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Clinical impact of mutation fraction in epidermal growth factor receptor mutation positive NSCLC patients

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Background: We examined clinical outcomes in a population-based cohort of *EGFR* mutant advanced NSCLC patients, exploring the potential role of factors including tumour *EGFR* mutation fraction and cellularity in predicting outcomes.

Methods: A cohort of patients with *EGFR* mutant advanced NSCLC was identified ($N=293$); clinical outcomes, pathologic and treatment details were collected. Tumour response was determined from radiology and clinical notes. Association between demographic and pathologic variables EGFR TKI response, time to treatment failure (TTF) and overall survival (OS) was examined using logistic regression and proportional hazards regression. EGFR TKI response rates were summarised by percent mutation fraction to explore their association.

Results: Higher mutation fraction was associated with greater EGFR TKI response rate (odds ratio 1.58, 95% CI = 1.21–2.07, $P=0.0008$), longer TTF (hazard ratio 0.80, 95% CI = 0.68–0.92, $P=0.003$) and better OS (hazard ratio 0.81, 95% CI = 0.67–0.99, $P=0.04$). However, even in patients with $\leqslant 5\%$ mutation fraction, response rate was 34%. Females had longer TTF ($P=0.02$).

Conclusions: *EGFR* mutation fraction in tumour samples was significantly associated with response, TTF and OS. Despite this, no lower level of mutation fraction was detected for which EGFR TKI should be withheld in those with activating *EGFR* mutations.

Epidermal growth factor receptor (*EGFR*) is one of the four closely related subgroup members of the human epidermal growth factor receptor (*HER*)-family (*EGFR* [*HER1/ErbB1*], *HER2* [*ErbB2*], *HER3* [*ErbB3*] and *HER4* [*ErbB4*]; Cadranel *et al*, 2013). The most common activating mutations involve short deletions in the tyrosine kinase (TK) domain in exon 19 (E746_A750) and point mutations in exon 21 (L858R) of the *EGFR* gene. These mutations result in constitutive activation of the TK domain and downstream pathway signalling activation, resulting in increased cell proliferation, decreased apoptosis and metastasis (Pao *et al*, 2005; Jackman *et al*, 2010). Multiple phase III trials in advanced non-small cell lung cancer (NSCLC) patients with *EGFR*-activating mutations

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have shown EGFR tyrosine kinase inhibitors (TKIs) have superior response, quality of life and progression-free survival compared with first-line platinum-based chemotherapy (Mok *et al*, 2009; Rosell *et al*, 2009; Maemondo *et al*, 2010; Mitsudomi *et al*, 2010; Zhou *et al*, 2011; Rosell *et al*, 2012; Yang *et al*, 2012; Inoue *et al*, 2013). The median time to progression (TTP) with EGFR TKIs in *EGFR* mutation-positive NSCLC is 9 to 13 months (Jackman *et al*, 2010; Cadranel *et al*, 2013). Secondary resistance often arises with the emergence of resistance mutations (Kobayashi *et al*, 2005; Pao *et al*, 2005; Sequist *et al*, 2011). An additional ten percent of patients present with primary resistance to EGFR TKIs at first evaluation despite the presence of *EGFR* mutant cells in their tumour (Cadranel *et al*, 2013). Primary resistance is likely a multifactorial process resulting from numerous genetic alterations (Ellis and Hicklin, 2009; Hammerman *et al*, 2009). Purported mechanisms of primary resistance include insertion mutations in exon 20 of the *EGFR* or *HER2* gene, loss of *PTEN*, *BRAF* and *KRAS* mutations; and increased levels of *MAPK*, *IGFR2*, *BCL-2* and *MET* amplification (Sequist *et al*, 2008; Ellis and Hicklin, 2009; Hammerman *et al*, 2009; Turke *et al*, 2010). It is not possible to predict which patients with activating mutations will not respond to EGFR TKI therapy, and the mechanism of primary resistance is poorly understood. This suggests that other factors in addition to *EGFR* mutation status may determine response to EGFR TKIs.

