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Prognostic role of FGFR1 amplification in early-stage non-small cell lung cancer

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Background: Recently, fibroblast growth factor receptor 1 (*FGFR1*) was discovered in squamous cell carcinomas (SCC) of the lung with *FGFR1* amplification described as a promising predictive marker for anti-FGFR inhibitor treatment. Only few data are available regarding prevalence, prognostic significance and clinico-pathological characteristics of *FGFR1*-amplified and early-stage non-small cell lung carcinomas (NSCLC). We therefore investigated the *FGFR1* gene status in a large number of well-characterised early-stage NSCLC.

Methods: *FGFR1* gene status was evaluated using a commercially available fluorescent *in situ* hybridisation (FISH) probe on a tissue microarray (TMA). This TMA harbours 329 resected, formalin-fixed and paraffin-embedded, nodal-negative NSCLC with a UICC stage I–II. The FISH results were correlated with clinico-pathological features and overall survival (OS).

Results: The prevalence of an *FGFR1* amplification was 12.5% (41/329) and was significantly ($P<0.0001$) higher in squamous cell carcinoma (SCC) (20.7%) than in adenocarcinoma (2.2%) and large cell carcinoma (13%). Multivariate analysis revealed significantly ($P=0.0367$) worse 5-year OS in patients with an *FGFR1*-amplified NSCLC.

Conclusions: *FGFR1* amplification is common in early-stage SCC of the lung and is an independent and adverse prognostic marker. Its potential role as a predictive marker for targeted therapies or adjuvant treatment needs further investigation.

Despite advances in diagnostics and treatment, lung cancer remains the leading cause of cancer-related deaths worldwide. It is estimated that 7% of the population will develop lung cancer during their lifetime (Howlader *et al*, 2012). During the past decade, the discovery of targetable oncogenic protein kinases, like epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) rearrangements (Shaw *et al*, 2013), have revolutionised diagnosis and treatment of non-small cell lung cancer (NSCLC) (Mok *et al*, 2009). However, predictive and targetable oncogenic mutations have mainly been found in adenocarcinomas (AC). The investigation of squamous cell carcinomas (SCC) has lagged behind, with no approved targeted drugs available thus far. Recently, several promising biological pathways and genomic alterations have been identified in SCC of the lung, including PIK3CA/AKT1, PTEN and fibroblast growth factor receptor 1 (*FGFR1*) alterations (Weiss *et al*, 2010; Hammerman *et al*, 2012). *FGFR1* amplification is one of the most promising findings in SCC due to the availability of *FGFR1* inhibitors and its association with response to FGFR inhibitor treatment, a result demonstrated in cell lines and xenograft mouse models, respectively (Weiss *et al*, 2010; Zhang *et al*, 2012). The *FGFR1* gene belongs to the FGFR family of tyrosine kinase receptors and is located on chromosome 8p11.23. The *FGFR1* receptor is a transmembrane protein kinase (Thisse and Thisse, 2005). Binding of the ligand to the extracellular domain induces dimerisation, auto-phosphorylation and activation of downstream

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pathways (Bae and Schlessinger, 2010). In this way *FGFR1* contributes to cell proliferation, differentiation and migration (Thisse and Thisse, 2005). Moreover, *in vivo* upregulation of *FGFR1* leads to cell transformation and carcinogenesis (Arbeit *et al*, 1996). Recently, it was shown in cell cultures and in xenograft mouse models that SCC harbouring *FGFR1* amplifications respond in up to 80% to anti-*FGFR1* treatment (Weiss *et al*, 2010; Zhang *et al*, 2012). Several small molecules targeting the *FGFR1* tyrosine kinase are now in clinical trials for the treatment of patients with SCC of the lung and of other solid malignant tumours (Gavine *et al*, 2012; Wolf *et al*, 2012). For most of these phase I and II clinical trials, the inclusion criterion is the verification of *FGFR1* amplification (ClinicalTrials.gov, 2013). Therefore, the assessment of *FGFR1* gene status might become increasingly important in the future for patients with SCC of the lung.

As for EGFR- and ALK-targeted treatment, success of *FGFR1* inhibitor treatment will be critically dependent on identification of an appropriate predictive marker and its assessment. In this context the knowledge of the prevalence of *FGFR1* amplification independent of treatment is crucial. Chemotherapy-naive patients with early-stage NSCLC treated with surgery only are therefore most suitable for evaluation of prognostic markers, as they are not confounded by the effects of different previous therapies. In addition, as about 30% of early-stage NSCLC relapse (El-Sherif *et al*, 2006), a prognostic marker could help stratify patients who might benefit from a more aggressive treatment.

Therefore, the aim of this study was to investigate the prevalence, the clinico-pathological characteristics and the prognostic significance of *FGFR1* gene status in a large cohort of early-stage NSCLC patients treated with surgery alone. The study was performed according to the REMARK guidelines (McShane *et al*, 2005).

