Lung Cancer: Targets and Therapy

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Impact of smoking on frequency and spectrum of *K-RAS* and *EGFR* mutations in treatment naive Indonesian lung cancer patients

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Background: Indonesia has the highest cigarette consumption in the world. We explored the clinical impact of smoking on the prevalence of *EGFR* and *K-RAS* mutations and survival in this prospective study.

Methods: 143 treatment naive lung cancer patients were recruited from Persahabatan Hospital, a national tertiary hospital. DNA from cytological specimens had been extracted and genotyped for both *EGFR* and *K-RAS* mutations using a combination of PCR high resolution melting, restriction fragment length polymorphism (RFLP) and direct DNA sequencing.

Results: *EGFR* mutation frequency in never smokers (NS) and ever smokers (ES) were 75% and 56% ($p = 0.0401$), respectively. In this cohort, the overall *K-RAS* mutation rate was 7%. Neither gender nor smoking history were associated with *K-RAS* mutation significantly. However, *K-RAS* transversion mutations were more common in male ES than transition mutations. Smoking history did not affect *EGFR* and *K-RAS* mutation frequencies in women. Concurrent *EGFR*/*K-RAS* mutation rate was 2.8% (4 of 143 patients). Four out of 91 *EGFR* mutation positive patients (4.4%) had simultaneous *K-RAS* mutation.

Conclusions: In region where cigarette consumption is prevalent, smoking history affected frequencies of *EGFR* and *K-RAS* mutations, mainly in males.

Keywords: lung cancer, Indonesia, *K-RAS* mutation, smoking

Introduction

Lung cancer is the most common and deadly cancer, contributing to 11.6% of total cancer and 18.4% of total cancer-related mortality. WHO estimates the incident and mortality rate of lung cancer in Indonesia is 12.4 and 10.9 per 100,000, respectively. In males, lung cancer shows higher incidence and mortality (19.4 and 17.4 per 100,000, respectively) than females (6.0 and 5.1 per 100,000, respectively).[1]

Epidermal growth factor receptor (*EGFR*) mutation is an important predictive biomarker in lung cancer targeted therapy. Common mutations such as deletion (del) of exon 19 and L858 substitution mutations in exon 21 predict tumor sensitivity to first-generation tyrosine kinase inhibitors (TKI) such as gefitinib and erlotinib.[2,3] There are also rare/uncommon mutations such as G719X and L861Q that confer variable therapeutic responses to TKI treatment.[4–6] On the other hand, there are some oncogenic mutations, such as *EGFR* T790M, insertions of exon 20 of *the EGFR* gene, and *K-RAS*, that contribute to primary and/or acquired resistance to TKI.[7,8] Moreover, baseline *K-RAS*

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mutation either alone or together with *EGFR* mutation may have negative[9] or neutral[10,11] outcomes to chemotherapy.

Gender, ethnicity, histology and smoking history are known factors affecting prevalent of *EGFR* and *K-RAS* mutations.[12,13] *EGFR* mutations generally occur in nonsmoker, female, East Asian, and adenocarcinoma patients. On the other hand, *K-RAS* mutations were observed mainly in western or European patients and may be associated with smoking history.[14] Moreover, *K-RAS* mutation is typically showing transversion purine to pyrimidine substitution subtypes a signature of smoking history.[15] *EGFR* and *K-RAS* mutations are thought to be mutually exclusive[16] although there are reports showing some cases of simultaneous mutations both in European and Asian patients albeit with various rates (0.4–1.1%).[17–21]

We have recently reported the frequency of *EGFR* mutations (44%) in a large retrospective study[22] of newly diagnosed lung cancer patients using cytological specimens. Smoking is highly prevalent among male Indonesians[23] and has contributed to a major proportion of lung cancer incidence.[24] However, the impacts of smoking to the prevalence of *EGFR* and *K-RAS* mutations in Indonesian lung cancer patients have not been analyzed.

Recent meta-analyses evaluating different histopathology, gender and ethnicities have described the likelihood of *ALK-EML4* translocations, and *EGFR* and *KRAS* mutations among lung cancer patients with or without smoking history. Never smoker (NS) patients tend to have higher rates of *EGFR* mutations and *ALK-EML4* translocations than ever smoker (ES) patients. On the other hand, NS patients are less likely to bear KRAS mutations than ES patients. Other factors, such as ethnicities, gender, and histopathology are also associated with key driver mutations in lung cancer.[13]

We aimed to evaluate the impact of smoking on the incidence and spectrum of *EGFR* and *K-RAS* gene mutations in lung cancer patients referred to Jakarta tertiary hospital.

Methods

Patients

A total of 143 newly diagnosed non-consecutive lung cancer patients with known *EGFR* mutation status were enrolled to participate in prospective disease monitoring study. DNA was also genotyped for baseline *K-RAS* mutations. Patients' age ranged from 26 to 84 years, with median of 55 years and average of 53.7 years. Ethical Committee of Faculty of Medicine Universitas Indonesia, Jakarta (396/UN2.F1/ETIK/2016) approved this study. The study was performed in accordance with the 1964 Helsinki declaration and its later Amendments. All patients had signed informed consent.