EGFR mutations are assessed with polymerase chain reaction (PCR) and have a 1–5% detection sensitivity (lowest percent reliably detectable (Gocke *et al*, 2000; Milbury *et al*, 2009; Kamel-Reid *et al*, 2012; Shiau *et al*, 2014). Unlike *HER2* amplification in breast and gastric cancer and *ALK* rearranged NSCLC where quantitative cut-offs have been established (Hirsch *et al*, 2002; Heinmoller *et al*, 2003; Camidge *et al*, 2010, 2013), it is unknown whether the level of *EGFR* mutation fraction affects response to EGFR TKIs. Previous investigation from our institution has shown that tumour cellularity was significantly associated with *EGFR* test success in NSCLC histology and cytology samples (Shiau *et al*, 2014). In another study, 75% of NSCLC samples with poor cellularity, but considered to be representative of tumour, were successfully tested with an *EGFR* mutation prevalence of 9% (Leary *et al*, 2012). The recent CAP/IASLC/AMP Molecular Testing Guideline for lung cancer highlights that an ideal test should be able to detect mutations in samples with tumour cellularity as low as 10% (Lindeman *et al*, 2013). The guidelines also recognise that while analytic sensitivity is important for smaller samples, ultrasensitive molecular assays may carry risks of false positive results. However, the impact of sample quality, including tumour cellularity, and *EGFR* mutation fraction on clinical outcome with EGFR TKI is unknown.

In this study, we describe clinical outcomes with EGFR TKI therapy, including response rate (RR), time to treatment failure (TTF) and overall survival (OS), in a population-based cohort of advanced *EGFR* mutation-positive NSCLC patients, and explore potential predictors of outcome including histopathologic correlates of tumour sample, *EGFR* mutation fraction, cellularity, sample and mutation type, and demographic variables. We also explore the relationship between different levels of mutation fraction and outcome, to identify a threshold associated with EGFR TKI response.

MATERIALS AND METHODS

The study protocol was approved by the research ethics boards of the eleven participating centres, along with data-sharing agreements. From March 2010 to March 2012, *EGFR* testing in the province of Ontario, Canada was conducted at a single centre (University Health Network, Toronto, Canada; UHN). The choice of 2010 to 2012 was due to the centralisation of EGFR testing to the UHN. Patients with *EGFR* mutation-positive samples were identified at each centre, and evaluated for *EGFR* mutation fraction. Standard protocol for *EGFR* mutation testing included an initial review of the haematoxylin- and eosin (HE)-stained section, prepared at the same time as unstained sections for DNA isolation, from the submitted tumour block. The slides and reports were reviewed by a pulmonary pathologist or cytopathologist. Sample-related parameters available in original reports or as assessed by pathologists were recorded. For histology samples, pathologists marked the tumour areas on the HE section to guide macrodissection by the molecular laboratory technologists.

EGFR mutation fraction was defined as the ratio between mutant *EGFR* and wild-type alleles in the macrodissected sample, but does not control for potential normal cell DNA contamination. Tumour cellularity was defined as the percentage of epithelial NSCLC tumour cells to all nucleated cells within the test sample (Shiau *et al*, 2014), and was performed on the same macrodissected sample, which allowed analysis to be performed in the same region. Mutation testing was conducted using fragment analysis (exon-19 deletions) and restriction fragment length polymorphism (exon-21 L858R) methods (Shiau *et al*, 2014). The same method of detection for EGFR exon 19 deletions and the L858R exon 21 mutation was used throughout the entire time period. The detection limit has been established at 1 to 5% by serial dilutions of relevant cell line DNA (Shiau *et al*, 2014). A reagent control, negative control and two positive controls were included with each run. Final test results were reported as (1) positive for exon-19 deletion, (2) positive for exon-21 L858R mutation or (3) negative for exon-19 deletion or exon-21 L858R mutation.

Clinical data were collected including demographic and tumour sample information, response to EGFR TKI, TTF and OS. Response assessment after EGFR TKI therapy was based on the best response reported in radiology and/or clinical reports. Response was defined as evidence of tumour regression, stable disease if there was no change in tumour size, mixed response if there was regression in some tumours but progression in others with continuation of EGFR TKI therapy and progressive disease in the case of tumour growth. TTF was calculated from the start of EGFR TKI treatment until the EGFR TKI treatment stoppage date or the date of death if the patient died on treatment. Patients were censored at last follow-up date if still on treatment or if lost to follow-up. OS was calculated from the start of EGFR TKI treatment until the date of death or censored at the last follow-up date.