MATERIAL AND METHODS

Patients. We searched for patients with early-stage (UICC stage IA to IIB), node-negative NSCLC treated with curative surgery only diagnosed at the Institute for Pathology, University Hospital Basel, Switzerland and at the Institute for Pathology, University Bern, Switzerland between January 1988 and August 2008. Patients' clinical and follow-up data were collected from hospital charts and questionnaires sent to the primary-care physicians. This retrospective study was approved by the ethical committee of Basel (EKBB Nr. 31/12).

The staging work up for all patients before surgery was performed according to institutional protocols including: history and physical examination, routine laboratory evaluation, bronchoscopy, chest/abdomen/pelvis computer tomography (CT) scan and bone scintigraphy. In 2006, integrated whole-body PET–CT scan was added for tumour staging and standardised intraoperative lymph node staging was performed according to the ESTS guidelines (Lardinois *et al*, 2006).

We identified 544 patients. Of those we excluded patients with neo-adjuvant treatment (*N*: 39, 7.4%), non-informative fluorescent *in situ* hybridisation (FISH) results (*N*: 92, 17%), rare lung cancer histologies (*N*: 5, <1%), patients with a wedge resection (*N*: 5, <1%) and with unknown surgical resection (*N*: 12, 2.2%), therefore the final patient number was 329 (Figure 1). The remaining patient cohort showed the expected prognosticators concerning age, gender, T-category, stage and histology (data not shown). The cohort included 244 (74.2%) male and 85 (25.8%) female patients. The median age at the time of surgery was 66.9 years (range: 42–83 years) and the median survival time was 93.8 months (range: 67.3–115.8 months). The patients showed the following stage distribution: 36 (11.0%) IA, 211 (64.1%) IB, 52 (15.8%) IIA and 30 (9.1%) IIB. A total of 239 (72.6%) patients had a smoking history (active smokers: $n=156$; ex-smokers: $n=83$), 18 (5.5%) patients were non-smokers and for 72 (21.9%) smoking history was not available. The median number of pack years was 50 (range: 5–150). The following distribution of histology was seen: 169 (51.4%) SCC, 137 (41.6%) AC and 23 (7.0%) large cell carcinoma (LC). The surgery procedures included: 263 (80.0%) lobectomies, 41 (12.4%) pneumonectomies and 25 (7.6%) bi-lobectomies. A complete surgical resection (R0) was achieved in 325 (98.8%) patients and an R1 status was diagnosed in 4 (1.2%) patients. The median number of extirpated lymph nodes was 9.5 (range 1–65) and the median tumour diameter was 40 mm (range 5–170 mm). The following T-categories were recorded: 36 (10.9%) T1a, 211 (64.1%) T1b, 52 (15.8%) T2a, 30 (9.1%) T2b and 0 (0%) T3. Adjuvant chemotherapy was administered in 12 (4.2%) and adjuvant radiotherapy in 6 (2.1%) patients (Table 1).

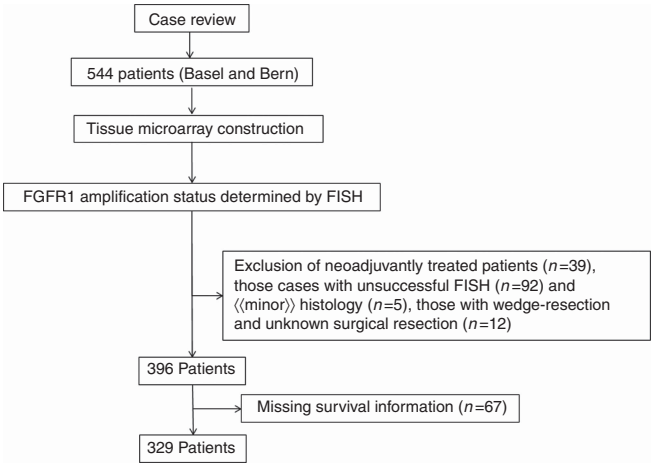

Figure 1. Patients and sample selection flow chart. Study design. In a first step, cases with stage I/II lung cancers of all histological subtypes were identified retrospectively and re-reviewed. Representative tissue blocks were selected and tissue microarray construction was undertaken. Fluorescent *in situ* hybridisation for FGFR1 was carried out. Patients with unsuccessful FGFR1 FISH, those with minor histologies (i.e., other than adenocarcinomas, large cell carcinomas or squamous cell carcinomas), neo-adjuvant-treated patients and patients with a wedge resection and unknown surgical resection as well as patients with missing survival data were excluded from the study. The final cohort consisted of 329 patients.

