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Fibroblast growth factor receptor 1 (*FGFR1*) copy number is an independent prognostic factor in non-small cell lung cancer

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ABSTRACT

Fibroblast growth factor receptor 1 (*FGFR1*) is an oncogene that can potentially be targeted by tyrosine kinase inhibitors. We aimed to investigate the prevalence and prognostic significance of alterations in *FGFR1* copy number in non-small cell lung cancer (NSCLC). *FGFR1* status was evaluated by chromogenic silver in situ hybridisation (ISH) in tissue microarray sections from a retrospective cohort of 304 surgically resected NSCLCs and results were correlated with the clinicopathological features and overall survival. High *FGFR1* gene copy number (amplification or high-level polysomy) was significantly more frequent in squamous cell carcinomas (SCC) (24.8%) and large cell carcinomas (LCC) (25%) compared to adenocarcinomas (11.3%) ($p = 0.01$ and $p = 0.03$ respectively). Among NSCLC there was no significant correlation between *FGFR1*-positive status and other clinicopathological features including age, gender, smoking history, tumour size, lymph node status, stage, grade, vascular, lymphatic or perineural invasion. *FGFR1*-positive patients showed a tendency to longer overall survival in univariate analysis ($p = 0.14$). Multivariate survival analysis using Cox regression model confirmed *FGFR1*-positive patients had a significant reduction in the risk of death compared to *FGFR1*-negative patients (HR 0.6; $p = 0.02$). High *FGFR1* gene copy number is a common finding in SCC and LCC and is an independent favourable prognostic factor.

1. Introduction

Our understanding of the molecular origins of non-small cell lung cancer (NSCLC) has progressed significantly over the past decade due to the identification of driver events such as mutations of *KRAS*, *EGFR*, *BRAF*, *PIK3CA*, *ALK* rearrangement, or *MET* amplification [1,2]. A growing number of targeted therapies, primarily tyrosine kinase inhibitors, are now in routine clinical use for lung adenocarcinomas (ADC) harbouring specific mutations, however, prospects for personalised and molecularly targeted therapy have not impacted on other subtypes of NSCLC [3]. While molecular abnormalities in specific subsets of lung adenocarcinomas have been well characterised [4], it is only recently that comprehensive genomic characterisation of squamous cell lung cancers has identified potentially targetable mutations [5]. Fibroblast growth factor receptor 1 (FGFR1) has been identified as one of the emerging molecular targets for the treatment of squamous cell carcinomas of the lung [6,7] and several early phase clinical trials of FGFR inhibitors are currently being undertaken in NSCLC [8,9].

FGFRs belongs to the super-family of receptor tyrosine kinases and are encoded by 4 genes (*FGFR1*, *FGFR2*, *FGFR3*, and *FGFR4*) [10]. FGFR acts as a cell-surface receptor for fibroblast growth factors and plays an essential role in the regulation of embryonic development, cell proliferation, differentiation and migration [11]. Alterations of the *FGFR* gene have been recognised in many epithelial malignancies including amplifications in gastric, breast, oral squamous cell, ovarian and bladder carcinomas [10,12,13] and more recently in squamous NSCLC [6], promising a novel therapeutic target in these tumours. Previous studies of *FGFR1* amplification in lung cancer have focussed on squamous cell carcinoma (SCC) [14] or SCC and ADC [6,7,15,16] with little data available on undifferentiated large

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cell carcinomas (LCC). We have undertaken a comprehensive evaluation of *FGFR1* gene copy status in the main subtypes of NSCLC in a study designed to investigate the prevalence and prognostic significance of alterations in *FGFR1* copy number in NSCLC. We also comprehensively report a comparison of *FGFR1* status in primary tumours and matched lymph node metastases.