Statistical analysis. Cox proportional hazard models (TTF, OS) and proportional odds logistic regression (tumour response) were used to assess the association between clinical outcomes and factors including *EGFR* mutation fraction, tumour sample cellularity, age, sex, smoking status, *EGFR* mutation type (exon 19 or 21), sample biopsy site (primary or metastatic) and EGFR TKI in the first-line *vs* second-line setting. Smoking status was ascertained from the medical notes recorded by the medical oncologist at the patient's first visit. Mutation fraction was analysed as a continuous variable in Cox regression and logistic regression analyses. The distribution of mutation fraction was right skewed; therefore, we performed a natural log transformation to achieve approximate normality. Cellularity was considered as a confounding factor, and it was included in all multivariable analyses to correct for this potential impact on biomarkers such as mutation fraction. Cellularity was dichotomised at its median (50%) as high *vs* low. All factors with $P<0.25$ in the univariable analysis were included in a stepwise variable selection procedure for the multivariable analysis, and those with $P<0.10$ were included in the final multivariable analysis. Hazard ratio (HR) and odds ratio (OR) with their 95% confidence interval (CI) were reported.

RESULTS

Patient and tumour sample characteristics. A total of 293 patients with activating *EGFR* mutations were identified at the 11 participating centres (Table 1). Of these, 253 received EGFR TKI treatment, 79% ($n=200$) as first-line treatment for NSCLC, 21% ($n=53$) as second-line treatment. Forty patients (14%) did not receive an EGFR TKI. The median age at diagnosis of metastatic disease was 65.2 years (range 26.2–95.5) in the cohort, with a predominance of females (72%). Most patients were never smokers (59%), 59% were Caucasian and 38% were Asian. The median follow-up time from the date of metastatic diagnosis was 24.4 months (range 0.03–69.9 months) and the median follow-up time from the date of EGFR TKI treatment initiation was 18.8 months (range 0–43.7 months).

The sample type submitted for *EGFR* testing was evenly split among resected samples (32%), fine-needle aspirate (FNA) or pleural fluid cytology samples (30%), and core lung biopsies (38%). Most (61%) had the primary sampled and submitted for *EGFR* testing. Half (53%) had an exon 19 mutation. The median cellularity of submitted samples was 50.0% (range 1.0–98.0%). The median mutation fraction was 27.2% (range 0.4–96.2%, 25–75% interquartile range 10–50%).

Table 1. Demographics and patient characteristics (TKI treated, $N=253$)

Characteristics	Number of patients (%)
Age	
Median (range)	65.2 years (26.2–95.5)
Sex	
Female	183 (72%)
Male	70 (28%)
Ethnicity	
White	173 (59%)
Asian	110 (38%)
Black	10 (3%)
Median tumour sample cellularity ($n=238$)	50.0% (range 1.0–98.0%)
Median *EGFR* mutation frequency ($n=246$)	29.7% (range 0.4–96.2%)
EGFR	
Exon 19	134 (53%)
Exon 21	119 (47%)
Smoking history	
Current	16 (7%)
Former	80 (34%)
Non-smoker	140 (59%)
Unknown	17
Best response to EGFR TKI	
Response	141(62%)
Stable/mixed	58 (25%)
Progression	30 (13%)
No assessment/unknown	24
Sample tested	
Resected sample	81 (32%)
Cytology sample	76 (30%)
Core biopsy	96 (38%)
Biopsy site	
Primary	154 (61%)
Metastases	99 (39%)
Received subsequent treatment after EGFR-TKI	
Second-line chemotherapy	50 (20%)
Platinum-based doublet	38 (15%)
Mean number of cycles of second-line chemotherapy	4 cycles
Third line chemotherapy	15 (6%)
Fourth line chemotherapy	1 (0.3%)
Another EGFR TKI or TKI trial	15 (6%)
Lost to follow-up	27 (11%)

Abbreviations: EGFR = epidermal growth factor receptor; TKI = tyrosine kinase inhibitor.

