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Prognostic and predictive effect of *KRAS* gene copy number and mutation status in early stage non-small cell lung cancer patients

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Background: In the current analysis, we characterize the prognostic significance of *KRAS* mutations with concomitant copy number aberrations (CNA) in early stage non-small cell lung cancer (NSCLC), and evaluate the ability to predict survival benefit from adjuvant chemotherapy.

Methods: Clinical and genomic data from the LACE (Lung Adjuvant Cisplatin Evaluation)-Bio consortium was utilized. CNAs were categorized as Gain (CN ≥ 2) or Neutral (Neut)/Loss; *KRAS* status was defined as wild type (WT) or mutant (MUT). The following groups were compared in all patients and the adenocarcinoma subgroup, and were correlated to survival endpoints using a Cox proportional hazards model: WT + Neut/Loss (reference), WT + Gain, MUT + Gain and MUT + Neut/Loss. A treatment-by-variable interaction was added to evaluate predictive effect.

Results: Of the 946 (399 adenocarcinoma) NSCLC patients, 41 [30] had MUT + Gain, 145 [99] MUT + Neut/Loss, 125 [16] WT + Gain, and 635 [254] WT + Neut/Loss. A non-significant trend towards worse lung cancer-specific survival (LCSS; HR =1.34; 95% CI, 0.83–2.17, P=0.232), DFS (HR =1.34; 95% CI, 0.86–2.09, P=0.202) and OS (HR =1.59; 95% CI, 0.99–2.54, P=0.055) was seen in *KRAS* MUT + Gain patients relative to *KRAS* WT + Neut/Loss patients. A negative prognostic effect of *KRAS* MUT + Neut/Loss was observed for LCSS (HR =1.32; 95% CI, 1.01–1.71, P=0.038) relative to *KRAS* WT + Neut/Loss on univariable analysis, but to a lesser extent after adjusting for covariates (HR =1.28; 95% CI, 0.97–1.68, P=0.078). *KRAS* MUT + Gain was associated with a greater beneficial effect of chemotherapy on DFS compared to *KRAS* WT + Neut/Loss patients (rHR =0.33; 95% CI, 0.11–0.99, P=0.048), with a non-significant trend also seen for LCSS (rHR =0.41; 95% CI, 0.13–1.33, P=0.138) and OS (rHR =0.40; 95% CI, 0.13–1.26, P=0.116) in the adenocarcinoma subgroup.

Conclusions: A small prognostic effect of *KRAS* mutation was identified for LCSS, and a trend towards

worse LCSS, DFS and OS was noted for *KRAS* MUT + Gain. A potential predictive effect of concomitant *KRAS* mutation and copy number gain was observed for DFS in adenocarcinoma patients. These results could be driven by the small number of patients and require validation.

Keywords: *KRAS* mutation; copy number aberration (CNA); non-small cell lung cancer (NSCLC)

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Introduction

Advances in next generation sequencing have led to the identification of molecular alterations in non-small cell lung cancer (NSCLC), including mutations, fusions, increased tumor mutation burden, and copy number aberrations (CNA), among others. However, understanding the complex genetic landscape in NSCLC remains challenging, as only a small proportion of these alterations likely have clinical significance.

Previous LACE-Bio studies evaluated the prognostic and predictive role of *KRAS* mutations, and *KRAS/TP53* co-mutations in early stage NSCLC (1,2). No clear prognostic or predictive role was detected, although a potentially negative predictive effect of *TP53/KRAS* co-mutation (2), and a possible deleterious effect of adjuvant chemotherapy in patients with a *KRAS* codon-13 mutation require further investigation (1). The *KRAS* oncogene is mutated frequently in NSCLC, particularly adenocarcinoma (3-5); however, the clinical impact of *KRAS* CNAs is unclear. Evaluation of the genome of primary lung adenocarcinomas by Weir *et al.* revealed areas of copy number amplification at 12p12.1, a region known to harbor the *KRAS* oncogene (6). Likewise, characterization of gene CNAs in resected NSCLC by the LACE-Bio consortium showed *KRAS* copy number gains; however, no significant association between *KRAS* CNA and survival was observed (7).

Few studies have shown that *KRAS* copy number gain might be associated with the presence of concomitant *KRAS* mutations (6,8,9). For instance, Modrek *et al.* showed that *KRAS*-mutated tumors appeared to have higher copy number compared to wild type (WT) tumors, with distinct patterns of gain across chromosome 12 (9). Moreover, even moderate *KRAS* copy number gains have been associated with increases in *KRAS* mRNA expression (8,9).

The prognostic and predictive role of concomitant *KRAS* mutations and CNA has not been well elucidated. Sasaki *et al.* showed that NSCLC patients with *KRAS* mutated

tumors and increased copy number had worse prognosis compared to those with *KRAS* WT tumors with no increase in copy number; however, their study was limited by a small number of patients (10). In the current study, we completed a pooled analysis of LACE-Bio data to characterize the prognostic effect of *KRAS* mutation status and concomitant CN gain in early stage NSCLC, and to determine the ability to predict survival benefit from adjuvant chemotherapy. We hypothesized that concomitant *KRAS* mutations and CN gain would be prognostic of worse survival compared to *KRAS* mutations alone and that patients with mutation and CN gain might have the greatest potential to benefit from adjuvant chemotherapy.

We present the following article in accordance with the REMARK reporting checklist (available at <http://dx.doi.org/10.21037/tlcr-20-927>).

Methods

Study design/patient characteristics

The LACE (Lung Adjuvant Cisplatin Evaluation)-Bio group conducts meta-analyses of data from 3 adjuvant randomized clinical trials [JBR.10, IALT (International Adjuvant Lung Cancer Trial), ANITA (Adjuvant Navelbine International Trialist Association)] comparing cisplatin-based chemotherapy versus observation, and CALGB-9633, which compared carboplatin-based chemotherapy versus observation (11-16). The LACE-Bio collaboration includes bio-banked tissue samples prospectively collected from JBR.10 and CALGB-9633, as well as retrospective samples from the IALT group (7,17). In this study, clinical and genomic data from the LACE-Bio consortium were used to evaluate the prognostic and predictive effect of concomitant *KRAS* mutation and gene CNAs in early stage NSCLC patients. All procedures performed in this study were in accordance with the Declaration of Helsinki (as revised in 2013).

