



BioCAST/IFCT-1002: epidemiological and molecular features of lung cancer in never-smokers

Sébastien Couraud^{1,2,3,4}, Pierre-Jean Souquet^{1,2}, Christophe Paris⁵, Pascal Dô⁶, Hélène Doubre⁷, Eric Pichon^{8,9}, Adrien Dixmier¹⁰, Isabelle Monnet¹¹, Bénédicte Etienne-Mastroianni^{12,13}, Michel Vincent¹⁴, Jean Trédaniel¹⁵, Marielle Perrichon¹⁶, Pascal Foucher¹⁷, Bruno Coudert¹⁸, Denis Moro-Sibilot^{19,20}, Eric Dansin²¹, Stéphanie Labonne^{1,2}, Pascale Missy²², Franck Morin²², Hélène Blanché²³ and Gérard Zalcman²⁴, on behalf of The French Cooperative Intergroup IFCT²⁵

Affiliations: ¹Dept of Respiratory Medicine, Lyon Sud Hospital, Hospices Civils de Lyon, Lyon, France. ²Faculty of Medicine Lyon-Sud Charles Mérieux, Lyon 1 University, Lyon, France. ³Centre for Clinical Epidemiology, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec, Canada. ⁴Dept of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Quebec, Canada. ⁵(CP) INGRES, EA7298, Lorraine University, Vandoeuvre Les Nancy, France. ⁶François Baclesse Cancer Institute, Caen, France. ⁷Pulmonology Unit, Foch General Hospital, Paris, France. ⁸Pulmonology Unit, University Hospital of Tours, Tours, France. ⁹François Rabelais University, Respiratory Diseases Study Centre, U1100/EA6305, Tours, France. ¹⁰Pulmonology Unit, Orleans Regional Hospital, Orléans, France. ¹¹Pulmonology Unit, Centre Hospitalier Intercommunal de Créteil, Créteil, France. ¹²Dept of Respiratory Medicine, Hôpital Louis Pradel, Hospices Civils de Lyon, Lyon, France. ¹³Claude Bernard University Lyon 1, Lyon, France. ¹⁴Pulmonology Dept, Saint Joseph-Saint Luc Hospital, Lyon, France. ¹⁵Pulmonology Dept, Paris - Saint Joseph Hospital, and Sorbonne Paris Cité University, Paris, France. ¹⁶Pulmonology Dept, Bourg-en-Bresse General Hospital, Bourg en Bresse, France. ¹⁷Thoracic Oncology, Dijon University Hospital, Dijon, France. ¹⁸Oncology Dept, Georges François Leclerc Cancer Centre, Dijon, France. ¹⁹Pulmonology and Thoracic Oncology Dept, Grenoble University Hospital, Grenoble, France. ²⁰Medicine Faculty, Joseph Fourier University, La Tronche, France. ²¹Oncology Dept, Oscar Lambret Cancer Centre, Lille, France. ²²Intergroupe Francophone de Cancérologie Thoracique, Paris, France. ²³Fondation Jean Dausset - CEPH, Paris, France. ²⁴Pulmonology and Thoracic Oncology Dept, Caen University Hospital, and UMR INSERM 1086, Caen Basse-Normandie University, Caen, France. ²⁵For collaborators of the BioCAST/IFCT-1002 study, please see the acknowledgements section.

Correspondence: Sébastien Couraud, Service de Pneumologie, Hospices Civils de Lyon, CH Lyon Sud, 165 Chemin du Grand Revoyet, F-69495 Pierre Bénite, France. E-mail: sebastien.couraud@chu-lyon.fr

ABSTRACT Lung cancer in never-smokers (LCINS) (fewer than 100 cigarettes in lifetime) is considered as a distinct entity and harbours an original molecular profile. However, the epidemiological and molecular features of LCINS in Europe remain poorly understood.

All consecutive newly diagnosed LCINS patients were included in this prospective observational study by 75 participating centres during a 14-month period. Each patient completed a detailed questionnaire about risk factor exposure. Biomarker and pathological analyses were also collected. We report the main descriptive overall results with a focus on sex differences.

384 patients were included: 65 men and 319 women. 66% had been exposed to passive smoking (significantly higher among women). Definite exposure to main occupational carcinogens was significantly higher in men (35% *versus* 8% in women). A targetable molecular alteration was found in 73% of patients (without any significant sex difference): *EGFR* in 51%, *ALK* in 8%, *KRAS* in 6%, *HER2* in 3%, *BRAF* in 3%, *PI3KCA* in less than 1%, and multiple in 2%.

We present the largest and most comprehensive LCINS analysis in a European population. Physicians should track occupational exposure in men (35%), and a somatic molecular alteration in both sexes (73%).