Specimen characteristics and tissue microarray (TMA). Paraffin-embedded (FFPE) tissue samples fixed in 10% neutral-buffered formalin were available for all the 329 patients from the archives of the Institute for Pathology, University Hospital Basel, Switzerland and at the Institute for Pathology, University Bern, Switzerland. For the TMA construction, the best-preserved and most suitable tissue samples were selected. One punch with a diameter of 0.6 mm per tumour was transferred from the donor tissue block to a receptor paraffin block as previously described (Bubendorf *et al*, 2001).

Two pathologist specialised in pulmonary diseases (SS and MG) reviewed all NSCLC for histology according to the current 2004 WHO recommendations. Staging was assigned according to the TNM classification (7th Edition of the UICC TNM Staging System).

Fluorescent *in situ* hybridisation. *FGFR1* gene status was evaluated using a commercially available FISH probe (*FGFR1*/CEN8; ZytoVision, Bremerhaven, Germany). In this probe, the *FGFR1* gene locus (*FGFR1*) is labelled with a green fluorochrome and the centromeric reference probe (CEN8) with an orange fluorochrome. Before hybridisation, samples were cut to 5 μm

Table 1. Clinico-pathological features and association *FGFR1* gene status

				FGFR1, *N* (%) (*n* = 329)		
Feature	**Freq analysis group (*n* = 329)**	**Freq missing group (*n* = 67)**	***P*-value**	**Negative, *n* = 288 (87.5%)**	**Amplified, *n* = 41 (12.5%)**	***P*-value**
Age (years), median (range)	66.9 (42–83)	67.3 (38–82)	0.5231	66.9 (42–82)	67.9 (51.8–76.7)	0.9539
Tumour size (mm), median (range)	40 (5–170)	35 (10–105)	0.1015	35 (5–170)	45 (15–110)	0.0047[a]
Gender, *n* (%)						
Female	85 (25.8)	15 (22.4)	0.5538	80 (94.1)	5 (5.9)	0.033
Male	244 (74.2)	52 (77.6)		208 (85.3)	36 (14.8)	
Smoker, *n* (%)						
Yes	239 (72.6)	24 (35.8)	0.8707	206 (86.2)	33 (13.8)	0.7472
No	18 (5.5)	1 (1.5)		17 (94.4)	1 (5.6)	
No information	72 (21.9)	42 (62.7)		65 (90.3)	7 (9.7)	
pT (UICC 7th) , *n* (%)						
pT1	36 (10.9)	5 (7.5)	0.2807	36 (100.0)	0 (0.0)	0.0504
pT2	263 (80)	59 (88.0)		227 (86.3)	36 (13.7)	
pT3	30 (9.1)	3 (4.5)		25 (83.3)	5 (16.7)	
Stage UICC 7th, *n* (%)						
Stage I	247 (75.1)	55 (82.1)	0.2188	224 (90.7)	23 (9.3)	0.0027
Stage II	82 (24.9)	12 (17.9)		64 (78.0)	18 (22.0)	
Histology, *n* (%)						
Adenocarcinoma	137 (41.6)	24 (35.8)	0.4961	134 (97.8)	3 (2.2)	<0.0001
Large cell	23 (7)	7 (10.5)		20 (87.0)	3 (13.0)	
Squamous cell	169 (51.4)	36 (53.7)		134 (79.3)	35 (20.7)	
Operation, *n* (%)						
Bilobectomy	25 (7.6)	5 (7.5)	0.1526	23 (92.0)	2 (8.0)	0.0003
Lobectomy	263 (80.0)	51 (76.1)		237 (90.1)	26 (9.9)	
Pneumonectomy	41 (12.4)	10 (14.9)		28 (68.3)	13 (31.7)	
Sublobar resection	0 (0.0)	1 (1.5)				
Recurrence (*n* = 127)						
No	58 (100.0)	0	—	53 (91.4)	5 (8.6)	0.1482
Yes	69 (100.0)	0		57 (82.6)	12 (17.4)	
Overall survival (months) (*n* = 329), median (95% CI)	93.8 (67.3–115.8)	—	—	103.1 (69.2–130.8)	43.9 (21.3-NE)	0.0205
Disease-free survival (months) (*n* = 127), median (95% CI)	43.2 (34.6–73.1)	—	—	52.4 (38–123.1)	22.5 (8.7–60.7)	0.0466

Abbreviations: CI = confidence interval; UICC = Union for International Cancer Control.
[a]See also Result section for detailed information.

sections, deparaffinised and pretreated with commercial pretreatment kit Vysis (Abbott Molecular, Des Plaines, IL, USA). Hybridisation was performed overnight in a humidified chamber at 37 °C. Afterwards slides were washed with Vysis washing solution and counterstained with Vysis DAPI (Abbott Molecular). Amplification was defined as an *FGFR1* to CEP8 signal ratio of $\geqslant 2.0$. An example of an amplified SCC is shown in Figure 2. The *FGFR* gene status was evaluated blinded from clinical or pathological data.

