BRIEF REPORT

# EGFR–Co-Mutated Advanced NSCLC and Response to EGFR Tyrosine Kinase Inhibitors

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**ABSTRACT**

**Objectives:** The evolution of *EGFR* tyrosine kinase inhibitors (TKIs) has changed the landscape of disease for a subset of patients with NSCLC. Most patients with an EGFR mutation respond to these drugs; however, a proportion show limited or no tumor response. We explored the impact of co-mutation (double or multiple mutation), compared with a single mutation, of the *EGFR* gene on response to TKIs in a series of patients with metastatic NSCLC.

**Methods:** We retrospectively analyzed the mutation profiles of nonsquamous NSCLC tested at Royal Prince Alfred Hospital between 2012 and 2015 by MassArray using the OncoCarta v1.0 panel. Patients with metastatic disease whose tumors had sensitizing *EGFR* mutation(s) were included. The primary end point was progression-free survival (PFS). We used the Kaplan-Meier method for PFS and overall survival; the log rank test was used to compare groups with and without co-mutation. Multivariable analysis was done for PFS; response rate was assessed using chi-square and logistic regression analysis.

**Results:** A total of 62 patients were included, and of these, eight (12.9%) had a co-mutation. The median PFS and overall survival times were 11.5 and 26.3 months, respectively. Patients with *EGFR* co-mutation had a significantly shorter median PFS than those with a single mutation (5.7 months versus 12.3 months, $p = 0.02$). The response rate to TKIs was significantly worse in those with co-mutation compared with in those without co-mutation (38% versus 89%, $p < 0.001$).

**Conclusions:** Taking into account the small number of patients in this study, PFS in patients with *EGFR* co-mutation appeared significantly shorter, and response rate significantly lower, than in patients with a single mutation. Data from multipanel testing may identify subgroups of patients who are likely to respond poorly to standard treatment. Clarification of these subgroups may improve patient care.



*Keywords:* EGFR; Co-mutation; Double mutation; Multiple mutation; Tyrosine kinase inhibitors

## Introduction

Lung cancer is the leading cause of cancer death in Australia, projected to account for 9.4% of cancer diagnoses and 18.9% of cancer deaths in 2015.[1] More than 60% of lung cancer diagnoses are made at an advanced

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stage and 5-year survival for metastatic NSCLC remains at 3%.[2] Platinum-based combination chemotherapy was found to prolong survival in metastatic disease nearly 30 years ago.[3] Only modest improvement in the treatment of metastatic NSCLC was made until molecular targeted agents were shown to improve patient survival in a subgroup of patients with NSCLC. A notable example is the use of EGFR tyrosine kinase inhibitors (TKIs) in patients whose tumors harbor a mutation in the tyrosine kinase domain of the *EGFR* gene.

The two most common *EGFR* mutations, a deletion in exon 19 (particularly E746-A750del) and a mutation in exon 21 (L858R), account for more than 80% of identified mutations and confer sensitivity to EGFR TKIs.[4] The response rate to first-line treatment with EGFR TKIs across all mutations approaches 70%,[5] comparing favorably with a 30% response rate to first-line chemotherapy. Responders ultimately progress while undergoing treatment after a median of 10 to 16 months[6] and the most frequent cause of this *secondary* resistance is acquisition of a resistant mutation, usually the T790M mutation, which is present in approximately 50% of cases.[7] Progress has been made in overcoming secondary resistance in patients with T790M mutation by using third-generation EGFR TKIs, which have clinical activity against this mutation.[8]

Despite the significant activity of EGFR TKIs in patients with *EGFR* mutations, up to 10% of patients with common and putative sensitizing mutations (exon 19 deletion and L858R) show no disease control.[9] The mechanisms for this primary resistance to EGFR TKIs are less well understood. Furthermore, sensitivity to EGFR TKIs in patients with co-mutation (double or multiple mutation) is not well described. Some studies to date have shown that co-mutation may be associated with higher rates of primary resistance to TKIs.[10,11]

We aimed to examine the relationship of *EGFR* co-mutation at diagnosis to patient outcomes in a series of patients with metastatic NSCLC treated with a first-line EGFR TKI.

## Methods

We reviewed the mutation profiles of patients with nonsquamous NSCLC who were tested at Royal Prince Alfred Hospital from March 2012 to March 2015 by MassArray using the OncoCarta v1.0 panel (Agena Bioscience, San Diego, CA). The OncoCarta reagents analyze for 238 variant targets within 19 oncogenes. After March 2015, the OncoFOCUS panel replaced the OncoCarta panel, providing a transition point for data cutoff.