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Occupational exposure and targetable mutation should be tracked in lung cancer of European never-smokers. <http://ow.ly/FB2WS>

Introduction

Although tobacco smoking is the strongest causal factor for lung cancer, 10–25% of lung cancer worldwide occurs in lifelong nonsmokers (300 000 deaths each year) [1–3]. A “never-smoker” is well-defined as an individual who has smoked fewer than 100 cigarettes during their lifetime. Lung cancer occurring in never-smokers (LCINS) is now considered a distinct entity. However, this disease appears strongly linked to geographical origins. For example, LCINS is drastically more common in Asia than in the USA or Europe [2]. This could be related to distinct features pertaining to inherited susceptibility, as well as to varied exposure to occupational and environmental carcinogens in different geographical areas. Thus, it is possible that LCINS is, in fact, globally a very heterogeneous disease [1, 2]. Although the disease is thought to be well characterised, very little data on LCINS are available in Europe [4], and nobody has concomitantly assessed most of the known risk factors for lung cancer.

LCINS is also known to hold an original spectrum of driver mutations. Thus, human epidermal growth factor receptor family (*EGFR* (also called *HER1*) and *HER2* (human epidermal growth factor receptor 2)) mutations appear more frequent, while *KRAS* (Kirsten rat sarcoma viral oncogene homologue) and *BRAF* (v-Raf murine sarcoma viral oncogene homologue B), thought to be related to tobacco carcinogens, are less common in this group [5–7]. Finally, *ALK* (anaplastic lymphoma kinase) rearrangements also occur slightly more frequently in never-smoking patients [8]. This spectrum is another argument favouring the hypothesis that separate genetic pathways lead to lung carcinogenesis in ever- and never-smokers [1].

Given that the clinical and molecular epidemiology of LCINS in Europe is still poorly understood, the French Collaborative Intergroup for Thoracic Cancer Research (IFCT) sought to investigate this specific entity through a prospective cohort of LCINS. The BioCAST (IFCT-1002) study’s main objective was to describe a French population of LCINS patients and especially the distribution of risk factor exposure and biomarker patterns. Here we report this study’s main descriptive results, focusing on potential sex differences among LCINS patients.

Population and methods

Population

Main inclusion criteria were: 1) being a self-declared never-smoker (*i.e.*, declaring having smoked fewer than 100 cigarettes during lifetime); 2) being newly diagnosed with a non-small cell lung cancer (NSCLC) by biopsy or by cytological sampling; 3) being aged at least 18 years; 4) speaking and understanding French (or having access to a relative able to translate); 5) having phone access, either at home or at the hospital; and 6) granting signed consent.

The IFCT sponsored the BioCAST study. The study was conducted in 75 participating centres throughout metropolitan France, from November 1, 2011 to January 31, 2013. Participating centres were asked to include all consecutive newly diagnosed LCINS cases. IFCT research staff members were in charge of administrative management and quality assurance (in compliance with international research standards) [9].

Ethics

The Sud-Est IV Lyon ethics committee approved the study protocol on September 13, 2011. The Advisory Committee on Information Processing for Health Research permitted use of a computerised database on September 8, 2011, and the National Commission for Data Protection was contacted on September 23, 2011, in accordance with French law. Blood sample collection was declared to the French Ministry of Research on July 1, 2011. The BioCAST study was registered on the US National Institute of Health website www.clinicaltrials.gov under the CTC ID NCT01465854.

Study design

Patients signed their consent after receiving information about the study from their physicians. Afterward, and before any anti-cancer treatment, each patient’s blood was sampled. Patients were then contacted by phone in order to schedule a dedicated phone interview and complete a standardised questionnaire. All

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patients had the opportunity to fully prepare this questionnaire before the pre-planned interview. Patients were also encouraged to seek assistance from a relative if needed (in cases of fatigue, deafness, memory deficiency or poor spoken French). Two trained research assistants performed all the interviews in order to control for reporting and interrogation biases. Additional medical data, as well as molecular testing and pathological reports were collected directly from participating physicians.

Patients' questionnaire

A 17-page questionnaire was delivered to patients upon inclusion. It included questions about demographics, socio-educative level and alternative tobacco consumption, as well as cannabis smoking, passive smoking exposure, occupational exposure, personal medical history, family history, alcohol intake and fried and stir-fried cooking exposure. Occupational exposures to bronchial carcinogens were assessed using a lifelong task-based questionnaire, the efficacy of which for detecting occupational exposure was recently published [10]. All patients were questioned about their home addresses and exposure to solid fuel for cooking or heating. Finally, women declared oral contraceptive and post-menopausal hormone replacement therapy intake, as well as other details on reproductive factors. Additional detailed information on recorded data and exposure measurements are provided in supplemental file S1.

