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

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Abstract

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.

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pathways (Bae and Schlessinger, 2010). In this way FGFR1 contributes to cell proliferation, differentiation and migration (Thise and Thiese, 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-lobection. 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).

**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 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
sections, deparaffinised and pretreated with commercial pre-treatment 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 \( \text{FGFR1} \) to CEP8 signal ratio of \( \geq 2.0 \). An example of an amplified SCC is shown in Figure 2. The \( \text{FGFR} \) gene status was evaluated blinded from clinical or pathological data.

### Statistical considerations

Differences between \( \text{FGFR1} \) 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 \( \text{FGFR1} \) 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).

### Table 1. Clinico-pathological features and association \( \text{FGFR1} \) gene status

| 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 (62–83)                  | 67.3 (38–82)                | 0.5231  | 66.9 (42–82)              | 67.9 (51.6–76.7)          | 0.9539  |
| Tumour size (mm), median (range) | 40 (5–170)                    | 35 (10–105)                 | 0.1015  | 35 (5–170)                | 45 (15–110)               | 0.0047* |

| 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.

*See also Result section for detailed information.*
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.

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 increases 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                        | 0.0367  | 1.00 (0.86–1.16) | 0.8668  | 1.05 (0.57–1.93) |
| Amplified                       |          | 2.06 (1.05–4.05) |          | 1.01 (0.99–1.02) |
| Smoking status                  |          |              |          |              |
| Non-smoker                      | 0.3209  | 1.68 (0.6–4.7) | Not included* | Not included* |
| Smoker                          |          |              |          |              |
| Tumour size                     |          |              |          |              |
| Baseline                        | 0.825   | 1.00 (0.98–1.02) | 0.5829  | 1.01 (0.99–1.02) |
| 1-unit increase                 |          | 1.00 (0.98–1.02) |          | 1.01 (0.99–1.02) |
| pT stage                        |          |              |          |              |
| pT1                              | 0.781   | 1.00 (1.44–2.95) | 0.8284  | 1.10 (0.48–2.48) |
| pT2                              |          |              |          |              |
| Disease-free survival            |          |              |          |              |
| FGFR1                           |          |              |          |              |
| Negative                        | 0.2539  | 1.00 (1.06–2.81) | 0.7973  | 1.12 (0.48–2.58) |
| Amplified                       |          | 1.46 (0.76–2.81) |          | 1.00 (1.03 (1.0–1.06) |
| Smoking status                  |          |              |          |              |
| Non-smoker                      | Not included* | Not included* | Not included* | Not included* |
| Smoker                          |          |              |          |              |
| Tumour size                     |          |              |          |              |
| Baseline                        | 0.1666  | 1.00 (0.99–1.04) | 0.0483  | 1.00 (1.03 (1.0–1.06) |
| 1-unit increase                 |          | 1.00 (0.99–1.04) |          | 1.00 (1.03 (1.0–1.06) |
| pT stage                        |          |              |          |              |
| pT1                              | 0.1277  | 1.00 (2.03 (0.82–5.0) | 0.871   | 1.10 (0.34–3.61) |
| pT2                              |          |              |          |              |

Abbreviations: CI = confidence interval; HR = hazard ratio; TNM = tumour–nodes–metastasis.

*Only 3 non-smokers and 96 smokers. No contribution to multivariate analysis possible.

**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.
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

| Author       | Number of NSCLC analysed | Tissue technique | FGFR1 amplification (%) | 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, IIA, IIB | 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, IIB, IIB, 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 | 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, IIB | 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, IIA, IIB | 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, IIB | 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 > 2.0 | IA, IB, IIA, IIB | Worse OS and worse DFS                | Difference between FGFR1-amplified 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.
ranges of FGFR1-amplified tumours (TMA: 6.5%–22% vs whole tissue section: 8.7%–17.3%) indicating that TMA 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.

FGFR1 amplification is discussed as a possible new therapeutic target for anti-FGFR 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 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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