

**Aim of the study:** Recent studies have suggested that k-RAS mutations are related to the response to epidermal growth factor receptor (EGFR) tyrosine-kinase inhibitors (TKIs) in advanced non-small cell lung cancer (NSCLC) treatment. The aim of this meta-analysis was to assess the relationship between smoking history and k-RAS mutations in NSCLC treated with TKIs.

**Material and methods:** We searched MEDLINE and Web of Science up to 15 March 2014. The pooled relative risk (RR) was estimated by using fixed effect model or random effect model, according to heterogeneity between studies. We also carried out power analyses.

**Results:** We identified 12 studies with 1193 patients, including 196 patients (16.4%) with k-RAS mutations. The pooled k-RAS mutations incidence was 22.8% (174/764) in patients with smoke exposure vs. 5.4% (23/429) in those with no smoke exposure. The pooled RR was 2.991 (95% CI: 1.884–4.746;  $Z = 4.65$ ,  $p = 0.000$ ). No publication bias was found (Begg's test:  $z = 1.09$ ,  $p = 0.274$  and Egger's test:  $t = 1.38$ ,  $p = 0.201$ ). In subgroup analyses, the pooled RR was 3.336 (95% CI: 1.925–5.779;  $Z = 4.30$ ,  $p = 0.000$ ) in the Caucasian subgroup, while in the Asian subgroup the pooled RR was 2.093 (95% CI: 0.909–4.822;  $Z = 1.73$ ,  $p = 0.083$ ), but the sample size was underpowered (0.465).

**Conclusions:** The current meta-analysis found that smoking was related to increased incidence of k-RAS mutations in non-small cell lung cancer treated with TKIs. This may be further evidence that smoking will lead to a worse prognosis in NSCLC patients treated with TKIs.

**Key words:** non-small cell lung cancer, smoking, k-RAS mutations, tyrosine-kinase inhibitors.

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# k-RAS mutations in non-small cell lung cancer patients treated with TKIs among smokers and non-smokers: a meta-analysis

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## Introduction

The latest cancer statistics in the United States show that the estimated number of deaths from lung and bronchial cancer was still higher than that of other cancers, which included 87,750 estimated deaths (29%) in males and 72,590 estimated deaths (26%) in females [1]. For advanced IIIB-IV non-small-cell lung cancer (NSCLC), biological therapy is a newly emerging treatment strategy. The biological agents include epidermal growth factor receptor (EGFR) family inhibitors, angiogenesis inhibitors, signal transduction inhibitors, apoptosis inducers, and eicosanoid pathway inhibitors [2]. EGFR tyrosine kinase inhibitors (TKIs) are most commonly used agents. These small molecules are reversible competitors with ATP for binding to the intracellular catalytic domain of the tyrosine kinase. They inhibit tyrosine kinase autophosphorylation in the intracellular domain and downstream intracellular signalling [2]. The TKIs include gefitinib and erlotinib.

Some phase III trials have already shown the therapy effectiveness of TKIs for NSCLC. The INTEREST study established non-inferior survival of gefitinib compared with docetaxel overall survival (hazard ratio [HR] 1.020, 96% CI: 0.905–1.150, meeting the predefined non-inferiority criterion; median survival 7.6 vs. 8.0 months), suggesting that gefitinib is a valid treatment for pretreated patients with advanced NSCLC [3]. SATURN showed that median progression-free survival (PFS) was significantly longer with erlotinib than with placebo: 12.3 weeks for patients in the erlotinib group vs. 11.1 weeks for those in the placebo group (HR 0.71, 95% CI 0.62–0.82;  $p < 0.0001$ ) [4]. However, most patients who initially respond to gefitinib and erlotinib eventually become resistant and experience progressive disease. Somatic activating mutations of the EGFR gene have been associated with response to TKIs [5]. The American Society of Clinical Oncology Clinical Practice Guideline update (2009) recommended the first-line use of gefitinib for patients with known EGFR mutations on chemotherapy for stage IV NSCLC [6].