Biomarker analyses

The French National Cancer Institute (INCa) launched a network of 28 molecular genetics platforms that provide routine cancer molecular testing for all patients [11]. Each BioCAST participant centre was asked to systematically order tests for somatic mutations in *EGFR* and *KRAS*, as well as *ALK* fusion gene, to its local molecular genetics laboratory. Investigator sites were also encouraged to request *BRAF*, *HER2*, and *PI3KCA* mutation analyses, which are also routinely performed (free of charge to the patient) at these platforms. All centres were advised to follow local policy, and were therefore allowed to forego further mutation testing if one mutation reputed to exclude the others was found. Final and detailed reports of these analyses were collected for each patient.

Actually, biomarker testing methods are not homogeneous throughout all centres. However, the ERMETIC study was designed to assess concordance of results between each centres (blinded cross validation study compared to an international reference lab) and between the different methods used in such centres (direct sequencing, PCR-based, Restriction fragment-length polymorphism, and high-resolution melting). This validation study showed good concordance rate suggesting that, despite some difference in sample processing and analyses, results are accurate [12, 13].

Blood samples bio-bank

Four tubes of each patient's blood were collected for further studies (two stored in EDTA, one dry and one ACD-citrated). Samples were transported to the BioCAST central laboratory (hosted at the Centre d'Étude du Polymorphisme Humain, Fondation Jean Dausset, Paris, France) at room temperature within 24 h of sampling and were then processed.

Statistics

Categorical variables were expressed as percentages. Comparisons of proportions used the Chi-squared test when the expected count in a given category was at least five, or Fisher's exact test otherwise. We used the one-sample Kolmogorov–Smirnov test in order to assess the plausibility of a normal distribution assumption for continuous variables. Normally distributed continuous variables were expressed as mean and standard deviation. Comparisons of means were conducted using the bilateral t-test. Differences in distribution of continuous variables between two independent samples were assessed using the Mann–Whitney U-test, and the Kruskal–Wallis one-way ANOVA was used to compare more than two independent samples. Some continuous variables were also categorised in quartiles, tertiles or clinically relevant categories according to their distribution in the overall population. Missing values were reported as such, and all tests were two-sided. All statistics were calculated using the SPSSv20 (IBM SPSS Statistics, New York, NY, USA).

Results

Population

Altogether, 384 consecutive French never-smokers were included during the study period. Of these, 336 (87.5%) completed the interview, 359 (93%) had at least one biomarker testing, and 381 (99.2%) underwent the correct blood sampling procedure (fig. 1).

Main demographic data

Table 1 gives the overall population's main features and a sex comparison. The sex ratio was 4.9 in favour of women. There was no patient-reported alternative smoking (water-pipe, cigar, pipe or gum) or cannabis

