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Prognostic value of *TP53*, *KRAS* and *EGFR* mutations in nonsmall cell lung cancer: the EUELC cohort

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ABSTRACT: Nonsmall cell lung cancer samples from the European Early Lung Cancer biobank were analysed to assess the prognostic significance of mutations in the *TP53*, *KRAS* and *EGFR* genes.

The series included 11 never-smokers, 86 former smokers, 152 current smokers and one patient without informed smoking status. There were 110 squamous cell carcinomas (SCCs), 133 adenocarcinomas (ADCs) and seven large cell carcinomas or mixed histologies. Expression of p53 was analysed by immunohistochemistry. DNA was extracted from frozen tumour tissues.

***TP53* mutations were detected in 48.8% of cases and were more frequent among SCCs than ADCs ($p<0.0001$). *TP53* mutation status was not associated with prognosis. G to T transversions, known to be associated with smoking, were marginally more common among patients who developed a second primary lung cancer or recurrence/metastasis (progressive disease). *EGFR* mutations were almost exclusively found in never-smoking females ($p=0.0067$). *KRAS* mutations were detected in 18.5% of cases, mainly ADC ($p<0.0001$), and showed a tendency toward association with progressive disease status.**

These results suggest that mutations are good markers of different aetiologies and histopathological forms of lung cancers but have little prognostic value, with the exception of *KRAS* mutation, which may have a prognostic value in ADC.

KEYWORDS: *EGFR*, *KRAS*, mutations, nonsmall cell lung cancer, prognosis, *TP53*

The development of nonsmall cell lung cancers (NSCLCs) is accompanied by multiple genetic and epigenetic alterations, with some differences according to aetiology and histological type [1, 2]. A survey of 139 NSCLC cell lines has identified a panel of frequently mutated genes that may be useful for NSCLC stratification on the basis of activating mutations. These genes include *KRAS, EGFR, ALK, MET, PDGFR, ROS, ERBB2, BRAF PI3K* and *MEK1* [3]. The most commonly mutated of these genes are *EGFR* and *KRAS* (the latter mostly in adenocarcinoma (ADC), and being mutually exclusive). *EGFR* encodes a transmembrane receptor for epidermal growth factor and related ligands, which contains an intracellular tyrosine kinase domain. Mutations are found almost exclusively in lung cancers of never-smokers, and cluster in the kinase domain and constitutively activate its activity and signal transduction. *KRAS* encodes a GTP/GDP exchange factor acting as a downstream effector of EGFR signalling that mediates the activation of growth promoting signalling cascades of kinases. Mutations mostly fall at codon 12, located in the GTP binding pocket, and prevent GTP hydrolysis.

In addition to these activating mutations, inactivating mutations in *TP53* are detected in the majority of NSCLCs. *TP53* encodes an all-round tumour suppressor transcription factor, p53, which mediates multiple anti-proliferative effects in response to a variety of stresses, including, in particular, DNA damage. Most known mutations fall within the DNA-binding domain and deactivate the suppressor by preventing DNA binding and transactivation. There is evidence that *TP53* or *KRAS* transversion mutations in NSCLC

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of smokers occur prevalently at G bases and are commonly the sites of adduct formation by metabolites of polycyclic aromatic hydrocarbons, one of the main family of tobacco carcinogens [4–6]. These observations suggest that at least some of these mutations may occur as the consequence of exposure to tobacco smoke and precede the development of cancer, therefore having an impact on molecular and biological patterns of lung carcinogenesis. However, the impact of these mutations on clinical prognosis remains a matter of debate.

The purpose of our study was to investigate the prognosis impact of mutations in *TP53*, *KRAS* or *EGFR* in resected, early-stage NSCLC and to evaluate their use as biomarkers of disease progression. We took advantage of the European Early Lung Cancer (EUELC) project and biobank [7, 8] to select a group of patients with good-quality frozen tissues. EUELC patients were recruited from 12 centres in eight European countries and were followed for 6 months after surgery. We show that *TP53* and *EGFR* mutations, although common in these cancers, have limited, if any, prognostic value, whereas *KRAS* mutations could be associated with progressive disease (PD) status.