DNA isolation

Cytological specimens were obtained as malignant pleural effusion as well as from fine needle aspirations, bronchoscopies, and transthoracic needle biopsies. Pathologists had marked areas with enriched tumor cells in the cytological specimens. Marked areas were then subjected to DNA isolation using QIAmp DNA Micro (Qiagen NV, Venlo, the Netherlands) according to the kit protocol.

EGFR mutation detection

The method used for mutation detection isPCR high resolution melting(PCR-HRM),restriction fragment length polymorphism(RFLP), and sequencing as described.[22] Briefly, PCR-HRM was used to screen for mutations in exon 18, 19, and 21. Suspected specimens showing mutation specific melting profiles were subjected to genotyping using direct sequencing (exon 18), fragment sizing (exon 19) and RFLP (exon 21 L858R and L861Q). Mutation detection in exon 20 was performed using direct sequencing.

PCR HRM of EGFR of exons 18, 19, and 21

PCR-HRM was performed in 25 µL reaction volume, containing 200 nM of each forward in reverse primer, 200 µM dNTP, 1 × buffer, 2.5 mM $MgCl_2$, 1.25 U of HotStarTaq (Qiagen) polymerase, 1 µL of template, 5 µM Syto-9 (Invitrogen) and PCR grade water. PCR-HRM analysis was performed on Rotor gene 6000^{TM} in the following conditions: 95°C (15 min), followed by 10 cycles of 95°C (10 s), 65°C (10 s) with touchdown for (1 cycle/1°C), 72°C (30 s), 40 cycles of 95°C (10 s), 55°C (10 s), 72°C (30 s), one cycle of 97°C (1 s). The HRM condition was: melt from 80°C to 90°C, rising 0.1°C per second.

PCR and RFLP of EGFR exon 21

RFLP was performed for exon 21, to detect point mutation in codon 858 and 861. The products of PCR-HRM were digested using MSC I and PVU II enzyme, to detect mutation on codon 858 and 861. To detect the mutation in codon 858, the reaction performed in 25 µL of reaction, consists of: 10 µL of PCR product, 5 U of MSC (NEB), 1 × buffer, and ddH_2O. Another reaction was performed to detect the mutation in codon 861 using 10 U of PVU II (NEB), 1 × buffer, 10 µL PCR product and then ddH_2O

added until the reaction volume reached 25 µL. RFLP was performed on 37°C for 3–16 h.

PCR and sequencing of exons 18 and 20

For exon 18, the product of PCR-HRM was purified using Exo Sap IT. Direct sequencing was performed using Applied Biosystems 3,500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

PCR of exon 20 was performed in 25 µL reaction volume, containing: 1 × buffer, 1.5 mM $MgCl_2$, 500 nM of forward primer and 500 nM reverse primer, 200 µM of dNTPs, 1.25 U of HotStar Taq polymerase (Qiagen), 1 µL of DNA template and PCR grade water. The PCR was performed using Veriti Thermal Cycler (Thermo Fisher Scientific) in the following conditions: 95°C (15 min), followed by 40 cycles of 95°C (30 s), 57°C (30 s), 72°C (30 s), one cycle of 72°C (7 min).

K-RAS mutation detection

PCR and direct sequencing of *K-RAS* mutations were performed as described.[25] Briefly, RAS SplitSCAN (KalgenDNA, Jakarta, Indonesia) HRM primers were used to screen a hotspot mutation of exon 2 of the *K-RAS* gene. Samples were ran using Rotor-Gene 6000TM (Corbett Life Science, Mortlake, Australia) or Rotor-gene Q (Qiagen). Melting curves were generated and scanned for the presence of split peaks predicting the presence of mutations. Samples showing putative mutated split peak patterns were then genotyped using direct DNA sequencing.

PCR HRM of KRAS exon 2

PCR HRM was performed in 20 µL reaction volume containing 200 nM of each forward in reverse primer, 200 µM dNTP, 1 × buffer, 2.5 mM $MgCl_2$, 1 U of HotStarTaq (Qiagen) polymerase, 1 µL of template, 5 µM Syto-9 and PCR grade water. HRM analysis was performed on the Rotor-Gene 6000^{TM} in the following conditions: 95°C (15 min), followed by 50 cycles of 95°C (10 s), 68°C (10 s), 72°C (20 s), one cycle of 95°C (1 s), one cycle of 95°C (1 s), melt from 72°C to 90°C, rising 0.1°C per second.