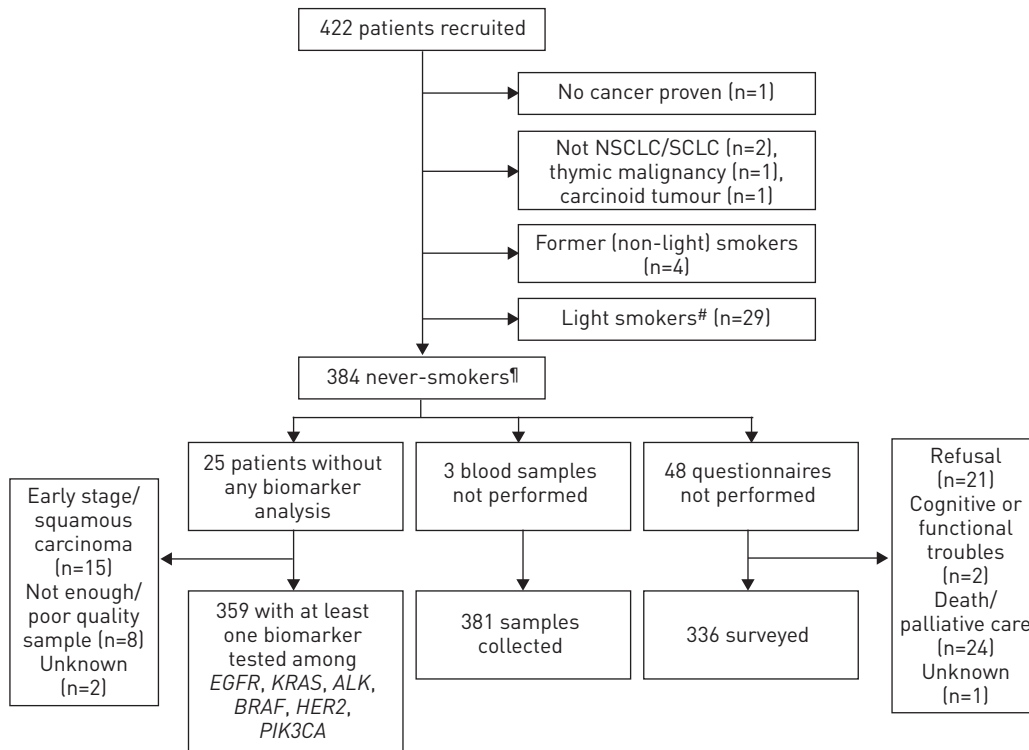


FIGURE 1 Flow chart of the BioCAST study. NSCLC: nonsmall cell lung cancer; SCLC: small cell lung cancer; *EGFR*: epidermal growth factor receptor; *KRAS*: Kirsten rat sarcoma viral oncogene homologue; *ALK*: anaplastic lymphoma kinase; *BRAF*: v-Raf murine sarcoma viral oncogene homologue B; *HER2*: human epidermal growth factor receptor 2; *PIK3CA*: phosphatidylinositol-3-kinase, catalytic subunit α . #: smoked fewer than 5 packs-year⁻¹ in lifetime and quit at least 15 years prior; ¶: smoked fewer than 100 cigarettes in lifetime.

use. 91% of patients originated from Europe: 276 from Western Europe, 22 from Southern Europe and six from Eastern Europe. Men were, on average, 4 years younger than women ($p=0.016$), and the proportion of patients aged <55 years old at diagnosis was significantly higher among males. In addition, men were better educated, more pre-obese and exhibited higher alcohol consumption than women.

Histology and stage of the disease

The main pathological lung cancer features found in our 384 patients are shown in table S2 in the online supplementary material. We found no significant difference between the sexes. Notably, the frequency of squamous cell carcinoma (SCC) was similar in both sexes (9% in women versus 7% in men; $p=0.606$). Most cases were adenocarcinoma (85%). The other histological types were SCC (8%), large cell carcinoma (4%), adenosquamous ($n=5$), sarcomatoid carcinoma ($n=4$) and carcinoma not otherwise specified ($n=2$). thyroid transcription factor 1 (TTF-1) immunostaining was positive in 76% of patients. Table S3 in the online supplementary material shows sample type and origin. Most were core biopsies (90%) from primitive tumours (69%) and were classified as stage IV (73%).

Occupational exposure

Data about exposure to occupational carcinogens was available in 334 patients, and results are presented in table 2. Altogether, 13% of patients were found to have been definitely exposed to at least one occupational carcinogen, with a striking difference between the sexes: 35% of men compared with only 8% of women ($p<10^{-4}$). The most frequent carcinogenic agent was polycyclic aromatic hydrocarbons, followed by asbestos, silica and diesel exhaust. Whatever the agent considered, men were in all cases significantly more exposed than women.

Passive smoke exposure

Overall, 219 (66%) of the 334 patients were ever exposed to passive smoking, either in a domestic setting (59%) or in the workplace (18%) (table 3). Domestic exposure to passive smoking was significantly more

TABLE 1 Main characteristics of the BioCAST population

	Lung cancer in never smokers (BioCAST)			Lung cancer in ever smokers [#]	
	Men	Women	p-value	All	
Total patients n				384	6246
Sex %					
Women		319 [83]			19
Men	65 [17]				81
Age	66.6±13.0	70.5±11.7	0.016 [¶]	69.8±12.0	65.5±11.3 ⁺
<55 years	12 [19]	30 [9]	0.033	42 [11]	
World region origin					
Missing data n	7	41		48	
Europe	51/58 [88]	253/278 [91]	0.468 [§]	304/336 [91]	
Africa	4/58 [7]	12/278 [4]		16/336 [5]	
Asia	1/58 [2]	9/278 [3]		10/336 [3]	
Caribbean	2/58 [3]	4/278 [1]		6/336 [2]	
Education level					
Missing data n	7	44		51	
High school and more	34/58 [59]	115/275 [42]	0.037	149/333 [45]	
Secondary school	13/58 [22]	66/275 [24]		79/333 [24]	
Never schooled/primary school	11/58 [19]	94/275 [34]		105/333 [32]	
Body mass index kg·m⁻²	25.4±4.8	23.7±5.4	0.015 ^f	24.2±5.5	
Missing data n	7	46		53	
Underweight/normal	27/58 [47]	165/273 [60]	0.087	192/331 [58]	
Pre-obese	24/58 [41]	74/273 [27]		98/331 [30]	
Obese	7/58 [12]	34/273 [13]		41/331 [12]	
Alcohol intake (in standard glass per day)					
Missing data n	7	43		50	
0-1	49/58 [85]	265/276 [96]	0.003 ^{##}	314/334 [94]	
≥2	9/58 [16]	11/276 [4]		20/334 [6]	