MATERIALS AND METHODS

Study subjects and tumours

The EUELC project is a collaboration involving 12 centres in France, Germany, Ireland, Italy, the Netherlands, Poland, Spain and the UK. This study recruited 762 patients with surgically resected primary lung cancers who were considered at very high risk of developing second primary lung cancers (SPLCs) and/or metastasis in relation to occupational or lifestyle risk factors. Among those, 739 were evaluated for disease progression and were followed up at 6-month intervals for up to 48 months (median 29 months). All patients completed a lifestyle and medical questionnaire at each follow-up visit. Patients with a history of a completely resected primary lung or head and neck cancer who developed a SPLC, either a recurrence or metastasis, or who died of the disease were grouped as PD. Patients who were alive and asymptomatic for the disease and who were not undergoing treatment by chemotherapy and/or radiotherapy at the time of the last follow-up were classified as disease-free (DF). Data on smoking and occupation were collected using a standardised lifestyle questionnaire. Instructions for interviewing and coding were developed, and training of research interviewers was carried out in each centre. All questionnaires were translated to ensure consistency across European Union partners.

A total of 306 samples were available for p53 detection by immunohistochemistry (fig. 1). 273 frozen tissues were found to be suitable for DNA extraction and mutation analysis. 250 patients with known follow-up status were finally selected for statistical analysis of *TP53* and *KRAS*. The series included 11 never-smokers (<100 cigarettes smoked in a lifetime), 86 former smokers (smoking cessation ⩾2 yrs before diagnosis), 152 current smokers (still smoking or <2 yrs since cessation) and one patient without known smoking status. There were 110 squamous cell carcinomas (SCCs), 133 ADCs and seven recorded as ''other histologies'' (large cell carcinoma or mixed histologies). *EGFR* mutations were analysed in 130 ADCs based on earlier reports that this gene is rarely mutated in types of NSCLC other than ADC and in smokers [9].

Mutation analysis

DNA previously extracted from frozen tissue was received and analysed for *TP53* (exons 4–10 including flanking splice sites) mutations by pre-screening with denaturing high-pressure liquid chromatography (dHPLC) followed by a second PCR and bi-directional automated sequencing as described elsewhere [10]. Specimens with matched dHPLC and sequencing results were considered to contain a mutation. *KRAS* mutations at codon 12 were analysed by mutant-enriched PCR as described elsewhere [10], allowing enrichment of the mutant sequence, and were sequenced.

EGFR mutations were detected using PCR-based direct sequencing of the four exons of the tyrosine kinase domain (exons 18–21) using primers and annealing conditions as described elsewhere [11].

Immunohistochemistry for p53 was performed as detailed previously [12] using the Ventana automated immunostainer (Ventana Corp., Tucson, AZ, USA) with specified procedures and reagents. Percentage of stained tumour cells was evaluated on a scale of 0–4 (0, absent; 1, <10%; 2, 10–50%; 3, 50–90%; and 4, >90%). Intensity of staining was assessed on a scale from 0 (absent) to 3 (marked). The results for percentage and intensity were summed up to generate a composite score as follows: sum of 0, no staining (score 0); sum of 1–3, slight staining (score 1); sum of 4–5, moderate staining (score 2); and sum of 6–7, marked staining (score 3).

