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ORIGINAL REPORT

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

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ABSTRACT

Purpose
We undertook this analysis of *KRAS* mutation in four trials of adjuvant chemotherapy (ACT) versus observation (OBS) to clarify the prognostic/predictive roles of *KRAS* in non–small-cell lung cancer (NSCLC).

Methods
KRAS mutation was determined in blinded fashion. Exploratory analyses were performed to characterize relationships between mutation status and subtype and survival outcomes using a multivariable Cox model.

Results
Among 1,543 patients (763 OBS, 780 ACT), 300 had *KRAS* mutations (codon 12, n = 275; codon 13, n = 24; codon 14, n = 1). In OBS patients, there was no prognostic difference for overall survival for codon-12 (mutation *v* wild type [WT] hazard ratio [HR] = 1.04; 95% CI, 0.77 to 1.40) or codon-13 (HR = 1.01; 95% CI, 0.47 to 2.17) mutations. No significant benefit from ACT was observed for WT-*KRAS* (ACT *v* OBS HR = 0.89; 95% CI, 0.76 to 1.04; $P = .15$) or codon-12 mutations (HR = 0.95; 95% CI, 0.67 to 1.35; $P = .77$); with codon-13 mutations, ACT was deleterious (HR = 5.78; 95% CI, 2.06 to 16.2; $P < .001$; interaction $P = .002$). There was no prognostic effect for specific codon-12 amino acid substitution. The effect of ACT was variable among patients with codon-12 mutations: G12A or G12R (HR = 0.66; $P = .48$), G12C or G12V (HR = 0.94; $P = .77$) and G12D or G12S (HR = 1.39; $P = .48$; comparison of four HRs, including WT, interaction $P = .76$). OBS patients with *KRAS*-mutated tumors were more likely to develop second primary cancers (HR = 2.76, 95% CI, 1.34 to 5.70; $P = .005$) but not ACT patients (HR = 0.66; 95% CI, 0.25 to 1.75; $P = .40$; interaction, $P = .02$).

Conclusion
KRAS mutation status is not significantly prognostic. The potential interaction in patients with codon-13 mutations requires validation. At this time, *KRAS* status cannot be recommended to select patients with NSCLC for ACT.

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INTRODUCTION

KRAS, a member of the *RAS* oncogene family, encodes a protein that binds guanine nucleotides, has GTPase activity, and is involved in signal transduction.[1] In non–small-cell lung cancer (NSCLC), *KRAS* mutations occur most frequently in codons 12 and 13, usually are found in cancers of smokers, and are most common in nonsquamous NSCLC. Mutations have been reported in ~30% of lung adenocarcinomas.[2,3] Slebos et al[4] were among the first to suggest that *KRAS* mutation was prognostic of 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 the prognostic effect.[5,6]

On the basis of early in vitro studies, it was postulated that *RAS* mutations might be associated with resistance to therapy.[7,8] However, subsequent clinical studies could not always confirm the preclinical effects.[9-11] The North American Intergroup trial

JBR.10 randomly assigned patients with completely resected stage IB-II NSCLC to receive adjuvant chemotherapy (ACT) with cisplatin/vinorelbine or observation (OBS) alone.[10,11] No differential overall survival (OS) benefit could be demonstrated in patients with wild-type (WT) *RAS* compared with those with mutations. However, disease-specific survival appeared to be prolonged with ACT in patients with WT-*RAS* (hazard ratio [HR] = 0.72; $P = .06$), with no apparent benefit in patients with mutations (HR= 1.07; $P = .82$). However, the study lacked power to demonstrate significant interaction.

In an attempt to clarify the impact of *KRAS* mutation on survival benefit from platinum-based ACT, we performed this pooled analysis of four randomized trials of ACT or OBS.

METHODS

Clinical Trials

This study used the LACE (Lung Adjuvant Cisplatin Evaluation) database of 3,533 patients from three LACE cisplatin-based ACT trials (IALT [International Adjuvant Lung Cancer Trial],[12,13] ANITA [Adjuvant Navelbine International Trialist Association],[14] JBR.10[10,11])[15] and Cancer and Leukemia Group B (CALGB) 9633, which used carboplatin-based ACT.[16] Scientists, clinicians, and statisticians from these trials represent the LACE-Bio Collaborative Group.

KRAS Mutation Analyses

KRAS mutation analyses were performed in one laboratory for ANITA and JBR.10 (M.-S.T.) and in the laboratories of P.A.J. and P.H. for CALGB-9633 and IALT, respectively. Scientists were blinded regarding study arm and outcome. Laboratory methods are provided (Data Supplement).