2. Materials and methods

2.1. Patient cohort and tissue microarray construction

This retrospective study was conducted in a cohort of 261 NSCLC patients who underwent resection of a primary NSCLC at the Royal Prince Alfred Hospital, Sydney, Australia. Surgery was performed by the one cardiothoracic surgeon (B.M.) from 1994 to 2002. In order to extend the sample size for correlative analyses between primary and lymph node metastatic tumours as well as clinicopathological features, an additional cohort of 43 NSCLC lymph node metastatic stage patients from 2010 to 2012 was also selected in a similar way, giving a total of 304 cases. Of these cases, *FGFR1* gene copy number was able to be evaluated in 264 cases (87%) (221 cases from the original cohort and all 43 additional cases). The additional cohort was excluded from survival analysis due to limited duration of follow up. The study was approved by the Royal Prince Alfred Ethics Review Committee (X02-0216, X06-0167 and X10-0278) with all tissue specimens de-identified.

Tumour subtype was determined using the World Health Organisation 2004 classification [17] by an expert lung pathologist (W.C.). Staging was determined using the 7th edition AJCC tumour, node, metastasis (TNM) classification [18]. In the whole cohort of 264 cases, there were 115 ADC, 101 SCC, 44 LCC, and 4 others (sarcomatoid carcinomas consisting of 2 pleomorphic carcinomas and 2 spindle cell carcinomas). In the 71 cases where primary tumours and paired lymph node metastases were available (28 from the original cohort and 43 additional cases), there were 40 ADC (56.3%), 20 SCC (28.2%), 8 LCC (11.3%) and 3 sarcomatoid carcinomas (4.2%).

Tissue microarrays were constructed using a manual tissue arrayer (MTA-1, Beecher Instruments Tissue Arrayer) with core size of 1 mm in diameter as previously described [19,20]. Each patient was represented by 2 to 4 tissue cores of normal bronchial epithelium obtained from bronchi or terminal bronchioles, 1 to 2 tissue cores of normal peripheral lung alveoli, and 3 to 6 tumour cores which were selected from areas previously marked by a specialist lung pathologist (W.C.). In case of lymph node metastatic disease, 1 to 6 tumour cores of each metastatic lymph node specimens were included.

2.2. Chromogenic silver in situ hybridisation analysis

Unstained 4 μm sections from each tissue microarrays were submitted to dual-colour ISH assay using a cocktail of *FGFR1* dinitrophenyl (DNP) probe (40 μg/mL) and chromosome 8 digoxigenin (DIG) probe (40 μg/mL), *ultra*View SISH DNP Detection Kit and *ultra*View Red ISH DIG Detection Kit (Roche, Basel, Switzerland). The ISH assay was performed on a BenchMark Ultra instrument (Roche) using an optimised protocol modified from a standard protocol provided by the manufacturer. Pretreatment was performed using ULTRA CC2 (Roche) at 80 °C for 3 cycles of 12 min and protease 3 for 20 min. Denaturation was performed at 80 °C for 8 min followed by probe hybridisation at 44 °C for 6 h, and 3 cycles of stringency washes with sodium chloride–sodium citrate buffer at 72 °C for 8 min. *FGFR1* DNP-labelled probe and chromosome 8 DIG-labelled probe bound to target sequence were incubated with anti-hapten antibody – silver (rabbit anti-DNP antibody) and anti-hapten antibody – red (mouse anti-DIG antibody) respectively for 24 min, then with goat anti-rabbit antibody with a horseradish peroxidise (HRP) enzyme and goat anti-mouse antibody with an alkaline phosphatase (AP) enzyme respectively, for 8 min.

Signals were enumerated in at least 50 non-overlapping tumour nuclei per core using a light microscope at 400× magnification. For each core, the mean copy number per cell of each probe target (*FGFR1* and centromere 8) and the percentage of cells with *FGFR1* signal clusters (more than 5 signals) was recorded for interpretation. Cases were considered as *FGFR1* amplified if one of the following conditions was satisfied: *FGFR1*/*CEP8* ratio ≥2.0; mean *FGFR1* signals per tumour cell ≥6.0; or percentage of tumour cells containing *FGFR1* clusters ≥10% [16]. Cases were considered as showing *FGFR1* copy number gain if the mean of *FGFR1* signals was between 4 and 6 or at least 50% of counted cells contained ≥4 *FGFR1* signals as described in previous studies [16,21,22]. Tumours were considered "*FGFR1* positive" if the *FGFR1* ISH showed *FGFR1* amplification or copy number gain.

