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ORIGINAL RESEARCH

Predictors of Acquired *T790M* Mutation in Patients Failing First- or Second-Generation *Epidermal Growth Factor Receptor*-Tyrosine Kinase Inhibitors

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Background: This study aims to determine the predictors of acquired *exon 20 T790M* mutation in advanced non-small cell lung cancer (NSCLC) patients harbouring sensitizing *epidermal growth factor receptor* (*EGFR)* mutation following the failure of first- or second-generation *EGFR*-tyrosine kinase inhibitor (TKI).
Methods: This is a retrospective observational study of NSCLC patients with sensitising *EGFR* mutation experiencing disease progression (PD) whilst on first- or second-generation *EGFR*-TKIs with subsequent investigations to detect acquired *T790M* mutation at the University of Malaya Medical Centre from 1st January 2015 to 31st December 2017.
Results: A total of 87 patients were included. Upon PD, acquired *T790M* mutation was found in 55 (63.2%) patients and was significantly more common in patients who achieved partial response (PR) whilst on the *EGFR*-TKIs (p = 0.008) or had new lung metastasis upon PD (p = 0.048). It was less frequent in patients who developed new symptomatic brain lesions (p = 0.021). Patients with *exon 19* deletion were more likely to acquire *T790M* mutation compared to those with *exon 21 L858R* point mutation (p = 0.077). Multivariate analysis revealed PR whilst on *EGFR*-TKI treatment was an independent predictor of acquiring *T790M* mutation (p = 0.021), whereas development of new symptomatic brain lesions (p = 0.034) or new lymph node metastases (p = 0.038) upon PD was independently against acquiring *T790M* mutation. Patients with *exon 19* deletion were more likely to acquire *T790M* mutation compared to those with *exon 21 L858R* point mutation (odds ratio: 2.3, 95% confidence interval: 0.84–6.25, p = 0.104).
Conclusion: The best tumour response of PR to first- or second-generation *EGFR*-TKI treatment independently predicts acquired *T790M* mutation. Patients with *exon 19* deletion are likely to acquire *T790M* mutation. This would prove useful for clinicians to prognosticate and plan subsequent treatments for patients with advanced NSCLC harbouring *EGFR* mutations.
Keywords: non-small cell lung cancer, epidermal growth factor receptor, acquired *T790M* mutation, independent predictor, tyrosine kinase inhibitor

Introduction

Lung cancer, 85% of which are non-small cell lung cancer (NSCLC), remains the leading cause of cancer mortality globally.[1] Upon diagnosis, the majority of NSCLC patients have locally advanced or metastatic disease. Conventional first-line chemotherapy in these patients confers a dismal median overall survival of 8–10 months and a 2-year survival rate of 11%.[2,3]

The discovery of mutations of the *epidermal growth factor receptor* (*EGFR*) has completely revolutionized the management of patients with advanced NSCLC.

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EGFR (*HER 1*) is a transmembrane tyrosine kinase receptor belonging to the *HER* family.[4] The binding of ligands consisting of transforming growth factor-α or epidermal growth factor to *EGFR* leads to auto-phosphorylation of key tyrosine residues.[5] This activates downstream signalling involving and mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt which promotes cellular proliferation and survival. The presence of *EGFR* mutation results in constitutive activation of the MAPK and PI3K/Akt pathway independent of ligand binding, subsequently leading to the development and progression of NSCLC.

EGFR-tyrosine kinase inhibitors (*EGFR*-TKIs) including gefitinib, erlotinib, afatinib, dacomitinib and osimertinib bind to the ATP-binding sites of *EGFR*, thereby inhibiting the activation of the *EGFR*, MAPK and PI3K/Akt pathway.[6,7] This results in reduced cellular proliferation and increased apoptosis. Several clinical trials have reported an impressive median progression-free survival (mPFS) of 9–13 months in NSCLC patients harbouring *EGFR*-TKI sensitising mutations treated with first-line gefitinib, erlotinib, or afatinib.[5]

Despite the obvious efficacy of these *EGFR*-TKIs, the majority of these patients develop drug resistance after a median treatment period of one year mainly due to the acquisition of the *exon 20 T790M* resistant mutation. This study aims to determine the predictors of acquiring *T790M* mutation as a resistance mechanism among NSCLC patients who develop disease progression whilst taking first- or second-generation *EGFR*-TKI treatment.

Methodology

Study Design and Patients

This is a retrospective observational study of NSCLC patients with sensitising *EGFR*-mutation who progressed while on first- or second-generation *EGFR*-TKI treatment with subsequent investigations to determine the mode of resistance at the University Malaya Medical Center (UMMC) from 1st January 2015 to 31st December 2017. All patients included had demonstrated an objective clinical benefit from the initial *EGFR*-TKI treatment as evidenced by either a complete response (CR), partial response (PR) or a minimum of six months of stable disease (SD) according to the Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1.[8] They were investigated for resistance mechanisms as soon as the monitoring computed tomography (CT)-scan detected a PD according to RECIST version 1.1 criteria,[9] without interruption of their *EGFR*-TKI treatment. Patients who had prior chemotherapy, inadequate tissue sample for resistance mechanism analysis, or incomplete medical records were excluded from this study. The study was conducted after receiving approval from the hospital medical ethics committee.