Data are presented as n (%), median±interquartile range or n/N available (%), unless otherwise stated. #: data from the KBP-CPHG study [14]; ¶: t-test; +: both smokers and never smokers (n=7051); not available for smokers only; §: computed between "European" and "non-European" categories; f: Mann-Whitney U test; ##: Fisher's exact test; all others are Chi-squared tests (for categorical variables).

common among women than men (64 versus 38%; p=0.0001), but this sex difference was not observed for workplace exposure. Moreover, of those exposed in a domestic setting, women were exposed for a significantly longer period than men. For patients exposed in a domestic setting, exposure began during childhood in 62% of patients. Men were more frequently exposed in childhood than women, suggesting that women were mainly exposed *via* their spouses.

Domestic pollution

Table S4 in the online supplementary material provides exposure to domestic pollution. Women more frequently reported having ever been exposed to cooking oil fumes (41 versus 18%; p=0.001). 26% of

TABLE 2 Definite exposure to occupational carcinogens (by task) according to sex

	Men	Women	p-value	All
Overall (at least one)	20 [35]	23 [8]	<10 ⁻⁴	43 [13]
PAH	15 [26]	15 [5]	<10 ⁻⁴	30 [9]
Asbestos	11 [19]	11 [4]	<10 ⁻⁴ #	22 [7]
Silica	10 [18]	5 [2]	<10 ⁻⁴ #	15 [4]
Diesel	6 [11]	2 [1]	<10 ⁻⁴ #	8 [2]
Chrome	5 [9]	1 [0]	0.001 [#]	6 [2]
Paint	4 [7]	3 [1]	0.018 [#]	7 [2]
Missing data	8	42		50

Data are presented as n (%), unless otherwise stated. PAH: polycyclic aromatic hydrocarbons. #: Fisher's exact test, others are Chi-squared tests.

TABLE 3 Passive smoking exposure according to sex

	Domestic exposure				Workplace				Overall			
	Men	Women	p-value	All	Men	Women	p-value	All	Men	Women	p-value	All
Missing n	7	43		50	7	43		50	7	43		50
Never exposed	36/58 (62)	100/276 (36)	<10 ⁻⁴	136/334 (41)	47/58 (81)	227/276 (82)	0.827	274/334 (82)	30/58 (52)	85/276 (31)	0.002	115/334 (34)
Ever exposed	22/58 (38)	176/276 (64)		198/334 (59)	11/58 (19)	49/276 (18)		60/334 (18)	28/58 (48)	191/276 (69)		219/334 (66)
Missing data		4		4		1		1		3		3
Length of exposure												
<20 years	14/22 (64)	59/172 (34)	0.008	73/194 (38)	8/11 (73)	23/48 (48)	NC	31/59 (53)	15/28 (54)	49/188 (26)	0.002	64/216 (30)
20–30 years	7/22 (32)	57/172 (33)		64/194 (33)	2/11 (18)	15/48 (31)		17/59 (29)	11/28 (39)	70/188 (37)		81/216 (38)
>30 years	1/22 (5)	56/172 (33)		57/194 (29)	1/11 (9)	10/48 (21)		11/59 (19)	2/28 (7)	69/188 (37)		71/216 (33)
Exposed in childhood	18/22 (82)	104/172 (59)	0.039	122/194 (62)								
Exposed in adulthood only	4/22 (8)	72/172 (41)		76/194 (38)								

Data are presented as n/N available (%), unless otherwise stated. NC: not computable; all p-values are computed with chi-squared tests.

patients reported to have been exposed for >50% of their lifetimes to solid fuel fumes; here there was no significant difference between men and women.

Personal and familial medical history

24% of patients reported having at least two biological first-degree relatives with lung cancer, and 17% reported a personal history of at least one other cancer. Medical histories of pertussis, tuberculosis and pneumonia were reported in 21%, 8% and 6% of patients, respectively. In addition, 13% had been diagnosed with a chronic bronchial disease. There were no differences between the sexes with respect to these variables (Table S5 in the online supplementary material).

Reproductive factors and hormone intake in women are reported in table S6 in the online supplementary material. Overall, 115 (42%) patients had used oral contraceptives, and 70 (25%) had undergone post-menopause hormonal replacement therapy.

