Association between lung cancer somatic mutations and occupational exposure in never-smokers

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Cite this article as: Paris C, Do P, Mastroianni B, *et al.* Association between lung cancer somatic mutations and occupational exposure in never-smokers. *Eur Respir J* 2017; 50: 1700716 [https://doi.org/10.1183/13993003.00716-2017].

ABSTRACT Occupational exposure constitutes a common risk factor for lung cancer. We observed molecular alterations in 73% of never-smokers, 35% of men and 8% of women were exposed to at least one occupational carcinogen. We report herein associations between molecular patterns and occupational exposure.

BioCAST was a cohort study of lung cancer in never-smokers that reported risk factor exposure and molecular patterns. Occupational exposure was assessed *via* a validated 71-item questionnaire. Patients were categorised into groups that were unexposed and exposed to polycyclic aromatic hydrocarbons (PAH), asbestos, silica, diesel exhaust fumes (DEF), chrome and paints. Test results were recorded for *EGFR, KRAS, HER2, BRAF* and *PIK3* mutations, and *ALK* alterations.

Overall, 313 out of 384 patients included in BioCAST were analysed. Asbestos-exposed patients displayed a significantly lower rate of *EGFR* mutations (20% *versus* 44%, p=0.033), and a higher rate of *HER2* mutations (18% *versus* 4%, p=0.084). *ALK* alterations were not associated with any occupational carcinogens. The DEF-exposed patients were diagnosed with a *BRAF* mutation in 25% of all cases. Chrome-exposed patients exhibited enhanced *HER2* and *PIK3* mutation frequency.

Given its minimal effects in the subgroups, we conclude that occupational exposure slightly affects the molecular pattern of lung cancers in never-smokers. In particular, asbestos-exposed patients have a lower chance of *EGFR* mutations.

This article has supplementary material available from erj.ersjournals.com

Received: April 05 2017 | Accepted after revision: Aug 03 2017

Clinical trial: The BioCAST study was registered at www.clinicaltrials.gov with identifier number NCT01465854.

Support statement: The BioCAST/IFCT-1002 study was supported by research grants from Astra Zeneca, Boehringer Ingelheim, Eli Lilly, Pfizer, Pierre Fabre and Roche. The funding sources had no role in the design, analysis and interpretation of the results, and thus the authors were independent of the funding source. Funding information for this article has been deposited with the Crossref Funder Registry.

Conflict of interest: Disclosures can be found alongside this article at erj.ersjournals.com

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Introduction

Exposure to occupational carcinogens constitutes a leading risk factor for lung cancer, beyond exposure to (active or passive) cigarette smoke or radon (which could also be occupational) [1–5]. Many agents used in an occupational setting are defined by the International Agency for Research on Cancer (IARC Group 1 [6]) as proven carcinogenic agents to humans. Many industries are known to present an excess risk of lung cancer, such as mining and quarrying, chemicals, asbestos production and use, metals, motor manufacturing, gas, painting, and construction [7].

Lung cancer in never-smokers is more frequent among women and Asian patients. Adenocarcinoma accounts for the vast majority. Molecular analysis shows a much higher proportion of *EGFR* and *HER2* mutations as well as *ALK* alterations compared to smokers, whereas *KRAS* mutations are less frequent in never-smokers [1, 8, 9].

The BioCAST/IFCT-1002 study was an observational, multicentre cohort, which aimed to assess exposure to several risk factors for lung cancer in lifelong never-smokers (<100 cigarettes), who were diagnosed with nonsmall cell lung cancer (NSCLC). As routine practice in France, somatic mutations in a pre-specified panel of key biomarkers were recorded [10]. The overall study results have been published, and indicated that there was definite occupational exposure in 35% of males and 8% of females [9].

Data on somatic alterations in NSCLCs regarding exposure to occupational carcinogens, or lack thereof, is scarce in the literature [2, 11–13]. We thus sought to report the molecular pattern of NSCLCs diagnosed in lifelong never-smokers from our BioCAST study, with respect to occupational carcinogens, or lack thereof.

Methods

Population

The BioCAST-IFCT1002 cohort dataset was employed, with the study design and overall results that have been previously reported [9, 14]. The BioCAST study was designed to better define the clinical, pathological and molecular epidemiology of NSCLC in lifelong never-smokers in France. This study enrolled consecutive, newly diagnosed NSCLC patients who claimed to be lifelong never-smokers (<100 cigarettes in total). Patients were surveyed using a standardised questionnaire in a scheduled phone interview with a study team member. The 17-page questionnaire requested information on demographics, occupational exposure and domestic pollution exposure, as well as personal and familial medical history, in addition to lifestyle-related and reproductive factors (women only).

This IFCT-sponsored study was conducted in 75 centres throughout metropolitan France, from November 1, 2011 to January 31, 2013. The study protocol was approved by the Sud-Est IV (Lyon, France) Ethics Committee on September 13, 2011. The Advisory Committee on Information Processing for Health Research (CCTIRS) authorised the study using a computerised database on September 8, 2011, and the National Commission for Data Protection (CNIL) was consulted on September 23, 2011. The BioCAST study was registered at www.clinicaltrials.gov, under the identifier NCT01465854.

The current analysis of the BioCAST database was restricted to those patients who both responded to the occupational questionnaire and were tested for at least one biomarker among the following: *EGFR, HER2/ERBB2, ALK, KRAS, BRAF* or *PI3K*.

Occupational exposure assessment

In our occupational exposure assessment, we considered only occupations that were practised for at least 1 year, using the 2008 edition of the International Standard Classification of Occupations (ISCO-2008) from the International Labour Organization [15] and 2008 edition of the French Classification of Activities (NAF-2008) [16], both used at their fourth levels. If the code was not fully recorded (*i.e.* not to the fourth level), we used free text in comment fields to complete it.