Statistical Methods

Analyses included only patients from the previously mentioned trials with *KRAS* mutation results. OS, the primary end point, was defined as the time from randomization to death from any cause or last follow-up in surviving patients. Disease-free survival (DFS) was defined as the time from randomization to recurrence or death from any cause or last follow-up in surviving patients. Second primaries were analyzed as a time-to-event end point from randomization to occurrence of a second primary.

A logistic model stratified by trial was used to study correlations between *KRAS* status and covariates. A Cox model stratified by trial and including the covariates of treatment, sex, age (< 55, 55 to 64, > 64 years), performance status (PS 1/2, PS 0), tumor stage (T1, T2, T3/4), nodal stage (N0, N1, N2), and histology (squamous, adenocarcinoma, other) was used to examine the prognostic value of *KRAS* status on OS, DFS, and second primary rate (Data Supplement). Prognostic analyses were performed in the OBS and ACT arms together, and in OBS patients alone when a potential treatment and *KRAS* interaction was identified. The interaction between treatment and *KRAS* status was assessed to determine the predictive value of mutation status. Prognostic and predictive effects of *KRAS* mutation subtypes were examined using the same methods. *KRAS* subtypes were grouped prospectively into three subgroups based on their potential association with tobacco carcinogens. To assess the effect of *KRAS* status by histologic subgroup, an interaction with histology was introduced in the models.

A test for heterogeneity was used to compare HRs among trials. Within pooled analyses, statistical significance was set at $P < .01$, and HRs were reported with 95% CIs. Survival curves were based on Kaplan-Meier methods and represented with adjusted/stratified HRs and *P* values. Statistical analyses were performed using SAS Software, version 9.2 (SAS Institute, Cary, NC). All *P* values are two-sided.

RESULTS

Patients

Of 3,532 patients randomly assigned (840 ANITA, 482 JBR.10, 1,867 IALT, 343 CALGB-9633; Data Supplement), 1,718 underwent *KRAS* testing, with mutation results for 1,543 (44% of randomized cases). Baseline demographics for patients with and without *KRAS* results and for patients with *KRAS* results by trial are shown in the Data Supplement.

KRAS Mutation Results

KRAS mutations (MUT) were identified in 300 specimens (19%). Mutations were more frequent in female patients (27% *v* 17% male; $P = .001$), patients with adenocarcinoma (34% *v* 6% squamous, 23% other; $P < .001$), and younger patients (trend $P = .0003$; Table 1). In the multivariable model, only age ($P = .044$) and histology ($P < .001$) remained significant.

Mutations were found at codon 12 in 275 patients (three double codon 12 mutations), codon 13 in 24, and codon 14 in one patient. The most frequent nucleotide change was a guanine>thymidine (G>T) transversion in 223 patients (Data Supplement). Amino acid substitutions are described in the Data Supplement. Mutations were found in 11 (1.6%) of 68 patients who were lifetime nonsmokers (codon 12, n = 10; codon 13, n = 1; Data Supplement). G>T transversions, considered the characteristic mutation from tobacco smoke, represented 78% (75 of 134) of codon 12 mutations in smokers and 50% (five of 10) in nonsmokers.

Prognostic Effect of KRAS Mutations

With 5.5 (95% CI, 5.3 to 5.7) years of median follow-up and 754 deaths (48.9%) among all 1,543 patients (Fig 1A), after accounting for the prognostic effect of baseline characteristics in multivariable analyses (Data Supplement), there was no significant difference in OS based on *KRAS* (multivariable HR MUT *v* WT, 1.17; 95% CI, 0.96 to 1.42; $P = .12$), with no heterogeneity among trials ($P = .47$). There was no prognostic effect in OBS patients (Fig 1B; HR = 1.04; 95% CI, 0.78 to 1.38; $P = .79$) or patients with adenocarcinoma (Fig 1C; HR = 1.00; 95% CI, 0.78 to 1.29; $P = .97$). Trends toward worse outcome were seen for MUT-*KRAS* in patients with nonadenocarcinoma (squamous HR = 1.41 [95% CI, 0.89 to 2.23]; other nonadenocarcinoma NSCLC HR = 1.86 [95% CI, 1.22 to 2.82], Data Supplement).

For DFS (Figs 1D, 1E, and 1F), similar results were found for all patients (HR = 1.15; 95% CI, 0.96 to 1.39; $P = .14$), OBS (HR = 1.05; 95% CI, 0.80 to 1.36; $P = .73$), and adenocarcinoma (HR = 0.98; 95% CI, 0.78 to 1.24; $P = .87$).

Because of potential treatment interactions, prognostic analyses for *KRAS* subtypes were performed in OBS patients alone. There was no significant prognostic effect on OS (Table 2) for codon 12 (HR *v* WT = 1.04; 95% CI, 0.77 to 1.40) or 13 mutations (HR *v* WT = 1.01; 95% CI, 0.47 to 2.17; $P = .96$). There was no significant difference in prognosis for codon 12 subgroups (Table 2) for OS ($P = .99$) or DFS ($P = .98$).