PCR and sequencing of KRAS exon 2

Conditions for first PCR reaction were 95°C (4 mins), followed by 25 cycles of 95°C (30 s), 50°C (30 s), 72°C (30 s), and 1 cycle of 72°C (7 min). PCR reaction performed in 25 µL reaction volume, containing: 1 × buffer, 1.5 mM $MgCl_2$, 500 nM of forward primer and 500 nM reverse primer, 200 µM of dNTPs, 1.25 U of Faststart Taq (Roche) polymerase, 1 µL of DNA template and PCR grade water. First PCR product diluted to 1:10, and 1 µL of the diluted product used as template for nested PCR. Nested PCR was performed in the following conditions: 95°C (4 min), followed by 35 cycles of 95°C (30 s), 55°C (30 s), 72°C (30 s), and 1 cycle of 72°C (7 min). PCR reaction performed in 25 µL reaction volume, containing 1 × buffer, 1.5 mM $MgCl_2$, 300 nM of forward primer and 300 nM reverse primer, 200 µM of dNTPs, 1.25 U of Faststart Taq (Roche) polymerase, DNA template and PCR grade water.

Statistical analysis

Categorical variables were summarized by frequency and percentage. Pearson's chi-squared test (or Fisher's exact test if cell frequencies less than 5 were expected) was used to test for associations between patient characteristics and mutation type and smoking status. A two-sided *p*-value of less than 0.05 was taken as statistically significant.

Results

Prevalence of *EGFR* and *K-RAS* mutations

A total of 143 newly diagnosed non-consecutive patients with adenocarcinoma histology had been genotyped for *EGFR* and *K-RAS* mutations. Most patients were males (71%), and the majority (64%) was smokers (Table 1). Common *EGFR* mutations (exon 19 indels and L858R) were the major (67%) subtypes, followed by uncommon mutations (19%, G719X, T790M, and L861Q), and mix or compound (14%) mutation subtypes. *K-RAS* mutation frequency was 7%, and *K-RAS* transversion mutation (60%) was slightly more common than transition mutation (40%, $p > 0.05$). Moreover, *K-RAS* mutation rate was slightly higher in females, 3 of 39 (7.6%, $p = 1.0$), than males, 6 of 101 (5.9%). The rate of concomitant or simultaneous *EGFR* and *K-RAS* mutations in the same individuals was 2.8% (or 4 out of 143 patients).

Impact of smoking history on *EGFR* mutation rate

When stratified according to smoking history, *EGFR* mutation was higher in NS (75%, $p = 0.0465$) than ES (57%) (Table 2). Furthermore, young NS had also more frequent *EGFR* mutation rate than ES patients (81% vs 53%, $p = 0.0343$). The trend toward higher *EGFR* mutation rate was also observed in NS males than ES males (82% vs 57%, $p = 0.06$), but did not reach statistical significance (see Figure 1). Similar EGFR mutation

Table 1 Demography

Characteristics	N (143)	Percent
Gender		
Male	102	71%
Female	41	29%
Age		
Median	55	
Average	53.7	
Range	26–84	
Smoking History		
Ever smokers (ES)	89	62%
Never smokers (NS)	51	36%
Unknown	3	
All adenocarcinoma		
EGFR Genotypes		
Wild type (normal)	52	36%
Mutations	91	64%
Common Mutations (Exon 19 Dels, L858R)	61	67%
Uncommon mutations (G719X, L861Q, T790M)	17	19%
Mix mutations	13	14%
***K-RAS* Genotypes**		
Wild type	133	93%
Mutations	10	7.0%
Transversion	6	60%
G12C	4	
G12A	1	
G12R	1	
Transition	4	40%
G12D	3	
G12S	1	
Mix *EGFR* and *K-RAS* concomitant mutations	4	2.8%
K-RAS mutations in 91 EGFR Mutant patients		4.4%
EGFR mutations in 10 K-RAS mutant patients		40%

frequency was also observed in females (71% in NS females vs 60% in ES females, $p = 0.63$ see Figure 1). Mix or multiple *EGFR* mutations containing both common and uncommon *EGFR* mutants in the same individuals seemed to be more prevalent in smoker (20%) vs nonsmoker (5%, $p = 0.0632$) patients (Table 2).

Impact of smoking history on *K-RAS* mutation rate

When stratified according to smoking history, *K-RAS* mutation was slightly higher in ES (7%, $p = 1.0$) than NS (6%) (Table 2). ES male patients had a tendency of a higher rate of *K-RAS* mutation (7.5%) than NS male patients (0%, $p = 0.56$) (Table 3, Figure 1). A signature of smoke-associated mutation, *K-RAS* mutation transversion type (mainly *K-RAS* G12C) was also consistently more frequent in ES than NS patients. On the other hand, *K-RAS* mutation rate and patterns (transversion or transition) among females were independent of smoking history (Table 3).