Biomarkers (somatic mutations)

EGFR mutations were tested in 340 patients, KRAS in 293, ALK rearrangements in 192, BRAF in 22, HER2 in 201, and PI3KCA in 187. Altogether, we found 220 molecular alterations in 208 patients (table 4). Six patients carried a somatic mutation within two (n=5) or three (n=1) genes simultaneously (multiple mutations), while five others hosted two simultaneous somatic mutations in the *EGFR* gene (table S7 in the online supplementary material). Alterations in the *EGFR* gene were the most common: 147 patients displayed 153 mutations, with deletions in exon 19 and substitution L858R in exon 21 being the most frequent (n=74 and 33, respectively). We also found one missense mutation T790M in exon 20 and one alteration never reported before in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (<http://cancer.sanger.ac.uk/cancergenome/projects/cosmic>) (c.2303_2305delinsTCT in exon 20). In addition, 24 gene fusions involving the *ALK* gene were observed in 23 patients. We also noted 20 *KRAS* mutations in 18 patients, 10 *BRAF* mutations in nine patients, eight *HER2* mutations in eight patients, and five *PI3KCA* mutations in three patients. Overall, an *EGFR* mutation was found in 43% of individuals, a *KRAS* mutation in 7%, a *BRAF* mutation in 5%, a *HER2* mutation in 4%, a *PI3KCA* (phosphatidylinositol-3-kinase, catalytic subunit α) mutation in 2%, and an *ALK* rearrangement in 13% of patients tested for the corresponding biomarker. We found no significant difference in the mutation frequency as well as in the mutation type according to the gender. For EGFR, women exhibit a higher frequency than men but the difference remains non-significant (39 versus 44% respectively; $p=0.438$). In addition, we found no difference among sex for the type of mutation found in each biomarker; but however, women tend to exhibit more *KRAS* transition mutations than men (table 5).

Taking all these data together, 77 (27%) patients were considered “pan-negative” (all biomarkers found were wild type; or at least wild-type *EGFR*, *KRAS* and *ALK* simultaneously). In the remaining 284 patients with complete data, the most common alteration found was an *EGFR* mutation (in 51% of patients), followed by an *ALK* rearrangement (8%), a missense mutation in *KRAS* (6%), *HER2* (3%), *BRAF* (3%) and *PI3KCA* (<1%). Only 2% carried multiple mutations (see fig. 2). Therefore, 73% of French never-smokers carried a targetable molecular alteration.

Discussion

Among French never-smokers with lung cancer, we found that occupational exposure to carcinogens was significantly higher in men than in women, whereas domestic exposure, both to passive smoking and to cooking-oil, was higher in women. Most of the tumours we studied (73%) carried a targetable mutation.

To our knowledge, our study is the largest and most comprehensive ever conducted that focused on LCINS in a European population. Indeed, most published cohort studies used a retrospective design, were dedicated to Asian populations and/or explored only a single or a small subset of risk factors simultaneously. In Europe, the EPIC (European Prospective Investigation into Cancer and Nutrition) cohort hosted 97 newly diagnosed lung cancer cases [15]. In Asia, a paper from Kim *et al.* [16], recently reported to be the largest and most comprehensive series on never-smokers, effectively analysed 229 Asian patients, using a retrospective review for *EGFR*, *KRAS* and *ALK*. Kawagushi *et al.* [17] also keep a prospective registry of never-smoker from Japan, Singapore, Korea as well as few patients in US, but they only reported their results on passive smoking exposure.

Our main findings are consistent with published literature. In fact, Clément-Duchêne *et al.* [18] have already shown, in 67 French never-smokers, that occupational exposure and passive smoking were differently distributed between the sexes. Indeed, some lung cancers are known to be related to domestic pollution exposure, although modestly, even in Europe [19]. Regarding biomarker distribution, we have shown that French never-smokers carry 73% of targetable mutations. This finding situates French never-smoker between Asian (more than 80%) and American (55%) never-smokers [1, 20, 21].