Predictive Effect of KRAS Mutation Status

The HR for death comparing ACT with OBS for patients with WT-*KRAS* tumors (Fig 2A) was 0.89 (95% CI, 0.76 to 1.05; $P = .15$) and 1.05 (95% CI, 0.76 to 1.46; $P = .77$; interaction $P = .37$) for patients with MUT-*KRAS* (Fig 2C). Results were not significantly

Table 1. Patient and Tumor Characteristics for Patients With *KRAS* Mutated and Wild-Type Tumors

Characteristic	*KRAS* Mutated (n = 300) No.	%	*KRAS* Wild Type (n = 1,243) No.	%	*P**
Sex					
Male	195	17	957	83	.001
Female	105	27	286	73	
Age, years					
< 55	107	25	325	75	.001 (.0003)
55-64	115	18	513	82	
> 64	78	16	405	84	
WHO performance status					
0	162	20	648	80	
1/2	137	19	592	81	.44
Unknown	1	25	3	75	
Smoking status					
No	10	17	48	83	
Yes	123	25	379	75	.20
Unknown†	167	17	816	83	
Stage‡					
I	175	23	590	77	.15 (.02)
II	96	18	430	82	
III	29	12	218	88	
Unknown	0	0	5	100	
N stage‡					
0	184	21	6,752	79	.50 (.21)
1	87	18	390	82	
2	28	14	171	86	
Unknown	1	13	7	87	
T stage‡					
1	33	19	141	81	.009 (.01)
2	252	22	912	78	
3/4	15	8	185	92	
Unknown	0	0	5	100	
Histology					
Squamous cell	43	6	664	94	< .001
Adenocarcinoma	204	34	401	66	
Other NSCLC	53	23	178	77	
Type of surgery					
Lobectomy/other	240	23	836	77	.001
Pneumonectomy	59	13	405	87	
Unknown	1	33	2	67	

Abbreviations: IALT, International Adjuvant Lung Cancer Trial; NSCLC, non–small-cell lung cancer.
*P values are calculated from univariate logistic regression stratified by trial in patients with no missing values. Test for trend in parentheses.
†No tobacco-related data collected in IALT trial (718 patients); this variable was not included in the multivariable models.
‡From 6th edition TNM staging classification.

different among trials $P = .52$ (Data Supplement). Results were similar for DFS (WT-*KRAS* HR = 0.86, 95% CI, 0.74 to 1.00, $P = .04$; MUT-*KRAS* HR = 0.93, 95% CI, 0.68 to 1.27, $P = .65$, interaction $P = .63$) and in patients with adenocarcinoma (interaction $P = .86$; Figs 2B and 2D, Data Supplement).

The predictive effects of codon 12 or 13 mutations are summarized in Table 2 (Figs 2E and 2F). Patients with tumors harboring codon 12 mutations seemed to derive no benefit from ACT (HR = 0.95; 95% CI, 0.67 to 1.35; $P = .77$). In contrast, the presence of codon 13 mutations was associated with significantly worse OS with ACT (HR = 5.78; 95% CI, 2.06 to 16.22; $P < .001$; interaction $P = .002$). Results were similar for DFS.

The predictive effects of specific codon 12 amino acid substitutions are summarized in Table 2. The effect of ACT was variable among patients with codon-12 mutations: G12A or G12R OS HR = 0.66 (95% CI, 0.21 to 2.10; $P = .48$), G12C or G12A HR = 0.94 (95% CI, 0.63 to 1.41; $P = .77$) and G12D or G12S HR = 1.39 (95% CI, 0.55 to 3.54; $P = .49$); the differences were not significant (comparison of four HRs, including WT, $P = .76$). Results were similar for DFS (interaction $P = .70$).

Second Primary Cancers

Second primary cancers were identified in 86 (6%) of 1,543 patients (not reported in ANITA). The analysis was therefore performed in 1,433 patients from the other trials. In OBS patients, the risk of developing a second primary was higher for patients with *KRAS*-mutated primary tumors (HR = 2.76; 95% CI, 1.34 to 5.70; $P = .005$; Table 3; Fig 3). In contrast, patients with *KRAS* mutations receiving ACT seemed to have a lower risk of second primaries (HR = 0.66; 95% CI, 0.25 to 1.75; $P = .40$; interaction $P = .02$).