KRAS gene copy number and mutation analyses

DNA extracted from formalin-fixed and paraffin-embedded (FFPE) sections was used for *KRAS* mutation and genome-wide copy number analyses using methods previously described (1,7). Briefly, *KRAS* mutation analyses were completed using allelic specific oligonucleotide hybridization with sequencing confirmation in JBR.10, and PCR amplification and direct sequencing of exon 2 in IALT and CALGB-9633 (1). Whole-genome copy number analyses were completed using the OncoScan CNV Plus Assay (ThermoFisher, Carlsbad, California, USA) (7). CNAs were categorized into two groups: Gain (2-fold higher CN) or Neutral (Neut)/Loss; and *KRAS* mutation status was defined as WT or mutant (MUT). The following groups were compared in all patients and the adenocarcinoma subgroup: WT + Neut/Loss (reference), WT + Gain, MUT + Gain, MUT + Neut/Loss.

Outcomes

The primary endpoint was lung cancer-specific survival (LCSS), defined as the time from randomization to death from lung cancer, censoring at the date of death from other causes and without lung cancer recurrence. Secondary endpoints included disease free survival (DFS), defined as the time from randomization to the time of first event (recurrence or death from any cause); and overall survival (OS), defined as the time from randomization to the date of death from any cause. Patients for whom no events had been observed were censored at the date of their last follow-up.

Statistical analysis

The Kaplan-Meier method and log-rank test were used to compare survival curves between the four groups mentioned above. *KRAS* mutation status, and concomitant *KRAS* CNA and mutational status were separately correlated to survival endpoints using a Cox proportional hazards model, which was stratified by trial in all models, adjusted for treatment in the unadjusted model, and adjusted for treatment, age, sex, tumor stage, nodal stage, histology, WHO performance status and surgery type in the fully adjusted model. For *KRAS* mutation status, the WT group was the reference category, and the HR was reported for MUT versus WT group. For the concomitant *KRAS* CNA and mutation status, WT + Neut/Loss was the reference category, and the HR for a given category (MUT + Gain, MUT + Neut/

Loss or WT + Gain) was interpreted as the relative hazard for that category versus the WT + Neut/Loss category. To evaluate the predictive role of concomitant *KRAS* CNA and mutation status, a treatment-by-variable interaction was added to the Cox models stratified by study. For each endpoint, we compared the treatment effect across four concomitant *KRAS* CNA and mutation status groups by using the ratio of HRs (rHR): the ratio of the HR for adjuvant chemotherapy versus observation in a given category 'c' (MUT + Gain, MUT + Neut/Loss or WT + Gain) to the HR for adjuvant chemotherapy versus observation in the WT + Neut/Loss category (rHR = HR(Chemo vs. Obs. in c)/HR(Chemo vs. Obs. in WT + Neut/Loss)). A rHR <1.0 indicates that the treatment effect size was greater for category 'c' than for WT + Neut/Loss patients. Given that *KRAS* mutations occur primarily in the adenocarcinoma group, analyses were also performed separately for this subtype. Statistical significance was set at P<0.05. All statistical analyses were performed using the 'Survival' and the 'survminer' packages in R software version 3.6.2.

Results

Patient characteristics

A total of 946 patients had complete clinical data including both *KRAS* mutation and CNA results (Figure 1): 41 MUT + Gain, 145 MUT + Neut/Loss, 125 WT + Gain, and 635 WT + Neut/Loss (reference group). There were 399 patients in the adenocarcinoma subgroup, with 30 MUT + Gain, 99 MUT + Neut/Loss, 16 WT + Gain, and 254 WT + Neut/Loss. The most common *KRAS* mutation subtypes were *KRAS* G12C (n=89; 47.8%), G12V (n=44; 23.6%) and G12D (n=18; 9.7%) (Table S1). Baseline patient characteristics for the total population are summarized in Table 1. Almost half of the patients were older than 60 years of age, with the majority (72.52%) being male. Data regarding smoking history were collected in the JBR.10 and CALGB-9633 trials, and summarized in Supplementary Table 2. Of these 440 patients, 397 (90.2%) were current/former smokers. Moreover, all *KRAS* MUT + Gain patients (n=25) were current/formers smokers (Tables S2,S3). Staging included 46.62% with stage I, 36.89% stage II and 16.49% stage III. There was a similar proportion of patients with adenocarcinoma (42.18%) and squamous cell carcinoma (44.08%). Adjuvant chemotherapy was given to half of patients. There were no significant differences in