Procedure

Patients who fulfilled the inclusion criteria were consecutively identified from the lung cancer registry of the Division of Respiratory Medicine, UMMC. Baseline demographic, clinical findings, treatment, pattern of PD, and investigation into resistance mechanisms were obtained from the electronic medical records.

At diagnosis, every patient underwent a baseline CT thorax, abdomen and pelvis (CT-TAP). CT-brain was only performed if there were neurological symptoms or signs. The initial tumor was staged according to the 7th edition of the American Joint Committee on Cancer system.[10] All patients were tested for the presence of *EGFR* mutation in their pre-treatment biopsy specimens. Gefitinib or erlotinib was given in the first-line setting while afatinib was given either in the first-line setting or as a second-line treatment when patients failed to respond to gefitinib or erlotinib. Gefitinib and erlotinib are first-generation *EGFR*-TKI that bind reversibly to *EGFR*/ErbB1; while afatinib is second-generation *EGFR*-TKI that binds irreversibly to all the ErbB family (*EGFR*/ErbB1, *HER2*/ErbB2, ErbB3, and ErbB4).[11,12] It was our standard practice to evaluate the tumor response by performing a repeat CT-TAP 4 weeks after initiation of *EGFR*-TKI and subsequently, once every three months. Tumor response was categorized according to RECIST version 1.1.[9]

Before 1st December 2016, tissue re-biopsy was the first-line investigation at PD unless the patient refused, was unfit or the procedure was not technically feasible, in which case the patient would be offered liquid biopsy (detection of *T790M* mutation from a blood sample) as an alternative. Starting 1st December 2016, liquid biopsy was the first-line investigation while tissue re-biopsy was offered if liquid biopsy failed to detect acquired *T790M* mutation. We did not repeat tissue biopsy for those already tested negative for acquired *T790M* mutation in their re-biopsy tissue sample to prevent delay in initiation of second-line treatment. Identification of *c-MET* amplification by fluorescent in-situ hybridisation (FISH) was only done in seven patients who were tested negative for acquired *T790M* mutation and histological transformation during PD, as part of a clinical trial.[13] Investigations for other resistance mechanisms were not available in Malaysia outside of clinical research during the period of this study.

Tissue Re-Biopsy

Tissue was obtained by either image-guided biopsy, endobronchial biopsy or excisional biopsy as clinically indicated. The histologic confirmation of lung cancer subtypes was based on tumor morphology on haematoxylin and eosin staining, complemented by immunohistochemical staining as needed to distinguish adenocarcinoma from squamous cell carcinoma. *T790M* mutation was detected by cobas® *EGFR* Mutation Test v2 (Roche Molecular Systems, New Jersey, USA), an allele-specific real-time polymerase chain reaction (PCR) assay.

Liquid Biopsy

Detection of *T790M* mutation in circulating cell-free tumor DNA obtained in the plasma using the QIAamp® Circulating Nucleic Acid kit (Qiagen, Hilden, Germany) was by the peptic nucleic acid-locked nucleic acid PCR (PNA-LNA PCR) clamp method (PANAGEN, Daejon, Korea) before 1st December 2016 and by droplet digital PCR (ddPCR) (Sanomics, Hong Kong, China) after that.

Statistical Analysis

Categorical variables were expressed as percentages while continuous variables were expressed as mean ± standard deviation (SD) or median with range. Differences in clinical variables were examined between patients with acquired *T790M* mutation versus those without. Differences in categorical variables were compared using the Chi-Squared test or Fisher Exact test. Differences in continuous variables were compared using independent *t*-test or Mann–Whitney *U*-test. Multivariate analyses were performed using logistic regression. A two-sided p-value of <0.05 was considered as statistically significant. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS for Windows version 23.0, SPSS Inc., Chicago, IL, USA).

Results

Incidence of Acquired *T790M* Mutation and Other Resistance Mechanisms

Of 122 patients with PD while on first- or second-generation *EGFR*-TKI, 87 patients who fulfilled the inclusion criteria were studied (Figure 1). At PD, acquired *T790M* mutation was found in 55 (63.2%) patients, two of whom (2.3%) also had concomitant small cell lung cancer (SCLC) transformation (Figure 2). Of the other patients, four (4.6%) had *c-MET* amplification, and one (1.1%) each had SCLC transformation and epithelial–mesenchymal transition (EMT), respectively. The resistance mechanism was unknown in 26 (29.9%) patients.

Baseline Demographic, Clinical and Treatment History

The patients' baseline demographic and clinical characteristics, as well as treatment history, are shown in Table 1. Of the initial sensitising *EGFR* mutations, 62.1% of patients had sensitizing *EGFR exon 19* deletion and the remaining patients had *EGFR exon 21 L858R* point mutation. For treatment in the first-line setting, 65.5% of patients received gefitinib, 16.1% received erlotinib and 18.4% received afatinib. Afatinib was also given in 12.6% of these patients as a second-line treatment before work-up for resistance mechanisms.