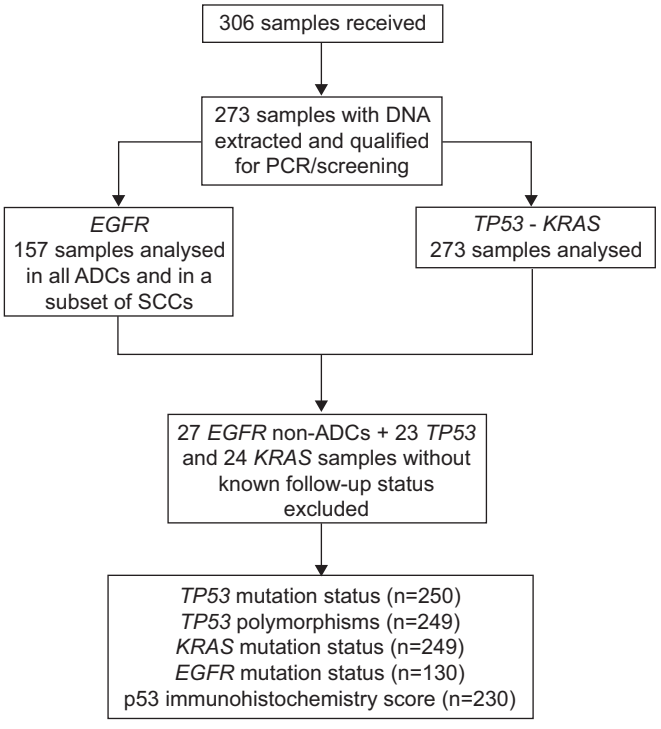

FIGURE 1. Flow chart of sample selection for mutational analysis. The initial number of tumour samples qualified for the study is indicated and the number of samples analysed for *TP53*, *EGFR* and *KRAS* is given. ADC: adenocarcinoma; SCC: squamous cell carcinomas.

TABLE 1 Characteristics of selected patients included for statistical analysis

Variable	Patients n
Sex	
Male	210
Female	40
Age yrs	
<60	89
60–65	82
65–70	30
⩾70	49
Education level	
No/primary level	181
Higher education	59
Missing	10
Histology	
ADC	133
SCC	110
Others	7
Asbestos exposure	
None	191
Yes	57
Missing	2
pT	
T1	76
T2	150
T3	15
T4	8
Missing	1
pN	
N0	173
N1	65
N2	2
NX	9
Missing	1
Past pulmonary illness	
No	110
Yes	138
Missing	2
Smoking status#	
Current smoker	152
Former smoker	86
Never-smoker	11
Missing	1
Total	250

ADC: adenocarcinoma; SCC: squamous cell carcinoma; pT: pathological tumour score; pN: pathological node score. #: former smokers were patients who had quit smoking at least 2 yrs before interview and current smokers were patients who were smokers in the last 2 yrs before interview.

TABLE 2 Single and multiple mutation prevalence in EUELC patients

Gene	Analysed samples n	Status	Patients n (%)
TP53	250	Wild-type	129 (51.6)
		Mutant (exons 4-9)	121 (48.4)
KRAS	249	Wild-type	203 (81.5)
		Mutant (codon 12)	46 (18.5)
		TP53 wild-type	35 (76.1)
		TP53 mutant	11 (23.9)
EGFR#	130	Wild-type	113 (86.9)
		Mutant	17 (13.1)
		TP53 wild-type	11 (64.7)¶
		TP53 mutant	6 (35.3)¶
		KRAS wild-type	16 (95)¶
		KRAS mutant	1 (5)¶

#: the group of cases analysed includes only adenocarcinoma; ¶: where 17 is 100%.

Statistical analysis

The Mantel–Haenszel Chi-squared test was used to test the association between clinical parameters and biomarkers, and also between biomarkers. The Fine and Gray model [13] was used to measure association between clinical variables and biomarkers with cancer progression. The model takes into account the presence of competing risks which in our study are patients who died from causes other than lung cancer. Hazard ratios (HRs) in the Fine and Gray model can be interpreted in the same way as relative risks. Bootstrapping was performed to obtain nonparametric confidence intervals for risk estimates. According to the distribution of follow-up duration, we censored the analysis at 48 months. Each biomarker was assessed one at a time in a multivariate model adjusted with the clinical variables significantly associated to the disease progression risk in the univariate analysis. Cumulative incidence plots were performed to illustrate the risk of disease progression through time according to the mutation status of the genes.

