CHARACTERISTICS OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)-MUTATED NON SMALL CELL LUNG CARCINOMA (NSCLC)-PATIENTS WHO DEVELOPED RESISTANCE TO FIRST OR SECOND GENERATION EGFR-TYROSINE KINASE INHIBITOR (TKI) THERAPY THROUGH T790M MUTATION

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THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF INTERNAL MEDICINE.

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Field of Study: Internal Medicine

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Dr.Vijayan Munusamy

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or special term	Explanation
ATP	Adenosine triphosphate
CRF	Case report form
CRO	Clinical research organization
DNA	Deoxyribonucleic acid
DOT	Duration of treatment
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal growth factor receptor
ESMO	European Society for Medical Oncology
IEC	Institutional Ethics Committee
ICD	International Classification of Disease
NCCN	National Comprehensive Cancer Network
NSCLC	Non-small cell lung cancer
PCR	Polymerase chain reaction
PFS	Progression-free survival
RECIST	Response Evaluation Criteria In Solid Tumours
TKI	Tyrosine kinase inhibitors
WHO	World Health Organisation

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ABSTRACT

Background:

The management of non-small cell lung cancer (NSCLC) has undergone a major revolution in diagnosis and treatment over the past few years. The detection of epidermal growth factor receptor (EGFR) mutations has enabled advanced NSCLC to be treated with EGFR-tyrosine kinase inhibitors (TKIs). However, most patients treated with first- or second-generation EGFR-TKIs inevitably develop acquired resistance and disease progression.

Objectives

This study was conducted to determine the incidence of T790M mutation as an acquired resistance mechanism in patients with advanced EGFR-mutant NSCLC who have developed disease progression while on first- or second-generation EGFR-TKI therapy, and the clinical characteristics of such patients while being treated at the University of Malaya Medical Centre. This study also aimed to compare the clinical characteristics of patients with acquired T790M mutation, causing resistance to first-line EGFR-TKI treatment, with that of patients who develop resistance to first-line EGFR-TKI treatment not due to acquired T790M mutation.

Methods:

This retrospective observational study evaluated EGFR-mutant advanced NSCLC patients on EGFR-TKI therapy who have developed disease progression during the period from 1st August 2015 to 2nd August 2017 at University of Malaya Medical Centre. Patients with disease progression, according to Jackman's criteria, underwent

liquid and/or tissue re-biopsy to detect the secondary T790M mutation in exon 20 of the EGFR gene.

Results:

The data from a total of 68 patients who met the study inclusion criteria were analysed. Six patients underwent only liquid biopsy, 36 patients underwent tissue re-biopsy and 15 underwent liquid biopsy and 11 tissue re-biopsy. Out of the 68 patients studied, T790M mutation was detected in 35 patients (51.4%). T790M mutation was detected in the plasma of 10 patients, in tissue re-biopsy specimens of 20 patients and in both the plasma and tissue re-biopsy specimens of 5 patients. From the total of 68 patients, 57 (84.0%) were treated with first-generation EGFR-TKI and 11 were treated with second-generation EGFR-TKI. Patients treated with first-generation EGFR-TKI were less likely to develop T790M mutation compared to those treated with second-generation EGFR-TKI (52.6% vs 45.5%, p=0.915).

The duration of first-line EGFR-TKI treatment was numerically longer in those patients who acquired T790M mutation (13.8 months) compared to those who did not (11.1 months) (p =0.21). A higher proportion of patients who did not acquire T790M mutation had a better ECOG performance status of 0-1 (60.6%) at the time of disease progression compared to those who developed progression due to acquired T790M mutation (28.6%) (p=0.028).

Conclusions:

T790M mutation was identified as the acquired resistance mechanism causing first-line EGFR-TKI treatment failure in 51.4% of our patients. There was no difference in the clinical and treatment characteristics between patients with or without acquired T790M

mutation as the cause of resistance to first-line EGFR-TKI treatment. At the time of disease progression, compared to patients with acquired T790M mutation, a significantly higher proportion of patients who did not have acquired T790M mutation had a better ECOG performance status.

The duration of first-line EGFR-TKI treatment was longer in those patients who acquired T790M mutation compared to those who did not.

1.0 INTRODUCTION

1.1 Background

Lung cancer which includes small cell and non-small cell lung cancer (NSCLC) is the second most commonly diagnosed type of cancer worldwide.[1,2] It is also the leading cause of cancer-related deaths worldwide, accounting for about 1 in 4 cancer deaths[1]. In the current era of 'theranostics' which combines therapeutics with diagnostics, personalized therapy has become the standard of care in advanced NSCLC patients with identifiable oncogenic drivers.[3] The detection of epidermal growth factor receptor (EGFR) mutation as an oncogenic driver has revolutionized the treatment approach in advanced NSCLC.[4] Several trials have demonstrated that first-line treatment with EGFR-tyrosine kinase inhibitors (TKIs) in patients with advanced NSCLC harbouring activating EGFR mutations translates to better progression-free survival (PFS) and overall survival (OS), compared to standard platinum-doublet chemotherapy.[5–7] However, most patients treated with first- or second-generation EGFR-TKIs inevitably develop acquired resistance and disease progression after a median treatment duration of 9 months to slightly over a year.[6,8–10]