Acquired *T790M* mutation was found significantly more frequently in patients who achieved PR as the best response to the initial *EGFR*-TKI treatment compared to those who only had SD as the best response (68.1% versus 40.0%, p = 0.008). Patients with *EGFR exon 19* deletion were more likely to acquire *T790M* mutation at PD compared to those with *EGFR exon 21 L858R* point mutation (70.4% versus 51.5%, p = 0.077). The mPFS was slightly longer in patients who acquired *T790M* mutation compared to those who did not acquire *T790M* mutation (12.6 months versus 11.6 months, p = 0.977) (Figure 3). The median duration from *EGFR*-TKI treatment initiation to re-biopsy was also longer in patients with acquired *T790M* mutation compared to those who did not (18.5 months versus 16.2 months, p = 0.321)

Pattern of Disease Progression

Upon PD, nearly half of the patients experienced enlargement of the lung primary, while 83.9% of the patients had new metastases (Table 2). The lung (48.3%) was the commonest site of new metastases, followed by intrathoracic lymph nodes (24.1%), bones (20.7%) and the liver

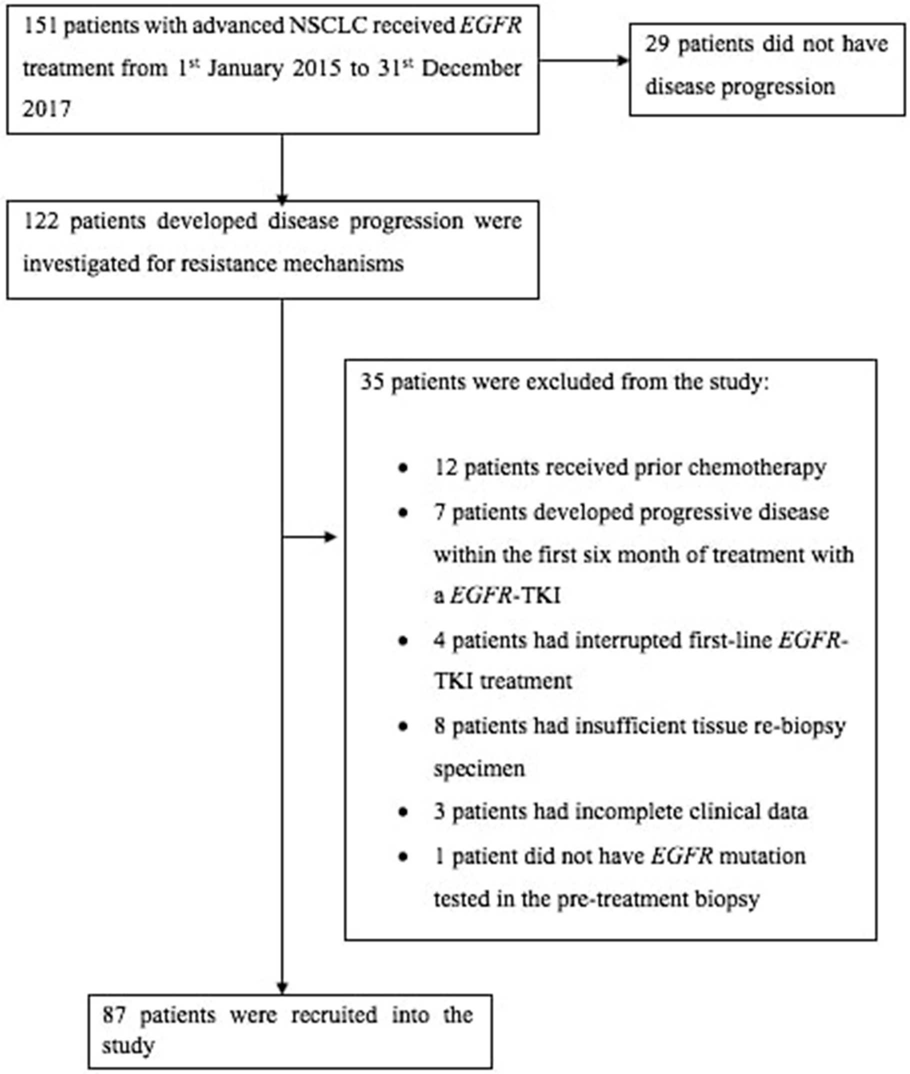

Figure 1 Algorithm of patient selection.
Abbreviations: NSCLC, non-small cell lung cancer, *EGFR-TKI epidermal growth factor receptor-tyrosine* kinase inhibitor.

(18.4%). The brain (11.5%) and adrenal glands (2.3%) were uncommon sites of new metastases.