Standard survival analysis was performed using the Cox proportional hazard model to assess association between overall death, lung cancer-specific death and biomarkers. Adjustment on clinical parameters associated with death was performed. All the analyses were stratified by centre. All statistical analyses were performed using SAS statistical software, version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Patients, mutation prevalence and associations with individual and pathological parameters

Selected characteristics of patients are shown in table 1 and mutation prevalence is shown in table 2. A total of 48.4% *TP53* mutations, including five silent mutations, were detected. *KRAS* mutations at codon 12 and *EGFR* mutations were detected in 18.5% and in 13.1% of samples, respectively. 18 patients had mutations in two genes (table 2), including 11 patients with both *TP53* and *KRAS* mutations and six patients with both *TP53* and *EGFR* mutations. One patient with *KRAS* mutation also had an *EGFR* silent mutation in exon 21 (codon 836, CGC>CGT Arg>Arg). No patient had mutations in all three genes.

The patterns of mutations in *TP53* are shown in online supplementary figure S1. *TP53* mutations and the codon distribution were in agreement with the known smoking patterns [4], with ~33% of G>C to T>A transversions and hotspots at codons

TABLE 3 Associations between mutations, patients' variables and clinical parameters

Variable[#]	Characteristics	Wild-type	Mutated	p-value[¶]
***TP53*[+]**				
Histology	ADC	85 (65.9)	48 (39.7)	**<0.0001**
	SCC/others	44 (31.8)	73 (57)	
pT (1)	T1	39 (30.5)	37 (30.6)	0.9810
	T2	76 (59.4)	74 (61.2)	
	T3	8 (6.3)	7 (5.8)	
	T4	5 (3.9)	3 (2.5)	
pN (1)	N0	89 (69.5)	84 (69.4)	0.3696
	N1	30 (23.4)	35 (28.9)	
	N2	2 (1.6)	0	
	Nx	7 (5.5)	2 (1.7)	
Smoking status (1)[§]	Current smoker	77 (60.2)	75 (62)	0.8287
	Former smoker	44 (34.4)	42 (34.7)	
	Never-smoker	7 (5.5)	4 (3.3)	
Asbestos exposure (2)	None	100 (78.7)	91 (75.2)	0.8007
	Yes	27 (21.3)	30 (24.8)	
***KRAS*[f]**				
Histology	ADC	93 (45.8)	41 (89.1)	**<0.0001**
	SCC/others	110 (54.2)	5 (10.9)	
pT (1)	T1	68 (33.7)	9 (19.6)	0.10
	T2, T3, T4	134 (66.3)	37 (80.4)	
pN (1)	N0	140 (69.3)	33 (71.7)	0.60
	N1, N2, Nx	62 (30.7)	13 (28.3)	
Smoking status (1)[§]	Current smoker	128 (63.4)	24 (52.2)	0.08
	Former smoker	64 (31.7)	21 (45.7)	
	Never-smoker	10 (5)	1 (2.2)	
Asbestos exposure	None	154 (76.2)	36 (80)	0.82
	Yes	48 (23.8)	9 (20)	
***EGFR*[##]**				
Sex	Male	92 (81.4)	11 (64.7)	0.35
	Female	21 (18.6)	6 (35.3)	
Smoking status[§]	Current smoker	64 (56.6)	8 (47.1)	0.11
	Former smoker	43 (38.1)	5 (29.4)	
	Never-smoker	6 (5.3)	4 (23.5)	
Sex/smoking status	Others	109 (96.5)	13 (76.5)	**0.0067**
	Never-smoking females	4 (3.5)	4 (23.5)	
Pack-yrs (1)	⩽40	61 (54.5)	13 (76.5)	0.15
	>40	51 (45.5)	4 (23.5)	

Data are presented as n (%), unless otherwise stated. pT: pathological tumour score; pN: pathological node score; ADC: adenocarcinoma; SCC: squamous cell carcinoma. [#]: values (1) and (2) are the number of patients with missing data for that variable; [¶]: Mantel–Haenszel test controlling for centre; [+]: wild-type n=129, mutated n=121; [§]: former smokers were patients who had quit smoking at least 2 yrs before interview and current smokers were patients who were smokers in the last 2 yrs before interview; [f]: wild-type n=203, mutated n=46; [##]: wild-type n=113, mutated n=17. p-values in bold are significant.