Clinical outcome of the *EGFR* mutation-positive patients treated with EGFR TKIs – Factors associated with response, TTF and OS

EGFR *TKI response.* The majority of patients (62%) had a response to EGFR TKIs (measured as any tumour regression); 25% of patients had stable disease or mixed response; and 13% demonstrated progression of disease on therapy. In multivariable analysis, mutation fraction was significantly associated with response (OR 1.58, 95% CI = 1.21–2.07, $P=0.0008$), even after correcting for the confounding effect of tumour cellularity (Table 2). However, even with ⩽5% mutation fraction, we saw a 34% response rate. Younger age was significant on univariable analysis (OR 0.75 per 10 years, $P=0.01$), but it was not significant on the multivariate analysis ($P=0.06$, Table 2).

Time to treatment failure. A total of 165 patients (64%) had experienced treatment failure at the time of analysis. The median TTF on EGFR TKI was 13.2 months (95% CI = 10.7–14.9 months), Figure 1. In the subgroup of patients who had response, TTF was 17.3 months (95% CI = 15.0–21.0 months) *vs* 9.2 months in those with stable disease/mixed response (95% CI = 7.5–14.7 months) and 2.3 months in the subgroup without response (95% CI = 1.9–NA or upper limit not reached). In multivariable Cox analysis, after correcting for tumour cellularity, higher mutation fraction (HR 0.80, 95% CI = 0.68–0.92, $P=0.003$) and female sex (HR 0.66, $P=0.02$) were significantly associated with a longer TTF (Table 3).

Overall survival from EGFR TKI initiation. One hundred and five patients (42%) had died at the time of analysis. The 1-year and

Table 2. Predictors associated with responses to EGFR-TKI treatment

	Best response (response *vs* mixed/stable *vs* progression)					
	Univariable			Multivariable		
	Odds ratio	95% CI	*P*-value	Odds ratio	95% CI	*P*-value
EGFR mutation frequency, in log scale	1.60	1.25–2.06	0.0002	1.58	1.21–2.07	0.0008
Tumour cellularity, high *vs* low	0.85	0.50–1.44	0.54	0.63	0.36–1.12	0.12
Age, per 10 years	0.75	0.60–0.94	0.01	0.79	0.62–1.01	0.06
Sex, female *vs* male	1.11	0.62–2.00	0.72			
Smoking, ever smoking *vs* other	0.61	0.35–1.06	0.08			
Mutation type, exon 19 *vs* exon 21	0.85	0.51–1.44	0.55			
Biopsy site, primary *vs* metastasis	0.96	0.56–1.64	0.89			
EGFR TKI, first line *vs* second line	0.92	0.49–1.76	0.81			

Abbreviations: CI = confidence interval; EGFR = epidermal growth factor receptor; TKI = tyrosine kinase inhibitor.

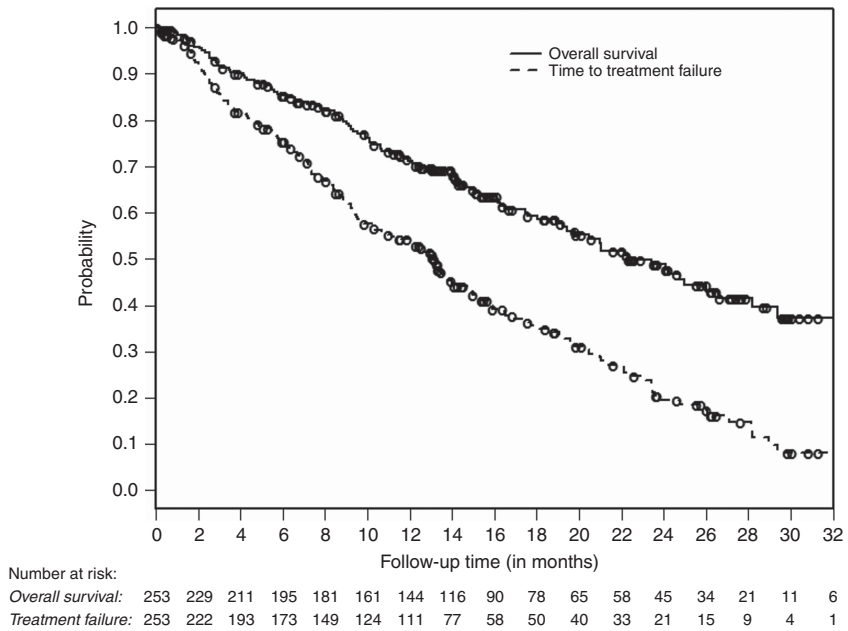

Figure 1. Time to treatment failure and overall survival in patients treated with EGFR-TKI.