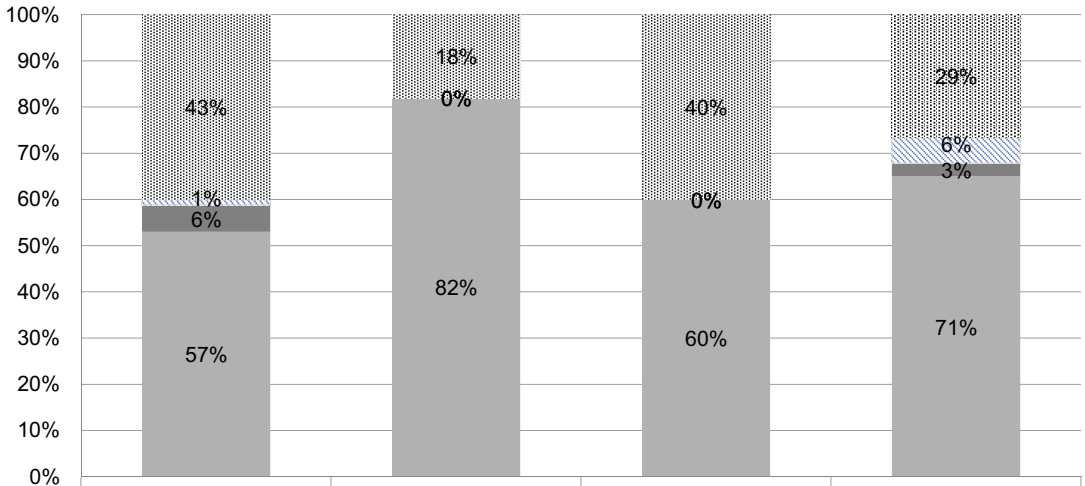
Double mutation of *EGFR* and *K-RAS* genes

Of the 143 patients, 4 (4.4%) had simultaneous *EGFR* and *K-RAS* gene mutations (Table 4, Figure 1). *K-RAS* mutation transversion types (G12C) were found with *EGFR* L858R mutation. Within our cohort, *EGFR* exon 19 deletion was never found together with *K-RAS* mutations. Although not statistically significant, frequency of

Table 2 Impact of smoking history to prevalence and spectrum of *EGFR* mutation

Characteristics			*EGFR* genotypes				*p-value	*EGFR* mutation types						*p-value
			Wild types		Mutants			Common		Uncommon		Mix		
Smoking history														
	Ever smokers	89	38	43%	51	57%	0.0465	33	65%	8	16%	10	20%	0.0632
	Never smokers	51	13	25%	38	75%		27	71%	9	24%	2	5%	
	Unknown	3												
Age 55 years old and younger														
	Ever smokers	51	24	47%	27	53%	0.0343	15	56%	7	26%	5	19%	0.139
	Never smokers	21	4	19%	17	81%		11	65%	6	35%	0	0%	
Age older than 55 years old														
	Ever smokers	38	14	37%	24	63%	0.6127	18	75%	1	4%	5	21%	0.421
	Never smokers	30	9	30%	21	70%		16	76%	3	14%	2	10%	
Male														
	Ever smokers	84	36	43%	48	57%	0.06	30	63%	8	17%	10	21%	0.429
	Never smokers	17	3	18%	14	82%		10	71%	3	21%	1	7%	
Female														
	Ever smokers	5	2	40%	3	60%	0.63	3	100%	0	0%	0	0%	1
	Never smokers	34	10	29%	24	71%		17	71%	6	25%	1	4%	

Notes: * *p*-value <0.05 were statistically significant.



	Male, ES (N=80)	Male, NS (N=15)	Female, ES (N=5)	Female, NS (N=33)
Wild types	43%	18%	40%	29%
EGFR + K-RAS mutants	1%	0%	0%	6%
K-RAS mutants	6%	0%	0%	3%
EGFR mutants	57%	82%	60%	71%

Figure 1 *EGFR* and *K-RAS* mutation rates in males and females with or without smoking history.
Abbreviations: ES, ever smokers; NS, nonsmokers.

double *EGFR* and *K-RAS* mutations was higher in young (3 of 52 or 6%) than old patients (1 of 91 or 1%, $p > 0.05$). Patients having uncommon or mix *EGFR* mutations also tend to harbor additional *K-RAS* gene mutation (3 of 30 or 10%) than those having *EGFR* common mutations only (1 of 61 or 1.6%, $p > 0.05$). Lastly, NS patients tend to have a higher frequency of concomitant *EGFR* and *K-RAS* mutations (2 of 51, 3.9%) than ES patients (1 of 89 or 1.1%, $p > 0.05$).

Discussion

A meta-analysis by Dearden et al showed that *EGFR* mutations occurred more frequently in Asian than Western patients (47.9% versus 19.2%), while *K-RAS* mutations are more frequent in Western than Asian patients (KRAS, 26.1% versus 11.2%).[26] The overall rates of *EGFR* and *K-RAS* mutations in this study confirmed the Asian profile. Patients with smoking history had 56% *EGFR* mutation rate, similar to other Asian studies,[27,28] confirming the importance of *EGFR* testing regardless of smoking history. In the current study, we also found a consistent trend of higher *EGFR* mutation in NS patients compared to ES patients (see Figure 1). However, the difference in *EGFR* mutation rates in NS vs ES female patients was not statistically significant. It is probably related to low numbers of female ES patients enrolled in this study (only 5 ES females out of a total of 39 patients). Nevertheless, a recent study enrolling a large number of ES female patients from seven Asian regions indeed demonstrated more frequent *EGFR* mutations in NS females (62%, N=358) than ES females (51%).[29] Another study by Hsiao et al also showed higher rates of EGFR mutations in NS females compared to ES females (60% vs 29%, N=426).[30]