TABLE 4 Mutation patterns observed in the BioCAST study

Mutation by biomarker and exon	Number of mutations in the same patient			Total n	% of gene	% of total
	One	Two	Three			
<i>BRAF</i>						
Exon 15						
V600E	7	1	1	9		
L597L		1		1		
Total				10		5%
<i>EGFR</i>						
Exon 18						
G719C	1	1		2		
G719A	1			1		
Unspecified	1	1		2		
Total				5	3%	
Exon 19						
Deletion	74	2		76		
Unspecified	12			12		
Total				88	58%	
Exon 20						
c.2303_2305delinsTCT	1			1		
T790M		1		1		
S768I		1		1		
Insertion	4			4		
Duplication	1			1		
Deletion	1			1		
Unspecified		1		1		
Total				10	7%	
Exon 21						
L858R	33	3		36		
L861Q	4	1		5		
Unspecified substitution	4			4		
P848L		1		1		
Unspecified	2	1		3		
Total				49	32%	
Unspecified	1			1		
Total				1	1%	
Total				153		70%
<i>PI3KCA</i>						
Exon 9						
E545K		2		2		
Unspecified substitution	1			1		
Unspecified			1	1	80%	
Exon 20						
Unspecified			1	1	20%	
Total				5		2%
<i>HER2</i>						
Exon 20						
Unspecified substitution	1			1		
Unspecified insertion	5			5		
Unspecified duplication	2			2		
Total				8		4%
<i>KRAS</i>						
Exon 2						
G13D	1			1		
G12V	7	1		8		
G12R	1			1		
G12G	1			1		
G12D	4			4		
G12C	1			1		
G12A	2			2		
Unspecified	1	1		2		
Total				20		9%
<i>ALK</i>						
Unspecified	23	1		24		
Total				24		11%
Total	197	20	3	220		100%

BRAF: v-Raf murine sarcoma viral oncogene homologue B; *EGFR*: epidermal growth factor receptor; *PIK3CA*: phosphatidylinositol-3-kinase, catalytic subunit α ; *HER2*: human epidermal growth factor receptor 2; *KRAS*: Kirsten rat sarcoma viral oncogene homologue; *ALK*: anaplastic lymphoma kinase.

TABLE 5 Mutation profile for each biomarker according to sex

	Lung cancer in never smokers (BioCAST)			All	Lung cancer in ever smokers [#]
	Men	Women	p-value		
Total n				384	7789
EGFR					
Patients n				340	
Wild type	35 (61)	158 (56)	0.438	193 (57)	
Mutation	22 (39)	125 (44)		147 (43)	5%
Data missing	1	6		7	
in exon 18	1/21 (5)	2/119 (2)	NC	3/140 (2)	
in exon 19	12/21 (57)	73/119 (61)		85/140 (61)	
in exon 20	0	7/119 (6)		7/140 (5)	
in exon 21	8/21 (38)	37/119 (31)		45/140 (32)	
KRAS					
Patients n				293	
Wild type	50 (94)	223 (93)	1.0 [¶]	273 (93)	
Mutation	3 (6)	17 (7)		20 (7)	32%
Data missing		2		2	
Transition	0	6/15 (40)	0.515 [¶]	6/18 (33)	
Transversion	3/3 (100)	9/15 (60)		12/18 (67)	
ALK					
Patients n				192	
Wild type	29 (94)	139 (86)	0.379 [¶]	168 (88)	
Mutation	2 (6)	22 (14)		24 (13)	4%
BRAF					
Patients n				222	
Wild type	34 (94)	178 (96)	0.667 [¶]	212 (95)	
Mutation	2 (6)	8 (4)		10 (5)	2%
HER2					
Patients n				201	
Wild type	32 (94)	161 (96)	0.625 [¶]	193 (96)	
Mutation	2 (6)	6 (4)		8 (4)	<1%
PI3KCA					
Patients n				187	
Wild type	30 (97)	153 (98)	0.519 [¶]	183 (98)	
Mutation	1 (3)	3 (2)		4 (2)	2%

Data are presented as n (%) or n/N available (%), unless otherwise stated. *EGFR*: epidermal growth factor receptor; *KRAS*: Kirsten rat sarcoma viral oncogene homologue; *ALK*: anaplastic lymphoma kinase; *BRAF*: v-Raf murine sarcoma viral oncogene homologue B; *HER2*: human epidermal growth factor receptor 2; *PI3KCA*: phosphatidylinositol-3-kinase, catalytic subunit α . #: Data from Biomarker France [22]; [¶]: Fisher's exact test; all other are Chi-squared test.

Our study does carry some limitations. First, due to financial constraints, we had no control patients. Thus, our cohort could not be used in order to estimate any risk ratios. However, our only aim was to assess the prevalence of known lung cancer risk factors and explore their actual distribution among certain subgroups. In order to put our findings into perspective, our data were compared to those of two recently published, nationwide epidemiological studies assessing the main features of French lung cancer smoker patients: 1) the KBP-CPHG (Cancer broncho-pulmonaire du Collège de Pneumologie des Hôpitaux Généraux) 2010 study, which includes 6246 lung cancers in smokers, for demographical, histological and staging data [14] (table 1); 2) the "Biomarker-France" study, sponsored by IFCT and funded by French NCI (INCa), of which preliminary results were reported after inclusion of the first 7789 lung cancers from smokers, for mutation profile data [22] (table 5). Indeed, our data perfectly mirror both the clinical and biological features of ever-smoker lung cancer patients, as reported in those two large series, conducted in the same French background, during the same period of time as BioCAST [14]. However, most of the collected variables had never been assessed in comparable epidemiological studies, or according to smoking status and, thus, are lacking of any comparable data in independent series of patients. Besides, our findings were congruous with existing literature: lung cancer in never smoker is more frequent among women, and adenocarcinoma. In addition, the mutation profile in never smoker strongly differs from smokers [1]. A second critical point is the retrospective reporting of risk factor exposures by patients themselves, without any possibility for a biological exposure objective assessment. However, we tried to minimise interrogation