Occupational exposure to lung carcinogenic agents was assessed *via* a previously published 71-item questionnaire [17]. Each item inquired about exposure to a specific carcinogen and/or specific activity. The number of years and frequency of exposure (1–5 Likert scale) were recorded. A pre-specified algorithm was subsequently applied to the dataset to define a probability of exposure to each carcinogenic agent for each task the patient performed [17]. The algorithm combined the different questions relative to each carcinogen assessed in this study to provide a unique probability of exposure. Five categories were defined regarding exposure intensity for each carcinogen, namely "unexposed", "doubt about an exposure", "possible exposure", "probable exposure" and "definite exposure". Given the low number of patients, we reclassified the population into two groups, namely, unlikely to be exposed (hereinafter referred to as unexposed), covering the "unexposed", "doubt", and "possible" categories, and likely to be exposed (hereinafter referred to as exposed), combining the "probable" and "definite" categories [9].

Biomarker testing

The BioCAST study comprised a recording of molecular aberration results, using the French NCI routine lung cancer panel used during the study (2012). Thus, each participating BioCAST physician was requested to order tests systematically for somatic mutations in *EGFR* and *KRAS*, as well as the *ALK* fusion gene [18, 19]. Investigators were also encouraged to request *BRAF*, *HER2* and *PI3KCA* mutation analyses. Centres were permitted to forego further mutation testing if a mutation was found. The final, detailed results of these analyses were collected for each patient, while consulting the Catalogue of Somatic Mutations in Cancer to categorise the observed *KRAS* mutations into transversion (G>T or G>C) or transition (G>A or T>C). Given that most mutations are mutually exclusive [20] and mutations are most frequently found in lung cancer in never-smokers [9], we considered samples that exhibited no mutations and tested for at least *EGFR*, *KRAS* and *ALK* to be "wild type/unknown".

Statistical analysis

Categorical variables were presented as percentages. Proportion comparison was conducted, using the Chi-squared test if the expected count in each category was at least five, or with the Fisher's exact test if not.

We applied a binary logistic regression model to assess the risk of mutation for each considered gene. For this purpose, we generated two models: 1) unadjusted (crude odds ratios (OR)); and 2) adjusted for gender (binary), age (continuous), duration of passive smoking exposure (expressed in cumulative duration of exposure (CDE) and computed as the sum of exposure years to passive smoking by each identified index smoker, as previously reported) [21], as well as body mass index (BMI) (continuous), since a recent study found that BMI correlated significantly with mutational pattern [22]. All tests were two-sided. A p-value <0.05 was considered statistically significant. All statistics were conducted using SPSS V20 software (IBM SPSS Statistics, New York, NY, USA).

Results

Population

Of the 384 patients included in BioCAST, 313 were tested for at least one biomarker, completed the questionnaire, and were thus included in the analysis. Among them, 297 were tested for *EGFR*, 173 for *HER2/ERBB2*, 255 for *KRAS*, 195 for *BRAF*, 171 for *ALK* and 163 for *PI3K*. Additionally, 250 patients were tested for at least *EGFR, KRAS* and *ALK* (figure 1).

Primary demographic data, occupational assessment findings, biomarker results and exposure to other risk factors are reported in table 1. As expected, 83% were female, 66% were exposed to passive smoking, and 87% were affected by adenocarcinoma. The mean age was 69.5±11.5 years, with 12% being under 55 years and 16% above 80 years. Regarding occupational carcinogens, 27 patients were exposed to polycyclic aromatic hydrocarbons (PAH), 21 to asbestos, 14 to silica, eight to diesel exhaust fumes (DEF), and six to chrome and paint, respectively. Overall, 40 patients were exposed to at least one occupational carcinogen: 19 were exposed to only one carcinogen, and 21 to two or more (figure 2). A detailed list of occupations and activities of all exposed patients is presented in supplementary table S1. Moreover, 187 somatic alterations were found, primarily in *EGFR* (n=127 mutations, 43%), in addition to 18 *KRAS*, 10 *BRAF*, eight *HER2/ERBB2*, four *PI3K* mutations and 20 *ALK* alterations. Overall, 10 patients showed a double

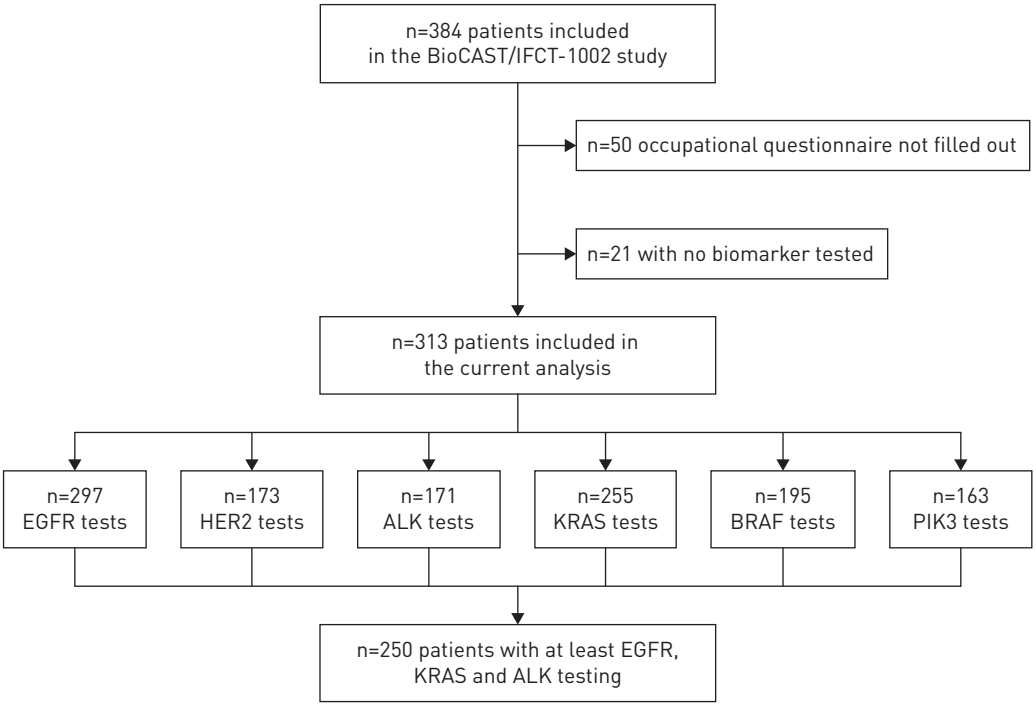

FIGURE 1 Flow chart of the current analysis.