2.3. Statistical analysis

Clinical and pathological features were correlated with *FGFR1* gene copy number status using Pearson Chi-square tests for categorical variables or Student *t*-test for continuous variables. Overall survival time was determined from the date of surgery to the date of patient death or last contact. The median follow-up time was 58.62 ± 47.61 (0.1–167.45) months for the 221 cases available for survival analysis. Overall survival analyses and outcome comparison were calculated using Kaplan–Meier method and log-rank tests, respectively. Multivariate analysis was performed using Cox proportional hazard regression model with a forward stepwise method. All statistical tests were two-sided, and statistical significance was set at $p < 0.05$ using IBM® SPSS® Statistics software version 21.0 (IBM, Chicago, IL).

3. Results

3.1. FGFR1 ISH analyses and clinicopathological correlations

A total of 304 cases were evaluated for *FGFR1* gene copy number in which 264 cases (87%) were scorable. The clinicopathological features of all 264 patients are summarised in Table 1. The majority of patients were male (64%), with early pathological stage tumours (35.6% stage I and 48.9% stage II), and a median age of 66.5 ± 8.7 years. Amongst all NSCLC, the mean *FGFR1* gene copy number was 2.88 (range 1.1–20.4) and mean CEP8 was 1.78 (range 1.0–3.9). Only 3% of tumours had mean CEP8 scores ≥ 3 suggesting chromosome 8 polysomy was a rare event. The frequencies of *FGFR1* amplification and copy number gain were 14% (37/264) and 4.5% (12/264), respectively amongst all NSCLC. The number of *FGFR1* amplified cases was significantly higher in SCC (21.8%) and LCC (20.5%) than in ADCs (5.2%) ($p < 0.001$) (Fig. 1 and Table 1). Differences in *FGFR1* copy number (normal, gain, or amplification) or status (negative or positive) were also observed between adenocarcinoma and non-adenocarcinoma ($p < 0.01$). Cases with amplified *FGFR1* included cases with clusters of *FGFR1* signals and multiple increased single copies (Fig. 2). We used several criteria for determining *FGFR1* amplification (*FGFR1*/*CEP8* ratio ≥2.0; mean of *FGFR1* signals per tumour cell ≥6.0; or percentage of tumour cells containing *FGFR1* clusters ≥10%) as suggested by Schildhaus et al. [16] but found a FGFR1/CEP8 ratio of ≥2.0 alone was sufficient for identifying all amplified cases (Table 2).

FGFR1 positive status (and *FGFR1* amplification) was more common in patients with SCC ≥ 65 years than younger patients ($p < 0.01$). There were no other significant associations between

Table 1
Clinicopathological characteristics of patients and *FGFR1* status.