Acquired *T790M* mutation was significantly more frequent in patients who developed new lung metastases than those who did not (73.8% versus 53.3%, p = 0.048) and significantly less frequent in patients who had new symptomatic brain metastases than those who did not (30.0% versus 67.5%, p = 0.021). Patients who developed new intrathoracic lymph node metastases tended to be less likely to acquire *T790M* mutation than those who did not (47.6 versus 68.2%, p = 0.089).

Investigations for Resistance Mechanisms

Equal proportions of patients underwent liquid biopsy (40.2%) and tissue re-biopsy (40.2%) as the initial investigation to detect acquired *T790M* mutation, with a positive detection rate of 57.1% and 74.3%, respectively (Table 3). Following an initial negative liquid biopsy, 19.6% of patients underwent a tissue re-biopsy, which detected *T790M* mutation in 52.9% of the cases.

Among patients who underwent liquid biopsy, PNA-LNA PCR was performed in 46.2% of them with a *T790M* mutation detection rate of 58.3%, while ddPCR was performed in the remaining patients with a *T790M* mutation detection rate of 53.6%. The detection rate of acquired *T790M* mutation from tissue biopsy was 81.8% in biopsies of lung metastatic lesions, 80.0% in biopsies of liver metastatic lesions, 60.7% in lung primary tumor biopsies and 50.0% in intrathoracic lymph node biopsies. The

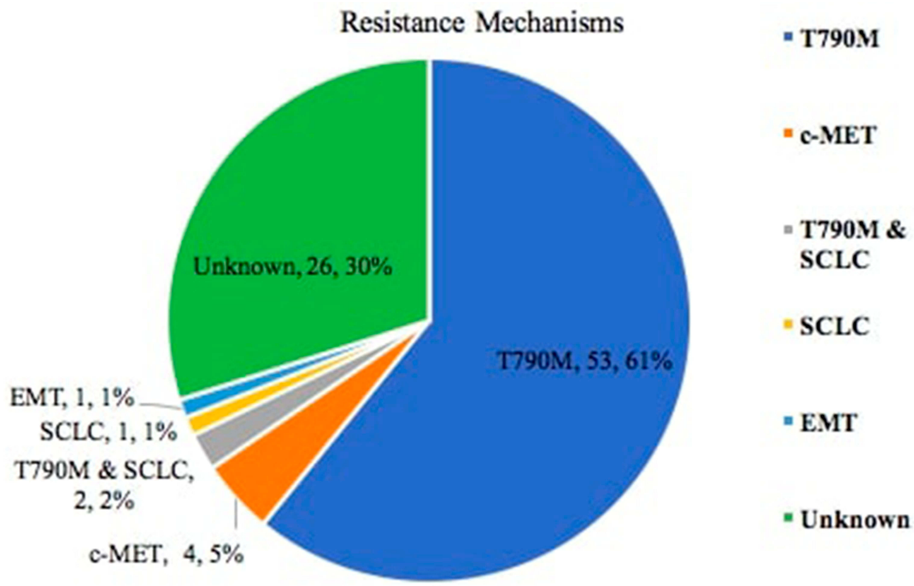

Figure 2 Incidence of acquired *T790M* mutation and other resistance mechanisms.
Abbreviations: SCLC, small cell lung cancer; EMT, epithelial-mesenchymal transition.

investigation methods and sites of tissue biopsy did not have a significant effect on the *T790M* mutation detection rate.

Independent Predictors of Acquired *T790M* Mutation

In multivariate analysis, compared to SD, PR with *EGFR*-TKI treatment was associated with a significantly higher rate of acquiring *T790M* mutation at PD (OR: 4.1, 95% CI: 1.24–13.50, p = 0.021) (Table 4); while the development of new symptomatic brain metastases or new intrathoracic lymph node metastases at PD was associated with significantly lower rates of acquired *T790M* mutation (OR: 0.2, 95% CI: 0.04–0.88, p = 0.034 and OR: 0.3, 95% CI: 0.11–0.94, p = 0.038, respectively). Patients with sensitizing *EGFR exon 19* deletion were more likely to acquire *T790M* mutation compared to those with sensitizing *EGFR exon 21 L858R* point mutation (OR: 2.3, 95% CI: 0.84–6.25, p = 0.104). Otherwise, the type of first-line *EGFR*-TKI received and having new lung metastases during PD did not have a significant effect on the *T790M* mutation rate.

Clinical and Treatment Characteristics of Patients Without PD

At the time of data cut-off, 29 patients continued to receive first- or second-generation *EGFR*-TKI without PD. 88.4% of these patients had *EGFR exon 19* deletion and the remaining patients had *EGFR exon 21 L858R* point mutation as the original sensitizing mutation. 48.2% of patients received gefitinib, 27.6% received erlotinib and 24.2% received afatinib as the choice of *EGFR*-TKI selected in the first-line setting. 82.7% of patients had PR and the remaining had SD as the best tumour response. The median follow-up period for this group of patients was 17.1 months (95% CI: 6.38–27.82).