DISCUSSION

More than 20 years have passed since the first reports that *KRAS* mutation was associated with poorer outcome in patients with lung adenocarcinoma.[2,4] Since then, most but not all studies have confirmed a prognostic effect.[6,17-20] However, there has been considerable heterogeneity among studies with respect to tumor type (some included only adenocarcinoma), stage, and treatment. Furthermore, many investigators reported only univariate comparisons without correcting for other prognostic variables.[6]

With more than 1,500 patients (300 with mutations), our study is the largest to report on the prognostic effect of *KRAS*. Furthermore, our study examines a homogeneous group of surgically staged patients with complete follow-up of more than 5 years duration. We could demonstrate only modest prognostic effects of *KRAS* mutation that were not significant in either univariate or multivariate adjusted analyses. Furthermore, when examining the adenocarcinoma subset (accounting for almost 70% of mutations), there was absolutely no prognostic effect.

In NSCLC, most *KRAS* mutations occur at codon 12, with many consisting of G>T transversions, the preferential mutation type induced by tobacco.[20] Our results agree, with more than 90% of mutations on codon 12, including mainly G>T transversions. In contrast, codon 13 is not known as a preferential site for tobacco carcinogen mutagenesis, and so *KRAS* codon 13 mutations might be expected to be more prevalent in cancers of nonsmokers. However, in our small nonsmoking subgroup, 10 of 11 mutations were on codon 12 and only one on codon 13. Riely et al[20] reported similar results with 10 of 12 mutations in nonsmokers being G12D mutations, with no codon13 mutations. They also reported that mutations in nonsmokers usually were transition G>A mutations rather than G>T or G>C transversions. G>A mutations were seen in only four of our nonsmoking population, with the rest being G>T or G> C.

Although codon 12 and 13 mutations differ in their structure, cellular, and molecular effects, they also have been shown to have quite different oncogenic effects, as reported by Prior et al.[3] In our study, the first to assess the potential for a differential prognostic effect in