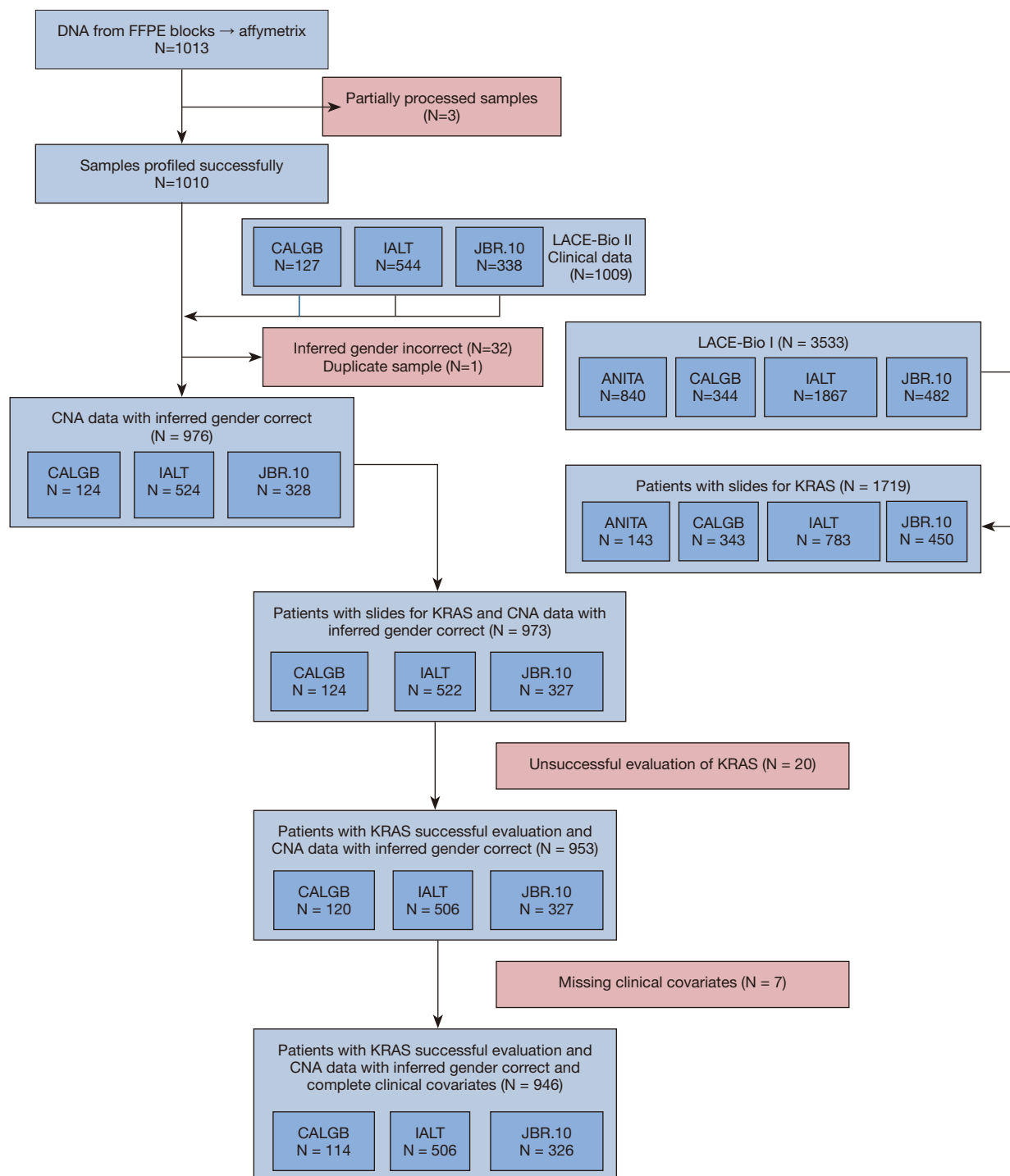


Figure 1 Consort diagram of all patients with complete clinical data, as well as *KRAS* mutational status and CNA data. CNA, copy number aberrations.

Table 1 Patient and tumor characteristics according to *KRAS* mutation and CNA

Variable	WT + Neut/ Loss(reference) (N=635)		WT + Gain (N=125)		MUT+ Gain (N=41)		MUT + Neut/Loss (N=145)		Total (N=946)	
	N	%	N	%	N	%	N	%	N	%
Age										
≤50	96	15.12	22	17.60	13	31.71	31	21.38	162	17.13
51–60	225	35.43	37	29.60	11	26.83	55	37.93	328	34.67
>60	314	49.45	66	52.80	17	41.46	59	40.69	456	48.20
Sex										
Male	467	73.54	105	84.00	26	63.41	88	60.69	686	72.52
Female	168	26.46	20	16.00	15	36.59	57	39.31	260	27.48
T-stage*										
T1	79	12.44	11	8.80	4	9.75	20	13.79	114	12.05
T2	468	73.70	99	79.20	35	85.37	119	82.07	721	76.22
T3/4	88	13.86	15	12.00	2	4.88	6	4.14	111	11.73
N-stage*										
N0	325	51.18	57	45.60	23	56.10	82	56.55	487	51.48
N1	221	34.80	50	40.00	15	36.58	49	33.79	335	35.41
N2	89	14.02	18	14.40	3	7.32	14	9.66	124	13.11
Stage*										
I	291	45.83	51	40.80	22	53.66	77	53.10	441	46.62
II	230	36.22	50	40.00	15	36.58	54	37.24	349	36.89
III	114	17.95	24	19.20	4	9.76	14	9.66	156	16.49
WHO Performance status										
0	328	51.65	55	44.00	21	51.22	80	55.17	484	51.16
≥1	307	48.35	70	56.00	20	48.78	65	44.83	462	48.84
Histology										
Squamous	302	47.56	92	73.60	4	9.76	19	13.10	417	44.08
Adenocarcinoma	254	40.00	16	12.80	30	73.17	99	68.28	399	42.18
Other NSCLC	79	12.44	17	13.60	7	17.07	27	18.62	130	13.74
Treatment										
Observation	328	51.65	60	48.00	14	34.15	70	48.28	472	49.89
Adjuvant chemotherapy	307	48.35	65	52.00	27	65.85	75	51.72	474	50.11

*, 6th Edition TNM Staging classification. NSCLC, non-small cell lung cancer; CNA, copy number aberration; WT, wild type; MUT, mutant; WHO, World Health Organization.

Table 2 Prognostic effect of *KRAS* mutation and copy number status on patient outcome in the total population (n=946)