Osimertinib After Failure of First- or Second-Generation *EGFR*-TKI

Nineteen (34.5%) patients with acquired *T790M* mutation received osimertinib soon after first- or second-generation *EGFR*-TKI failure. The care of three of these patients were transferred to another center and further details about their response to osimertinib were not available. The second mPFS for the remaining 16 patients treated with osimertinib was 8.0 months (95% CI: 1.75–14.25).

Discussion

The present study identified acquired *T790M* mutation as the commonest resistance mechanism causing treatment failure to first- or second-generation *EGFR*-TKI in Malaysian patients with sensitising *EGFR*-mutant NSCLC. Having the best tumour response of PR while on first-line *EGFR*-TKI was the only significant independent predictor of acquiring *T790M* mutation causing PD. Acquired *T790M* mutation was more common in patients with tumors harbouring *exon 19*

Table 1 Baseline Demographic, Clinical and Treatment Characteristics of Patients with Acquired *T790M* Mutation

Characteristics	All Patients (n = 87)	Patients with T790M (n = 55)	p value*
Age, year			
Mean (± SD)	61.7 ± 9.7	61.5 ± 10.1	0.693
Gender, No. (%)			
Female	50	33 (66.0)	0.532
Male	37	22 (59.5)	
Ethnicity, No. (%)			
Chinese	68	41 (60.3)	0.294
Malay	12	10 (83.3)	
India	7	4 (57.1)	
Smoking history, No. (%)			
Never smoker	70	44 (62.9)	0.887
Former or current smoker	17	11 (64.7)	
Baseline ECOG, No. (%)			
0–1	80	52 (65.0)	0.244
2–4	7	3 (42.9)	
Tumour stage, No. (%)			
IIIB	8	5 (62.5)	0.822
IVA	28	19 (67.9)	
IVB	51	31 (60.9)	
Histology, No. (%)			
Adenocarcinoma	86	55 (64.0)	0.187
Large cell carcinoma	1	0 (0)	
***EGFR* mutation subtype, No. (%)**			
Exon 19 deletion	54	38 (70.4)	0.077
Exon 21 *L858R* point mutation	33	17 (51.5)	
First-line *EGFR*-TKI treatment, No. (%)			
Gefitinib	57	40 (70.2)	0.134
Erlotinib	14	6 (42.9)	
Afatinib	16	9 (56.3)	
***EGFR*-TKI treatment before biopsy, No. (%)**			
1st generation *EGFR*-TKI only	60	38 (63.3)	0.683
2nd generation *EGFR*-TKI only	16	9 (56.3)	
1st followed by 2nd generation *EGFR*-TKI	11	8 (72.7)	
Best tumour response to *EGFR*-TKI, No. (%)			**0.008**
Partial response	72 (82.8)	49 (68.1)	
Stable disease	15 (17.2)	6 (40.0)	
Progression-free survival on *EGFR*-TKI, months			
Median (95% CI)	12.4 (10.9–13.9)	12.6 (9.4–15.8)	0.977
Duration from *EGFR*-TKI initiation to re-biopsy, months			
Median (95% CI)	17.3 (12.2–22.4)	18.5 (13.1–23.9)	0.321

(*Continued*)

Table 1 (Continued).

Notes: *Chi-Square test for categorical variables; independent *t*-test for continuous variables. Bold p value was statistically significant.

Abbreviations: ECOG, Eastern Cooperative Oncology Group; *EGFR, epidermal growth factor receptor*; TKI, tyrosine kinase inhibitor.

deletion than those with *exon 21 L858R* point mutation as their original sensitizing mutation. On the other hand, the presence of new symptomatic brain metastases or intrathoracic lymph node metastases upon PD was significant independent predictors against acquiring *T790M* mutation. The yield of detecting *T790M* mutation from either tissue or liquid biopsy in the present study was high. Performing reflex tissue re-biopsy after the failure of liquid biopsy to detect *T790M* mutation could detect this resistance mutation in half of the cases.

The incidence of acquired *T790M* mutation in the present study corresponds to that of 45.1% to 62.0% reported in the literature.[14–22] Several studies have highlighted a longer mPFS with *EGFR*-TKI treatment as the most important independent predictor of acquired *T790M* mutation.[18,19,21-24] Even though the mPFS of our patients who acquired *T790M* mutation was slightly longer than the mPFS of those who did not acquire *T790M* mutation, the difference was not statistically significant because of the small number of patients. The exclusion of a number of prolonged *EGFR*-TKI responders who had not experienced PD in the present study could be a confounding factor because previous studies suggested these patients are more likely to acquire *T790M* mutation at PD.[18,19,21-23]

A few studies have shown that initial *exon 19* deletion mutation is another predictor of acquiring *T790M* mutation as a resistance mechanism.[19–21,25] The present study too showed a trend favoring acquired *T790M* mutation among patients with initial sensitizing *EGFR exon 19* deletion compared to those with *EGFR exon 21 L858R* point mutation. The lack of statistical significance could be attributed to the small number of patients in our study. Matsuo et al reported a significant association of better tumour response to *EGFR*-TKI with acquired *T790M* mutation (objective response rate: 84.7% versus 60.0%, p = 0.001) which is also shown by the present study.[19] Oxnard et al and Hata