157 and 158. Mutations in *EGFR* were spread among the four exons tested (4% in exon 18, 3% in exon 19, 3% in exon 20 and 5% in exon 21) and were all previously reported in the Catalogue of Somatic Mutations in Cancer (COSMIC) mutation database [14]. Table 3 shows the associations between mutations and selected pathological or individual variables. *TP53* mutations were less frequent in ADC (39.7%) than SCC (57%); $p<0.0001$ (table 3). *KRAS* mutations were preferentially found in ADC (89.1%) than SCC (10.9%) ($p<0.0001$) (table 3). None of these mutations were associated with either T or N status of the TNM classification of tumours. *TP53* mutations were marginally more common in subjects who reported a past history of pulmonary illness or a familial history of lung cancer, but these associations were not statistically significant (online supplementary tables S1b and S1c; $p=0.1505$ and $p=0.1620$, respectively). Neither smoking status nor history of asbestos exposure was associated with *TP53* or *KRAS* mutation status. No significant association was found between *TP53* mutation and smoking duration, age at smoking initiation, consumption in pack-yrs, time since quitting smoking or cigarette type (data

not shown). Among *EGFR* mutations, 23.5% were found among never smoking females (p=0.007) (table 3).

Association between TP53 *mutations and p53 expression*

Missense *TP53* mutations may lead to nuclear accumulation of mutant p53 protein. Information on both mutation status and p53 immunohistochemistry was available for 230 patients. There was a strong correlation between mutation status and p53 immunohistochemistry (p<0.0001; online supplementary table S3). Among tumours with mutations, 62% were highly positive for p53 protein. Among tumours with wild-type *TP53*, however, 25% had widespread, high expression of p53 across the tumour, suggesting that p53 may be widely expressed in a subset of lung cancers without missense mutations in exons 4–9.

Prognostic significance of mutations

There were 26.4% PD and 73.6% DF patients. The following parameters were significantly associated with PD status (data not shown): T status of TNM (T1 *versus* T2 or more; p<0.0001), N status of TNM (N0 *versus* N1, N2 or NX; p<0.0001). *TP53*, *KRAS* or *EGFR* mutation status, however, were not associated with prognosis (fig. 2). No prognostic value was found when mutations were grouped into different categories according to their predicted effects on p53 protein structure or function [15]. G>T transversions were marginally more common among PD patients than DF (table 4), but this effect was not statistically significant (adjusted HR 1.49, 95% CI 0.66–3.36; p=0.13).

Likewise, p53 immunohistochemistry positive status was not associated with prognosis (p=nonsignificant). As there were important disparities in the recruitment of patients among countries and centres, we repeated these analyses on the largest homogenous subgroup, comprising the 103 patients from the French centres (Nancy and Grenoble). Again, in this subgroup, neither *KRAS* nor *TP53* mutations had prognostic value (results not shown). However, patients with tumours containing both mutations had a marginally significantly higher risk of developing a PD (adjusted HR 3.30, 95% CI 1.08–10.0; p=0.036).

TP53 *mutations in relation with* TP53 *polymorphisms*

The *TP53* gene is highly polymorphic and there is evidence that mutations may occur at different rates on different *TP53* alleles. We analysed the distribution of three common polymorphisms located within a 312-basepair region of the *TP53* gene encoding the N-terminus of p53, in relation with *TP53* mutation status. These three polymorphisms are PIN2 (G to C, intron 2, rs1642785), PIN3 (16-basepair duplication, intron 3, rs17878362) and PEX4 (nonsilent G to C, codon 72, R to P; rs1042522). Results (table 5) show that there was a tendency for more mutations to occur in subjects who were carriers of two PEX4 C alleles encoding P at codon 72 (85.7% as compared with 43.9% and 46.6% in G–C heterozygotes and G–G homozygote, respectively; p=0.05). The two other polymorphisms did not appear to be associated with significant differences in mutation prevalence (data not shown).