Patients with metastatic disease whose tumors had sensitizing *EGFR* mutation(s) and received first-line treatment with an EGFR TKI were included and their clinical characteristics, tumor histopathologic features, and treatment and response details were obtained and analyzed retrospectively. Some patients were referred to Royal Prince Alfred Hospital upon progression and underwent repeat biopsy for entry into a trial of third-generation EGFR TKIs. These patients were excluded, as their samples were not representative of de novo *EGFR* mutations. Those patients with a complete response (CR) or partial response (PR) were classified as responders; those with stable or progressive disease were classified as nonresponders. Response was determined by the treating physicians and based on radiological assessment using the Response Evaluation Criteria in Solid Tumors.[12] The Human Research Ethics Committee at Sydney Local Health District approved this retrospective study and granted waiver of consent.

Progression-free survival (PFS) and overall survival (OS) were calculated from date of TKI commencement to date of radiological progression or death and date of death, respectively, by using the Kaplan-Meier method; the log rank test was used to compare groups with and without co-mutation. Multivariable analysis was done for PFS. The interval between computed tomography scans was determined by the treating doctor and was typically performed trimonthly. Chi-square test and logistic regression were used to compare response rates between groups. A $p$ value less than 0.05 was considered statistically significant. We used IBM SPSS Statistics version 21 (IBM Corp., Armonk, NY) for statistical analysis.

## Results

### *Patient and Tumor Characteristics*

A total of 62 patients were included in the study (see the CONSORT diagram in Fig. 1): the median age was 70 years (range 40–88) and 37 patients (60%) were female. Most patients (n = 53) had adenocarcinoma, whereas eight had NSCLC not otherwise specified and one had adenosquamous carcinoma. There were eight patients with co-mutation (12.9%).

At the time of analysis, 25 patients (40%) had died and a further 15 (24%) had progressed. The median Eastern Cooperative Oncology Group performance status at time of start of treatment was 1 (range 0–2) and eight patients (13%) participated in a clinical trial. A range of EGFR TKIs were used as first-line treatment: gefitinib in 51.6% of patients, erlotinib in 40.3%, afatinib in 6.5%, and dacomitinib in 1.6%. The main patient characteristics are listed in Table 1. The median PFS and OS times were 11.5 and 26.3 months, respectively.

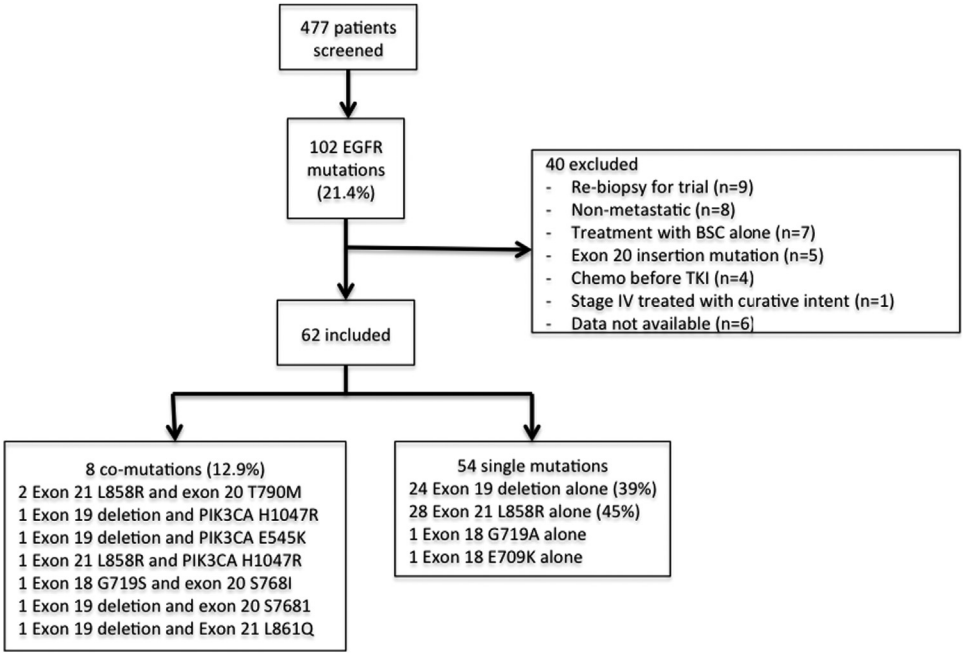


**Figure 1.** CONSORT diagram. BSC, best supportive care; TKI, tyrosine kinase inhibitor; IV, intravenous; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene.