Within our cohort, there was a trend toward more frequent uncommon *EGFR* mutations among ES patients, although statistically not significant, which is consistent with other Asian studies.[4,31] On the other hand, a significant association of *EGFR* uncommon mutations with smoking has been described amongst European lung cancer patients.[32]

Overall *K-RAS* mutation rate in this study (7%) was also similar to other Asian studies (around 8–10%), which is generally lower than Western patients (25–30%).[26,33] Our results also showed a consistent tendency of a high frequency of *K-RAS* mutations among smokers. Smoking history association with *K-RAS* mutations has been shown in many studies,[33] with G12C or transversion mutation type being most common in smokers. We also observed that *K-RAS* mutation G12C (transversion) types were frequent among male ES. However, our study and others have also shown that the association between smoking history and *K-RAS* mutation may not necessarily be strict.[34] For instance, we

Table 3 Impact of smoking history to prevalence and spectrum of *K-RAS* mutation

			K-RAS WT		*K-RAS* mutation		*p-value	Transversion		Transitions	
		Total	n	Rate	n	Rate		n	Rate	n	Rate
		140	131	94%	9	6%					
Smoking history											
	Ever smokers	89	83	93%	6	7%	1.00	4	67%	2	33%
	Never smokers	51	48	94%	3	6%		1	33%	2	67%
	Unknown	3									
55 years and under											
	Ever smokers	51	47	92%	4	8%	1.00	2	50%	2	50%
	Never smokers	21	19	90%	2	10%		0	0%	2	100%
Older than 55 years											
	Ever smokers	38	36	95%	2	5%	1.00	2	100%	0	0%
	Never smokers	30	29	97%	1	3%		1	100%	0	0%
Male		101	95	94%	6	6%					
	Ever smokers	84	78	93%	6	7%	0.56	4	67%	2	33%
	No smokers	17	17	100%	0	0%		0	-	0	-
Female		39	36	93%	3	7%					
	Ever smokers	5	5	100%	0	0%	1.00	0	-	0	-
	Never smokers	34	31	91%	3	9%		1	33%	2	67%

Table 4 Clinical characteristic of *EGFR* and *K-RAS* double mutations

Gender	Age (years)	Smoking history	*EGFR* in cytology	*K-RAS* mutation	*K-RAS* mutation types
Female	84	Never smoker	L858R	G12C	Transversion
Male	46	Unknown	T790M, G719S, L858R	G12C	Transversion
Male	42	Ever smoker	G719S	G12S	Transition
Female	41	Never smoker	L861Q, T790M	G12D	Transition

did find some *K-RAS* mutations among NS patients having concurrent *EGFR* and *K-RAS* mutations (Figure 1).

The significance of *K-RAS* mutations in women with smoking history has been described. The study suggests that women are more susceptible to smoking-related lung cancer because they have higher rates of *K-RAS* G12C mutations occurring in a younger age of diagnosis and with fewer pack-years of smoking than men with the same mutations.[35] However, in our cohort, we did not find any *K-RAS* mutations in ES females. Instead, *K-RAS* G12D types mutations were found in NS females. Therefore, future studies may characterize driver mutations in Indonesian ES females.

Recent review[36] shows a higher tendency of *K-RAS* mutation frequencies in smokers (25%) and male patients (22%) than nonsmokers (6%) and female patients (20%). In our study, the mutation frequency among ES patients and male gender in this study was modest (7%). Asian studies in Japan, Korea, and China show that the rates of *K-RAS* mutations among male patients are 14%, 23%, and 33%, respectively.[17,37,38] This relatively low frequency of KRAS mutations in male and ES patients was unexpected, given the high prevalence of cigarettes consumptions among Indonesian men.[39] Between 85% and 90% of all cigarettes smoked in Indonesia are kreteks, a type of clove cigarette with high tar content.[23,40]

In addition, we speculate that the extensive use of woods as solid fuels for cooking that are prevalent among up to 50% of the Indonesian population[41] may affect the rate of *K-RAS* mutation as described in a Mexican study.[42] Lung cancer patients exposed to wood smokes have a low frequency of *K-RAS* mutations regardless of cigarettes smoking history. Therefore, future studies may explore lifestyle and environmental related *K-RAS* gene and possibly other oncogenic driver mutations in Indonesia.