Characteristic	Total	*FGFR1* Negative	*FGFR1* Positive	*p*-Value
Gender				
Female	95 (36)	80 (84.2)	15 (15.8)	0.39
Male	169 (64)	133 (79.9)	34 (20.1)	
Age (years)				
<65	98 (37.1)	82 (83.7)	16 (16.3)	0.47
≥65	166 (62.9)	133 (80.1)	33 (19.9)	
Smoking history				
Never smoked	9 (3.4)	9 (100)	0 (0)	
Ex-or current smoker	165 (62.5)	129 (78.2)	36 (21.8)	0.12
Unknown	90 (34.1)			
Stage				
IA	46 (17.4)	42 (91.3)	4 (8.7)	
IB	48 (18.2)	35 (72.9)	13 (27.1)	
IIA	97 (36.7)	80 (82.5)	17 (17.5)	0.87[a]
IIB	32 (12.1)	25 (78.1)	7 (21.9)	
III	41 (15.5)	33 (80.5)	8 (19.5)	
Histology				
ADC	115 (43.6)	102 (88.7)	13 (11.3)	
SCC	101 (38.3)	6 (75.2)	25 (24.8)	0.02
LCC	44 (16.7)	33 (75.0)	11 (25.0)	
Other[c]	4 (1.5)	4 (100)	0 (0)	
Tumour differentiation (grade)				
Well	25 (9.5)	24 (96.0)	1 (4.0)	
Moderate	122 (46.2)	98 (80.3)	24 (19.7)	0.47[b]
Poor	117 (44.3)	93 (79.5)	24 (20.5)	
Blood vessel invasion				
Absent	221 (83.7)	180 (81.4)	41 (18.6)	0.99
Present	43 (16.3)	35 (81.4)	8 (18.6)	
Lymphatic invasion				
Absent	219 (83.0)	182 (83.1)	37 (16.9)	0.13
Present	45 (17.0)	33 (73.3)	12 (26.7)	
Perineural invasion				
Absent	247 (93.6)	200 (81.0)	47 (19.0)	0.46
Present	17 (6.4)	15 (88.2)	2 (11.8)	
TTF1 expression in LCC[d]				
Negative	17 (40.5)	10 (58.8)	7 (41.2)	0.06
Positive	25 (59.5)	22 (88.0)	3 (12.0)	

[a] Stage I–II vs III.
[b] Well and moderately differentiated tumours versus poorly differentiated tumours.
[c] Other tumours consisted of sarcomatoid carcinomas (2 spindle cell and 2 pleomorphic carcinomas).
[d] TTF1 expression results unavailable in 2 cases due to lack of tumour tissue.

FGFR1 status (negative versus positive or amplification versus non-amplification) and patient age, gender, smoking status, tumour size, lymph node status, stage, grade, blood vessel invasion, lymphatic invasion or perineural invasion amongst all NSCLC (Table 1) and amongst NSCLC subgroups (data not shown). All 9 never-smokers were *FGFR1* negative. There was a trend for TTF1 positive LCCs to be *FGFR1* negative but this was not statistically significant ($p = 0.06$, Fisher's exact test) (Table 1).

3.2. FGFR1 status in primary tumours versus lymph node metastases

In an effort to further understand the biological characteristics of *FGFR1*, a paired comparison of *FGFR1* gene copy status between primary tumours and their matched lymph node metastasis was undertaken and showed significant concordance (Pearson's correlation = 0.54; $p < 0.001$) (Table 3), however, 6 cases with normal *FGFR1* copy number and 5 cases with *FGFR1* copy number gain in the primary tumour showed amplification in matched lymph node metastases.

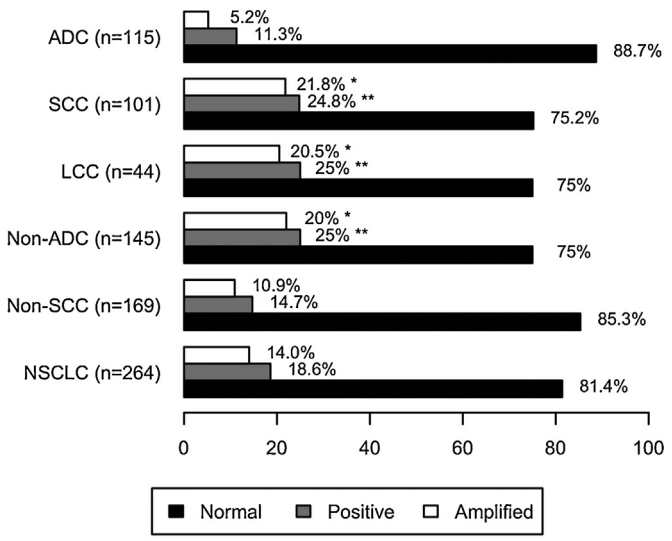

Fig. 1. Frequency of *FGFR1* gene alteration in different subtypes of NSCLC. *FGFR1* amplification, copy number gain or positive status (amplification or copy number gain) were more frequent in SCC, LCC, and non-ADC than in ADC. * and **: p-value < 0.01 for two-tailed Chi-square tests, compared to *FGFR1* amplification (*) or positive (**) in ADC subgroup.