But even in EGFR-mutation patients, there were some still resistant to TKIs. The response rate was 55% (95% CI: 33–70) [7]. A meta-analysis reported that the objective response rate (ORR) of NSCLC patients with mutant k-RAS was 3% (6/210), whereas the ORR of NSCLC patients with wild-type k-RAS was 26% (287/1125). The overall pooled RR for ORR was 0.29 (95% CI: 0.18–0.47;  $p < 0.01$ ) [8]. Another systematic review and meta-analysis also found that the presence of k-RAS mutations was significantly associated with an absence of response to TKIs (sensitivity = 0.21 [95% CI: 0.16–0.28], specificity = 0.94 [0.89–0.97]; +LR = 3.52; –LR = 0.84). Somatic mutation of the k-RAS oncogene is another mechanism of resistance to TKIs in patients with NSCLC [9].

Which factors contributed to k-RAS gene mutation? Smoking is the most well known factor that closely relates to lung cancer aetiology and prognosis. A meta-analysis has already found that the incidence of EGFR mutations in NSCLC differs according to cigarette-smoking history, with an OR for the EGFR mutation in non-smokers relative to smokers of 4.829 (95% CI: 3.598–6.482;  $p < 0.001$ ) [10]. But the relationship between cigarette-smoking and k-RAS gene mutation has not been investigated. The aim of this meta-analysis was to assess the relationship between smoking history and k-RAS mutations in NSCLC treated with TKIs.

## Material and methods

### Databases and literature search

We searched MEDLINE (PubMed, <http://www.ncbi.nlm.nih.gov/pubmed/>) and Web of Science (<http://webofknowledge.com/>) up to 15 March 2014. The search terms included of “non-small cell lung cancer”, “tyrosine-kinase inhibition”, “KRAS”, and “smoke”. The search detail in MEDLINE was “Carcinoma, Non-Small Cell Lung” [MeSH] AND (“tyrosine-kinase inhibition” [tiab] OR “TKI” [tiab] OR “gefitinib” [tiab] OR “erlotinib” [tiab] OR “iressa” [tiab] OR “tarceva” [tiab]) AND (“KRAS” [tiab] OR “K-ras” [tiab]) AND smok\* [tw]. In Web of Science, the search detail used was as follows: (TS = (non-small cell lung cancer) OR TS = NSCLC) AND (TS = (tyrosine-kinase inhibition) OR TS = TKI OR TS = gefitinib OR TS = erlotinib OR TS = iressa OR TS = tarceva) AND (TS = KRAS OR TS = K-ras) AND TS = smok\*. We supplemented our searches by manually reviewing the references of all relevant studies. Only studies published in English were included.

### Eligibility criteria

The following inclusion criteria had to be fulfilled: 1) investigated patients with non-small cell lung cancer who were treated with TKIs and chemotherapy agents or TKIs alone; 2) k-RAS mutations were tested on all or some of the patients in the studies; 3) providing sufficient data to construct the two-by-two contingency tables to calculate relative risk (RR) of k-RAS mutation rate comparing a smoking exposure population and an unexposed population in the studies. We excluded case reports, case series, and reviews.

### Data extraction

The following data were abstracted onto standardised forms: first author, publication year, country, number of patients, ethnicity, study design, gender of patients, age of patients, tumour stage, tumour histology, type of TKI, and outcome results. Data extraction was carried out independently by two reviewers. Disagreements were resolved by discussion between the two reviewers.

### Statistical analysis

Pooled RR with 95% CI was used to assess the strength of an association between cigarette smoking and k-RAS mutation. RR with 95% CI was calculated for each mea-

surement. Overall effects were determined using the Z test. Statistical heterogeneity was explored by  $\chi^2$  and inconsistency ( $I^2$ ) statistics; an  $I^2$  value of 50 per cent or more represented substantial heterogeneity [11]. In the absence of significant heterogeneity, studies were pooled using a fixed-effect model. If heterogeneity was observed, a random-effects model was used. An estimate of potential publication bias was carried out by the funnel plot, the Egger regression test, and Begg adjusted rank correlation test. Sensitivity analyse was conducted by removal of a retrospective case-control study. Subgroup analysis was carried out by different ethnicity. The meta-analysis was performed with Stata software, version 12.0 (Stata Corp, College Station, Texas).

Because the sample size was still low after pooled data from included studies, we also carried out power analyses. Power analyses were estimated using the number of members of the smoking exposure population and that of the unexposed population, and the incidence of k-RAS mutations in the unexposed smoking population. Power  $\geq 0.8$  is a threshold for acceptable power. Calculations were performed with PS software (version 3.0.43) [12].