KRAS mutation and CNA	LCSS	DFS	OS
	HR [95% CI], P value	HR [95% CI], P value	HR [95% CI], P value
Unadjusted model			
KRAS _{MUT} , n=186	1.31 [1.04–1.66], 0.023	1.18 [0.94–1.47], 0.145	1.11 [0.87–1.40], 0.406
KRAS _{WT} , n=760 (reference)	1.00	1.00	1.00
KRAS _{MUT} + Gain, n=41	1.42 [0.89–2.27], 0.140	1.34 [0.86–2.06], 0.192	1.39 [0.88–2.19], 0.163
KRAS _{MUT} + Neut/Loss, n=145	1.32 [1.01–1.71], 0.038	1.13 [0.88–1.45], 0.326	1.03 [0.79–1.34], 0.841
KRAS _{WT} + Gain, n=125	1.11 [0.84–1.48], 0.463	0.96 [0.73–1.26], 0.770	0.92 [0.69–1.23], 0.567
KRAS _{WT} + Neut/Loss, n=635 (reference)	1.00	1.00	1.00
Fully adjusted model			
KRAS _{MUT} , n=186	1.27 [0.98–1.63], 0.065	1.18 [0.93–1.49], 0.180	1.18 [0.91–1.52], 0.212
KRAS _{WT} , n=760 (reference)	1.00	1.00	1.00
KRAS _{MUT} + Gain, n=41	1.34 [0.83–2.17], 0.232	1.34 [0.86–2.09], 0.202	1.59 [0.99–2.54], 0.055
KRAS _{MUT} + Neut/Loss, n=145	1.28 [0.97–1.68], 0.078	1.14 [0.88–1.47], 0.333	1.08 [0.82–1.42], 0.597
KRAS _{WT} + Gain, n=125	1.19 [0.88–1.60], 0.256	0.99 [0.75–1.31], 0.946	0.88 [0.65–1.19], 0.412
KRAS _{WT} + Neut/Loss, n=635 (reference)	1.00	1.00	1.00

HR, hazard ratio; CI, confidence interval; CNA, copy number aberration; LCSS, lung cancer specific survival; DFS, disease free survival; OS, overall survival; WT, Wild-type; KRAS_{MUT}, KRAS mutant; Gain, copy number gain; Neut/Loss, copy number neutral/loss.

baseline patient or tumor characteristics among the four subgroups.

Prognostic effect

Kaplan-Meier analyses showed no significant differences in terms of LCSS, DFS or OS among the four groups based on *KRAS* CNAs and mutational status in the total population (Figure 2), or in the adenocarcinoma subgroup (data not shown). Univariable analyses showed that *KRAS* MUT and *KRAS* MUT + Neut/Loss were associated with poor LCSS outcome: the hazard ratio for patients with a *KRAS* MUT was 1.31 (95% CI, 1.04–1.66, P=0.023) relative to the *KRAS* WT group, and the hazard ratio for patients with *KRAS* MUT + Neut/Loss was 1.32 (95% CI, 1.01–1.71, P=0.038) relative to the *KRAS* WT + Neut/Loss category (Table 2). However, this was no longer significant after adjusting for clinical covariates (*KRAS* MUT HR =1.27; 95% CI, 0.98–1.63, P=0.065; and *KRAS* MUT + Neut/Loss HR =1.28; 95% CI, 0.97–1.68, P=0.078, respectively) (Table 2). In both univariable and multivariable analyses including all patients, the worst outcomes consistently were observed in the *KRAS* MUT + Gain subgroup for LCSS (HR =1.34;

95% CI, 0.83–2.17, P=0.23), DFS (HR =1.34; 95% CI, 0.86–2.09, P=0.20,) and OS (HR =1.59; 95% CI, 0.99–2.54, P=0.055), compared to WT + Neut/Loss (Table 2). When limiting analyses to *KRAS* MUT + Gain compared directly to *KRAS* WT + Neut/Loss, there was a significant negative prognostic effect of *KRAS* MUT + Gain for OS (HR =1.66; 95% CI, 1.03–2.67, P=0.037; Table 3).

In the adenocarcinoma subgroup, there was no significant prognostic effect of *KRAS* mutation and CNA on LCSS, DFS or OS (Table S4). However, there was a non-significant trend towards worse OS (HR =1.51; 95% CI, 0.84–2.72, P=0.169) when *KRAS* MUT + Gain was compared directly to *KRAS* WT + Neut/Loss in adenocarcinoma patients (results not shown).

Predictive effect

Results of the univariable and multivariable analysis did not show any significant predictive effect of *KRAS* CNA and mutation status in the overall population (Figure 3A,B,C and Table 4) (interaction P=0.829, 0.670 and 0.579 for LCSS, DFS and OS, respectively, in multivariable analysis). In the adenocarcinoma subgroup, univariable analysis showed a