Table 3. Predictors associated with time to treatment failure and overall survival

	Time to treatment failure						Overall survival					
	Univariable			Multivariable			Univariable			Multivariable		
	Hazard ratio	95% CI	*P*-value	Hazard ratio	95% CI	*P*-value	Hazard ratio	95% CI	*P*-value	Hazard ratio	95% CI	*P*-value
EGFR mutation frequency, in log scale	0.81	0.70–0.94	0.005	0.80	0.68–0.92	0.003	0.80	0.67–0.95	0.01	0.81	0.67–0.99	0.04
Tumour cellularity, high *vs* low	0.89	0.64–1.22	0.46	1.00	0.72–1.38	0.99	1.31	0.89–1.96	0.17	1.38	0.92–2.09	0.12
Age, per 10 yrs	1.08	0.95–1.23	0.21				1.23	1.04–1.45	0.01	1.20	0.99–1.44	0.06
Sex, female *vs* male	0.7	0.51–0.98	0.04	0.66	0.48–0.93	0.02	0.77	0.51–1.17	0.23			
Smoking, ever smoking *vs* others	1.31	0.96–1.80	0.09				1.67	1.12–2.48	0.01			
Mutation type, exon 19 *vs* exon 21	0.9	0.66–1.22	0.49				0.99	0.67–1.45	0.95			
Biopsy site, primary *vs* metastases	0.86	0.62–1.18	0.34				0.89	0.60–1.33	0.57			
EGFR TKI, first line *vs* second line	0.93	0.65–1.33	0.67				1.04	0.66–1.66	0.86			

Abbreviations: CI = confidence interval; EGFR = epidermal growth factor receptor; TKI = tyrosine kinase inhibitor.

2-year survival rates in the cohort were 71.2% (95% CI = 65.5%–77.4%) and 48.9% (95% CI = 41.6%–57.4%), respectively (Figure 1). Median survival for patients who received EGFR TKI in the first-line setting was 21.0 months (95% CI = 18.9–28.2 months) from the start of therapy, and 26.0 months (95% CI = 12.5–NA; $P = 0.86$) for those receiving EGFR TKI as second-line therapy. In multivariable analysis, higher mutation fraction was associated with longer OS (HR 0.81, 95% CI = 0.67–0.99, $P = 0.04$), after correcting for the effect of cellularity (Table 3). Increasing age was associated with shorter OS on the univariable analysis, but not on the multivariable Cox regression ($P = 0.06$).

Subsequent treatment following progression on EGFR TKI. Although approximately one-third of patients in the cohort remained on EGFR TKI, most (32%) did not receive any additional systemic therapy after EGFR TKI failure. Twenty-one percent received subsequent therapy after progression on EGFR TKI therapy, most commonly (76% of cases) platinum-based doublet chemotherapy for a median of four cycles (range 1–6). Of these, 15 (30%) had a response (defined as any tumour regression) to second-line treatment (first-line chemotherapy), another 40% had stable disease and 28% progressive disease. Only 15 patients received third-line treatments. Median duration of third-line therapy was 3.5 cycles; two of 15 achieved response, six had stable disease and three progressed. Fifteen patients received a second-generation EGFR TKI or participated in a randomised trial of a second-generation EGFR TKI (NCIC Clinical Trials Group BR.26 dacomitinib *vs* placebo; NCT01000025).

***EGFR* mutation-positive patients not treated with EGFR TKI.** Forty patients (14%) in the cohort with *EGFR* mutations did not receive an EGFR TKI. The most common reasons for non-treatment were that patients were too unwell or had died before testing results and/or EGFR TKI funding approval. Other reasons included loss to follow-up and minimal disease burden on observation. Median survival in those untreated was 3.6 months (95% CI = 2.4–NR).