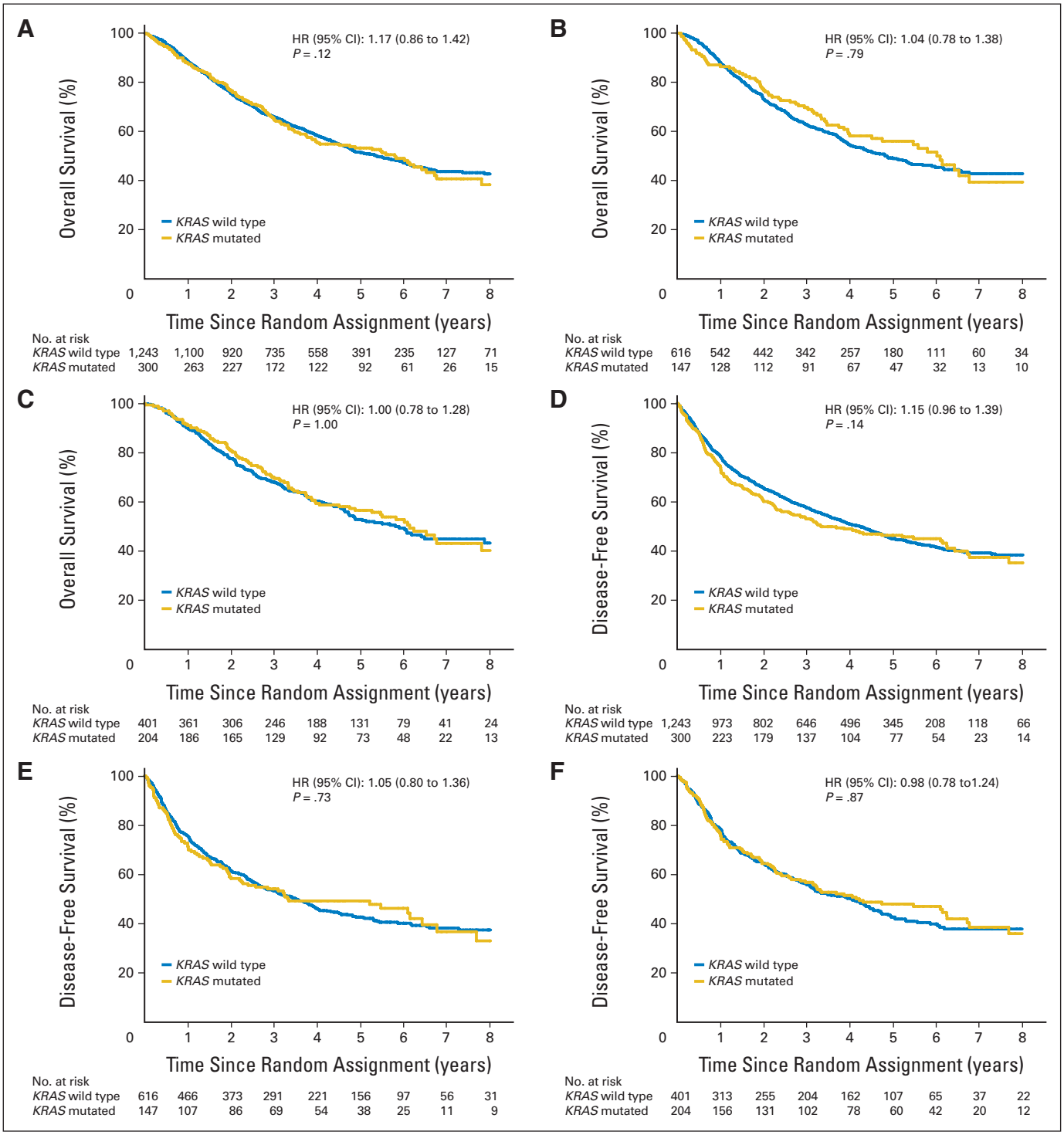

Fig 1. Overall survival (A, B, C) and disease-free survival (D, E, F) for patients with *KRAS* wild-type tumors compared with patients with *KRAS* mutated tumors. (A) All patients; (B) patients in observation arm; (C) all patients with adenocarcinoma; (D) all patients; (E) patients in observation arm; (F) all patients with adenocarcinoma. HR, hazard ratio.

NSCLC, we could identify no difference in prognosis based on codon. In colorectal cancer, Abubaker et al[21] reported significantly poorer survival ($P = .0009$) in patients with *KRAS* mutations, with codon 12 mutation associated with the worst 5-year survival (64.4%; $P = .0025$) compared with codon 13 (75.8%) or WT-*KRAS* (78.2%). Among 195 patients with advanced colorectal cancer with *KRAS* results treated on the OBS arm of National Cancer Institute of Canada Clinical Trials Group CO.17, which randomly assigned patients to single-agent cetuximab or supportive care alone, 82 (42%) had mutations (13 G13D, 69 other). In contrast to the results of Abubaker et al,[21] patients

Table 2. Summary of the Prognostic and Predictive Effects of Codon 12 and 13 *KRAS* Mutations and of Codon 12 Amino Acid Substitutions

Variable	Survival Measure	Effect	*KRAS*	Comparison	HR	95% CI	*P*
Codon-specific *KRAS* mutation	OS	Prognostic*					.96
				Codon12 mutation *v* WT	1.04	0.77 to 1.40	.79
				Codon13 mutation *v* WT	1.01	0.47 to 2.17	.98
		Predictive	WT	ACT *v* OBS	0.89	0.76 to 1.04	.15
			Codon12 mutation	ACT *v* OBS	0.95	0.67 to 1.35	.77
			Codon13 mutation	ACT *v* OBS	5.78	2.06 to 16.22	< .001
		Interaction test					.002
	DFS	Prognostic*					.93
				Codon12 mutation *v* WT	1.05	0.80 to 1.39	.71
				Codon13 mutation *v* WT	1.00	0.49 to 2.04	1.0
		Predictive	WT	ACT *v* OBS	0.86	0.73 to 1.00	.04
			Codon12 mutation	ACT *v* OBS	0.86	0.62 to 1.19	.36
			Codon13 mutation	ACT *v* OBS	3.88	1.44 to 10.46	.007
		Interaction test					.01
Specific amino acid† substitutions on *KRAS* codon 12	OS	Prognostic*					.99
				G12C or G12V *v* WT	1.04	0.74 to 1.46	.81
				G12D or G12S *v* WT	0.95	0.50 to 1.81	.87
				G12A or G12R *v* WT	1.08	0.49 to 2.37	.86
		Predictive	WT	ACT *v* OBS	0.89	0.76 to 1.05	.16
			G12C or G12V	ACT *v* OBS	0.94	0.63 to 1.41	.77
			G12D or G12S	ACT *v* OBS	1.39	0.55 to 3.54	.49
			G12A or G12R	ACT *v* OBS	0.66	0.21 to 2.10	.48
		Interaction test					.76
	DFS	Prognostic*					.98
				G12C or G12V *v* WT	1.04	0.76 to 1.42	.82
				G12D or G12S *v* WT	1.03	0.57 to 1.85	.94
				G12A or G12R *v* WT	1.15	0.55 to 2.39	.72
		Predictive	WT	ACT *v* OBS	0.86	0.74 to 1.00	.04
			G12C or G12V	ACT *v* OBS	0.89	0.61 to 1.30	.55
			G12D or G12S	ACT *v* OBS	1.11	1.46 to 2.64	.82
			G12A or G12R	ACT *v* OBS	0.48	0.15 to 1.47	.20
		Interaction test					.70