DISCUSSION

Many studies have investigated the prognostic value of *TP53* or *KRAS* mutations in lung cancer. There is evidence that both the pattern and frequency of mutations vary according to risk factors such as tobacco smoke. However, it remains unclear whether mutations are associated with an increased risk of disease progression and of unfavourable outcome. Here we have used the setup of a large European collaborative study, EUELC, to assess the prognostic value of *TP53* and *KRAS* mutations in a series of 250 NSCLC cases with detailed follow-up information. We have analysed the relationships between *TP53* mutations and several common *TP53* polymorphisms. Finally, we have assessed *EGFR* mutations in 130 ADCs, as

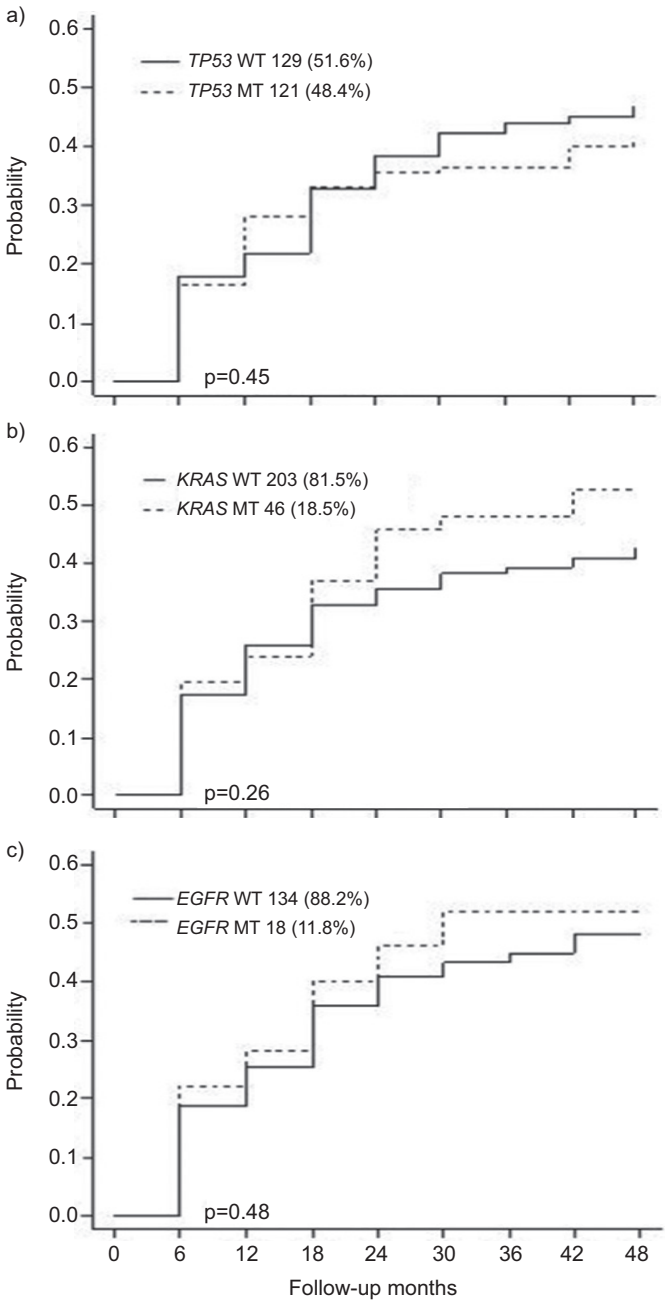

FIGURE 2. Cumulative incidence plots of the progressive disease (PD) risk for a) *TP53*, b) *KRAS* and c) *EGFR* mutations showing the proportion of subjects with PD detected during follow-up of a maximum 48 months after complete resection of the primary tumour. The numbers of cases with and without mutations and percentage are given. p-values are from the univariate Fine and Gray model. WT: wild-type; MT: mutant.