## Results

### Characteristics of the included studies

We identified 12 studies [13–24] that met our inclusion criteria for meta-analysis. The detailed steps of our literature search are shown in Figure 1. Of these studies, three were multi-centred [13, 14, 19]. The studies were conducted in the Netherlands, France, the United Kingdom, Switzerland, Italy, the USA, Korea, Taiwan, and China. The ethnicity consisted mainly of Caucasians and Asians. The studies included 11 prospective cohort studies and a retrospective case-control study [18]. The tumour stage was almost IIIB-IV. Table 1 shows the characteristics of the 12 identified studies.

### Main meta-analysis

A total of 1193 patients were analysed, including 196 patients (16.4%) with k-RAS mutations. The pooled k-RAS mutations incidence was 22.8% (174/764) in patients with smoke exposure, while in patients with no smoke exposure the pooled k-RAS mutation incidence was 5.4% (23/429). There was no heterogeneity in the studies ( $I^2 = 0.0\%$ ,  $p = 0.962$ ). The pooled RR was 2.991 (95% CI: 1.884–4.746;  $Z = 4.65$ ,  $p = 0.000$ ) by fixed-effect model (Fig. 2). This sample had 1.000 power to detect the RR of 2.991. The funnel plot is shown in Fig. 3, and either the Begg’s test ( $Z = 1.09$ ,  $p = 0.274$ ) or the Egger’s test ( $t = 1.38$ ,  $p = 0.201$ ) suggested no publication bias. Upon removal of a retrospective case-control study, the pooled RR was 3.039 (95% CI: 1.901–4.858;  $Z = 4.64$ ,  $p = 0.000$ ) by fixed-effect model.

### Subgroup analysis by ethnicity

Two ethnicity subgroups (Caucasian and Asian) were found in the included studies. Eight studies [13–15, 17–19, 21, 22] were included in the Caucasian subgroup, and the other four studies [16, 20, 23, 24] were included in the

**Table 1.** Main characteristics of the studies included in the meta-analysis

First author, Year	Country	Patients (N)	Ethnicity	Study design	Gender (M/F)	Age (year)	Stage	Histology		TKI	k-RAS mutation (tested)		k-RAS wild-type (tested)	
								Ad	Sq		Smoke	Never smoke	Smoke	Never smoke
Giaccone <i>et al.</i> 2006 [13]	Netherlands; France; UK; Switzerland	53	Caucasian	cohort	22/31	60 (30–80)	IIIB–IV	24	8	erlotinib	10	0	10	5
Cappuzzo <i>et al.</i> 2007 [14]	Italy; USA	42	Caucasian	cohort	11/31	60.9 (43–80)	III–IV	32	1	gefitinib	0	1	6	30
Eberhard <i>et al.</i> 2005 [15]	USA	264	Caucasian	cohort	153/111	64 (24–82)	IIIB–IV	120	38	erlotinib	55	0	186	23
Han <i>et al.</i> 2006 [16]	Korea	69	Asian	cohort	39/30	59 (30–82)	IIIB–IV	43	14	gefitinib	7	2	27	33
Massarell <i>et al.</i> 2007 [17]	USA	70	79% Caucasian	cohort	29/41	Median 57.5–65	IIIB–IV	47	11	gefitinib	15	1	38	16
van Zandwijk <i>et al.</i> 2007 [18]	Netherlands	15	Caucasian	case control	7/8	37–71	locally advanced or metastatic	9	4	gefitinib	3	0	10	2
Hirsch <i>et al.</i> 2007 [19]	Italy; USA	204	Caucasian	cohort	116/88	NA	III–IV	99	26	gefitinib	33	3	80	22
Wu <i>et al.</i> 2012 [20]	China	116	Asian	cohort	62/54	66.0 (27.9–91.1)	NA	NA	NA	erlotinib or gefitinib	2	3	23	49
Bonanno <i>et al.</i> 2010 [21]	Italy	67	Caucasian	cohort	35/32	64 (35–81)	IIIB–IV	52	15	erlotinib	8	4	16	32
Boldrini <i>et al.</i> 2009 [22]	Italy	411	Caucasian	cohort	235/176	65.7 (37–88)	NA	411	0	erlotinib or gefitinib	37	4	115	42
Wu <i>et al.</i> 2008 [23]	China	237	Asian	cohort	137/100	62 (28–84)	I–IV	159	64	erlotinib or gefitinib	4	5	69	142
Wang <i>et al.</i> 2008 [24]	China	24	Asian	cohort	14/10	24–71	NA	18	2	gefitinib	0	0	10	10