Statistical considerations. Differences between *FGFR1* gene status and categorical clinico-pathological features were determined using the chi-square test or Fisher's exact test, where appropriate. Continuous variables such as tumour size were analysed using the non-parametric Wilcoxon's rank sum test. Overall survival (OS; date of operation to date of death from any cause or last date of follow-up) and disease-free survival (DFS; date of operation to date of any sign of tumour relapse – local, regional or distant) were the primary endpoints. Patients without the event were censored at the date of last follow-up. Differences in survival time were analysed using the log-rank or Wilcoxon's test and plotted using Kaplan–Meier curves. In addition, Cox regression analysis in multivariable setting was employed to determine the effect of *FGFR1* gene status on survival time after adjustment for possible confounding factors (smoking status, tumour size and stage). Assumption of proportional hazards was met. The hazard ratio (HR) and 95% CI were used in this setting with a value of 1.0 considered baseline. *P*-values <0.05 were considered statistically significant. All analyses were carried out using SAS (V9.2; The SAS Institute, Cary, NC, USA).

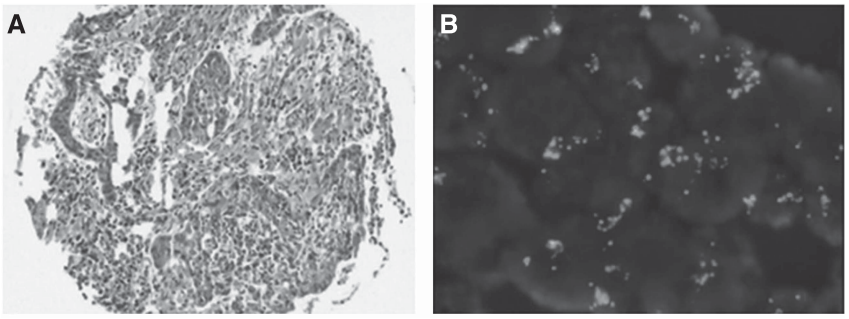

Figure 2. Squamous cell carcinoma (SCC) with *FGFR1* amplification. (**A**) Poorly differentiated SCC on the tissue microarray (haematoxylin and eosin staining, original magnification × 200). (**B**) Fluorescent *in situ* hybridisation of the same SCC shows *FGFR1* amplification (*FGFR1*/CEP8 ratio: >2.0). The *FGFR1* gene is labelled in green and the centromeric CEP8 reference probe in red. The full colour version of this figure is available at *British Journal of Cancer* online.

RESULTS

***FGFR1* amplification and clinico-pathological features.** *FGFR1* amplification was detected in 12.5% (41/329) of all NSCLC. Amplification was detected in 20.7% (35/169) of SCC, 13% (3/23) LC and 2.2% (3/137) AC. Hence, *FGFR1* amplification was significantly associated with SCC histology ($P<0.0001$).

No *FGFR1* amplification was seen in T1 NSCLC (⩽3 cm; 0/36). The remaining associations with *FGFR1* amplification were as follows: T2 (>3 cm to <7 cm) 13.7% (36/263) and T3 (>7 cm tumour size) 16.7% (5/30). *FGFR1* amplification was significantly associated with a higher T-category ($P=0.0504$) and larger tumour size ($P=0.0047$) (Table 1).

Stage I (IA and IB) NSCLC was amplified in 9.3% (23/247) and stage II (IIA and IIB) in 22% (18/82), resulting in a significant ($P=0.0027$) correlation with higher tumour stage.

FGFR1 amplification was significantly more frequent in male patients (14.8%; 36/244) compared with female patients (5.9%; 5/85) ($P=0.033$). The prevalence of smoking history was higher in males (76.3%) with a median of 50 pack-years (py; range: 5–150 py) compared with females (23.7%) with a median of 40 py (range: 15–100 py). *FGFR1* amplification was seen in 13.8% (33/239) of active smokers and in 5.6% (1/18) of non-smokers.

FGFR1 amplification and prognosis

Overall survival. Overall survival was available for all 329 patients included in the analysis. Overall survival at 5 and 10 years were significantly worse in patients with *FGFR1* amplification $P=0.0204$ and $P=0.0205$, respectively (Figure 3). All AC and LC patients with *FGFR1* amplification died within the first 5 years resulting in a significantly ($P<0.05$) worse OS compared with patients with a non-amplified tumour.