Abbreviations: ACT, adjuvant chemotherapy; DFS, disease-free survival; HR, hazard ratio; OBS, observation; OS, overall survival; WT, wild type.
*In OBS arm only.
†Amino acids are designated as follows: A, alanine; C, cystine; D, aspartic acid; G, glycine; R, arginine; S, serine; V, valine.

with G13D mutations had significantly shorter survival compared with those with other *KRAS* mutations.[22] However, on multivariable analysis, the differences were not significant. These colorectal cancer studies with conflicting results emphasize the potential pitfalls of drawing conclusions from small sample sets (only 28 codon 13 mutations from the two studies and 24 in our study). Furthermore, although *KRAS* mutations in lung and colorectal cancer are seen predominately on codons 12 and 13, the amino acid substitutions vary considerably between the two cancers, and so comparisons of prognostic effects across cancer types may not be appropriate.

This study is the first to examine the prognostic effect of different *KRAS* codon 12 amino acid substitutions in lung cancer. As expected, the majority (81%) were smoking-related G12C and G12V mutations. We could identify no prognostic effect for any mutation subtype. In colorectal cancer, the international RASCAL-II (Kirsten Ras in Colorectal Cancer Collaborative Group) study examined the effect of *KRAS* codon 12 and 13 mutations in 4,268 patients.[23] Only G12V mutations (in 8.6% of patients), showed a significant prognostic effect on failure-free survival (HR = 1.3; $P = .004$) and OS (HR = 2.9; $P = .008$).

With respect to the predictive effects of *KRAS*, patients with WT tumors demonstrated a trend to greater survival benefit from ACT as compared with those with mutations. However, even in this relatively well-powered study, the test for interaction was not significant. Furthermore, in the adenocarcinoma subset, modest but nonsignificant effects in favor of ACT were seen in patients with both mutated and WT tumors.

Our observations concerning the predictive effects of codon 12 and 13 mutations are intriguing. Despite a total lack of prognostic effect, we found that, even after accounting for other prognostic variables, patients with codon 13 mutations seemed to derive significant harm from ACT (HR = 5.78; $P < .001$; interaction $P = .002$). Only 24 patients had codon 13 mutations, and so our results, although statistically significant, must be interpreted with caution and require validation. Although in vitro and clinical studies suggest that *KRAS* predicts for poorer response to chemotherapy and radiation,[7-9] we could identify no other reports of predictive studies for chemotherapy based on *KRAS* codon in NSCLC. Tejpar et al[24] reported shorter survival in patients treated with chemotherapy alone for colorectal cancer if their tumors had G13D mutations compared with WT-*KRAS* or other MUT-*KRAS*. This study did not examine untreated patients, and so the predictive effect of mutation subtype for chemotherapy could not be confirmed; however, the poorer outcome associated with chemotherapy and G13D mutation may support our observations.