mutation; four in the same biomarker (*EGFR*) and six in different biomarkers (1.9%) (supplementary table S2).

Somatic mutation frequency in relation to occupational exposure

Mutation frequencies of all biomarkers in relation to occupational carcinogen exposure are presented in table 2 and in figure 3 (by agent), supplementary figure S1 (by biomarker) and supplementary figure S2 (overall pattern by agent).

We observed a significantly lower *EGFR* mutation frequency in asbestos-exposed than asbestos-unexposed patients (20% *versus* 44%, respectively, p=0.033). In contrast, though marginally significant, the frequency of *HER2* mutations proved higher in asbestos-exposed patients than in unexposed patients (18% *versus* 4%, respectively, p=0.084). Whereas *KRAS* exhibited a similar frequency in both groups, *BRAF* mutations appeared slightly more frequent in asbestos-exposed patients than in unexposed patients (13% *versus* 4%, respectively; p=NS (nonsignificant)). The *ALK* alterations were found exclusively in unexposed patients.

Patients exposed to silica demonstrated a very similar molecular profile to those exposed to asbestos. Moreover, 10 patients were exposed to both carcinogens (figure 2), accounting for 48% (10 out of 21) of patients exposed to asbestos, and 71% (10 out of 14) of patients exposed to silica.

Although not statistically significant, patients exposed to DEF were diagnosed with *BRAF* mutations in 25% of cases, whereas no *HER2, KRAS*, and *PIK3* mutations, or *ALK* alterations were detected in this group. Patients exposed to chrome displayed a high frequency of *HER2* and *PIK3* mutations (33% each; p=NS), with no *KRAS* or *BRAF* mutations, or *ALK* alterations. Patients exposed to paint showed high frequencies of *KRAS* (25%) and *PI3K* mutations (33%), but no alterations in *HER2*, *BRAF* or *ALK*.

The *ALK* alterations (n=20) did not correlate with exposure to any of the agents under study, except for PAH. *ALK* rearrangement frequency was similar in PAH-exposed and unexposed patients, occurring in only one exposed patient (n=1 out of 27). The *PIK3* mutations (n=4) were higher in exposed patients, as compared to unexposed patients, irrespective of the occupational carcinogen (supplementary figure S2). Similarly, *HER2/ERBB2* mutations (n=8) were higher in patients exposed to PAH, asbestos, silica and chrome, but null in those exposed to DEF and paints (supplementary figure S1).

Final molecular diagnosis of the full dataset of 250 patients is presented in supplementary figure S2. Patients exposed to DEF, chrome and paints were all diagnosed with one mutation (no wild type), whereas cases of multiple mutations (⩾2 mutations found in different biomarkers) were found only in unexposed patients.

TABLE 1 Main demographics, exposure to risk factors, observed mutations, and definite occupational exposure findings in the analysed population

Variable	Included in the current analysis
Subjects n	313
Female sex	260 (83.1)
Age at diagnosis	69.46±11.46
Age <55 years	36 (12)
Age >80 years	50 (16)
BMI	
Underweight	14 (4.5)
Normal	164 (53.1)
Pre-obese	91 (29.4)
Obese	40 (12.9)
Education level	
High school and higher	139 (44.8)
Secondary school	69 (22.3)
Primary school or no schooling	102 (32.9)
ETS exposure "yes"	206 (66.0)
Cumulative duration of ETS exposure years	30.03±21.09
Family history of lung cancer[#]	73 (23.4)
Personal history of cancer[¶]	57 (18.2)
Patients exposed to solid fuels from heating or cooking for over 50% of his/her lifetime	62 (25.7)
Personal history of respiratory disease[+]	46 (14.7)
Personal history of respiratory infection[§]	103 (32.9)
Histology	
Squamous cell carcinoma	22 (7.0)
Adenocarcinoma	273 (87.2)
Other and NOS	18 (5.8)
TTF-1 status by immunohistochemistry	
Positive	240 (76.7)
Negative	51 (15.3)
Unknown	25 (8.0)
Mutation results	
EGFR	127 (42.8)
KRAS	18 (7.1)
ALK	20 (11.7)
BRAF	10 (5.1)
HER2	8 (4.6)
PIK3	4 (2.5)
TNM 7th edition stage	
Stage I	25 (8.1)
Stage II	21 (6.8)
Stage III	36 (11.6)
Stage IV	228 (73.5)
Exposure (definite/probable) to occupational carcinogens	
Asbestos	21 (6.7)
PAH	27 (8.6)
Chrome	6 (1.9)
DEF	8 (2.6)
Paint	6 (1.9)
Silica	14 (4.5)
Number of occupational agent exposures per patient	
0	273 (87.2)
1	19 (6.1)
2	9 (2.9)
3	4 (1.3)
4	7 (2.2)
5	1 (0.3)

Data are presented as mean±SD or n (%), unless otherwise stated. BMI: body mass index; ETS: environmental tobacco smoke; NOS: not otherwise specified; TTF-1: thyroid transcription factor-1; TNM: tumour, node, metastasis; PAH: polycyclic aromatic hydrocarbons; DEF: diesel exhaust fumes. [#]: two or more relatives affected by lung cancer; [¶]: at least one case of cancer; [+]: asthma/emphysema/chronic obstructive pulmonary disease/bronchiectasis; [§]: pertussis/tuberculosis/pneumonia.