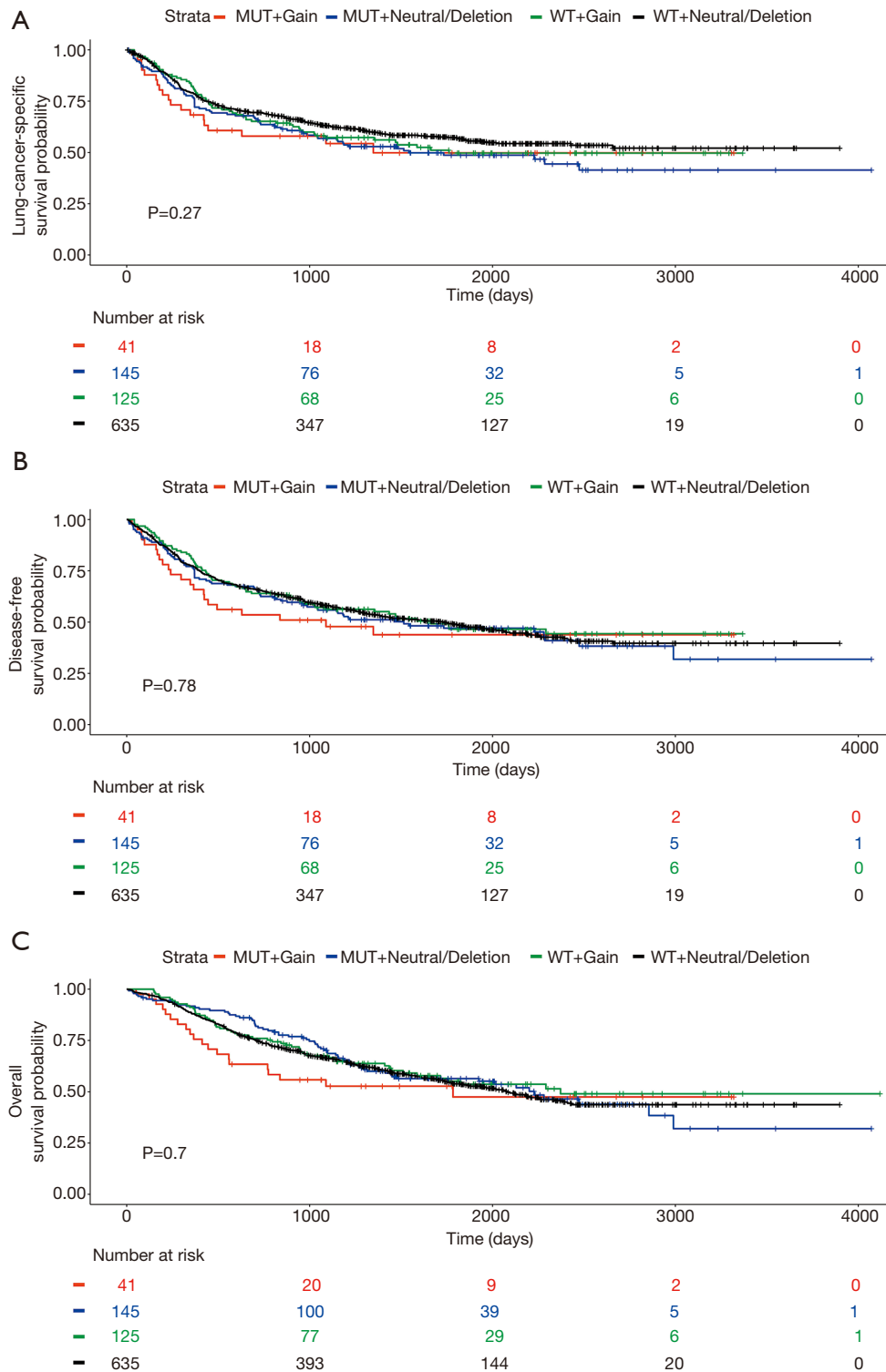
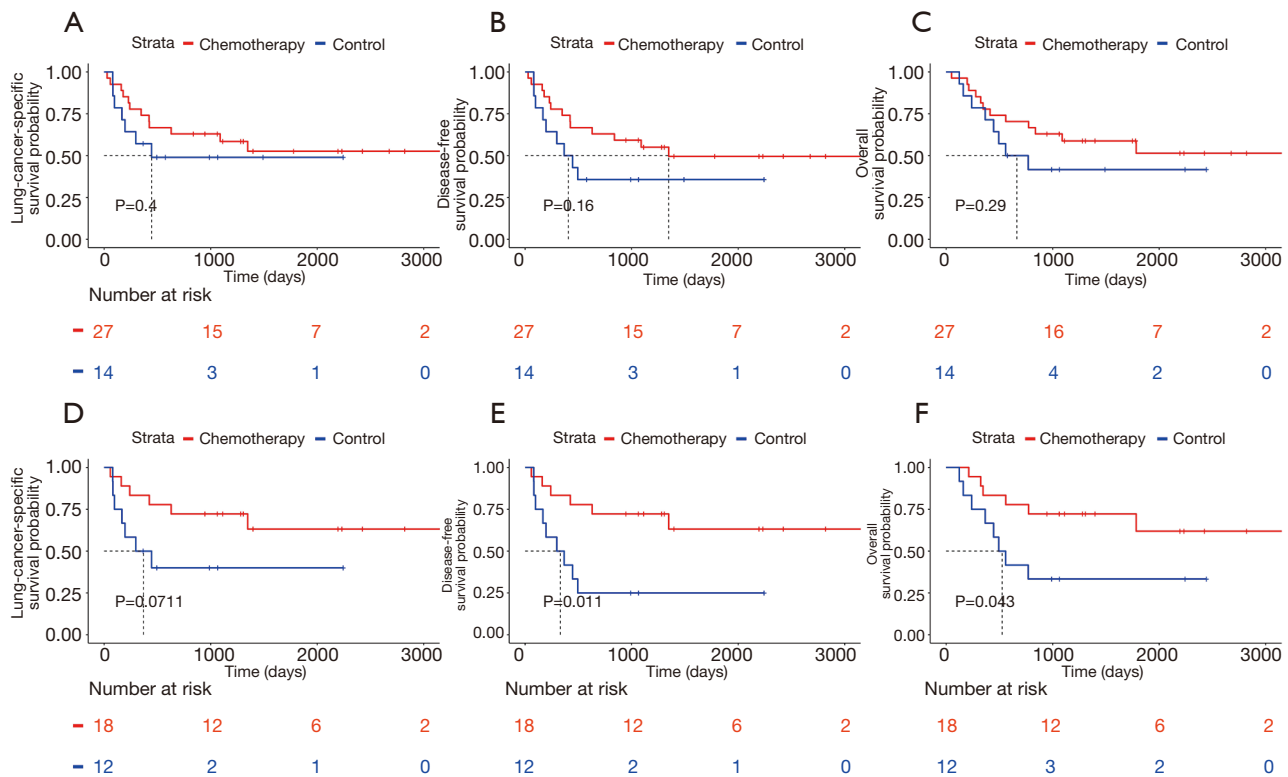


Figure 2 Unadjusted Kaplan-Meier survival curves comparing the prognostic effect of MUT + Gain, MUT + Neut/Loss, WT + Gain, and WT + Neut/Loss for (A) Lung-cancer-specific survival, (B) Disease-free survival and (C) Overall survival in the total population. MUT, mutant; WT, wild-type; Gain, copy number gain; Neut/Loss, copy number neutral/loss.

Table 3 Prognostic effect of *KRAS* mutation and copy number status on patient outcome, limited to the *KRAS* MUT + Gain and WT + Neut/Loss population (fully adjusted models)

KRAS mutation and CNA	LCSS	DFS	OS
	HR [95% CI], P value	HR [95% CI], P value	HR [95% CI], P value
KRAS _{MUT} + Gain, n=41	1.33 [0.81–2.16], 0.255	1.37 [0.87–2.14], 0.171	1.66 [1.03–2.67], 0.037
KRAS _{WT} + Neut/Loss, n=635 (reference)	1.00	1.00	1.00



significant predictive effect of *KRAS* CNA and mutational status for OS (interaction $P=0.038$), although this was no longer significant after adjusting for clinical covariates (interaction $P=0.076$; *Table 5*). However, there appeared to be a beneficial effect of chemotherapy on DFS and OS based on Kaplan-Meier analysis in *KRAS* MUT + Gain adenocarcinoma patients when compared to observation (*Figure 3D,E,F*).