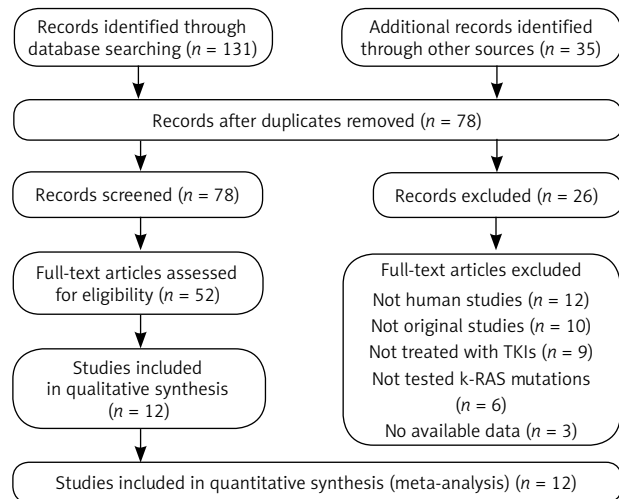
Ad – adenocarcinoma; Sq – squamous cell carcinoma; Ot – others; TKI – tyrosine kinase inhibitor

Asian subgroup. In the Caucasian subgroup, the pooled RR was 3.336 (95% CI 1.925 to 5.779;  $Z = 4.30, p = 0.000$ ) by fixed-effect model. This sample had 1.000 power to detect the RR of 3.336. While in the Asian subgroup the pooled RR was 2.093 (95% CI 0.909 to 4.822;  $Z = 1.73, p = 0.083$ ) by fixed-effect model. This sample had 0.465 power to detect the RR of 2.093. The funnel plot is shown in Fig. 4.

**Discussion**

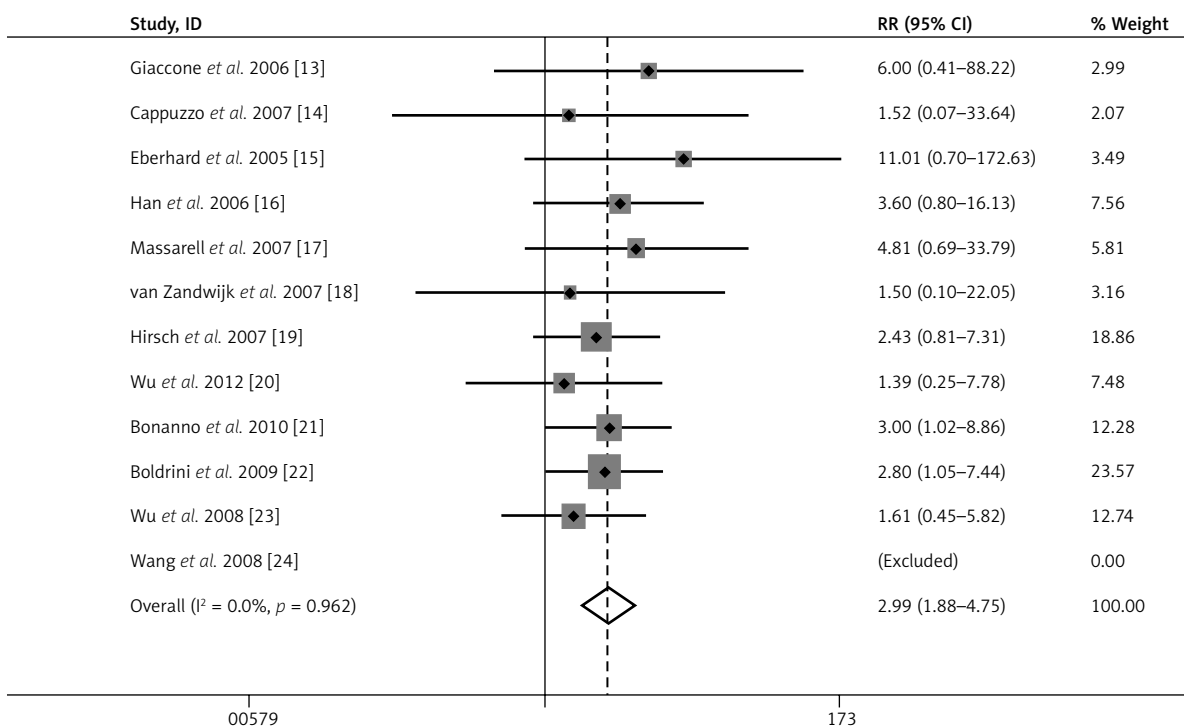
k-RAS is a member of the Ras family of small G proteins involved in intracellular signalling [25]. Studies have confirmed that activation of k-RAS mutations will result in the constitutive activation of downstream signalling pathways, and confers resistance to inhibition of cell surface receptor tyrosine kinases of EGFR. [26] Our meta-analysis indicated that smoking was related to increased incidence of k-RAS mutations in NSCLC treated with TKIs, in which the pooled RR was 2.991 (95% CI 1.884–4.746;  $Z = 4.65, p = 0.000$ ). Riely *et al.* have already found k-RAS transition mutations (G→A) were more common in patients who had never smoked cigarettes. In contrast, transversion mutations (G→T or G→C) were more common in former/current smokers. These data suggest that some mutations in k-RAS are associated with cigarette smoking [27].