3.3. Survival analyses

Patients with "*FGFR1*-positive" NSCLC (*FGFR1* amplified or copy number gain) showed a tendency for better survival than those who were *FGFR1*-negative in univariate analysis, although the difference was not significant (median overall survival 60.3 months versus 41.8 months; $p = 0.14$) (Fig. 2E). There were no statistically significant differences in overall survival by *FGFR1* amplification (amplified versus not amplified) (Fig. 2F) or copy number gain in the NSCLC population as a whole or 5-year survival in separate subtypes of NSCLC (ADC, SCC, or LCC) (data not shown). Multivariate analysis was performed using Cox proportional hazard model, including age, vascular, lymphatic, or perineural invasion, grade, *FGFR1* status (negative or positive), and stage. The multivariate model confirmed that lymphatic invasion and stage were independent, poor prognostic factors ($p < 0.01$) but furthermore showed "*FGFR1*-positive" status was an independent favourable prognostic factor (HR = 0.6, $p = 0.02$) (Table 4).

4. Discussion

There is increasing interest in characterisation of key molecular alterations in lung cancers that may provide opportunities for targeted therapeutic interventions. Deregulation of the FGFR signalling pathway has been described in many epithelial tumours,

Table 2
Correlation between criteria for evaluation of *FGFR1* amplification.

FGFR1	*FGFR1*/*CEP8* ratio		Mean of *FGFR1* signals		Clusters	
	<2	≥2	<6 copies	≥6 copies	<10% cells	≥10% cells
Non-Amplification	227 (100%)	0 (0%)	227 (100%)	0 (0%)	227 (100%)	0 (0%)
Amplification[a]	0 (0%)	37 (100%)	18 (48.6%)	19 (51.4%)	17 (45.9%)	20 (54.1%)

[a] Amplification defined as *FGFR1*/*CEP8* ratio ≥2.0; mean of *FGFR1* signals per tumour cell ≥6.0; or percentage of tumour cells containing *FGFR1* clusters ≥10%.