DISCUSSION

The introduction of EGFR TKI therapies and discovery of *EGFR* mutations in the last decade has significantly changed the approach to the treatment of NSCLC (Antonicelli *et al*, 2013; Cadranel *et al*, 2013). However, as many as 30 to 40% of patients with activating *EGFR* mutations do not have a major response to EGFR TKI therapy (Cadranel *et al*, 2013). This suggests that additional factors may influence *EGFR* signalling, including dysregulation of other genes and pathways. We assessed potential factors including *EGFR* mutation fraction and cellularity affecting clinical outcomes in patients with *EGFR*-activating mutations treated with EGFR-TKIs. In our study, there was an increase in response with increasing *EGFR* mutation fraction; however, response for those patients with $\leqslant$5% mutation fraction was still considerable, with 34% of patients experiencing tumour regression. Therefore any level of mutation fraction should be tested for an *EGFR* mutation, as long as it is within the reliable lower limit of detection of the *EGFR* testing method, and all patients with an activating mutation detected in their tumours should be offered an EGFR TKI. No *EGFR* mutation fraction cut-off level was identified at which it would be considered reasonable to withhold treatment. However clinicians should be mindful that lower mutation fraction levels may be associated with lesser response, shorter TTF and OS as demonstrated in this study. Variables associated with a longer TTF included increasing *EGFR* mutation fraction and female sex. Following multivariable analysis, factors associated with improved response and OS included increasing mutation fraction. Increasing age was associated with a poorer response and worse OS in univariate analysis, but was not significant following multivariable analysis. From our study, we found mutation fraction to be a useful measure which was associated with survival outcomes and is a parameter that can be used by clinical labs globally. However this measurement does have limitations, as it cannot rule out the potential for normal cell inclusion.

With the heterogeneous nature of cancer, it is reasonable to expect that not all cells within a tumour will have mutant *EGFR* alleles. *EGFR* mutation fraction may be a reflection of the proportion of EGFR TKI-sensitive cells in a tumour, but may be biased because of sampling or other issues, such as the presence of *EGFR* amplification. There is increasing evidence that intratumour heterogeneity (ITH), as defined by the 'presence of cell subpopulations harbouring distinct biologic properties', results in the emergence of resistant subclones and has a role in the resistance to therapies (Snuderl *et al*, 2011; Swanton, 2012). Intratumour heterogeneity has demonstrated spatial and temporal expression within a single lesion (Crockford *et al*, 2014). Intratumour heterogeneity has been demonstrated in a number of cancers including glioblastoma multiforme (GBM). Sottoriva *et al* (2013) collected spatially distinct tumour fragments from 11 GBM patients and identified copy number alterations in EGFR/CDKN2A/Bp14ARF as early driver events, and aberrations in PDGFRA and PTEN as later events during cancer progression. Previous investigation in NSCLC patients with *EGFR* mutations identified tumours with heterogeneous populations of both *EGFR* mutated and non-mutated cancer cells resulting in reduced response to gefitinib (Taniguchi *et al*, 2008). Therefore, a putative alteration in *EGFR* mutation fraction numbers could be proposed to exist throughout the tumour. To demonstrate this, it would require sampling multiple sites of tumour tissue for *EGFR* mutation fraction. However, our data demonstrated that the response rate was 34% even in patients whose tumours contained $\leqslant$5% mutation fraction and so they too should be offered treatment with an EGFR-TKI. Therefore testing multiple tumour sites would be unlikely to affect the management decision to treat with an EGFR-TKI in patients with an activating *EGFR* mutation.