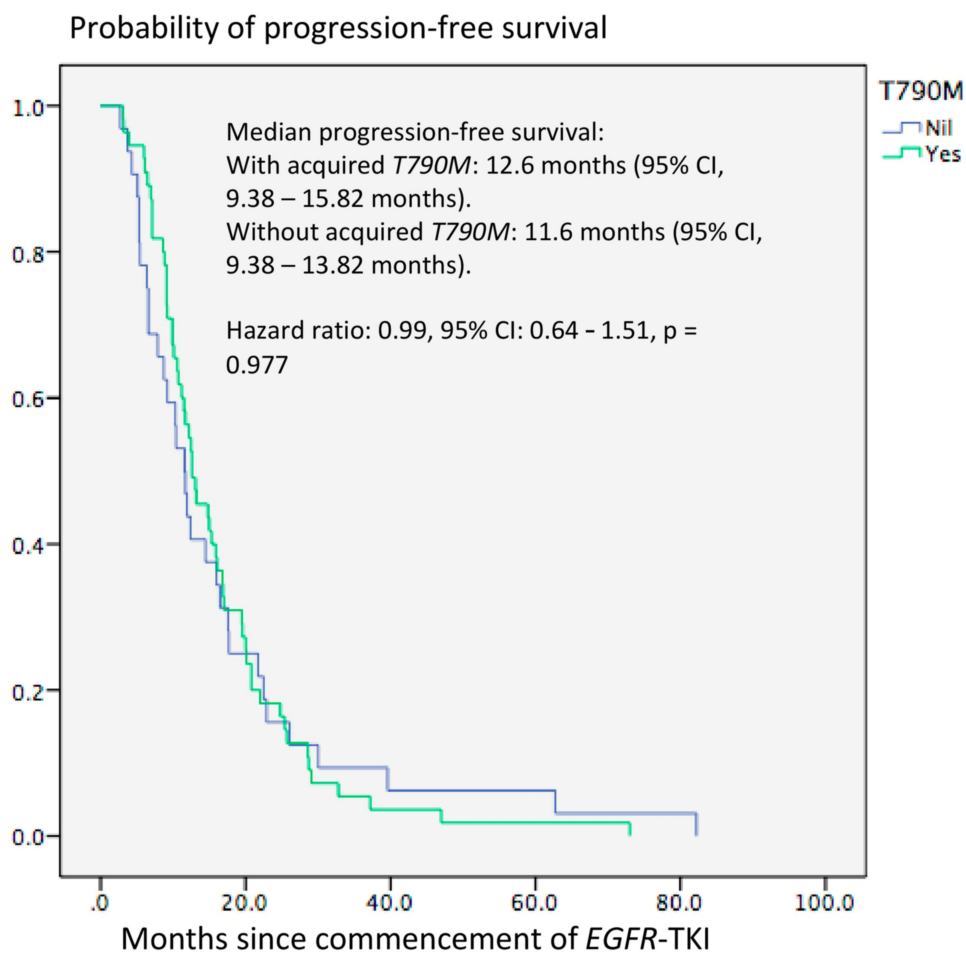

Figure 3 Kaplan–Meier plot for progression-free survival of patients according to acquired *T790M* mutation status.

et al, respectively, reported extra-thoracic disease progression (p = 0.014) and new brain metastases (p = 0.042) as features against acquiring *T790M* mutation as a resistance mechanism.[15,23] Our finding is in agreement with the latter observation. A recent study by Del Re et al reported a higher incidence of *T790M* mutation detected by plasma biopsy at PD among patients receiving first-line gefitinib or erlotinib compared to afatinib.[26] Otherwise, the present study and other studies consistently observe that gender, smoking status, proportion of stage IV disease and the site of re-biopsy do not have a significant effect on the frequency of acquired *T790M* mutation.[21,23,27,28]

The substitution of methionine by threonine at the gatekeeper residue in position 790 of the *EGFR* kinase domain leads to *T790M* mutation which confers resistance to first- or second-generation *EGFR*-TKI by steric hindrance to TKI, increased ATP-binding affinity and increased in auto-phosphorylation levels.[29–31] The emergence of *T790M* mutation during *EGFR*-TKI treatment could be by selection or by acquisition.[32–34] In the selection hypothesis, a small proportion of *T790M* resistant clone is already present even before *EGFR*-TKI therapy. This *T790M* clone proliferates when the sensitive clones are successfully eradicated by *EGFR*-TKI therapy. This hypothesis is supported by the discovery of de novo *T790M* mutation in 31.5–38.0% of patients with sensitising *EGFR*-mutant NSCLC prior to treatment.[35–37] On the other hand, the acquisition hypothesis suggests that the tumor cells develop novel genetic or epigenetic defects as a consequence of prolonged *EGFR*-TKI treatment.[38–40] In the light of these two hypotheses, the higher incidence of acquired *T790M* mutation among patients who achieve PR with *EGFR*-TKI can be explained by the selection model while the higher incidence of acquired *T790M* mutation among patients with longer mPFS and longer duration on *EGFR*-TKI treatment before biopsy can be explained by the acquisition model.