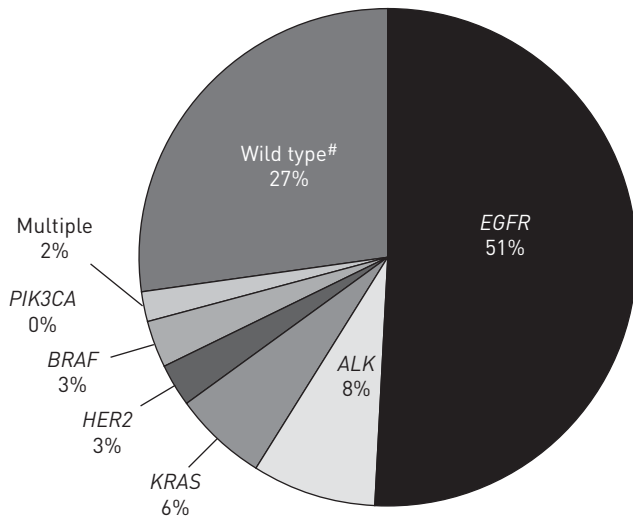


FIGURE 2 Final diagnosis of biomarker analysis in the 284 patients with complete data. #: all biomarkers were wild-type or at least EGFR (epidermal growth factor receptor) and KRAS (Kirsten rat sarcoma viral oncogene homologue) and ALK (anaplastic lymphoma kinase) were wild-type. Double EGFR mutations are categorised under the EGFR category; missing data not shown.

and reporting biases (by using a standardised questionnaire with only two trained, dedicated interviewers), as well as memorisation bias (by delivering the questionnaire prior to the interview to provide more time to answer all questions, and by allowing post-interview revisions and additions). However, our study remains submitted to recall bias. The third critical point is our modest accrual. Although our cohort is one of the largest in this specific field, comparative subgroup analysis was only based on small-number subsets. Therefore, our study lacked sufficient power for estimating differences between patient subsets. For instance, with only 65 patients in the male subgroup, we were able to detect a 23% difference with 80% power and a 0.05 alpha risk (using a bilateral test) [23]. This point is especially critical for mutations since all, except EGFR, deal with small numbers. In addition, our study emphasised exposure patterns that have occurred over the last decades, and it thus did not reflect current occupational and social changes that may result in distinct exposure profiles. Finally, our analysis is restricted to biomarkers tested in the routine setting in France. Thus, some important biomarkers commonly expressed in never smokers such as ROS1 (6% in never smokers) and RET rearrangements were not included in this study [24, 25].

In spite of these limitations, our study carries some strength. First, the never-smoker inclusion criterion was very strict although consensual (fewer than 100 cigarettes in the lifetime) and was checked by physician at inclusion and by staff during interview. Indeed, many studies may be biased, by assessing the “non-smoker” status retrospectively from medical charts, with a risk of overlapping between former- and never-smoker. We also checked for alternative smoking and can thus ensure the lack of contamination from any former (or even very light) smokers. Another noteworthy strength is our comprehensive approach. We collected data on possible exposures to most known or supposed risk factors (including a detailed investigation of occupational exposure), as well as medical data and comprehensive information from tumour biomarker analysis.

This paper reports descriptive finding of the large cohort of European LCINS. Based on these data, we will provide in future papers the main differences in clinical and molecular epidemiology regarding exposure to main risk factors such as passive-smoking, occupational exposure, domestic pollution or reproductive factors [26]. In addition, some new data will result from collaboration with French national institutions for assessment of radon exposure and atmospheric pollution exposure into the BioCAST cohort. Lastly, the population-attributable fraction for most known risk factors of LCINS will be computed taking all this data into account [27].

In conclusion, this study, the largest and most comprehensive analysis of LCINS in a European population, reports differences in age and carcinogen exposure distribution by gender, and emphasizes the crucial role of targetable somatic mutations in this specific population. Physicians facing a never-smoker with a lung cancer should keep in mind that two-thirds of men underwent an occupational exposure and that three-quarters of patients may carry a targetable mutation. The BioCAST/IFCT-1002 study is also an interesting tool for investigating biomarker profiles according to numerous risk exposures and further results are expected soon.

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