In addition, univariable Cox regression models in the adenocarcinoma population showed that the beneficial

effect of chemotherapy on DFS was significantly more pronounced for the *KRAS* MUT + Gain group compared to WT + Neut/Loss patients (rHR = 0.32; 95% CI, 0.11–0.94, $P=0.039$), with non-significant trends for better LCSS (rHR = 0.44; 95% CI, 0.14–1.39, $P=0.161$) and OS (rHR = 0.33; 95% CI, 0.11–1.03, $P=0.057$). Similarly, multivariable analysis showed a significantly higher treatment effect size in *KRAS* MUT + Gain patients for DFS (rHR = 0.33; 95% CI, 0.11–0.99, $P=0.048$) in adenocarcinoma patients (*Table 5*), with a non-significant trend also seen for LCSS

Table 4 Predictive effect of *KRAS* mutation and copy number status on patient outcome in the total population (n=946)

KRAS mutation and CNA	LCSS		DFS		OS	
	rHR [95% CI], P value	P value*	rHR [95% CI], P value	P value*	rHR [95% CI], P value	P value*
Unadjusted Model						
KRAS _{MUT} , n=186	1.15 [0.72–1.83], 0.559	0.559	1.05 [0.68–1.63], 0.828	0.828	1.21 [0.75–1.93], 0.435	0.435
KRAS _{WT} , n=760 (reference)	1.00		1.00		1.00	
KRAS _{MUT} + Gain, n=41	0.80 [0.30–2.11], 0.653	0.716	0.62 [0.26–1.49], 0.281	0.558	0.61 [0.24–1.53], 0.291	0.360
KRAS _{MUT} + Neut/Loss, n=145	1.28 [0.76–2.16], 0.350		1.20 [0.73–1.97], 0.722		1.41 [0.83–2.40], 0.203	
KRAS _{WT} + Gain, n=125	1.71 [0.66–2.07], 0.588		1.15 [0.67–1.98], 0.390		1.18 [0.66–2.12], 0.569	
KRAS _{WT} + Neut/Loss, n=635 (reference)	1.00		1.00		1.00	
Fully adjusted Model						
KRAS _{MUT} , n=186	1.06 [0.66–1.70], 0.795	0.795	0.98 [0.63–1.53], 0.934	0.934	1.16 [0.72–1.86], 0.552	0.552
KRAS _{WT} , n=760 (reference)	1.00		1.00		1.00	
KRAS _{MUT} + Gain, n=41	0.92 [0.35–2.43], 0.861	0.829	0.70 [0.29–1.71], 0.439	0.670	0.73 [0.29–1.84], 0.501	0.579
KRAS _{MUT} + Neut/Loss, n=145	1.15 [0.68–1.95], 0.600		1.09 [0.66–1.80], 0.722		1.30 [0.76–2.22], 0.341	
KRAS _{WT} + Gain, n=125	1.27 [0.71–2.26], 0.420		1.27 [0.74–2.19], 0.390		1.26 [0.70–2.26], 0.445	
KRAS _{WT} + Neut/Loss, n=635 (reference)	1.00		1.00		1.00	

*, interaction between variable and treatment. rHR, ratio of Hazard Ratio (rHR = HR_(Chemo vs. Obs. in c)/HR_(Chemo vs. Obs. in reference)); CNA, copy number aberration; CI, Confidence interval; LCSS, Lung cancer specific survival; DFS, disease free survival; OS, overall survival; WT, Wild-type; KRAS_{MUT}, KRAS mutant; Gain, copy number gain; Neut/Loss, copy number neutral/loss.

(rHR =0.41; 95% CI, 0.13–1.33, P=0.138) and OS (rHR =0.40; 95% CI, 0.13–1.26, P=0.116). Similar trends were also noted in the total population (Table 4), with a suggestion of more benefit to improve DFS (rHR =0.70; 95% CI, 0.29–1.71, P=0.439) and OS (rHR =0.73; 95% CI, 0.29–1.84, P=0.501) with adjuvant chemotherapy in the KRAS MUT + Gain group relative to WT + Neut/Loss patients; this higher benefit was likely driven by the adenocarcinoma subset.

Discussion

The *KRAS* oncogene frequently is mutated in NSCLC, particularly adenocarcinoma, and the biologic heterogeneity of *KRAS*-mutated lung cancer has been well characterized. The majority of *KRAS* mutations occur at

codon 12 (18), and different *KRAS* point mutations are associated with diversity in biological behavior, response to therapy, and clinical outcome (5). However, the role of gene CNAs in *KRAS*-mutated tumors is not as well characterized. A correlation between *KRAS* mutations and copy number gain has been reported in NSCLC (6,8,9); however, these studies have been limited by small numbers of patients. A study by Modrek *et al.* found concomitant *KRAS* mutations and copy number gain in 11 out of 166 (6.6%) NSCLC patients (9). Likewise, Sasaki *et al.* evaluated 172 surgical tissue samples from NSCLC patients and identified 8 patients (4.6%) with concomitant *KRAS* mutation and gene copy number gain (10). In our pooled analysis of 946 patients, we were able to identify 41 patients (4.3%) in the total population who had a *KRAS* mutation with concomitant copy number gain, which is consistent with

Table 5 Predictive effect of *KRAS* mutation and copy number status on patient outcome in the adenocarcinoma subgroup (n=399)