### *Response to Treatment*

A total of 51 patients (82.3%) responded to treatment, with five CRs and 46 PRs. Those patients who achieved a CR have been receiving treatment for 17 to 77 months; all remain on treatment. Cancers showing a CR harbored either a single exon 19 deletion (two) or L858R mutation (three) and were treated with either erlotinib (two), gefitinib (two), or dacomitinib (one).

Eleven patients (17.8%) did not respond to treatment, comprising four with progressive disease and seven with stable disease as their best response.

There were eight patients whose tumors harbored a co-mutation. The co-mutation occurred as follows: T790M (two patients), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene (*PIK3CA*) (two patients), exon 20 (two patients), and exon 21 (one patient).

**Table 1.** Patient Characteristics

| | Single Mutation (n = 54) | | Co-mutation (n = 8) | | All (N = 62) | |
|---|---|---|---|---|---|---|
| Characteristic | No. Patients | % | No. Patients | % | No. Patients | % |
| Age, y | | | | | | |
| Median | 70 | | 67 | | 70 | |
| Range | 40-88 | | 55-80 | | 40-88 | |
| Sex | | | | | | |
| Male | 21 | 38.9 | 4 | 50 | 25 | 40.3 |
| Female | 33 | 61.1 | 4 | 50 | 37 | 59.7 |
| Performance status | | | | | | |
| 0-1 | 43 | 79.6 | 7 | 87.5 | 50 | 80.6 |
| 2 | 11 | 20.4 | 1 | 12.5 | 12 | 19.4 |
| Histologic subtype | | | | | | |
| Adenocarcinoma | 45 | 83.3 | 8 | 100 | 53 | 85.5 |
| Other | 9 | 16.7 | 0 | 0 | 9 | 14.5 |
| Treatment | | | | | | |
| Gefitinib | 28 | 51.9 | 4 | 50 | 32 | 51.6 |
| Erlotinib | 21 | 38.9 | 4 | 50 | 25 | 40.3 |
| Afatinib | 4 | 7.4 | 0 | 0 | 4 | 6.5 |
| Dacomitinib | 1 | 1.9 | 0 | 0 | 1 | 1.6 |

There was de novo progressive disease in two of two T790M cases and one of three *PIK3CA* cases and stable disease in the exon 21 case (see Supplementary Table 1). The two patients with *PIK3CA* co-mutation who showed a PR had PFS times of 10.3 months (exon 19 del and *PIK3CA* H1047R) and 11.1 months (exon 19 del and *PIK3CA* E545K). The two patients with exon 20 co-mutation who achieved PR had PFS times of 13.1 months (exon 18 G719S and exon 20 S7681) and 11.2 months (exon 19 deletion and exon 20 S7681). Overall, patients with co-mutation had significantly shorter PFS than those with a single mutation (median PFS 5.7 months [95% confidence interval (CI): 0–16.3] versus 12.3 months [95% CI: 9.4–15.2], respectively; $p = 0.02$) (Fig. 2). This remained significant in the multivariate analysis, with a hazard ratio of 2.6 ([95% CI: 1.0–6.7], $p = 0.04$). No other factors analyzed, including age, sex, performance status, and histologic type, were predictive of PFS (see Supplementary Table 2). The data for OS are not mature; however, there was a shorter OS in co-mutated patients without statistical significance being reached (OS = 14.5 months versus 26.3 months, $p = 0.42$).

Response rate to a first-line TKI was significantly better in those with a single *EGFR* mutation versus co-mutation (89% versus 38%, $p < 0.001$). In the multivariate logistic regression, patients with a single mutation were more than 20 times more likely to respond to treatment than their co-mutated counterparts. Other factors, including age, sex, performance status, and histologic type, were not significantly associated with treatment response (see Supplementary Table 3).

There is some evidence that de novo T790M is a predictor of poor response[13,14]; an exploratory analysis excluding these patients was done. A total of 60 patients were analyzed: six with (non-T790M) co-mutation versus 54 with a single mutation. PFS remained shorter in patients with co-mutation, without statistical significance being reached (median PFS = 10.3 months [95% CI: 0.3–20.4] versus 12.3 months [95% CI: 9.4–15.2], $p = 0.14$). OS remained shorter in co-mutated patients, with the difference not statistically significant (14.5 months versus 26.3 months [95% CI: 9.6–19.4], $p = 0.4$). Response rates remained significantly better in those with a single *EGFR* mutation (89% versus 50%; $p = 0.04$). The multivariate analysis showed that patients with a single mutation were more than10 times more likely to respond to treatment.