Some phase III trails have already shown that never-smoking patients with advanced NSCLC have a better prognosis when treated with TKIs. The TRIBUTE trial reported that patients who reported never smoking experienced improved overall survival (OS) in the erlotinib arm (22.5 vs. 10.1 months for placebo) [28]. The Tarceva trial reported no differences in OS, time to disease progression (TTP), response rate (RR), duration of response, and quality

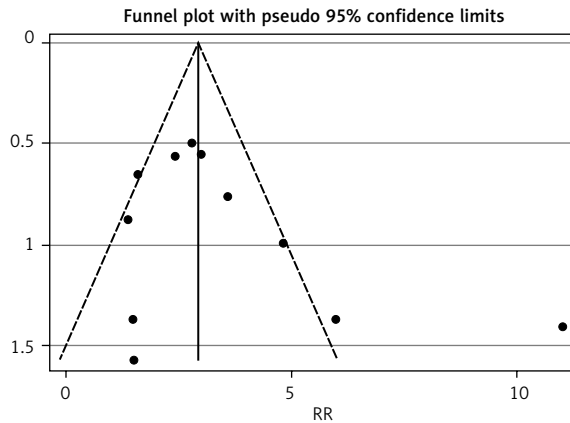


**Fig. 1.** Flow diagram showing selection of studies

of life (QoL) between erlotinib and placebo groups. However, in a small group of patients who had never smoked, OS and progression-free survival were increased in the erlotinib group [29]. The SAKK trial also reported that never smokers tend to have a better outcome, with a disease stabilisation rate (DSR) of 56% and a median OS of 20.2 months when treated with first-line gefitinib monotherapy in advanced NSCLC [30]. A meta-analysis has already found that cigarette-smoking history was related to decreased incidence of EGFR mutations, which increases the rate of resistance [10]. This may be an important reason why never-smoking patients with advanced NSCLC have a better prognosis when treated with TKIs. Our meta-anal-



**Fig. 2.** Meta-analysis of k-RAS mutations in non-small cell lung cancer treated with TKIs among smokers and non-smokers



RR – relative risk; SE – standard error

**Fig. 3.** Funnel plot for publication bias analysis

ysis indicated that smoking was related to increased incidence of k-RAS mutations in NSCLC treated with TKIs, and this may be further evidence that never-smoking patients will have a worse prognosis.

Because k-RAS mutations lead to poor prognosis after TKI treatment in advanced NSCLC, some novel strategies to circumvent KRAS-mutated tumours have been designed, such as farnesyl transferase inhibitors, and some of them have been used in clinical trials [31]. It also indicates that patients with NSCLC, who have a smoking history, will benefit from these agents in TKI treatment.