Disease-free survival. Information on tumour progression was available in 38.6% ($n=127$) of patients with the following histologies: 46.5% ($n=59$) SCC, 45.7% ($n=58$) AC and 7.9% ($n=10$) LC. Patients with an *FGFR1*-amplified tumour showed a significant difference in DFS at 5 years ($P=0.0466$) compared with patients with a non-amplified tumour and there was a trend towards worse DFS in *FGFR1*-amplified tumours at 10 years ($P=0.0965$) with a median of 22.5 months (8.7–60.6) compared with 52.4 months (38–123.1) in non-amplified tumours (95% CI).

Recurrence. Data for recurrence were available in 38.6% ($n=127$) patients. Of 17 amplified tumours with informative results, 12/17 (70.6%) had recurrence in comparison to 57/110 (51.8%) non-amplified tumours with recurrence. The results are summarised in Table 1.

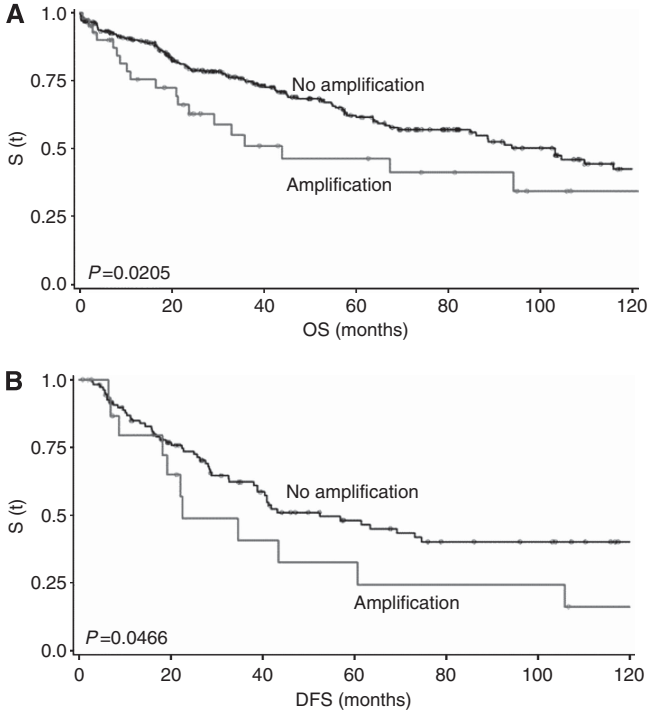

Figure 3. *FGFR1* gene status and overall and progression-free survival. (**A**) Patients ($n=329$) with an *FGFR1*-amplified NSCLC show a significantly worse OS at 5 ($P=0.0204$) and 10 years ($P=0.0205$) (purple curve) compared with patients with a non-amplified NSCLC (black curve). (**B**) Patients ($n=127$) with *FGFR1*-amplified NSCLC (purple curve) have worse disease-free survival at 5 years ($P=0.0466$) and 10 years ($P=0.00965$) compared with patients with a non-amplified NSCLC (black curve). The full colour version of this figure is available at *British Journal of Cancer* online.

Overall survival of SCC. Overall survival of patients with SCC showed within the first 4 years significant worse survival compared with patients with non-amplified tumour (2 years $P=0.024$, 3 years $P=0.0208$, 4 years $P=0.0422$, 5 years $P=0.0685$).

Multivariate analysis and prognosis. To control possible confounding factors, we included smoking status, tumour size and pathological tumour stage in multivariate logistic regression analysis. The multivariate analysis confirmed that *FGFR1* amplification is an independent poor prognostic factor ($P=0.0367$; HR: 2.06 (95% CI: 1.05–4.05)). Analysing *FGFR1* status with tumour size and histological subtype (squamous *vs* other) as independent variables, tumour size shows only a marginal significant

relationship with amplification ($P=0.0854$; OR (95% CI): 1.01 (0.99–1.03)), whereas histological subtype is significantly associated with *FGFR1* amplification ($P<0.0001$; OR (95% CI): 7.32 (2.77–19.3)). In a second analysis with smoking status and tumour size, again a marginal significance for size was noticed ($P=0.0763$; OR (95% CI): 1.02 (0.99–1.04)), whereas smoking status was not associated with *FGFR1* amplification ($P=0.3890$; OR (95% CI): 2.5 (0.31–20.1)).

The results are shown in Table 2.

DISCUSSION

Different pathways are activated due to *FGFR1* upregulation such as, for example, mitogen-activated protein kinase (MAPK) signalling (Weiss *et al*, 2010) a key pathway in carcinogenesis and maintenance of lung cancer (Neuzillet *et al*, 2014). Therefore, disruption of tumorigenesis by targeting this kinase cascade is of great clinical value. A promising novel target is the tyrosine kinase *FGFR1*, especially for *FGFR1*-amplified NSCLC. Information on the prevalence of *FGFR1* amplification in NSCLC and its correlations with clinico-pathological features is important for further clinical studies and was the subject of this study.