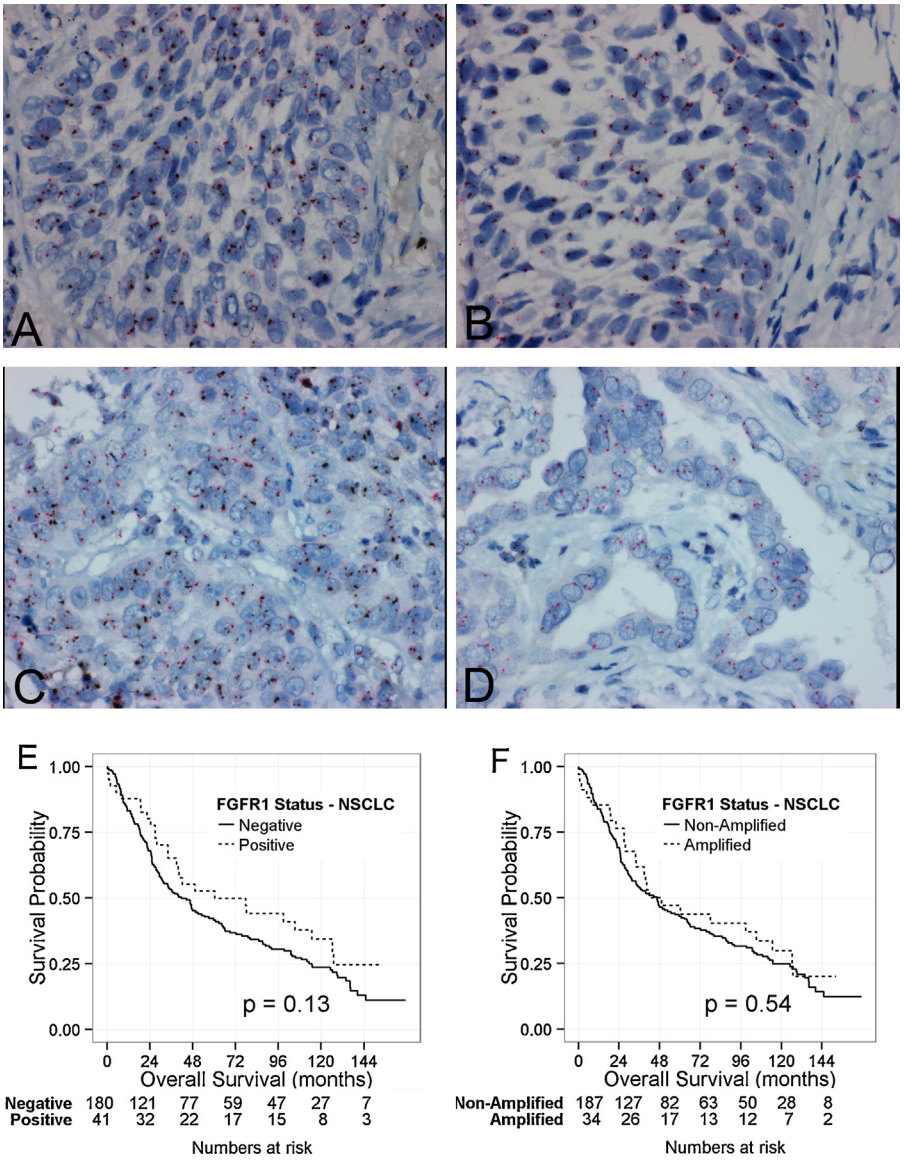

Fig. 2. (A) *FGFR1* chromogenic ISH with black *FGFR1* signals and red chromosome 8 signals in an SCC with amplified *FGFR1* forming clusters and (B) corresponding lymph node metastasis also showing *FGFR1* amplification. (C) LCC with amplified *FGFR1* showing clusters and increased single gene copies. (D) Adenocarcinoma with normal *FGFR1* copy number (magnification 400×). (E) Overall survival rates of NSCLC patients by *FGFR1* status. "*FGFR1* positive" (*FGFR1* amplification or copy number gain) vs. "*FGFR1* negative"; and (F) *FGFR1* amplified vs. non-amplified.

predominantly through receptor overexpression or more rarely through activating somatic mutations of the receptor gene [10]. *In vitro* and *in vivo* studies have shown FGFR1-mediated signalling plays an important role in NSCLC cell growth, survival and migration and that FGFR1 specific small molecule inhibitors selectively have a growth inhibitory effect in *FGFR1* amplified tumours [6,7,23–25]. While *FGFR1* is frequently amplified in human NSCLC, sequencing of the *FGFR1* gene has shown that mutations are extremely rare or absent in lung SCC and ADC [6,7] and so are unlikely to play a significant role in lung cancer. In this study, we analysed *FGFR1* gene copy number in a large cohort of early stage NSCLC with corresponding nodal metastases and evaluated the clinicopathological features of *FGFR1* amplified tumours and prognostic features.

To our knowledge, this is for the first report of *FGFR1* copy number evaluation using chromogenic silver in situ hybridisation

Table 3
Correlation of *FGFR1* status between primary tumour and corresponding lymph node metastases.

FGFR1 copy number		Metastatic lymph node (%)			Total (%)	Pearson's *r*	*p*-Value
		Normal	Gain	Amplified			
Primary tumour (%)	Normal	58 (86.6)	3 (4.5)	6 (9.0)	67 (85.9)	0.54	<0.001
	Gain	2 (20.0)	3 (30.0)	5 (50.0)	10 (12.8)		
	Amplified	0 (0.0)	0 (0.0)	1 (100)	1 (1.3)		
Total (%)		60 (76.9)	6 (7.7)	12 (15.4)	78 (100)		

Table 4
Univariate and multivariate analyses of overall survival in the NSCLC cohort.

