53_{MUT}$ (n = 94)	20/45	25/49	0.73 [0.40 to 1.33] (HR_3); $P = .31$
$TP53_{MUT}$ *v* double WT [95% CI]	0.90 [0.53 to 1.51]; $P = .68$	0.78 [0.49 to 1.24]; $P = .28$	1.16 [0.58 to 2.30]*; $P = .68$
Double mutant (n = 18)	4/9	6/9	0.55 [0.15 to 2.01 (HR_4); $P = .36$
Double mutant *v* double WT [95% CI]	0.81 [0.28 to 2.29]; $P = .69$	0.94 [0.40 to 2.17]; $P = .88$	0.86 [0.22 to 3.32]*; $P = .83$
Test for interaction ($HR_1 = HR_2 = HR_3 = HR_4$): $P = .74$			

NOTE. Five patients were excluded because of missing values for some covariates. Heterogeneity across trials: $P = .29$.
Abbreviations: ACT, adjuvant chemotherapy; HR, hazard ratio; $EGFR_{MUT}$, *EGFR* mutant; OBS, observation; $TP53_{MUT}$, *TP53* mutant; WT, wild-type.
*HR of interaction between treatment and two mutation categories (double WT and $EGFR_{MUT}$, double WT and $TP53_{MUT}$, double WT and double mutant). This HR of interaction and the corresponding test compare the treatment effect (chemotherapy *v* observation) across two mutation categories.

Table A9. Univariable Cox Proportional Hazard Regression Model Presenting Relations Between Analyzed Covariates and Overall Survival for the *TP53* and *KRAS* Combination

Variable	No. of Deaths/No. of Patients	HR [95% CI]	*P*
Age, years			.06
< 55	139/330	Ref	
55-64	233/478	1.24 [1.01 to 1.53]	
≥ 65	188/373	1.28 [1.03 to 1.59]	
Sex			< .001
Male	457/888	Ref	
Female	103/293	0.65 [0.52 to 0.80]	
T stage*			< .001
T1	54/132	Ref	
T2	409/907	1.22 [0.91 to 1.63]	
T3/4	95/139	1.96 [1.40 to 2.75]	
N stage*			< .001
N0	243/631	Ref	
N1	196/389	1.72 [1.39 to 2.12]	
N2	118/156	3.23 [2.51 to 4.16]	
Histology			.04
Squamous	239/508	Ref	
Adenocarcinoma	227/497	1.04 [0.87 to 1.26]	
Other NSCLC	94/176	1.36 [1.07 to 1.73]	
TP53 × *KRAS*			.03
$TP53_{WT}$ and $KRAS_{WT}$	252/570	Ref	
$TP53_{WT}$ and $KRAS_{MUT}$	84/186	1.12 [0.87 to 1.44]	
$TP53_{MUT}$ and $KRAS_{WT}$	198/376	1.26 [1.04 to 1.52]	
$TP53_{MUT}$ and $KRAS_{MUT}$	26/49	1.59 [1.06 to 2.39]	
Treatment arm			.57
Observation	284/587	Ref	
Adjuvant chemotherapy	276/594	0.95 [0.81 to 1.13]	

Abbreviations: NSCLC, non–small-cell lung cancer; $KRAS_{MUT}$, *KRAS* mutant; $TP53_{MUT}$, *TP53* mutant.
*Using 6th edition TNM staging classification.

Table A10. Univariable Cox Proportional Hazards Regression Model Presenting Relations Between Analyzed Covariates and Overall Survival for the *TP53* and *EGFR* Combination

Variable	No. of Deaths/No. of Patients	HR [95% CI]	*P*
Age, years			.37
< 55	53/140	Ref	
55-64	66/147	1.24 [0.86 to 1.78]	
≥ 65	54/117	1.29 [0.88 to 1.89]	
Sex			.0002
Male	125/247	Ref	
Female	48/157	0.53 [0.38 to 0.74]	
T stage*			.37
T1	26/64	Ref	
T2	137/323	1.16 [0.74 to 1.82]	
T3/4	9/15	1.74 [0.80 to 3.77]	
N stage*			< .001
N0	84/251	Ref	
N1	63/117	2.28 [1.56 to 3.35]	
N2	24/33	5.83 [3.22 to 10.58]	
WHO performance status			.004
0	85/229	Ref	
≥ 1	87/173	1.55 [1.15 to 2.09]	
TP53 × *EGFR*			.95
$TP53_{WT}$ and $EGFR_{WT}$	114/260	Ref	
$TP53_{WT}$ and $EGFR_{MUT}$	13/31	1.02 [0.57 to 1.81]	
$TP53_{MUT}$ and $EGFR_{WT}$	39/95	0.93 [0.64 to 1.34]	
$TP53_{MUT}$ and $EGFR_{MUT}$	7/18	0.85 [0.39 to 1.83]	
Treatment arm			.08
Observation	100/213	Ref	
Adjuvant chemotherapy	73/191	0.76 [0.56 to 1.03]	

Abbreviations: NSCLC, non–small-cell lung cancer; $EGFR_{MUT}$, *EGFR* mutant; $TP53_{MUT}$, *TP53* mutant.
*Using 6th edition TNM staging classification.