Table 2 Pattern of Disease Progression in Patients with Acquired *T790M* Mutation

Characteristics	All Patients (n = 87)	Patients with T790M (n = 55)	p value*
Number of progression sites, No. (%)			
1	46	31 (67.4)	0.260
2	21	13 (61.9)	
3	17	8 (47.1)	
4	3	3 (100)	
Progression site, No. (%)			
Enlarged lung primary	14	10 (71.4)	0.242
New metastases	47	32 (68.1)	
Enlarged primary and new metastases	26	13 (50.0)	
Enlarged lung primary, No. (%)			
Yes	40	23 (57.5)	0.308
No	47	32 (68.1)	
New lung metastases, No. (%)			**0.048**
Yes	42	31 (73.8)	
No	45	24 (53.3)	
New symptomatic brain metastases, No. (%)			**0.021**
Yes	10	3 (30.0)	
No	77	52 (67.5)	
New liver metastases, No. (%)			
Yes	16	10 (62.5)	0.947
No	71	45 (63.4)	
New adrenal metastases, No. (%)			
Yes	2	2 (100.0)	0.275
No	85	53 (62.4)	
New intrathoracic lymph node metastases, No. (%)			
Yes	21	10 (47.6)	0.089
No	66	45 (68.2)	
New bone metastases, No. (%)			
Yes	18	13 (72.2)	0.374
No	69	42 (60.9)	

Notes: *Chi-square test for categorical variables. Bold p-value was statistically significant.

Table 3 Investigations for Resistance Mechanisms in Patients with Acquired *EGFR* Exon 20 *T790M* Mutation

Resistance Mechanism Investigation	All Patients (n = 87)	Patients with T790M (n = 55)	p value*
Type of biopsy, No. (%)			
Liquid biopsy	35	20 (57.1)	0.205
Tissue re-biopsy	35	26 (74.3)	
Liquid followed by tissue re-biopsy	17	9 (52.9)	
Type of liquid biopsy	**All patients (n = 52)**	**Patients with *T790M* (n = 29)**	**p value***
Liquid biopsy platform, No. (%)			
PNA-LNA PCR	28	15 (53.6)	0.730
ddPCR	24	14 (58.3)	
Tissue re-biopsy	**All patients (n = 52)**	**Patients with *T790M* (n = 35)**	**p value***
Site of re-biopsy, No. (%)			
Lung primary	28	17 (60.7)	0.323
Lung metastasis	11	9 (81.8)	
Brain metastasis	1	0	
Liver metastasis	10	8 (80.0)	
Lymph node metastasis	2	1 (50.0)	

Note: *Chi-square for categorical variables.
Abbreviations: PNA-LNA PCR, peptic nucleic acid-locked nucleic acid polymerase chain reaction; ddPCR, droplet digital polymerase chain reaction.

Compared to patients with *exon 21 L858R* point mutation, better tumour response and longer mPFS among patients with *exon 19* deletion may reflect the higher incidence of acquired *T790M* mutation in patients with the latter mutation.[41] Secondary *T790M* mutation is less likely in patients who experience new brain metastases because first- or second-generation *EGFR*-TKI do not cross the blood-brain barrier well to promote *T790M* mutation resistance by selection or acquisition mechanisms.

The findings of our study further complement the result of existing literature. While ethnicity, smoking status and histologic subtypes of lung cancer are associated with initial *EGFR* mutation, the present study and other studies show that initial *EGFR* mutation subtypes, tumor response to *EGFR*-TKI treatment and sites of PD are predictors of

Table 4 Multivariate Analysis of Predictors of Acquiring *T790M* Mutation

Characteristics	All Patients (n = 87)	Patients with T790M (n = 55)	Multivariate Analysis, OR (95% CI), p value
***EGFR* mutation subtypes, No. (%)**			
Exon 19 deletion	54	38 (70.4)	2.3 (0.84–6.25), 0.104
Exon 21 L858R point mutation[#]	33	17 (51.5)	
First-line *EGFR*-TKI, No. (%)			
Gefitinib	57	40 (70.2)	1.6 (0.41–6.38), 0.491
Erlotinib	14	6 (42.9)	0.5 (0.09–2.70), 0.424
Afatinib[#]	16	9 (56.3)	
Best tumour response, No (%)			4.1 (1.24–13.50), **0.021**
Partial response	72	49 (68.1)	
Stable disease[#]	15	6 (40.0)	
New lung metastases, No. (%)			
Yes	42	31 (73.8)	1.6 (0.54–4.55), 0.404
No[#]	45	24 (53.3)	
New symptomatic brain metastases, No. (%)			0.2 (0.04–0.88), **0.034**
Yes	10	3 (30.0)	
No[#]	77	52 (67.5)	
New intrathoracic lymph nodes metastases, No. (%)			0.3 (0.11–0.94), **0.038**
Yes	21	10 (47.6)	
No[#]	66	45 (68.2)	