Variable	Category	Univariate			Multivariate		
		HR	95% CI	*p*	HR	95%CI	*p*
Age	<65 years/≥65 years	NT	NT	0.18	NT	NT	NT
Grade	I–II/III	NT	NT	0.45	NT	NT	NT
Blood vessel invasion	Absent/Present	1.73	1.17–2.56	<0.01	NT	NT	NT
Perineural invasion	Absent/Present	2.19	1.24–3.88	<0.01	NT	NT	NT
Lymphatic invasion	Absent/Present	2.54	1.62–3.98	<0.01	2.23	1.40–3.57	<0.01
FGFR1	Negative/Positive	NT	NT	0.14	0.60	0.40–0.91	0.02
Lymph node	Negative/Positive	2.08	1.46–2.95	<0.01	1.83	1.27–2.64	<0.01
Stage	I–II/III	2.66	1.60–4.42	<0.01	2.46	1.46–4.12	<0.01

Total $n = 221$; events = 168; censored = 53. NT: not tested.

technique for lung cancer tissue. We found the technique was suitable for *FGFR1* analysis with 87% of cases being evaluable, a result comparable to that reported in fluorescent in situ hybridisation (FISH) studies of *FGFR1* [14–16]. Chromogenic silver ISH provides excellent concordance with FISH [26] but has the advantage of enabling cell morphology to be assessed simultaneously with gene copy number, ensuring only tumour cells are scored. In addition, the assay is automated, there is a shorter processing time than FISH (less than 1 day), staining is visualised using standard light microscopy and slides can be stored at room temperature [26].

In order to have a broader view of *FGFR1* gene copy number alteration, this study had been designed to include all common subtypes of NSCLC (ADC, SCC, and LCC). We found high *FGFR1* gene copy number in 24.8% of SCCs, similar to previous FISH studies where 16% [14], 20% [16] and 22% of SCCs were amplified [6]. Another study using FISH found high *FGFR1* gene copy numbers in only 10.5% of their SCCs using a lower threshold of ≥4 gene copy numbers to define amplification and they did not find any cases of high level amplification (copy number ≥9) [15]. We found high *FGFR1* was significantly more common in SCCs and LCCs than ADC. Others have also found high *FGFR1* copy number to be more frequent in SCCs compared to ADCs [6,16,27], but the reported frequency of *FGFR1* amplification in ADCs is variable. Our study identified a small proportion of ADC with *FGFR1* gene copy number aberration (5.2% amplification and 6.1% copy number gain) in agreement with that found by Kohler et al. (4.7%) [15]. Similarly in a study using SNP array analysis amplification of 8p11-12 (containing *FGFR1*) was found in 21% of SCC and 3.4% of lung ADC [7]. By contrast, Weiss et al. [6] reported *FGFR1* gene copy number alteration in ADCs as a rare event using FISH and SNP array data and *FGFR1* amplifications have only rarely been reported in genome wide analyses of lung adenocarcinomas [28]. In the study by Schildhaus et al. [16], none of 100 ADCs had *FGFR1* amplification and only 1 of 6 undifferentiated LCCs was amplified. Interestingly, our study also found that *FGFR1* gene copy alteration was as frequent in LCC, a subtype of NSCLC rarely included in other studies, as in SCC (25% versus 24.8%, $p = 0.86$). This is in keeping with *in vitro* evidence for FGFR1 autocrine signalling in LCCs as well as SCCs [23]. Our results suggest that *FGFR1* amplification is frequent in a range of NSCLCs, and that inhibition of the protein should be considered not only in SCC but also in LCC and a small proportion of ADC. Our finding of *FGFR1* amplification in 5.2% of adenocarcinomas is similar to the reported prevalence of *ALK* rearrangements in approximately 5% of lung adenocarcinomas [29]. Thus, *FGFR1* testing should not be restricted to SCCs only as this may miss a significant number of potentially druggable changes in LCCs and a smaller proportion of ADCs, based on the WHO 2004 classification of NSCLC. We found a non-significant trend for LCCs expressing the ADC lineage marker TTF1 to have normal *FGFR1* copy number but 3 "*FGFR1* positive" tumours expressed TTF1 suggesting neither the ADC-lineage marker TTF1 nor ADC histology completely excludes the possibility of increased *FGFR1* copy number.