Patients receiving an irreversible TKI (afatinib or dacomitinib) had a trend for longer PFS than those receiving a reversible TKI (erlotinib or gefitinib): a median PFS of 23.5 months (95% CI: 15.1–31.8) versus 11.3 months (95% CI: 9.9–12.6) ($p = 0.11$) (see Supplementary Fig. 1).

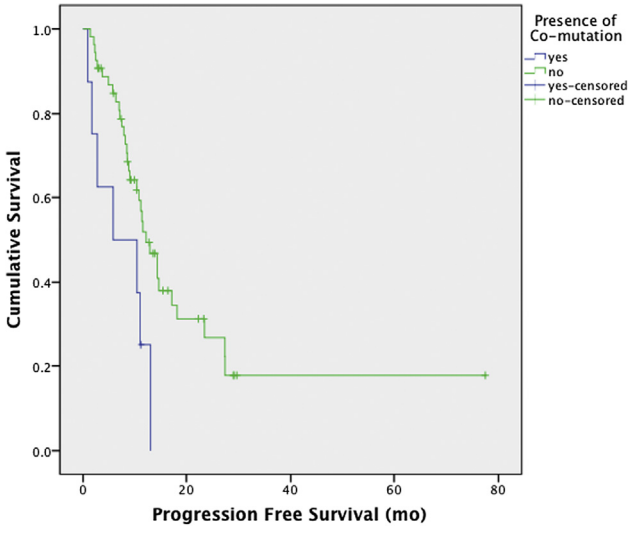


**Figure 2.** Kaplan Meier curves for PFS demonstrating longer survival in patients without a co-mutation compared to patients with a co-mutation.

## Discussion

Our data represent all patients whose lung cancers were tested for *EGFR* mutation at a major Sydney laboratory over a 3-year period. The patient characteristics, tumor histopathologic features, mutation types, PFS, and OS (immature) are consistent with the known pattern of disease. The rate of co-mutation at diagnosis in our cohort was 12.9%. This is higher than in previous large trials showing rates of *uncommon* mutation around 10% and rates of *co-mutation* closer to 3% overall.[15] Reasons for this discrepancy may include comparison with trial data, which predominantly analyze only the *EGFR* domain of the cell (excluding *PIK3CA*, for example). An analysis of resected early lung adenocarcinoma using the same OncoCarta Panel as our study showed a rate of co-mutation closer to 9%.[16] Rates of detection of more than one mutation in the *EGFR* gene and co-mutations in *EGFR* and other oncogenes will vary according to which genes are analyzed and which mutation testing technique is used. This particularly applies to de novo T790M, with its reported prevalence varying from less than 1%[13] to more than 25%[14] depending on the sensitivity of the detection method. Coincidental mutations in nondriver genes such as tumor protein p53 gene (*TP53*) are relatively high (>5%) in the *EGFR*-mutated population and are associated with higher tumor grade and shorter PFS/OS during EGFR TKI therapy.[17,18] These mutations lie outside the *EGFR* gene but may influence patient outcomes.

This study shows that co-mutations are not uncommon and that knowledge of co-mutations, particularly those involving de novo *EGFR* T790M and *PIK3CA*, could

provide clinically important information predicting a poor response to most EGFR TKIs. Currently, any additional mutations identified during *EGFR* molecular testing are not used to guide first-line treatment beyond presence or absence of a sensitizing mutation. There is evidence that different *EGFR* mutations represent distinct disease entities that respond differently to treatment with TKIs.[19] Deciphering which tumors are likely to gain benefit from which TKI is a developing field.

The limitations of our study include its retrospective design and the relatively small number of patients. Patients analyzed in this study underwent computed tomography at the discretion of their treating oncologist. It was trimonthly in most cases; however, early imaging is more likely to have been performed in poor responders, potentially skewing to shorter PFS in this group.

Treatment options for *EGFR*-mutated lung cancer are evolving and alternatives to first-generation agents are likely to become available in the future. Comparative information about TKI efficacy within the *EGFR* co-mutated group is needed to optimize treatment in this subset of patients who have poor outcomes with current therapies. Furthermore, given that co-mutation may be found in more than 12% of patients and appears to portend poor response to TKI, it is important to accurately identify this group before commencing treatment. Routine use of a sensitive, multiplex panel method for mutation testing may more reliably identify this group and allow tailoring of care accordingly (for example, with more frequent review). More studies are needed to confirm our findings and enable accurate subtyping of patients. This will provide patients with stage IV *EGFR*-mutated lung cancer maximal opportunity to maintain quality of life, avoid unwarranted drug toxicity, and reduce expenditure on ineffective treatment.

## Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at http://dx.doi.org/10.1016/j.jtho.2016.09.001.

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