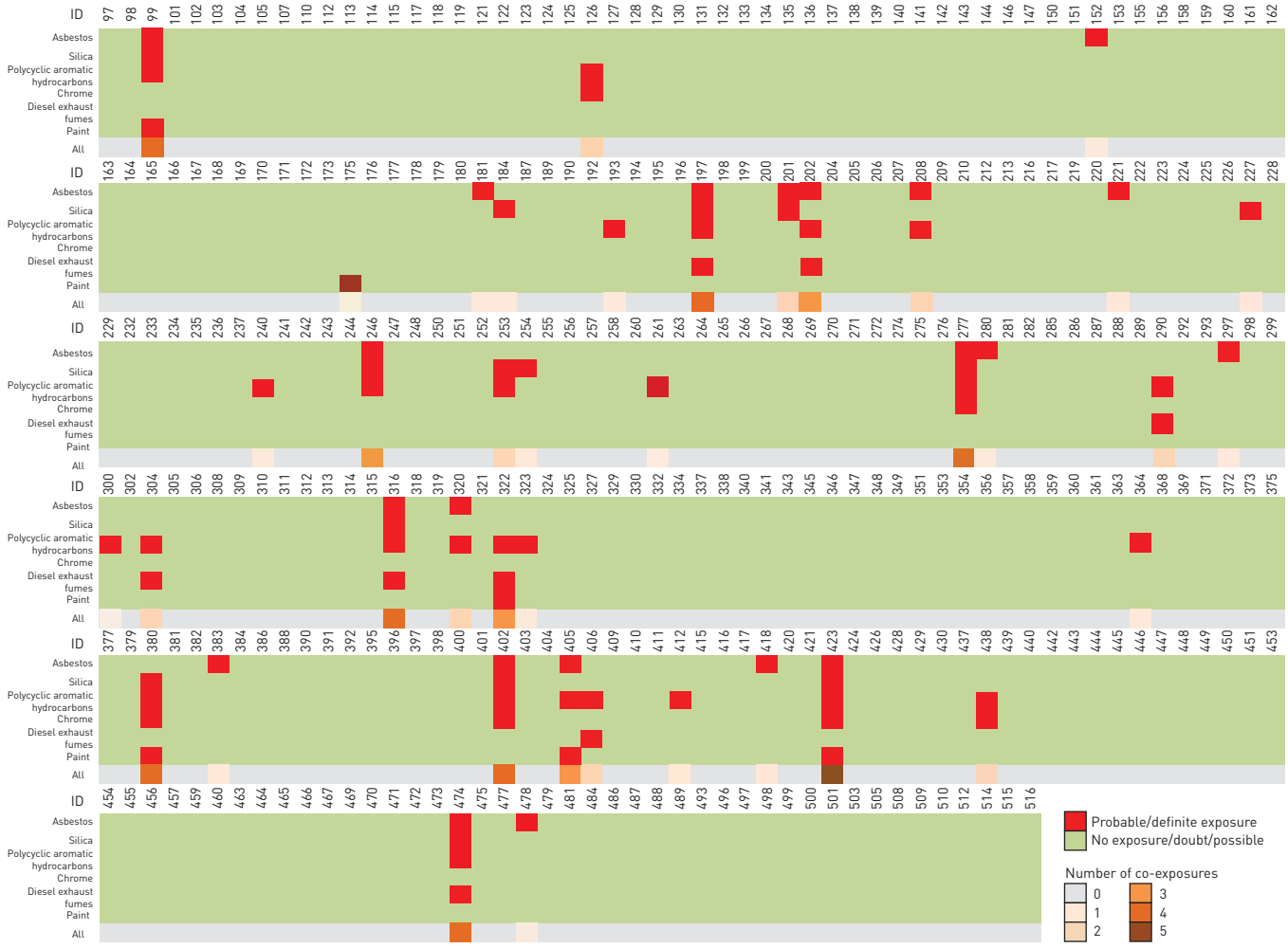

FIGURE 2 Exposure(s) to occupational carcinogens per patient.

We found that thyroid transcription factor-1 (TTF1) status was associated with *EGFR* and *KRAS*. The *EGFR* mutations occurred more frequently in TTF1 positive tumours than in TTF1 negative tumours (48.9% *versus* 15.2%, respectively, p<0.0001). In contrast, *KRAS* mutations were higher in TTF1 negative tumours compared to TTF1 positive tumours (18.6% *versus* 5.2, respectively, p=0.007) (supplementary table S3).

KRAS *and* EGFR *mutation types in relation to occupational exposure*

No significant differences were noted regarding occupational exposure in relation to distribution of transversion or transition *KRAS* mutations (data not shown). The *EGFR* mutation type patterns differed slightly in relation to occupational exposure, with a slightly lower number of exon 19 mutations in exposed patients, with the exception of DEF-exposed patients (table 3).

Logistic regression analysis

Univariate analysis results are shown in table 4. The incidence of *EGFR* mutations was significantly reduced by 69% in patients exposed to asbestos (95% CI 0.102–0.960, p=0.042); whereas the incidence of *HER2* mutations was increased by 5.8% (95% CI 1.019–32.775, p=0.048). Moreover, patients exposed to chrome exhibited a close to significant association with an increased risk of *HER2* mutations (OR 11.643, 95% CI 0.940–144.249, p=0.056). However, many odds ratios were not computable, due to small sample sizes.