Notes: Bold p values are statistically significant. [#]Parameters as the reference group.
Abbreviations: *EGFR, epidermal growth factor receptor*; TKI, tyrosine kinase inhibitor; OR, odds ratio; 95% CI, 95% confidence interval.

acquired *T790M* mutation.[18,19,21-23,25,42,43] The initial *EGFR* mutation subtypes and best tumor response while on *EGFR*-TKI treatment are the clinically more relevant predictors of acquired *T790M* mutation compared to PFS and sites of PD. This is because these information enable the treating clinicians to predict the likelihood of their patients developing *T790M* mutation before the actual PD, and therefore allows early prognostication and management planning. Patients with tumors harbouring *exon 19* deletion and patients who achieve PR as the best tumor response while on *EGFR*-TKI shall have a better overall survival because they are more likely to acquire *T790M* mutation as a cause of PD. This is supported by several studies that report a significantly longer post-progression survival in patients with acquired *T790M* mutation compared to those without.[15,20,23]

The importance of planning tissue sampling carefully when patients fail *EGFR*-TKI is also highlighted in the current study. First, acquired *T790M* mutation could be detected in tissue biopsy in half of the patients who were initially tested negative for *T790M* mutation in their plasma. Second, the yield of acquired *T790M* mutation from different sites of tissue biopsied was not the same. Third, even though PR with *EGFR*-TKI treatment was a predictor for acquiring *T790M* mutation, this resistance mutation was detected in two-fifths of patients with SD as the best tumor response.

The strength of this study lies in the fact that only patients who were treated with first- or second-generation *EGFR*-TKI were included, therefore excluding the potential tumorigenic effect of chemotherapy. In addition, this study concurrently explores the association of acquired *T790M* mutation with patient baseline demographics and clinical characteristics, treatment history, pattern of disease progression and investigation methods.

However, we do acknowledge that the study has several limitations. First, it was performed in a single centre, thus limiting the generalizability of the results. Second, this was a retrospective study with attendant limitations. Third, patients who experienced PD within six months of initial *EGFR*-TKI treatment or had interrupted *EGFR*-TKI treatment were excluded. Such patients were not uncommon in real-life practice. Fourth, patients with rare or complex *EGFR* mutation were not included because none of them had objective clinical benefit from initial *EGFR*-TKI treatment. Fifth, the acquired *T790M* mutation in patients who develop new symptomatic brain metastases on PD might have been underreported because brain biopsy was rarely performed. Sixth, the post-progression survival and overall survival of these patients were not assessed because of the heterogeneity or absence of subsequent lines of treatment.

Conclusions

This study concludes that acquired *T790M* mutation is the most common resistance mechanism leading to first- or second-generation *EGFR*-TKI treatment failure in Malaysian patients. The best tumor response of PR was an independent predictor of *T790M* mutation as a resistance mechanism. Patients with tumour harboring *exon 19* deletion mutation as the original sensitizing mutation are more likely to acquire *T790M* mutation causing PD. These information are useful for clinicians to prognosticate and plan subsequent treatments for patients with advanced NSCLC harbouring *EGFR* mutations.

Abbreviations

NSCLC, non-small cell lung cancer; *EGFR, epidermal growth factor receptor*; PI3K, phosphatidylinositol 3-kinase; MAPK, mitogen-activated protein kinase; TKIs, tyrosine kinase inhibitors; mPFS, median progression-free survival; PD, disease progression; UMMC, University Malaya Medical Center; CR, complete response; PR, partial response; SD, stable disease; CT, computed tomography; RECIST, Response Evaluation Criteria in Solid Tumours; TAP, thorax, abdomen and pelvis; FISH, fluorescent in-situ hybridisation; PCR, polymerase chain reaction; PNA-LNA PCR, peptic nucleic acid-locked nucleic acid PCR; ddPCR, droplet digital PCR; SD, standard deviation; SCLC, small cell lung cancer; EMT, epithelial–mesenchymal transition; ECOG, Eastern Cooperative Oncology Group; OR, odds ratio; 95% CI, 95% confidence interval.

Ethic Approval and Informed Consent

This study was performed in accordance with the Declaration of Helsinki. It was a retrospective study and all the data used for the statistical analysis were anonymous. Therefore, informed consent from the enrolled patients was waived. The Institutional Review Board and Medical Ethic Committee of UMMC approved this study, with ethic number of MECID. No 2,018,224-6046.

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Chong-Kin Liam, Chee-Shee Chai, Mau-Ern Poh et al Acquired T790M mutation in patients failing treatment with first or second-Generation EGFR-tyrosine kinase inhibitors. JTO 2019; 14(10):S1038.[44]

Author Contributions

All authors have contributed substantially to this study, including the conception and design, acquisition of data, or analysis and interpretation of data; drafting the article or revising it critically for important intellectual content; giving of their final approval of the version to be published; and agreement to be accountable for all aspects of the work.

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