EGFR and *K-RAS* mutations are thought to be mutually exclusive.[33] However, in our cohort, *EGFR* and *K-RAS* co-mutation frequency was 2.8% overall or 4.6% (of *EGFR* mutation positive cases). In Asia the rate is 0.25% (6 *K-RAS* mutation cases of 2,387 *EGFR* mutation positive) or 1.5% (6 *EGFR* mutations of 398 *K-RAS* mutation positive),[43] 1.5% (29 of 1,854 *EGFR* mutation cases) or 6.7% (29 of 429 cases).[19]

In Taipei, *K-RAS* mutation rate was 8.3%, while concomitant *K-RAS* and *EGFR* mutation rate was 1.4% (1 out of 69 *EGFR* mutation positive).[18] In a Chinese study, 1 *K-RAS* (0.3%) mutation has been found in 320 *EGFR* mutation positive patients.[44] In Western patients overlapping *EGFR* and *K-RAS* mutations represented 6.8% (3 of 44) and 3.2% (3 of 92) of the population with single *EGFR* or *K-RAS* mutations, respectively.[45] Among Asians, our rate of concurrent *K-RAS* and *EGFR* gene mutations seemed to be the highest. Moreover, other studies[19,46] show that Del19 EGFR mutations occur together with KRAS mutations, which are not observed in our cohort. In a recent large study, concurrent mutation is associated with non-smoking patients and may affect progression-free survival, but not overall survival.[47] These accumulated data may reconsider a given dogma of *EGFR* and *K-RAS* mutually exclusive mutations in lung cancer.[47] Furthermore, conflicting prognostic and/or predictive utility of baseline *K-RAS* mutations to chemotherapy may question the clinical utility of *K-RAS* genotyping in lung cancer.[48] Interestingly, a recent commentary on promising results of selumetinib, a potent inhibitor of mitogen-activated protein kinase 1 (*MEK1*) and *MEK2* has pointed out an association of *K-RAS* G12C mutations and good response rate.[49] Therefore, future studies may clarify the roles of *K-RAS* mutation in the routine management of lung cancer patients.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;49(suppl8):509. doi:10.3322/caac.21492
2. Gazdar AF. Activating and resistance mutations of EGFR in non-small-cell lung cancer: role in clinical response to EGFR tyrosine kinase inhibitors. *Oncogene*. 2009;28 Suppl 1(S1):S24–S31. doi:10.1038/onc.2009.198
3. Cabanero M, Sangha R, Sheffield BS, et al. Management of EGFR-mutated non-small-cell lung cancer: practical implications from a clinical and pathology perspective. *Curr Oncol*. 2017;24(2):111–131. doi:10.3747/co.24.3524
4. Wu JY, Yu CJ, Chang YC, Yang CH, Shih JY, Yang PC. Effectiveness of tyrosine kinase inhibitors on "uncommon" epidermal growth factor receptor mutations of unknown clinical significance in non-small cell lung cancer. *Clin Cancer Res*. 2011;17(11):3812–3821. doi:10.1158/1078-0432.CCR-10-3408
5. Chen K, Yu X, Wang H, et al. Uncommon mutation types of epidermal growth factor receptor and response to EGFR tyrosine kinase inhibitors in Chinese non-small cell lung cancer patients. *Cancer Chemother Pharmacol*. 2017;80(6):1179–1187. doi:10.1007/s00280-017-3464-9
6. Pilotto S, Rossi A, Vavalà T, et al. Outcomes of first-generation EGFR-TKIs against non-small-cell lung cancer harboring uncommon EGFR mutations: a post hoc analysis of the BE-POSITIVE study. *Clin Lung Cancer*. 2018;19(1):93–104. doi:10.1016/j.cllc.2017.05.016
7. Morgillo F, Corte Della CM, Fasano M, Ciardiello F. Mechanisms of resistance to EGFR-targeted drugs: lung cancer. *ESMO Open*. 2016;1(3):e000060–10. doi:10.1136/esmoopen-2016-000060
8. Tetsu O, Hangauer MJ, Phuchareon J, Eisele DW, McCormick F. Drug resistance to EGFR inhibitors in lung cancer. *Chemotherapy*. 2016;61(5):223–235. doi:10.1159/000443368
9. Nygaard AD, Garm Spindler K-L, Pallisgaard N, Andersen RF, Jakobsen A. The prognostic value of KRAS mutated plasma DNA in advanced non-small cell lung cancer. *Lung Cancer*. 2013;79(3):312–317. doi:10.1016/j.lungcan.2012.11.016
10. Camps C, Jantus-Lewintre E, Cabrera A, et al. The identification of KRAS mutations at codon 12 in plasma DNA is not a prognostic factor in advanced non-small cell lung cancer patients. *Lung Cancer*. 2011;72(3):365–369. doi:10.1016/j.lungcan.2010.09.005
11. Yu HA, Sima CS, Shen R, et al. Prognostic impact of KRAS mutation subtypes in 677 patients with metastatic lung adenocarcinomas. *J Thorac Oncol*. 2015;10(3):431–437. doi:10.1097/JTO.0000000000000432
12. Soh J, Toyooka S, Matsuo K, et al. Ethnicity affects EGFRand KRASgene alterations of lung adenocarcinoma. *Oncol Lett*. 2015;10(3):1775–1782. doi:10.3892/ol.2015.3414