Multivariate analysis did not substantially affect the value of association for most odds ratios, some of which were increased after adjustment (*EGFR*/DEF AOR=2.54 and *BRAF*/DEF AOR=11.03), although not significantly. Nevertheless, a reduction in the number of *EGFR* mutations in asbestos-exposed patients was observed (OR 0.376, p=0.099), as well as an increase in *HER2* mutations in asbestos-exposed subjects (OR 5.089, p=0.10).

TABLE 2 Exposure to occupational lung carcinogens and biomarker mutation frequency

		PAH			Asbestos			Silica			DEF			Chrome			Paint		
		Unexposed	Exposed	p-value	Unexposed	Exposed	p-value	Unexposed	Exposed	p-value	Unexposed	Exposed	p-value	Unexposed	Exposed	p-value	Unexposed	Exposed	p-value
EGFR[¶]	WT	157 (57)	13 (57)	0.942	154 (56)	16 (80)	0.033	161 (57)	9 (69)	0.371	167 (57)	3 (50)	0.703[#]	167 (57)	3 (60)	1.0[#]	167 (57)	3 (60)	1.0[#]
	Mt	117 (43)	10 (43)		123 (44)	4 (20)		123 (43)	4 (31)		124 (43)	3 (50)		125 (43)	2 (40)		125 (43)	2 (40)	
HER2[+]	WT	155 (96)	10 (91)	0.415	1 56 (96)	9 (82)	0.084[#]	160 (96)	5 (83)	0.251	162 (95)	3 (100)	1.0[#]	163 (96)	2 (67)	0.133[#]	164 (95)	1 (100)	1.0[#]
	Mt	7 (4)	1 (9)		6 (4)	2 (18)		7 (4)	1 (17)		8 (5)	0 (0)		7 (4)	1 (33)		8 (5)	0 (0)	
KRAS[§]	WT	219 (92)	18 (100)	0.624[#]	221 (93)	16 (94)	1.0[#]	226 (93)	11 (92)	0.593[#]	233 (93)	4 (100)	1.0[#]	232 (93)	5 (100)	1.0[#]	234 (93)	3 (75)	0.255[#]
	Mt	18 (8)	0 (0)		17 (7)	1 (6)		17 (7)	1 (8)		18 (7)	0 (0)		18 (7)	0 (0)		17 (7)	1 (25)	
BRAF[f]	WT	170 (95)	15 (94)	0.584[#]	172 (96)	13 (87)	0.174[#]	175 (95)	10 (91)	0.448[#]	182 (95)	3 (75)	0.191[#]	181 (95)	4 (100)	1.0[#]	183 (95)	2 (100)	1.0[#]
	Mt	9 (5)	1 (6)		8 (4)	2 (13)		9 (5)	1 (9)		9 (5)	1 (25)		10 (5)	0 (0)		10 (5)	0 (0)	
ALK[##]	WT	142 (88)	9 (90)	1.0[#]	142 (88)	9 (100)	0.601[#]	143 (88)	8 (100)	0.598[#]	148 (88)	3 (100)	1.0[#]	149 (88)	2 (100)	1.0[#]	150 (88)	1 (100)	1.0[#]
	Al	19 (12)	1 (10)		20 (12)	0 (0)		20 (12)	0 (0)		20 (12)	0 (0)		20 (12)	0 (0)		20 (12)	0 (0)	
PI3K[¶¶]	WT	146 (98)	13 (93)	0.304[#]	149 (98)	10 (91)	0.246[#]	154 (98)	5 (83)	0.141[#]	155 (97)	4 (100)	1.0[#]	157 (98)	2 (67)	0.072[#]	157 (98)	2 (67)	0.072[#]
	Mt	3 (2)	1 (7)		3 (2)	1 (9)		3 (2)	1 (17)		4 (3)	0 (0)		3 (2%)	1 (33)		3 (2%)	1 (33)	

Data are presented as n (%), unless otherwise stated. PAH: polycyclic aromatic hydrocarbons; DEF: diesel exhaust fumes; WT: wild type; Mt: mutation; Al: alteration.
[#]: two-sided Fisher tests, others are two-sided Chi-squared tests; [¶]: n=297; [+]: n=173; [§]: n=255; [f]: n=195; [##]: n=171; [¶¶]: n=163.

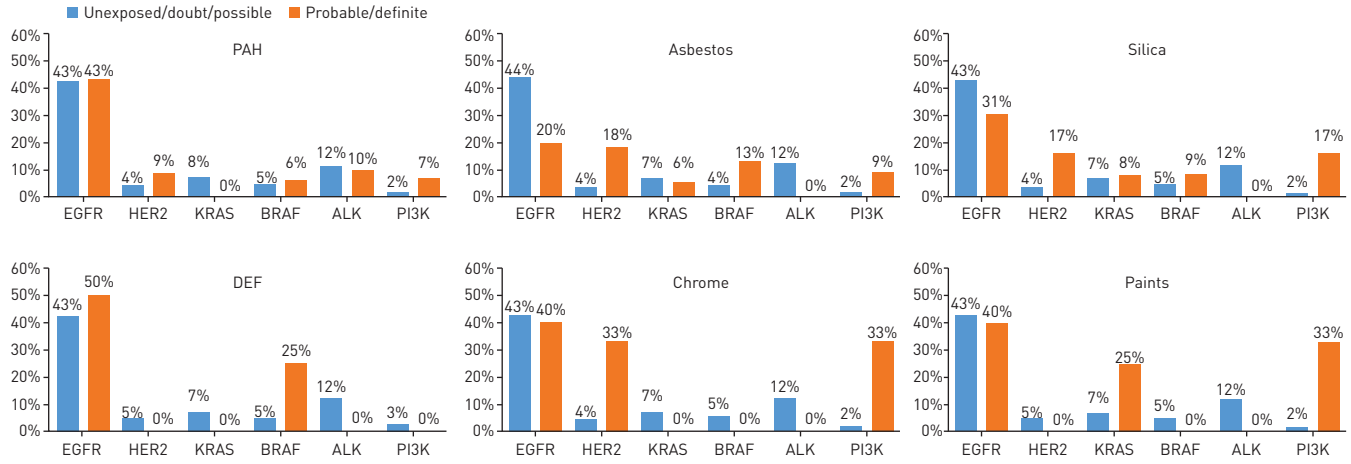

FIGURE 3 Biomarker mutation frequency in relation to exposure to occupational lung carcinogens: exposure to polycyclic aromatic hydrocarbons (PAH), asbestos, silica, diesel exhaust fumes (DEF), chrome and paints.