FGFR1 amplification detected by FISH is common in early-stage SCC (20.7%) and in LC (13%) but rare in AC (2.2%). This finding is in line with previous studies (Table 3). Not surprisingly, *FGFR1* amplification was mostly seen in active smokers or ex-smokers of male gender as most SCC are diagnosed in men with a smoking history. Some studies assumed that *FGFR1* might contribute to carcinogenesis in SCC (Freier *et al*, 2007). Whether smoking causes *FGFR1* alterations or whether smoking and *FGFR1* alterations are independent and trigger combined tumorigenesis in NSCLC has to be determined in further studies.

It is hypothesised that *FGFR1* amplification is a driver mutation (Weiss *et al*, 2010) and subsequently should therefore be seen early on in small tumours. However, we were unable to see any *FGFR1* amplification in small tumours (T1); therefore this hypothesis is difficult to substantiate with our results. A possible explanation of our results could be that *FGFR1* amplification is acquired later during tumour growth, which would explain why *FGFR1* amplification was not detected in small tumours (T1). The hypothesis of *FGFR1* amplification as passenger mutation is supported by the observation that the prevalence of *FGFR1* amplification does increase with tumour size (T1: 0%, T2: 13.7%; T3: 16.7%). It has previously been shown that *FGFR1* amplification can be focal (Heist *et al*, 2012; Tran *et al* 2013), resulting in

Table 2. Multivariate overall and disease-free survival analyses of *FGFR1* gene status adjusting for smoking status, tumour size and TNM stage

	All patients		Squamous cell carcinoma patients	
	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**
Overall survival				
FGFR1				
Negative Amplified	0.0367	1 2.06 (1.05–4.05)	0.8668	1 1.05 (0.57–1.93)
Smoking status				
Non-smoker Smoker	0.3209	1 1.68 (0.6–4.7)	Not included[a]	
Tumour size				
Baseline 1-unit increase	0.825	1 1.0 (0.98–1.02)	0.5829	1 1.01 (0.99–1.02)
pT stage				
pT1 pT2	0.781	1 1.14 (0.44–2.95)	0.8284	1 1.1 (0.48–2.48)
Disease-free survival				
FGFR1				
Negative Amplified	0.2539	1 1.46 (0.76–2.81)	0.7973	1 1.12 (0.48–2.58)
Smoking status				
Non-smoker	Not included[b]		Not included[a]	
Smoker				
Tumour size				
Baseline 1-unit increase	0.1666	1 1.01 (0.99–1.04)	0.0483	1.0 (1.03 (1.0–1.06)
pT stage				
pT1 pT2	0.1277	1 2.03 (0.82–5.0)	0.871	1.1 (0.34–3.61)

Abbreviations: CI = confidence interval; HR = hazard ration; TNM = tumour–nodes–metastasis.
[a]Only 3 non-smokers and 96 smokers. No contribution to multivariate analysis possible.
[b]To prevent over-fitting, smoking status was removed from the analysis. It was not associated with DFS in univariate analysis, whereas size and pT were.

Table 3. Selected lung cancer studies analysing the *FGFR1* gene status in lung cancer patients using FISH technique

Author	Number of NSCLC analysed	Tissue technique	*FGFR1* amplification (%)	*FGFR1* amplification and histology	ISH probe used	Definition of *FGFR1*amplification	Tumour stages included in the study	Overall survival whole patient cohort	Overall survival early stage (I and II)
Weiss *et al*, 2010	153	TMA (two to three cores)	22	only SCC analysed	FGFR1 locus: BAC Reference: none	FGFR1 >9 signals	IA, IB, IIB, IIIA, IIIB (available from $n=15$)	No data	No data
Heist *et al*, 2012	226	Whole tissue sections	16	only SCC analysed	FGFR1 locus: BAC Reference: commercially available CEP8	Ratio FGFR1/CEP⩾2.2	IA; IB; IIA; IIB; IIIA; IIIB; IV	No statistical difference between *FGFR1*-amplified and non-amplified	No difference between *FGFR1*-amplified and non-amplified ($n=155$)
Kohler *et al*, 2012	260	TMA (one core)	6.5	10.5% SCC, 4.7% AC	Commercially available dual-colour FISH probe	FGFR1⩾4 signals	T1; T2; T3; T4; N0; >N0	No impact	No data
Schildhaus *et al*, 2012	407	Whole tissue sections	17.3	20% SCC and 1x ASCC and 1x LC	Commercially available dual-colour FISH probe	FGFR1/CEN8 ratio ⩾2.0 or average number of FGFR1 signals per tumour cell nucleus is ⩾6 or the percentage of tumour cells containing ⩾15 FGFR1 signals or large clusters in ⩾10% or the percentage of tumour cells containing ⩾5 FGFR1 signals in ⩾50%	No data	No impact (preliminary data)	No data
Zhang *et al*, 2012	127	Whole tissue sections	8.7	12.5% SCC, 7% AC	FGFR1 locus: BAC Reference: commercially available CEP8	Ratio FGFR1/CEP⩾2.0 or ⩾10%gene cluster	I, II, III, IV	No data	No data
Craddock *et al*, 2013	121	TMA (three cores)	18.2	only SCC analysed	Commercially available dual-colour FISH probe	>5 Copies of FGFR1	IA, IB, IIA, IIB, IIIA, IIIB, IV	No significant difference	No data
Kim *et al*, 2013	262	TMA (three cores)	13	Only SCC analysed	Commercially available dual-colour FISH probe	FGFR1 signal ⩾9 (high-level amplification)	I; II; IIIA; IIIB	High-level FGFR1 amplified shorter survival and DFS	No data
Tran *et al*, 2013	264	TMA (three to six cores)	14	21.8% SCC, 20.5%LC, 5.2% AC	Commercially available dual-colour FISH probe	According to Schildhaus *et al*, 2012	IA, IB, IIA, IIB, III	*FGFR1*-amplified tumours better prognosis	No data
Current study	329	TMA (one core)	12.5	20.7% SCC, 2.2% AC, 13% LC	Commercially available dual-colour FISH probe	FGFR1 to CEP8 signal ratio of ⩾2.0.	IA, IB, IIA, IIB	Worse OS and worse DFS	Difference between *FGFR1*-amlified and non-amplified tumours ($n=329$)