TABLE 4 Associations between biomarkers and disease progression

Variable	Items	DF	PD	HR (95% CI)	p-value#	Adjusted HR (95% CI)	p-value¶
***TP53* status**	Wild-type	70 (49.3)	59 (54.6)	1	0.45	1	0.64
	Mutated	72 (50.7)	49 (45.4)	0.86 (0.59–1.27)		0.91 (0.62–1.40)	
Type	0 – others	124 (88.6)	84 (80.8)	1	0.19	1	0.14
	1 – all G>T	16 (11.4)	20 (19.2)	1.4 (0.9–2.3)		1.46 (0.89–2.41)	
***KRAS* status**	Wild-type	118 (84.3)	85 (78)	1	0.26	1	0.46
	Mutated	22 (15.7)	24 (22)	1.30 (0.82–2.06)		1.19 (0.75–1.90)	
***KRAS/TP53* status**	Otherwise	138 (97.9)	102 (93.6)	1	0.07	1	0.21
	Both mutated	3 (2.1)	7 (6.4)	2.08 (0.95–4.57)		1.67 (0.74–3.77)	
p53 haplotype	GNA-CDP	33 (23.6)	23 (21.1)	1.07 (0.64–1.76)	0.95	1.16 (0.69–1.96)	0.93
	GNA-CNP	17 (12.1)	15 (13.8)	1.15 (0.63–2.07)		1.52 (0.63–2.11)	
	Others	65 (46.4)	49 (45)	1.15 (0.69–1.92)		1.11 (0.66–1.89)	
	GNA-GNA	25 (17.9)	22 (20.2)	1		1	
***EGFR* status+**	Wild-type	62 (87.3)	51 (86.4)	1	0.48	1	0.68
	Mutated	9 (12.7)	8 (13.6)	1.31 (0.62–2.80)		0.97 (0.67–1.38)	

Data are presented as n (%), unless otherwise stated. DF: disease free; PD: progressive disease; HR: hazard ratio. #: Fine and Gray model with centre stratification; ¶: Fine and Gray model with centre stratification adjusted on pT and pN; +: the group of cases analysed included 130 adenocarcinomas.

mutations in this gene have been reported to be rare in histologies of other lung cancers [16, 17].

Results show that *TP53* mutations were present in 48.4% and *KRAS* mutations in 18.5% of the cases. For both genes, the codon distribution showed a high proportion of G to T transversions in agreement with the well-documented prevalence of this mutation type in lung cancers of smokers. We also observed differences between the two main histological forms of NSCLC, SCC and ADC. *TP53* mutations were detected in 57% of SCCs *versus* 39.7% in ADCs. In contrast, *KRAS* mutations were detected in 89.1% of ADC *versus* 10.9% of SCC. As shown in other case series, *KRAS* mutations tended to be more common in lung cancers of ever- than former or never-smokers (52.2%, 45.7% and 2.2%, respectively). Among clinical and aetiological factors, only histology was statistically associated with mutation prevalence, while never-smoking status was significantly associated with *EGFR* mutation (p=0.0067). One tumour contained both *EGFR* and *KRAS* mutation, an extremely rare occurrence according to the literature. Interestingly, the *EGFR* mutation in this tumour was a silent one (codon 836 CGC>CGT Arg>Arg) and was thus not supposed to lead to tyrosine kinase activation.

TABLE 5 Associations between *TP53* mutation and polymorphisms

Genotype	*TP53* status		p-value#
	Wild-type	Mutated	
PIN2			
CC	7 (5.5)	15 (12.8)	0.21
GC	58 (45.3)	44 (37.6)	
GG	63 (49.2)	58 (49.6)	
PIN3			
DD	4 (3.1)	8 (6.8)	0.16
ND	43 (33.6)	29 (24.8)	
NN	81 (63.3)	80 (68.4)	
PEX4			
CC	2 (1.6)	12 (10.3)	**0.05**
CG	55 (43.0)	43 (36.8)	
GG	71 (55.5)	62 (53.0)	

Data are presented as n (%), unless otherwise stated. #: Mantel–Haenszel Chi-squared test controlling for centre. p-value in bold is significant.