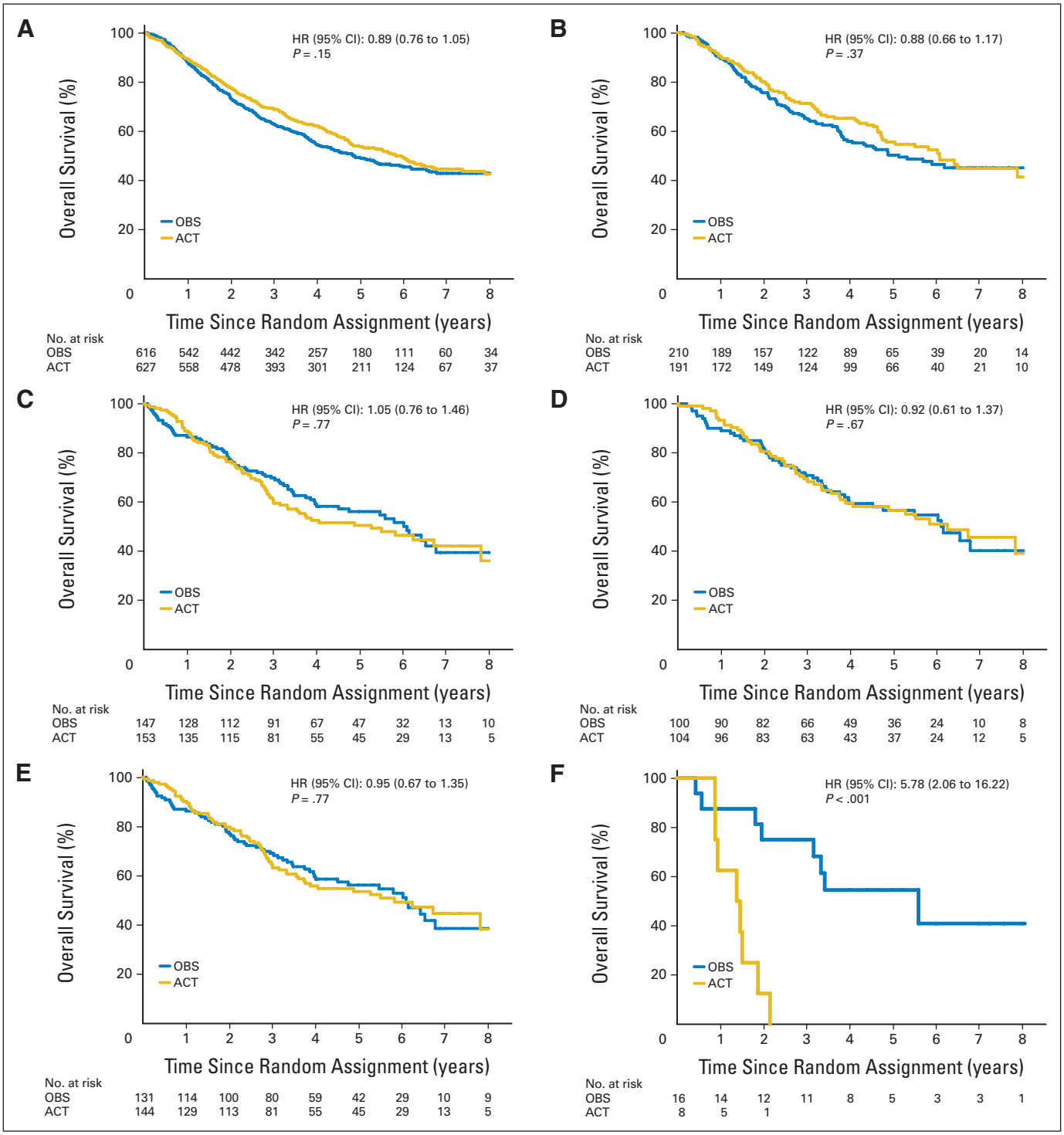

Fig 2. Overall survival benefit from adjuvant chemotherapy (ACT) compared with observation (OBS) in (A) patients with *KRAS* wild-type tumors and (C) patients with *KRAS*-mutated tumors, for patients with (B) adenocarcinoma and *KRAS* wild-type tumors and (D) adenocarcinoma with *KRAS*-mutated tumors, and for patients with (E) *KRAS* codon 12 mutations and (F) codon 13 mutations. (A) *KRAS* wild type; (B) adenocarcinoma *KRAS* wild type; (C) *KRAS* mutated; (D) adenocarcinoma *KRAS* mutated; (E) *KRAS* codon 12 mutated; (F) *KRAS* codon 13 mutated. HR, hazard ratio.

The predictive effect of *KRAS* for response to epidermal growth factor receptor (EGFR) inhibitors has been studied more extensively, with several reports suggesting poorer outcomes in response to tyrosine kinase inhibitors (TKIs) in NSCLC.[25-31] Most studies did not differentiate between codon 12 and 13 mutations. Recently, however, Metro et al[32] reported significantly shorter progression-free survival and OS in response to EGFR TKIs for patients with NSCLC whose tumors had *KRAS* codon 13 mutations compared with those with codon 12 mutations, a result that is similar to our observation with chemotherapy.

Table 3. Risk of Developing a Second Primary Malignancy in Patients With *KRAS* Mutated and *KRAS* Wild-Type Tumors in CALGB-9633, JBR.10, and IALT Trials

	ACT Arm		OBS Arm		ACT *v* OBS		
	No. of Second Primaries	No. of Patients	No. of Second Primaries	No. of Patients	HR	95% CI	*P*
KRAS wild type, n = 1,146	38	581	25	565	1.34	0.81 to 2.22	.26
KRAS mutated, n = 276	5	143	13	133	0.32	0.11 to 0.91	.03
Mutated *v* wild type							
HR	0.66		2.76		0.24*	0.08 to 0.76	
95% CI	0.25 to 1.75		1.34 to 5.70				
P	.40		.005				

NOTE. Eleven patients were excluded because of missing covariates. HR of interaction *KRAS**treatment *P* = .02.
Abbreviations: ACT, adjuvant chemotherapy; CALGB, Cancer and Leukemia Group B; HR, hazard ratio; IALT, International Adjuvant Lung Cancer Trial; OBS, observation.