High *FGFR1* copy number or amplification was found to be associated with older patients in those with SCC but not with other tumour subtypes. Consistent with previous reports, our data showed no association between any other clinical features and *FGFR1* gene copy status [7] demonstrating that clinical features are unlikely to be suitable for selection of patients expected to harbour *FGFR1* aberrations. In particular, we did not find *FGFR1* levels were associated with patient smoking status while others have described an association between *FGFR1* amplification and smokers [6,27] and male gender [27]. Although the proportion of never-smokers in our study was small (4.1%), the tumours from all 9 of these patients were *FGFR1* negative. The smoking status was unknown in a relatively high proportion of cases in our study (21.2%), however, making it difficult to draw conclusions on this factor.

We found significant but not perfect concordance between *FGFR1* gene copy status in primary tumours and their corresponding lymph node metastasis. Only one previous study has directly investigated the concordance of *FGFR1* amplification status between primary tumours and metastases and they found complete correlation in 39 cases with regional lymph node metastases using FISH [14]. Our findings suggest *FGFR1* gene copy number alteration may be more frequent with disease progression, a phenomenon that is recognised with *EGFR* amplifications in lung adenocarcinoma [30].

Our data showed *FGFR1* positive status was an independent predictor of improved overall survival in NSCLC patients. However, no relationship with survival was found when *FGFR1* amplification (rather than positivity which includes cases with copy number of between 4 and 6) was assessed or when histological subtypes such as SCC alone were analysed. Previous studies have failed to show a difference in survival outcome by *FGFR1* gene copy status but have described a non-significant trend towards improved survival in NSCLC with high *FGFR1* copy number [27] or poorer survival in SCCs with high *FGFR1* copy number [15]. In breast cancers, *FGFR1* amplification is associated with poor patient outcome [12]. Interestingly, the study by Kohler et al. [15], reported a trend for better survival amongst ADCs with amplified *FGFR1* but the number of cases was very small ($n = 3$). Larger studies are required to confirm our findings of *FGFR1* being a favourable prognostic factor in NSCLC patients.

Comparison of results from studies evaluating alteration in gene copy number using ISH techniques are frequently hampered by differences in criteria for establishing amplification. Due to the lack of standardised criteria for scoring *FGFR1* amplification we chose to investigate a number of different thresholds as described in a previous detailed study of patterns of *FGFR1* gene copy number alterations [16] as well as more established criteria for other amplified genes critical for determination of targeted therapy (e.g. *HER2*) [21] although it is possible that the biological impact of gene copy number may differ for specific genes. Increased gene copy number can occur as a result of regional specific amplification of the gene of interest or due to polysomy of the whole chromosome. We found polysomy of chromosome 8 as assessed by the CEP8

probe, which harbours *FGFR1* was infrequent with 97% of NSCLC having CEP8 scores <3 while others have reported the majority of SCCs displayed polysomy [16]. It has been suggested, however, that *FGFR1*/chromosome 8 ratios alone are insufficient for determining amplification due to heterogeneously distributed clusters of amplified cells [16]. We did not observe this, however, and analysis of the different scoring parameters used in our study, however, showed that the *FGFR1*/*CEP8* ratio was sufficient to identify amplified cases with other scoring parameters not identifying any additional cases. As in our study, others have also described intra-tumoral uniformity in *FGFR1* amplification within tumour cells [14].

We have shown *FGFR1* gene copy number alterations are not only common in lung SCC but also in LCCs and that there is concordance between gene copy number changes in primary tumours and corresponding nodal metastases. These results provide promise for a potential therapeutic target in NSCLC, particularly SCC and LCC, in which current therapeutic targets are limited. Our findings suggest ADCs should not be excluded from consideration of *FGFR1* inhibition as a small but not insignificant proportion of ADCs also have high level *FGFR1* copy number changes.

Conflict of interest statement

S. O'Toole has received honoraria for participating in an Advisory Board for Roche.

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