13. Chapman AM, Sun KY, Ruestow P, Cowan DM, Madl AK. Lung cancer mutation profile of EGFR, ALK, and KRAS: meta-analysis and comparison of never and ever smokers. *Lung Cancer*. 2016;102:122–134. doi:10.1016/j.lungcan.2016.10.010
14. Boch C, Kollmeier J, Roth A, et al. The frequency of EGFR and KRAS mutations in non-small cell lung cancer (NSCLC): routine screening data for central Europe from a cohort study. *BMJ Open*. 2013;3(4):e002560. doi:10.1136/bmjopen-2013-002560
15. Jackman DM, Miller VA, Cioffredi L-A, et al. Impact of epidermal growth factor receptor and KRAS mutations on clinical outcomes in previously untreated non-small cell lung cancer patients: results of an online tumor registry of clinical trials. *Clin Cancer Res*. 2009;15(16):5267–5273. doi:10.1158/1078-0432.CCR-09-0888
16. Takamochi K, Oh S, Suzuki K. Differences in EGFR and KRAS mutation spectra in lung adenocarcinoma of never and heavy smokers. *Oncol Lett*. 2013;6(5):1207–1212. doi:10.3892/ol.2013.1551
17. Zheng D, Wang R, Zhang Y, et al. The prevalence and prognostic significance of KRAS mutation subtypes in lung adenocarcinomas from Chinese populations. *Ott*. 2016;9:833–843. doi:10.2147/OTT.S96834
18. Wen Y-S, Cai L, Zhang X-W, et al. Concurrent oncogene mutation profile in Chinese patients with stage Ib lung adenocarcinoma. *Medicine*. 2014;93(29):e296–10. doi:10.1097/MD.0000000000000296
19. Li S, Li L, Zhu Y, et al. Coexistence of EGFR with KRAS, or BRAF, or PIK3CA somatic mutations in lung cancer: a comprehensive mutation profiling from 5125 Chinese cohorts. *Br J Cancer*. 2014;110(11):2812–2820. doi:10.1038/bjc.2014.210
20. Lohinai Z, Hoda MA, Fabian K, et al. Distinct epidemiology and clinical consequence of classic versus rare EGFR mutations in lung adenocarcinoma. *J Thorac Oncol*. 2015;10(5):738–746. doi:10.1097/JTO.0000000000000492
21. Sun J-M, Hwang DW, Ahn JS, Ahn M-J, Park K. Prognostic and predictive value of KRAS mutations in advanced non-small cell lung cancer. Gorlova OY, ed. *PLoS One*. 2013;8(5):e64816. doi:10.1371/journal.pone.0064816
22. Syahruddin E, Wulandari L, Sri Muktiati N, et al. Uncommon *EGFR* mutations in cytological specimens of 1,874 newly diagnosed Indonesian lung cancer patients. *Lctt*. 2018;9:25–34. doi:10.2147/LCTT.S154116
23. Aditama TY. Smoking problem in Indonesia. *Med J Indones*. 2002;11(1):56–65. doi:10.13181/mji.v11i1.52
24. Kristina SA, Endarti D, Prabandari YS, Ahsan A, Thavorncharoensap M. Burden of cancers related to smoking among the Indonesian population: premature mortality costs and years of potential life lost. *Asian Pac J Cancer Prev*. 2015;16(16):6903–6908.
25. Levi M, Prayogi G, Sastranagara F, et al. Clinicopathological associations of K-RAS and N-RAS mutations in Indonesian colorectal cancer cohort. *J Gastrointest Canc*. 2018;49(2):124–131. doi:10.1007/s12029-016-9901-x
26. Dearden S, Stevens J, Wu Y-L, Blowers D. Mutation incidence and coincidence in non small-cell lung cancer: meta-analyses by ethnicity and histology (mutMap). *Ann Oncol*. 2013;24(9):2371–2376. doi:10.1093/annonc/mdt205
27. Tseng C-H, Chiang C-J, Tseng J-S, et al. EGFR mutation, smoking, and gender in advanced lung adenocarcinoma. *Oncotarget*. 2017;8(58):98384–98393. doi:10.18632/oncotarget.21842
28. Shi Y, Li J, Zhang S, et al. Molecular epidemiology of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology - Mainland China subset analysis of the PIONEER study. Chai KX, ed. *PLoS One*. 2015;10(11):e0143515. doi:10.1371/journal.pone.0143515
29. Shi Y, Au JS-K, Thongprasert S, et al. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). *J Thorac Oncol*. 2014;9(2):154–162. doi:10.1097/JTO.0000000000000033
30. Hsiao S-H, Lin S-E, Chou Y-T, et al. Histological subtype and smoking status, but not gender, are associated with epidermal growth factor receptor mutations in non-small-cell lung cancer. *Mole Clin Oncol*. 2014;2(2):252–258. doi:10.3892/mco.2013.232
31. Kauffmann-Guerrero D, Huber RM, Reu S, et al. NSCLC patients harbouring rare or complex EGFR mutations are more often smokers and might not benefit from first-line tyrosine kinase inhibitor therapy. *Respiration*. 2018;95(3):169–176. doi:10.1159/000484175
32. Lohinai Z, Ostoros G, Moldvay J, Dome B, Hegedus B. Differences in the epidemiology of rare EGFR mutations in different populations. *J Thorac Oncol*. 2016;11(1):e19–e20. doi:10.1016/j.jtho.2015.09.003
33. Kempf E, Rousseau B, Besse B, Paz Ares L. KRAS oncogene in lung cancer: focus on molecularly driven clinical trials. *Eur Respir Rev*. 2016;25(139):71–76. doi:10.1183/16000617.0071-2015