In colorectal cancer,[22,24,33,34] EGFR monoclonal antibody therapy is restricted to patients with WT- *KRAS* tumors. Recently however, some but not all studies suggest that patients with G13D-mutated tumors may respond to EGFR monoclonal antibodies.[22,24,33] In colorectal cancer, the situation also may be complicated further by mutations in other genes, such as *BRAF*, which may have a significant effect on pathways downstream from RAS.[32,34] *BRAF* mutations are rare in NSCLC, and the interaction of *BRAF* or other potential markers and *KRAS* has not been studied. In contrast, *KRAS* was not shown to be a predictor of a differential outcome in patients with NSCLC treated with chemotherapy and cetuximab.[35,36]

Our results concerning codon 12 amino acid substitutions are inconclusive, with HRs varying from 0.66 to 1.39. The lack of statistical significance may, in part, be due to low numbers in some of the subsets. Clearly, a large international collaboration (akin to RASCAL-II) would be required to provide sufficient numbers for statistical power. This may not be possible, though, because prospective ACT studies in NSCLC will no longer have a no-treatment control arm, and many of the older trials did not collect samples for correlative studies.

There is evidence that structural differences between various codon 12 and 13 mutations may affect GTPase activity and binding of effector proteins.[37,38] Certainly, different amino acid substitutions have been shown in lung and other cancers to be associated with greater or lesser carcinogenic potential and downstream signaling effects,[3] and so the nonsignificant differences we observed potentially can be explained. Recently Ihle et al[36] reported that patients whose tumors had G12C or G12V mutations had worse survival than patients with other MUT-*KRAS* or WT-*KRAS*. Their in vitro NSCLC cell line studies showed that mutant *KRAS* G12A cell lines had activated phosphatidyl-inositol 3-kinase and mitogen-activated protein/extracellular signal-regulated kinase signaling. In contrast, cell lines with mutant *KRAS* G12C or G12V had activated Ral signaling and decreased growth factor-dependent Akt activation. These different downstream effects may result in a differential response to therapy.

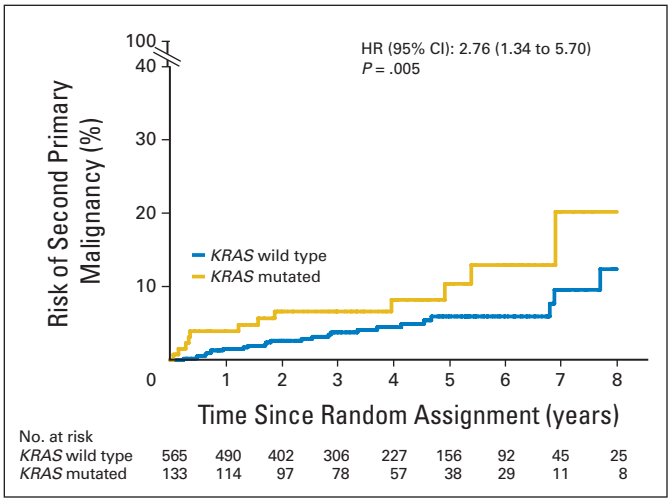

Fig 3. The risk of developing a second primary malignancy in observation patients with *KRAS*-mutated tumors. HR, hazard ratio.

Second primary cancers have been reported in approximately 15% of patients with NSCLC who undergo curative resection.[39,40] The most frequent malignancy is a second lung cancer, with two-fold higher risk in patients who continue to smoke. Other smoking-related cancers also are seen frequently, including head and neck cancers and malignancies of the esophagus, stomach, and bladder.[39] Because smoking is so strongly associated with *KRAS* mutation in NSCLC, and because of the field effect of this carcinogen in the upper aero-digestive tract, we postulated that *KRAS* mutation, an early event in lung carcinogenesis, might correlate with the development of second primaries. Indeed, in our observation cohort, the risk of developing a second primary cancer was almost three-fold greater in patients with *KRAS*-mutant tumors. To our knowledge, this is the first report of such an association, and our observation may have implications for follow-up in this high-risk group. Our finding of a reduced risk for second cancers in the ACT group was unexpected and unexplained. We theorize that ACT may have had a therapeutic effect on subclinical deposits of *KRAS* mutant cells in lung or other organs, and that these cells with *KRAS*-activated growth pathways may have been sensitive to the effects of and, potentially, were even eradicated by the ACT. Our observation requires validation in other NSCLC cohorts.

In summary, *KRAS* is not a significant prognostic marker in patients with resected NSCLC. Overall, it is not significantly predictive of a differential benefit from ACT, particularly in patients with adenocarcinoma, and at this time, it cannot be recommended as a tool to select patients for ACT. Although our results suggest a potentially detrimental effect from chemotherapy in patients with codon 13 mutations, validation studies will be critical. However, large international collaborations will be necessary to confirm these results and also to explore the potential for a differential treatment effect in patients with

various amino acid substitutions. Postoperative follow-up of patients with lung cancer is variable, with no clear guidelines, and only low level evidence concerning the use of computed tomography or positron emission tomography scans. *KRAS* mutation status may identify a cohort of patients who, in the absence of ACT, may be at particularly high risk of developing second primary cancers and who potentially might benefit from more intense follow-up.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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