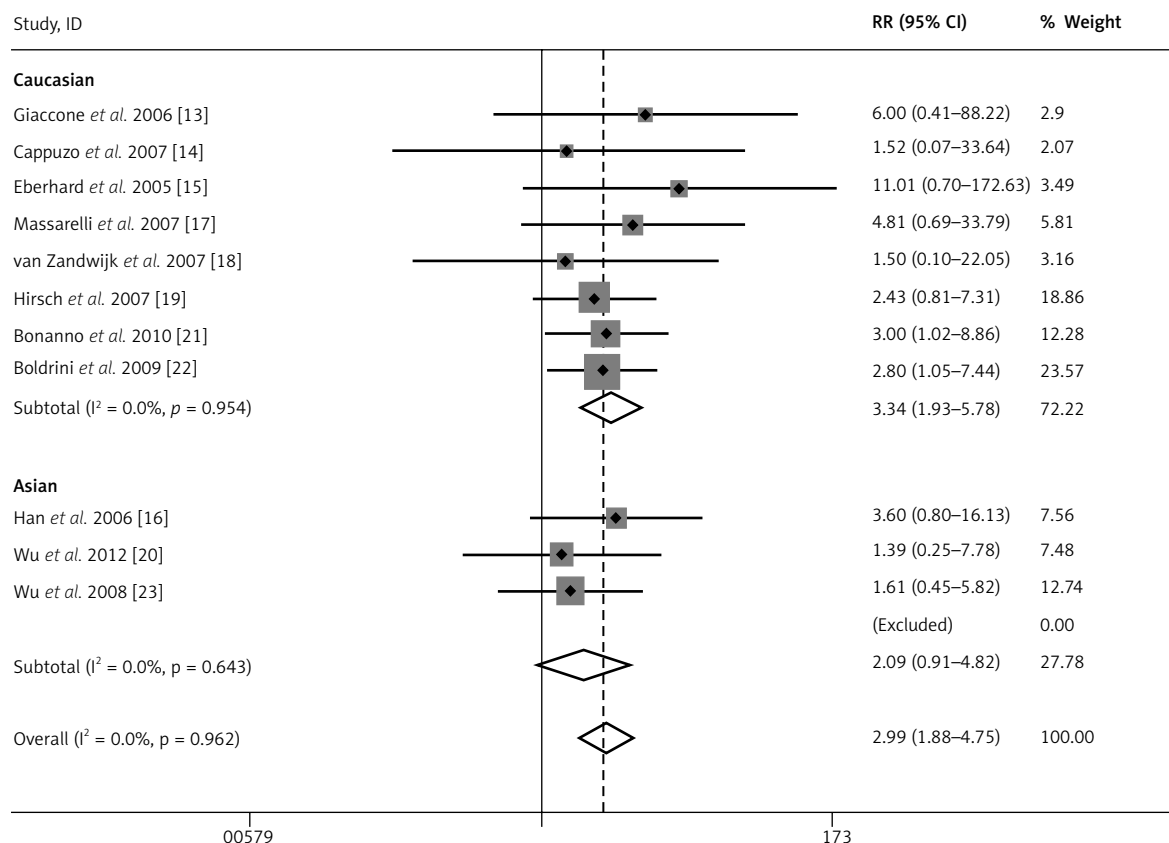
Our meta-analysis had several limitations. First, the sample size was still low after the data was pooled from the included studies. In the Asian subgroup, the sample only had 0.465 power, which did not meet the threshold to detect RR. Further studies with large samples are needed to confirm this conclusion, especially in the Asian population. Second, our result was based on unadjusted estimates. Notably, k-RAS accounts for 90% of mutations in lung adenocarcinomas, and is uncommon in lung squamous cell carcinomas [32]. k-RAS mutations can also be affected by other factors. Results will be more precise when adjusted by histology, stage, and other factors.

In conclusion, our meta-analysis showed that smoking was related to increased incidence of k-RAS mutations in non-small cell lung cancer treated with tyrosine-kinase inhibitors. This may be further evidence that smoking results in a worse prognosis in non-small cell lung cancer patients treated with tyrosine-kinase inhibitions.

*The authors declare no conflict of interest.*

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**Fig. 4.** Subgroup analysis by ethnicity for k-RAS mutations in non-small cell lung cancer treated with TKIs among smokers and non-smokers

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