In the present case series, mutation of none of the three genes analysed appeared to carry a significant prognostic value in the cohort, either as a whole or in specific histological subgroups. Given the multicentric character of the study and the possibility of a bias due to different recruitment centres, we performed a separate analysis on the largest and most homogeneous subgroup, which revealed a borderline effect in patients carrying both *TP53* and *KRAS* mutations (HR 3.26, 95% CI 1.07–9.90; p=0.038). The value of these analyses is constrained by the relatively small sample size and it will be important to verify this interpretation in larger cohorts.

Similar to our results, a study on Japanese patients with surgically resected ADC did not identify any prognostic implication for *TP53* or *KRAS* mutations [18]. The authors detected a significant association between *EGFR* mutation and longer survival, while none of the gene mutations appeared to be an independent prognosis marker. It is noteworthy that, in that Japanese series, 49% of the patients had *EGFR* mutations, a much higher rate than in the present Caucasian series (13.1%). It is well documented that mutations in *EGFR* are associated with never-smoking status, female sex and Asian ethnicity [16–18]. The relatively low prevalence of *EGFR* mutations in our series may reflect the characteristics of the patients recruited in EUELC, *i.e.* Caucasian, 84% males and 95.2% ever-smokers. Given these characteristics, the *EGFR* mutation showed a higher than expected rate, and it was not restricted to NSCLC of

never-smokers, as it was detected in ~10% of former (five out of 48) or current (eight out of 72) smokers.

Based on these results, the conservative conclusion is that mutation status does not predict short-term outcomes in completely resected lung cancers and, given the overall poor prognosis of lung cancer over a period of 5–8 yrs, it remains to be determined whether it may be a prognostic factor for longer-term outcomes.

From a biological viewpoint, *TP53* and *KRAS* mutations may represent very early events in lung carcinogenesis, occurring before tumour onset as the result of genetic damage by tobacco components. Although these mutations do participate in launching bronchial cells towards transformation and progression, it is likely that the tumour behaviour may be dictated by specific, additional events occurring after initiation by tobacco carcinogens. We found that tumours carrying both *TP53* and *KRAS* mutations might have a worse prognosis, and this underlines a possible higher exposure to tobacco carcinogens or a particular susceptibility to their mutagenic effects. These patients may have increased risk of acquiring additional mutations, which, in turn, may be responsible for their poorer prognosis. Thus, presence of both *TP53* and *KRAS* mutations in the same lesion may act as a marker to identify a small group of tumours that are genetically unstable and prone to the accumulation of mutations, which may accelerate disease progression and/or escape from therapy. Further studies are needed to identify the targets of such genetic instability in NSCLC. Candidate markers may involve genes with activating mutations, making it possible to treat these cancers using selective pharmacological inhibitors [3], and epigenetic changes in DNA methylation patterns and in microRNA expression, which may distinguish different NSCLC subgroups [19].

Our data on *TP53* polymorphisms show that *TP53* mutations tend to occur at different rates on different *TP53* alleles. Although the group of patients was small, patients with two PEX4 C alleles tended to more frequently have a *TP53* mutation than patients with at least one G allele. This suggests that the *TP53* C allele may be intrinsically more ''mutable'' than the G allele, perhaps as a result of subtle differences in the functional properties of p53 proteins. Experimental studies have identified such functional differences, including a greater ability to induce apoptosis for 72P than for 72A [20]. This observation is in agreement with results from MECHANIC *et al*. [21], who found that common genetic variation in *TP53* could modulate lung cancer pathways, as suggested by the association of *TP53* codon 72 polymorphism with lung cancer in African-Americans and with somatic *TP53* mutation frequency in lung tumours. Thus, in future studies, it may be important to take into account both *TP53* mutation and *TP53* haplotypes in assessing the prognostic and predictive significance of *TP53* gene status in lung cancer.

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STATEMENT OF INTEREST

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