34. Riely GJ, Kris MG, Rosenbaum D, et al. Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. *Clin Cancer Res*. 2008;14(18):5731–5734. doi:10.1158/1078-0432.CCR-08-0646
35. Dogan S, Shen R, Ang DC, et al. Molecular epidemiology of EGFR and KRAS mutations in 3,026 lung adenocarcinomas: higher susceptibility of women to smoking-related KRAS-mutant cancers. *Clin Cancer Res*. 2012;18(22):6169–6177. doi:10.1158/1078-0432.CCR-11-3265
36. Mao C, Qiu L-X, Liao R-Y, et al. KRAS mutations and resistance to EGFR-TKIs treatment in patients with non-small cell lung cancer: a meta-analysis of 22 studies. *Lung Cancer*. 2010;69(3):272–278. doi:10.1016/j.lungcan.2009.11.020
37. Sasaki H, Okuda K, Kawano O, et al. Nras and Kras mutation in Japanese lung cancer patients: genotyping analysis using LightCycler. *Oncol Rep*. 2007;18(3):623–628.
38. Lee B, Lee B, Han G, Kwon MJ, Han J, Choi Y-L. KRAS mutation detection in non-small cell lung cancer using a peptide nucleic acid-mediated polymerase chain reaction clamping method and comparative validation with next-generation sequencing. *Korean J Pathol*. 2014;48(2):100–107. doi:10.4132/KoreanJPathol.2014.48.2.100
39. Ng M, Freeman MK, Fleming TD, et al. Smoking prevalence and cigarette consumption in 187 Countries, 1980-2012. *Jama*. 2014;311(2):183–192. doi:10.1001/jama.2013.284692
40. Benhamou S, Benhamou E, Auquier A, Flamant R. Differential effects of tar content, type of tobacco and use of a filter on lung cancer risk in male cigarette smokers. *Int J Epidemiol*. 1994;23(3):437–443.
41. Huboyo HS, Lestari P, Tohno S. Modeling indoor PM2.5 air pollution, estimating exposure, and problems associated with rural Indonesian households using wood fuel. In: McLellan B, editor. *Sustainable Future for Human Security*. Vol. 14. Singapore: Springer Singapore; 2017:287–300. doi:10.1007/978-981-10-5433-4_20
42. Arrieta O, Campos-Parra AD, Zuloaga C, et al. Clinical and pathological characteristics, outcome and mutational profiles regarding non-small-cell lung cancer related to wood-smoke exposure. *J Thorac Oncol*. 2012;7(8):1228–1234. doi:10.1097/JTO.0b013e3182582a93
43. Lee T, Lee B, Choi Y-L, Han J, Ahn M-J, Um S-W. Non-small cell lung cancer with concomitant *EGFR, KRAS*, and *ALK* mutation: clinicopathologic features of 12 cases. *J Pathol Transl Med*. 2016;50(3):197–203. doi:10.4132/jptm.2016.03.09
44. Hu W, Liu Y, Chen J. Concurrent gene alterations with EGFR mutation and treatment efficacy of EGFR-TKIs in Chinese patients with non-small cell lung cancer. *Oncotarget*. 2017;8(15):25046–25054. doi:10.18632/oncotarget.15337
45. Ulivi P, Chiadini E, Dazzi C, et al. Nonsquamous, non-small-cell lung cancer patients who carry a double mutation of EGFR, EML4-ALK or KRAS: frequency, clinical-pathological characteristics, and response to therapy. *Clin Lung Cancer*. 2015:1–7. doi:10.1016/j.cllc.2015.11.004.
46. Choughule A, Sharma R, Trivedi V, et al. Coexistence of KRAS mutation with mutant but not wild-type EGFR predicts response to tyrosine-kinase inhibitors in human lung cancer. *Br J Cancer*. 2014;111(11):2203–2204. doi:10.1038/bjc.2014.401
47. Guibert N, Barlesi F, Descourt R, et al. Characteristics and outcomes of patients with lung cancer harboring multiple molecular alterations: results from the IFCT Study Biomarkers France. *J Thorac Oncol*. 2017;12(6):963–973. doi:10.1016/j.jtho.2017.02.001
48. Roberts PJ, Stinchcombe TE. KRASMutation: should we test for it, and does it matter? *JCO*. 2013;31(8):1112–1121. doi:10.1200/JCO.2012.43.0454
49. Kaufman J, Stinchcombe TE. Treatment of KRAS-mutant non-small cell lung cancer: the end of the beginning for targeted therapies. *JAMA*. 2017;317(18):1835–1837. doi:10.1001/jama.2017.3436