Discussion

We demonstrated the mutational pattern of NSCLCs in never-smokers to be slightly associated with occupational exposure. More specifically, exposure to asbestos appeared to be correlated with a lower frequency of *EGFR* mutations (20% *versus* 44% in the unexposed) and a higher frequency of *HER2/ERBB2* mutations. Exposure to primary occupational carcinogens suggests a particular distribution of *KRAS, BRAF, PI3K* and *ALK* alterations.

Only a few studies have dealt with the molecular profile of lung adenocarcinomas in relation to asbestos exposure. Andujar *et al.* [13] performed a comparative study of 50 cases of asbestos-exposed NSCLC and 50 unexposed cases. Overall, the authors observed a higher *EGFR* mutation rate in unexposed patients (12% *versus* 4% in exposed patients; p=ns). In never-smokers, the respective proportions were 50% and 14% (p=ns). In addition, *KRAS* mutations were found in 10% and 16% of exposed and unexposed cases, respectively (0% and 17% for never-smokers). In a cohort of 510 Finnish NSCLC patients, Mäki-Nevala *et al.* [11] observed no differences in terms of *EGFR* mutations between 46 asbestos-exposed and 198 unexposed patients (10.6% *versus* 9.6%, respectively). The same team performed exome sequencing of lung adenocarcinoma (n=26, nine of which were asbestos-exposed) [12]. In the adenocarcinoma cases, 42% exhibited a *KRAS* mutation in both exposed and unexposed patients, with no activating *EGFR* mutation observed. Unlike our findings, only two *BRAF* mutations were found in that study, both of which were observed in unexposed patients. In a recent paper, the same team reported no association between asbestos

TABLE 3 Distribution of EGFR mutation type (substitution in exon 21/deletion in exon 19/other or NOS) in relation to occupational exposure

Agent	Exposure	EGFR mutation type				Total
		Substitution in Exon 21[#]	Deletion in Exon 19[¶]	p-value exon 21 *versus* 19 (Fisher)	Other/NOS	
Asbestos	Unexposed	35 (29.7)	72 (61.0)	0.6	11 (9.3)	118 (100)
	Exposed	2 (50.0)	2 (50.0)		0 (0.0)	4 (100)
PAH	Unexposed	33 (29.2)	70 (61.9)	0.437	10 (8.8)	113 (100)
	Exposed	4 (44.4)	4 (44.4)		1 (11.1)	9 (100)
Chrome	Unexposed	36 (30.0)	74 (61.7)	0.333	10 (8.3)	120 (100)
	Exposed	1 (50.0)	0 (0.0)		1 (50.0)	2 (100)
Diesel	Unexposed	36 (30.3)	72 (60.5)	1.0	11 (9.2)	119 (100)
	Exposed	1 (33.3)	2 (66.7)		0 (0.0)	3 (100)
Paints	Unexposed	35 (29.2)	74 (61.7)	0.109	11 (9.2)	120 (100)
	Exposed	2 (100.0)	0 (0.0)		0 (0.0)	2 (100)
Silica	Unexposed	36 (30.5)	72 (61.0)	1.0	10 (8.5)	118 (100)
	Exposed	1 (25.0)	2 (50.0)		1 (25.0)	4 (100)

Data are presented as n (%), unless otherwise stated. NOS: not otherwise specified; PAH: polycyclic aromatic hydrocarbons. [#]: L858R/L861Q/P848L/Exon 21 subst. NOS; [¶]: Del 19/Exon 19 NOS.

TABLE 4 Binary logistic regression analysis in univariate and adjusted models for biomarker mutation occurrence in relation to occupational exposure (binary variable)[#]

Biomarker	Occupational exposure	Crude OR	(95% CI)	p-value	AOR[¶]	(Lower 95% CI)	p-value
EGFR	PAH	1.032	(0.437–2.436)	0.942	1.340	(0.533–3.369)	0.533
	Asbestos	0.313	(0.102–0.960)	0.042	0.376	(0.118–1.201)	0.099
	Silica	0.582	(0.175–1.933)	0.377	0.703	(0.197–2.515)	0.588
	DEF	1.347	(0.267–6.786)	0.718	2.541	(0.392–16.463)	0.328
	Chrome	0.891	(0.147–5.411)	0.900	1.011	(0.156–6.536)	0.991
	Paint	0.891	(0.147–5.411)	0.900	0.875	(0.141–5.421)	0.886
HER2	PAH	2.214	(0.248–19.799)	0.477	1.644	(0.153–17.656)	0.681
	Asbestos	5.778	(1.019–32.775)	0.048	5.089	(0.732–35.395)	0.100
	Silica	4.571	(0.469–44.538)	0.191	3.297	(0.239–45.395)	0.373
	Chrome	11.643	(0.940–144.249)	0.056	7.779	(0.488–124.078)	0.147
KRAS	Asbestos	0.813	(0.102–6.501)	0.845	0.867	(0.102–7.354)	0.896
	Silica	1.209	(0.147–9.925)	0.860	1.363	(0.144–12.871)	0.787
	Paint	4.588	(0.453–46.507)	0.197	5.072	(0.452–56.942)	0.188
BRAF	PAH	1.259	(0.149–10.622)	0.832	1.136	(0.116–11.116)	0.913
	Asbestos	3.308	(0.636–17.203)	0.155	3.411	(0.565–20.595)	0.181
	Silica	1.944	(0.224–16.895)	0.547	1.697	(0.153–18.855)	0.667
	DEF	6.741	(0.636–71.395)	0.113	11.103	(0.507–243.270)	0.126
ALK	PAH	0.830	(0.100–6.923)	0.864		NC	