Abbreviations: AC = adenocarcinoma; BAC = bacterial artificial chromosome; FISH = fluorescent *in situ* hybridisation; LC = large cell carcinoma; NSCLC = non-small cell lung carcinoma; SCC = squamous cell carcinoma; TMA = tissue microarray.

false-negative findings in T1 cancers using our TMA. This could be true mainly for tumours with a small tumour-cell population with *FGFR1* amplification. However, such heterogeneity of *FGFR1* amplification would apply for all stages and tumour sizes, and can hardly explain the differences in *FGFR1* amplification between stages. A study addressing the issue of tumour heterogeneity and tumour marker validation on TMAs advised four to five cores for best results. However, accurate results of heterogeneously distributed tumour markers using TMAs are dependent on many factors such as for example, complexity of the staining assessment or the amount of positivity of a certain marker. Investigation of larger patient cohorts helps in getting appropriate results (Goethals *et al*, 2006). Interestingly, looking at studies using TMAs *vs* studies using whole tissue sections (Table 3), both groups had similar

ranges of *FGFR1*-amplified tumours (TMA: 6.5%–22% *vs* whole tissue section: 8.7%–17.3%) indicating that TMAs can be suitable to validate *FGFR1* gene status on large patient samples. However, some of the variability in the *FGFR1* gene status seen between the different studies might be explained as well by other factors such as ethnicity. Hence, our result of 20.7% amplified SCC correlates best with the Weiss study that included nearly the same ethnic group (mostly Swiss patients) and showed 22% amplified SCC.

In our study, patients with an *FGFR1*-amplified NSCLC showed a significantly worse 5-year ($P=0.0204$) and long-term (10-year) OS ($P=0.0205$), more frequent recurrence (70.6% *vs* 51.8%) and significantly shorter long-term DFS ($P=0.0466$). These results indicate that *FGFR1* amplification is a negative prognostic marker in early-stage NSCLC. However, it is still controversially discussed whether *FGFR1* amplification is associated with worse OS. Some previous studies showed unfavourable prognosis (Kim *et al*, 2013), some showed no difference (Heist *et al*, 2012; Craddock *et al*, 2013) and some have concluded that *FGFR1* amplification could be a favourable prognostic marker (Tran *et al*, 2013). The reason for this conflicting data might be the mixed patient cohorts including early and advanced lung cancer patients and the different criteria used to determine *FGFR1* amplification (see Table 3). The one study using similar cut-off criteria for *FGFR1* amplification and with available survival data on early-stage lung cancer patients showed no significant difference in OS between patients with an amplified and non-amplified tumour (Heist *et al*, 2012). However, this study included only SCC lung cancer patients and it is not known whether the cohort of early-stage lung cancer patients did as well include neo-adjuvant-treated patients. As we analysed in our cohort SCC lung cancers only, we could clearly see a significantly worse OS of patients with an *FGFR1*-amplified tumour within the first 4 years (Figure 4). After this time point *FGFR1* amplification had no prognostic significance in patients with SCC lung cancer may be because all patients with an aggressive, *FGFR1*-amplified tumour died early. This seems to be true especially for AC and LC as these patients contributed substantially to the significantly worse OS in our multivariate analysis. Our study focused on a well-defined patient group of node-negative early-stage NSCLC treated with surgery alone and that might have resulted in a more accurate survival data. Reasons for worse OS and worse DFS in patients with *FGFR1*-amplified tumours could be due to the activation of proliferation as it was shown in lung cancer cell lines (Weiss *et al*, 2010). This could explain our finding that *FGFR1*-amplified tumours show a significant higher T-category ($P=0.0504$) and larger tumour size ($P=0.0047$). However, we do not have data on proliferation and *FGFR1* gene status to support this theory. We can, however, conclude that *FGFR1* amplification is an indicator of more aggressive tumour biology and that patients with an *FGFR1*-amplified tumour would need special treatment.