KRAS mutation and CNA	LCSS		DFS		OS	
	rHR [95% CI], P value	P value*	rHR [95% CI], P value	P value*	rHR [95% CI], P value	P value*
Unadjusted model						
KRAS _{MUT} , n=129	1.07 [0.58–1.99], 0.827	0.827	0.92 [0.51–1.63], 0.768	0.768	1.08 [0.58–2.03], 0.807	0.807
KRAS _{WT} , n=270 (reference)	1.00		1.00		1.00	
KRAS _{MUT} + Gain, n=30	0.44 [0.14–1.39], 0.161	0.214	0.32 [0.11–0.95], 0.039	0.065	0.33 [0.11–1.03], 0.057	0.038
KRAS _{MUT} + Neut/Loss, n=99	1.43 [0.72–2.82], 0.306		1.29 [0.68–2.44], 0.439		1.60 [0.79–3.24], 0.191	
KRAS _{WT} + Gain, n=16	1.92 [0.52–7.04], 0.325		2.16 [0.62–7.45], 0.225		2.60 [0.68–9.90], 0.161	
KRAS _{WT} + Neut/Loss, n=254 (reference)	1.00		1.00		1.00	
Fully adjusted model						
KRAS _{MUT} , n=129	1.02 [0.54–1.92], 0.947	0.947	0.91 [0.50–1.64], 0.745	0.745	1.23 [0.64–2.34], 0.535	0.535
KRAS _{WT} , n=270 (reference)	1.00		1.00		1.00	
KRAS _{MUT} + Gain, n=30	0.41 [0.13–1.33], 0.138	0.287	0.33 [0.11–0.99], 0.048	0.135	0.40 [0.13–1.26], 0.116	0.076
KRAS _{MUT} + Neut/Loss, n=99	1.38 [0.68–2.79], 0.367		1.27 [0.65–2.45], 0.486		1.76 [0.85–3.62], 0.126	
KRAS _{WT} + Gain, n=16	1.46 [0.38–5.51], 0.580		1.60 [0.45–5.69], 0.471		2.12 [0.53–8.37], 0.286	
KRAS _{WT} + Neut/Loss, n=254 (reference)	1.00		1.00		1.00	

*, interaction between variable and treatment. rHR, ratio of Hazard Ratio ($rHR = HR_{(Chemo\ vs.\ Obs.\ in\ c)}/HR_{(Chemo\ vs.\ Obs.\ in\ reference)}$); CNA, copy number aberration; CI, Confidence interval; LCSS, Lung cancer specific survival; DFS, disease free survival; OS, overall survival; WT, Wild-type; KRAS_{MUT}, KRAS mutant; Gain, copy number gain; Neut/Loss, copy number neutral/loss.

previous reports. Overall, the proportion of patients who harbor concomitant *KRAS* mutation and copy number gain is quite small (approximately 4–6%), suggesting that this is an uncommon occurrence.

To our knowledge, this study is the largest to report on the prognostic and predictive roles of concomitant *KRAS* mutation and copy number gain. We observed a consistent but non-significant trend towards worse LCSS (HR =1.34; 95% CI, 0.83–2.17, P=0.232), DFS (HR =1.34; 95% CI, 0.86–2.09, P=0.202) and OS (HR =1.59; 95% CI, 0.99–2.54, P=0.055) in *KRAS* MUT + Gain patients relative to the WT + Neut/Loss group in the total population. The adenocarcinoma subgroup accounted for 73.2% (n=30) of the MUT + Gain patients, and although there was no significant prognostic effect noted, there appeared to be a similar trend towards worse OS (HR =1.40; 95% CI, 0.78–2.51, P=0.255). Sasaki *et al.* previously reported worse prognosis in NSCLC patients with both *KRAS* mutations and increased gene copy number when compared to *KRAS* WT patients with no increase in copy number; however, the study was limited by

a small number of patients (n=8) (10). When we completed a similar comparison limited to only the two subgroups of *KRAS* MUT + Gain compared to WT + Neut/Loss, we also observed a significantly worse OS (HR =1.66; 95% CI, 1.02–2.67, P=0.037) in *KRAS* MUT + Gain patients.

Singh *et al.* showed a significant correlation between *KRAS* gene amplification and *KRAS*-dependency. Moreover, increased gene copy number in *KRAS*-mutated tumors has been associated with elevated *KRAS* mRNA and protein expression (9,19). Perhaps there is a selection for copy number gain in tumors that already harbor a *KRAS* mutation, thereby conferring dependency on the *KRAS* oncogene for growth and survival. Studies suggest that *KRAS* oncogene addiction might play a role in early tumor initiation, with preclinical data showing development of early onset lung cancer in mice harboring mutations in *KRAS* (20). However, although *KRAS* mutations alone have been shown to promote lung tumor formation, additional genomic alterations are likely required for ongoing tumor progression (21). It is plausible that gene amplification

is acquired in *KRAS*-dependent tumors to aid in tumor progression. Newnham *et al.* compared resected and recurrent NSCLC tumors and showed differential gene expression suggesting that concomitant *KRAS* mutations with CN gain might be implicated in tumor recurrence clinically (3). Moreover, preclinical models show that copy number gain in *KRAS*-mutated tumors appears to select for enhanced glycolytic metabolism (a compensatory mechanism to promote survival in tumors with mitochondrial defects), and might also be involved in increasing metastatic potential in lung cancer cells (21). Interestingly, we previously showed an increased risk (HR =2.76; 95% CI, 1.34–5.70, P=0.005) of developing a second primary cancer in patients with *KRAS*-mutated tumors (1). The impact of concomitant *KRAS* mutation and gene copy number gain on cell survival, tumor recurrence, or the development of second primary cancers is unclear, and should be further investigated to determine if this might explain the observed trend towards worse survival in *KRAS* MUT + Gain patients.

Variable responses to chemotherapy have been reported in *KRAS*-mutated tumors, and may depend on the subtype of the *KRAS* mutation (22,23). Garassino *et al.* reported a decreased response to cisplatin but increased response to paclitaxel and pemetrexed in *KRAS* G12C mutated tumors, whereas, *KRAS* G12D mutations appeared to be resistant to paclitaxel, and G12V mutations were highly sensitive to cisplatin but slightly more resistant to pemetrexed (23). Shepherd *et al.* previously reported variability in the effect of adjuvant chemotherapy on survival based on *KRAS* mutation subtypes, with worse DFS (HR =3.88; 95% CI, 1.44–10.46, P=0.007, interaction P=0.01) and OS (HR =5.78; 95% CI, 2.06–16.2, P=0.001, interaction P=0.002) observed in patients with *KRAS* codon 13 mutations who received adjuvant chemotherapy (1). In addition, a potential deleterious effect of adjuvant chemotherapy was seen in patients with *KRAS/TP53* co-mutations when compared to *WT/WT* tumors (HR =2.49; 95% CI, 1.10–5.64, P=0.03) (2). However, due to small patient numbers, these data require further validation. In contrast, we found that *KRAS* MUT + Gain was associated with a higher beneficial effect of chemotherapy on DFS (rHR =0.33; 95% CI, 0.11–0.99, P=0.048) compared to the WT + Neut/Loss group in adenocarcinoma patients, with a non-significant trend also seen for LCSS (rHR =0.41; 95% CI, 0.13–1.33, P=0.14) and OS (rHR =0.40; 95% CI, 0.13–1.26, P=0.12) on multivariable analysis. A similar higher beneficial effect of chemotherapy on DFS and OS was also noted in *KRAS* MUT + Gain patients compared