AOR: adjusted odds ratio; PAH: polycyclic aromatic hydrocarbons; DEF: diesel exhaust fumes; NC: not computable. [#]: for all categories, the unexposed patient group is the reference (not shown). [¶]: adjusted for sex (binary), age at diagnosis (continuous), cumulative duration (years) of passive smoking exposure (continuous) and body mass index (continuous). Others are not computable.

exposure and mutation patterns (for *EGFR, KRAS, BRAF, ALK, HER2, PI3K* and others like, *MET, TP53, PTEN* or *NRAS*) in 425 Finnish NSCLC patients, including 8.9% never-smokers and 29 subjects (6.8%) exposed to asbestos [23]. In another study conducted in the USA, 84 male patients (95% of whom were smokers) with NSCLC, who were also subjected to asbestos exposure, exhibited a higher frequency of *KRAS* mutations (crude OR 4.8, 95% CI 1.5–15.4) [24]. No association was established however, between *KRAS* mutations and asbestos exposure in 105 NSCLC patients (all of whom were smokers) [25]. The *ALK* alterations were investigated in relation to asbestos exposure in only one malignant mesothelioma (MM) cohort (n=63), with none of the samples exhibiting *ALK* alteration [26]. Overall, these data highlight the scarcity of literature regarding mutational patterns of driver oncogenes in relation to occupational exposure. Whereas the study of Andujar *et al.* [13] reported similar results to the present study concerning *EGFR* mutations and asbestos exposure, other articles reported no such correlation in a similar setting. These studies nonetheless, used relatively small samples, and were not conducted specifically in never-smokers. For these reasons, active smoking status is possibly too strong a factor to determine differences in *EGFR* mutation frequency.

Our study has some limitations. The main limitation is that of the very low number of subjects in some subgroups. The number of patients exposed to certain occupational carcinogens was therefore very small (ranging from 27 exposed to PAH, to six each exposed to paints and chrome). In addition, the number of alterations observed in this cohort proved to be very small for some driver genes (ranging from 20 *ALK* alterations to four *PI3K* mutations, with the exclusion of *EGFR* mutations). Altogether, we obtained some very small subgroups, such as four *EGFR* mutations in asbestos-exposed patients and three *BRAF* wild type in DEF-exposed patients (see table 2). The interpretation of patterns by carcinogen (or biomarker) must therefore be done with caution. Moreover, the majority of patients (21 out of 40, 53%) had been affected by simultaneous exposure to at least two occupational carcinogens (ranging from two to five, table 1 and figure 2), and it is therefore difficult to differentiate the role of each occupational exposure to carcinogens. Although nonsignificant, multivariate models adjusted for age, gender, BMI and passive smoking did not affect the trends observed in our primary results. Thus, our findings seem to be independent of these adjusting factors. Another limitation arose from the definition of occupational exposure that was based exclusively on a self-administered questionnaire, without biological evidence, and without measured metrological data of such exposure. Nonetheless, mineralogical analyses are not required for MM management [27, 28]. A third limitation of the current analysis is the lack of consideration of the exposure to environmental radon, which is a leading risk factor of lung cancer, and which might be associated with *EGFR* and *ALK* molecular patterns [29]. Another limitation arose from the heterogeneity of assays and techniques used for biomarker assessment. Indeed, each platform is itself able to determine which

panel (although a minimal is expected), and which assay is to be used. The IFCT-ERMETIC study [19] was conducted to investigate the accuracy of *EGFR* and *KRAS* mutation detection in 15 platforms. That study found a favourable agreement between centres, underlining the accuracy of such analysis [19]. In addition, as clinicians were allowed to forego biomarker testing if one mutation was found, this might have introduced a selection bias in the case of multiple mutations. However, multiple mutations in lung cancer are uncommon. The IFCT-Biomarker France study (n=17 664 patients), found only 1% of patients with multiple mutations. Patients with multiple alterations were more likely to be never-smokers [30]. In the American Lung Cancer Mutation Consortium Experience, the rate of multiple mutations appeared to be slightly higher (2.9% among 1007 specimens), but the panel used was the widest in comparison to other studies [31]. In the current analysis, we found an intermediate rate of 1.9%, which illustrates that selection bias was probably not a major factor. Finally, the design of our study might have also generated selection bias. It is possible that some physicians were more alert to track the never-smoker status in particular settings, such as with younger subjects or women. However, our overall results were comparable to those in the literature within a similar setting [9]. In addition, our study is a case-only single cohort study without comparison to smokers, and without an independent cohort to validate our findings. Our study also exhibits certain strengths. To the best of our knowledge, this is the only cohort to address six driver oncogenes and six occupational carcinogens simultaneously in a unique cohort of lifelong never-smokers with NSCLC. We therefore report fully comprehensive findings. In particular, smoking did not interfere as a confounder, unlike most studies addressing occupational lung cancers. In addition, we used a standardised questionnaire, delivered during a phone interview by dedicated staff, to limit redaction bias, as well as memorisation bias, and to minimise the occurrence of missing values. Finally, we used an internationally recognised definition of never-smoker to avoid contamination bias [9].