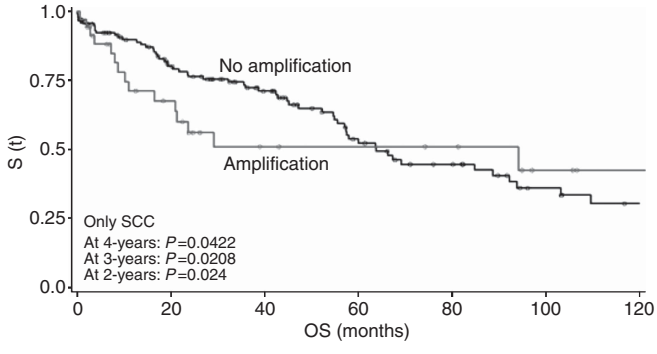

Figure 4. *FGFR1* gene status and overall survival in squamous cell carcinoma (SCC). Patients ($n=169$) with an *FGFR1*-amplified SCC show a significantly worse overall survival at 2 ($P=0.024$), 3 ($P=0.0208$) and 4 years ($P=0.0422$) (purple curve). After 5 years no difference between patients with an *FGFR1*-amplified and non-amplified SCC was seen (black curve). The full colour version of this figure is available at *British Journal of Cancer* online.

FGFR1 amplification is discussed as a possible new therapeutic target for anti-*FGFR1* inhibitors (Weiss *et al*, 2010); therefore, we feel that *FGFR1* amplification status should be evaluated prospectively in patients with SCC lung cancer and LC, and might be as well in AC. As patients with SCC lung cancer have only limited options regarding systemic therapies, they might profit from a targeted therapy. In this context it is also worth noting that *FGFR1* gene status in primary and lymph node metastases are reported as highly concordant (97.7%). Therefore, *FGFR1* gene status might be determined on both sites (Craddock *et al*, 2013). Whether *FGFR1* is not only involved in tumorigenesis but also might as well in lymphatic spread has to be determined in further studies.

In a recently published study, it was shown that increased expression of *FGFR1* was observed in cell lines resistant to gefitinib (Terai *et al*, 2013) and in another study it was observed that patients with amplified *FGFR1* status benefit from adjuvant chemotherapy (Kim *et al*, 2013). These findings revealed further the potential role of *FGFR1* as a predictive marker not only for anti-FGFR therapy but also might as well for other lung cancer treatments.

In summary, we showed that *FGFR1* amplification is a negative prognostic marker in patients with early-stage NSCLC treated with surgery. Moreover, our data challenged the hypothesis of *FGFR1* as a driver mutation as we were not able to detect any *FGFR1* amplification in small tumours (T1). These findings may be of value when planning future prospective or translational studies on *FGFR1* and NSCLC. However, we have to consider that our results are based on retrospective data, and clinical data of recurrence or tumour progression were not available for all patients. A disadvantage of our study is that some of the patients with surgery before 2006 had only limited lymph node staging as standardisation of intraoperative lymph node staging was introduced in 2006 (Lardinois *et al*, 2006). Using a TMA with only one core per tumour can be limiting, and we might have missed some of the tumours with a heterogeneous *FGFR1* amplification. As discussed, however, we feel strongly that our assessment of *FGFR1* gene status in this large cohort is accurate.

To conclude, *FGFR1* amplification is frequent in early-stage NSCLC, especially in SCC, and it appears to be a marker of poor clinical outcome in these patients. Whether it can be used as a prognostic marker has to be elucidated in further clinical studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

NC drafted the manuscript and provided intellectual input. S Savic was involved in the study design, data analysis and interpretation, reviewed the lung cancer samples and provided intellectual input. S Schneider evaluated the *FGFR1* gene status. IA and MB-N were involved in the TMA construction, data collection and approved of the final manuscript. RAS was involved in tissue collection and approved the final manuscript. DL was involved in tissue collection, approved the final manuscript and provided intellectual input. MG provided intellectual input, reviewed the lung cancer samples and approved the final manuscript. LB provided intellectual input and approved the final manuscript. IZ performed

the statistical analysis, provided intellectual input, edited and approved the final manuscript. CT conducted the study, edited and approved the final manuscript.

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