to WT + Neut/Loss patients in the total population, but this benefit may be driven by the adenocarcinoma subset. It is possible that *KRAS* MUT + Gain patients have an increased dependency on *KRAS* activation, which might lead to increased susceptibility to chemotherapy, leading to a decrease in risk for tumor recurrence. Whether this hypothesis might explain the higher beneficial effect of chemotherapy on DFS observed in our study is still to be determined, and additional studies are required to elucidate the mechanism by which *KRAS* MUT-Gain patients might be more responsive to chemotherapy.

There are limitations in this study. The *KRAS* mutation analyses were pooled from separate trials; therefore, there were differences in technique and laboratories used for testing. Secondly, we were unable to evaluate the predictive or prognostic role of different *KRAS* mutation subtypes in patients with *KRAS* mutations and CNA due to the small numbers in each group. This further highlights the need for pooled analyses to ensure adequate sample numbers for robust analyses for less common genetic alterations. Finally, we did not have complete smoking history data from all trials included in the current study; therefore, survival analyses adjusting for smoking status could not be completed due to the small sample size.

Conclusions

In summary, the current LACE-Bio analysis showed that concomitant *KRAS* mutations and copy number gain are rare, occurring in approximately 4% of NSCLC patients. Although not significant, there was a trend towards worse LCSS, DFS and OS in *KRAS* MUT + Gain patients relative to WT + Neut/Loss patients. In addition, concomitant *KRAS* mutation and CN gain appeared to predict for better DFS in adenocarcinoma patients treated with adjuvant chemotherapy, with a trend towards better LCSS and OS as well. However, these results could be driven by the small number of patients and require further validation.

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Footnote

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Ethical Statement: The authors are 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. All procedures performed in this study were in accordance with the Declaration of Helsinki (as revised in 2013).

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Table S1 *KRAS* mutation subtype according to *KRAS* copy number aberration (CNA) status

KRAS Mutation subtype	KRAS CNA status				Total (N=186)	
	Gain (N=41)		Neutral/Loss (N=145)		N	%
	N	%	N	%		
G12A	3	7.32	7	4.83	10	5.38
G12C	23	56.09	66	45.52	89	47.85
G12D	3	7.32	15	10.34	18	9.68
G12D, G12V	0	0.00	1	0.69	1	0.54
G12R	0	0.00	3	2.07	3	1.62
G12S	2	4.88	3	2.07	5	2.68
G12V	6	14.63	38	26.20	44	23.65
G13C	2	4.88	4	2.76	6	3.22
G13D	2	4.88	6	4.14	8	4.30
G13R	0	0.00	1	0.69	1	0.54
Missing	0	0.00	1	0.69	1	0.54

CNA, copy number aberration; Gain, copy number gain; Neut/Loss, copy number neutral/loss.

Table S2 Smoking status according to combined *KRAS* mutation and CNA status (n=440)

Smoking status	WT + Neut/Loss (reference) (N=275)		WT + Gain (N=58)		MUT + Gain (N=25)		MUT + Neut/Loss (N=82)		Total (N=440)	
	N	%	N	%	N	%	N	%	N	%
	Smokers (Current/former)	244	88.73	54	93.10	25	100.0	74	90.24	397
Non-smokers	31	11.27	4	6.90	0	0.00	8	9.76	43	9.77

Table S3 Smoking status according to *KRAS* mutation and CNA status (n=440)

Smoking status	KRAS Mutation status				KRAS CNA status				Total (N=440)	
	Wild Type (N=397)		Mutant (N=107)		Gain (N=83)		Neutral/Loss (N=357)		N	%
	N	%	N	%	N	%	N	%		
Smokers (Current/former)	298	89.49	99	92.52	79	95.18	318	89.08	397	90.23
Non-smokers	99	10.51	8	7.48	4	4.82	39	10.92	43	9.77

Table S4 Prognostic effect of *KRAS* mutation and copy number status on patient outcome in adenocarcinoma subgroup (n=399) (fully adjusted models)

KRAS mutation and CNA	LCSS	DFS	OS
	HR [95% CI], P value	HR [95% CI], P value	HR [95% CI], P value
KRAS _{MUT} , n=129	1.04 [0.76-1.43], 0.800	1.00 [0.74-1.35], 0.993	1.03 [0.75-1.42], 0.851
KRAS _{WT} , n=270 (reference)	1.00	1.00	1.00
KRAS _{MUT} + Gain, n=30	1.00 [0.55-1.83], 1.000	1.06 [0.60-1.84], 0.851	1.40 [0.78-2.51], 0.255
KRAS _{MUT} + Neut/Loss, n=99	1.09 [0.77-1.54], 0.610	1.02 [0.74-1.41], 0.892	0.98 [0.69-1.40], 0.933
KRAS _{WT} + Gain, n=16	1.59 [0.81-3.13], 0.176	1.56 [0.82-2.96], 0.177	1.41 [0.72-2.77], 0.318
KRAS _{WT} + Neut/Loss, n=254 (reference)	1.00	1.00	1.00

LCSS, Lung cancer specific survival; DFS, disease free survival; OS, overall survival; HR, Hazard Ratio; CI, Confidence interval; WT, Wild-type; KRAS_{MUT}, KRAS mutant; Gain, copy number gain; Neut/Loss, copy number neutral/loss.