Tumours involving the colon, breast, brain and pancreas have an average of 33 to 66 genes that display subtle somatic mutations, resulting in altered protein products (Vogelstein *et al*, 2013). The majority of these mutations are single-base substitutions, and to a lesser extent deletions or insertions. In addition, gene amplification has been demonstrated to have a role in other cancers. For example, breast cancer patients with overexpression of HER2 amplification have shorter disease-free interval and poorer OS than patients whose cancer do not overexpress HER2 (Mayer, 2009). It has been proposed that the variation in survival outcomes seen in patients with *EGFR*-activating mutations may be a result of tumour heterogeneity (Shan *et al*, 2015). There is evidence that increased EGFR copy number is associated with better response to EGFR-TKIs (Hirsch *et al*, 2002; Cappuzzo *et al*, 2005). A recent study assessing the concurrence of EGFR amplification and sensitizing mutations with survival outcomes from EGFR-TKI therapy identified that patients with *EGFR* gene amplification had a significantly longer PFS than those without (Shan *et al*, 2015). We did not assess *EGFR* amplification in our study; however, given this recent data, assessment of *EGFR* copy number and its association with *EGFR* mutation fraction and its effect on clinical outcome should be assessed in future studies.We now know that some mutations such as the T790M mutation, a rare exon 20 mutation, is associated with resistance to EGFR-TKIs (Yu *et al*, 2014). At the time of our study, it was not common practice to test for mutations other than exon 19 and 21. Future investigation to assess for other mutations, including resistance mutations, and their association with EGFR mutation fraction would be interesting and may provide further insight into the clinical response that was seen with the different mutation fraction groups. In this study, we used fragment analysis and RFLP which was the available technology in our institution at the time to measure EGFR mutation status. Since then, there are many alternative platforms including real-time PCR and next-generation sequencing, which allow quantitative testing of multiple mutations. However, as tumours are heterogeneous, attaining a representative sample of tumour is an important consideration in all these techniques. Recently, platforms such as Sequined or Snapshot can assess for multiple genetic abnormalities (Korpanty and Leighl, 2012). In addition, platforms such as FoundationOne also incorporate the detection of gene rearrangement and changes in gene copy number (Korpanty and Leighl, 2012). Future work assessing the use of *EGFR* mutation fraction in *EGFR* mutation positive tumours should be assessed with alternative diagnostic platforms.

Median survival for patients treated with EGFR TKIs in the first-line setting in this community-based population was 21.0 months (95% CI = 18.9–28.2 months), similar to survival outcomes reported in clinical trials. It is interesting that the majority of patients (80%) did not receive second-line chemotherapy after EGFR TKI progression. However, the majority (70%) of those who did receive further therapy, had evidence of clinical benefit and a similar number of cycles as that which most patients receive first-line. Although our study did not collect data as to the reasons behind not pursuing further therapy after EGFR TKI failure, it is important for clinicians to educate patients that there is a role for second-line chemotherapy if performance status is adequate.

In our study, pre-treatment tumour cellularity was not associated with survival outcomes. Assessment of tumour cellularity change has been investigated in neoadjuvant studies. Radiological response to EGFR TKI treatment in NSCLC patients treated preoperatively with gefitinib for 28 days was related to loss of tumour cellularity and cell proliferation (Rajan *et al*, 2004; Lara-Guerra *et al*, 2012). Reduction in tumour cellularity has also been noted with neoadjuvant chemotherapy in breast cancer and changes were variable between different response categories (Rajan *et al*, 2004). It is unknown whether these changes correlate into a survival advantage. Tumour cellularity changes in the non-

neoadjuvant setting may be assessed with repeat biopsy at the time of progression, which are increasingly being performed for patients entering clinical trials.

CONCLUSIONS

From the current study, no evidence exists to use a lower limit of detection beyond what is technically required for *EGFR* mutation fraction or cellularity to exclude or select *EGFR* mutation-positive NSCLC patients for EGFR TKI therapy. The presence of *EGFR* mutant cells in a tumour sample, irrespective of proportion, using a clinical laboratory improvement amendments (CLIA) approved testing method is associated with response in our study. However, it is clear that mutation fraction is associated with outcome, with those patients with higher *EGFR* mutation fractions having higher response rates, longer time to treatment failure and survival. Therefore, clinicians should be aware of *EGFR* mutation fraction and consider closer follow-up for patients with lower *EGFR* mutation fraction. A greater understanding of both primary and secondary resistance is required to identify patients who will not respond to EGFR TKIs. This may allow identification of new treatments and tailoring of these on an individual basis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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