Whereas exposure to passive smoking does not appear to affect the molecular pattern in a French never-smoker cohort [21], occupational exposure seems to be slightly associated with specific patterns. In particular, *EGFR* and *HER2/ERBB2* appear to have opposite levels of association with asbestos exposure; although these findings were limited by the small sample size. Such results highlight the crucial step of assessing occupational exposure in lung cancer patients, especially in male never-smokers [9]. These original results could contribute to the formulation of hypotheses to design further studies and have a better understanding of the oncogenic pathways driven by occupational carcinogens.

Acknowledgements

Collaborators to the BioCAST/IFCT-1002 study: Pierre-Jean Souquet, HCL, Hôpital Lyon Sud, Lyon; Radj Gervais, Centre François Baclesse, Caen; Hélène Doubre, Hôpital Foch, Suresnes; Eric Pichon, CHU de Tours; Adrien Dixmier, CH d'Orléans; Isabelle Monnet, CHI de Créteil; Bénédicte Mastroianni, Hcl, Hôpital Louis Pradel, Lyon; Michel Vincent, Hôpital Saint-Joseph, Lyon; Jean Tredaniel, Hôpital Saint Joseph, Paris; Marielle Perrichon, CH de Bourg-En-Bresse; Pascal Foucher, CHU Bocage, Dijon; Bruno Coudert, Centre Georges-François Leclerc, Dijon; Denis Moro-Sibilot, CHU de Grenoble; Eric Dansin, Centre Oscar Lambret, Lille; Patrick Dumont, CH de Chauny; Lionel Moreau, CH de Colmar; Didier Debieuvre, CH de Mulhouse; Jacques Margery, HIA de Percy, Clamart; Élisabeth Quoix, CHU de Strasbourg, Nouvel Hôpital Civil; Bernard Duvert, CH de Montélimar; Laurent Cellerin, CHU de Nantes, Hôpital Nord Laennec; Nathalie Baize, CHU d'Angers; Bruno Taviot, CM Nicolas de Pontoux, Chalon-Sur-Saône; Marie Coudurier, CH Chambéry; Jacques Cadranel, AP-HP, Hôpital Tenon, Paris; Patrick Chatellain, CH d'Annemasse; Jérôme Virally, CHI d'Aulnay-Sous-Bois; Virginie Westeel, CHU de Besançon; Sylvie Labrune, Ap-Hp, Hôpital Ambroise Paré, Boulogne; Laureline Le Maignan de Kerangat, CHG Le Mans; Jean-Marc Dot, HIA Desgenettes, Lyon; Sébastien Larive, CH de Mâcon; Christos Chouaid, Ap-Hp, Hôpital Saint-Antoine, Paris; Daniel Coëtmeur, CHG de Saint-Brieuc; Clarisse Audigier-Valette, CHI de Toulon; Jean-Pierre Gury, CHI de Vesoul; Luc Odier, CH de Villefranche Sur Saône; Tsellina Desfemmes-Baleyte, CHU de Caen; Yannick Duval, CH de Cannes; Patrick Merle, CHU de Clermont-Ferrand; Gilles Devouassoux, Hcl, Hôpital de La Croix Rousse, Lyon; Reza Azarian, CH de Versailles; Patricia Barre, CH de Cahors; Olivier Raffy, CH de Chartres; Philippe Masson, CH de Cholet; Stéphanie Dehette, CH de Compiègne; Caroline Toussaint Batbedat, CH de Lagny-Sur-Marne; Gérard Oliviero, CH de Longjumeau; Marc Derollez, Polyclinique du Parc, Maubeuge; Nadine Paillot, CHR de Metz; Jérôme Dauba, CH de Mont de Marsan; Dominique Herman, CH de Nevers; Jean-Michel Rodier, Ap-Hp, Hôpital Bichat, Paris; Suzanna Bota, CHU de Rouen; Philippe Brun, CH de Valence; Geneviève Letanche, Clinique de Vénissieux; Mohamed Khomsi, CH d'Annonay; Béatrice Gentil-Lepecq, CH de Bourgoin-Jallieu; Philippe Ravier, Cabinet de Pneumologie, Dijon; Yassine Hammou, Clinique Mutualiste, Lyon; Fabrice Barlesi, AP-HM, Hôpital Nord, Marseille; Hélène Laize, CH de Rambouillet; Pierre Fournel, Institut de Cancérologie de La Loire, Saint-Priest En Jarez; Christelle Clement-Duchene, CHU de Nancy, Vandoeuvre-Les-Nancy; Joël Castelli, CHD Castelluccio, Ajaccio; Sophie Schneider, CH de Bayonne; Antoine Levy, CH Jacques Cœur, Bourges; Jérôme Dauba, CH de Dax; Geneviève Jolimoy, Centre d'Oncologie et de Radiothérapie du Parc, Dijon; Hervé Pegliasco, Fondation Hôpital Ambroise Paré, Marseille; Michel Poudenx, Centre Antoine Lacassagne, Nice; Alain Prevost, Institut Jean-Godinot, Reims; Philippe Romand, CH de Thonon-Les-Bains; Laurence Bigay-Game, CHU de Toulouse; Etienne Suc, Clinique St Jean Languedoc, Toulouse.

CH, CHG, CHI: secondary public hospital; CHU: University Hospital; CHR: Primary Hospital; HIA: Army Hospital; CM: Private hospital; HCL: Hospices Civils de Lyon (Lyon University Hospital); AP-HP: Assistance Publique – Hôpitaux de Paris (Paris University Hospital); AP-HM: Assistance Publique – Hôpitaux de Marseille (Marseille University Hospital).

Authors thank William Lebossé and Stéphanie Labonne, who performed interviews with patients; Quan Tran and Antoine Deroy (Data Manager, IFCT); Pascale Missy (IFCT), who provided administrative support; Gabrielle Cremer

for expert English rewording; the French League Against Cancer; and all investigators in the 75 BioCAST participating centres; the patients and their families, who greatly contributed to this work by giving their time to prepare the questionnaire and participate in the interview.

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