Acquired resistance to EGFR-TKIs can be defined by the Jackman's criteria[8] which include the following:

- Previous treatment with single-agent EGFR-TKI
- Either of the following: (a) a tumour that harbours an EGFR mutation known to be associated with drug sensitivity; (b) objective clinical benefit from treatment with an EGFR-TKI
- Systemic progression of disease while on continuous treatment with an EGFR-TKI within the last 30 days

- No intervening systemic therapy between cessation of EGFR-TKI therapy and initiation of new therapy.

Several mechanisms of acquired resistance to EGFR-TKIs have been elucidated. Acquiring a secondary T790M mutation in exon 20 of the EGFR gene is the most common resistance mechanism which is reported in about 50-60% of resistant cases.[11,12] One of the proposed mechanisms by which the T790M mutation confers resistance involves an increase in the adenosine triphosphate (ATP) affinity for binding to the tyrosine kinase active site, thereby decreasing the potency of ATP-competitive EGFR-TKIs such as the first- and second-generation EGFR-TKIs.[13] Another proposed mechanism is the steric hindrance caused by the structural substitution of threonine with a bulkier methionine at amino acid position 790 in the EGFR tyrosine kinase domain in T790M mutation. T790M is an important binding site for TKIs, and this steric hindrance in T790M mutation may interfere with the binding affinity of some EGFR-TKIs, resulting in resistance to these drugs.[14]

The introduction of novel third-generation EGFR-TKIs targeting T790M mutation such as osimertinib has been revolutionary in the personalized therapy for those who failed first-line treatment with first- or second-generation EGFR-TKIs. Clinical trials have demonstrated the efficacy of these drugs in the treatment of EGFR-mutant NSCLC patients with this acquired resistance, thus highlighting the importance of testing for the T790M mutation in cases of disease progression with first- or second-generation EGFR-TKIs.[15]

In EGFR-mutant NSCLC patients with disease progression, despite targeted therapy with first- or second-generation EGFR-TKIs, retesting after TKI therapy for acquired resistant mutations has been proven to be a feasible approach. Re-biopsy has been found to detect acquired T790M mutation in about 50% of such cases by other researchers.[16-17]

The above studies were conducted in Japan and The United Kingdom. It is unclear what is the incidence rate of T790M mutation in a Malaysian setting.

Liquid biopsy is a reliable method for detecting resistant mutations in this setting.[18-19] However, the sensitivity of liquid biopsy is generally lower than tissue biopsy in detecting T790M mutation, with a relatively high false negative rate in the region of 30%.[18–20] In some cases, T790M-negative in first post-TKI biopsies have been found to be T790M-positive in later re-biopsies. Thus, underlining the importance of sequential re-biopsies in advanced NSCLC patients with disease progression despite appropriate therapy.[16,21]

The National Comprehensive Cancer Network (NCCN) version 8.2017[20] clinical practice guidelines for NSCLC recommend conducting a biopsy to determine the mechanism of acquired resistance in EGFR-positive NSCLC cases progressing on EGFR-TKI therapy.[20] Both the NCCN and European Society for Medical Oncology (ESMO) [21] clinical practice guidelines recommend initiation of treatment with a third-generation TKI in cases with acquired T790M mutation. These guidelines recommend a liquid biopsy to detect T790M in circulating cell-free DNA (cfDNA) in the plasma as the first-line investigation for patients who develop resistance to first-line EGFR-TKI treatment. If T790M is not detected in cfDNA by the liquid biopsy, then reflex tissue re-biopsy should be performed because of the high false negative rate of liquid biopsy. Liquid biopsy is suggested as the first-line diagnostic method in the guidelines because it is less invasive compared to tissue biopsy especially in the setting of disease progression.

1.2 Research Questions

The research questions of this study include the following:

1. Is the incidence of T790M mutation, as a mechanism of acquired resistance, causing disease progression in EGFR-mutant advanced NSCLC patients treated with first-line EGFR-TKI therapy?

2. What are the clinical characteristics of patients with acquired T790M mutation which causes resistance to first-line EGFR-TKI treatment, compared to the clinical characteristics of patients who develop resistance to first-line EGFR-TKI treatment not due to acquired T790M mutation?

1.3 Research Objectives

1. To determine the incidence of T790M mutation as a mechanism of acquired resistance, causing disease progression in EGFR-mutant advanced NSCLC patients treated with first-line EGFR-TKI therapy at the University of Malaya Medical Centre.

2. To compare the clinical characteristics of patients with acquired T790M mutation, causing resistance to first-line EGFR-TKI treatment, with that of patients who develop resistance to first-line EGFR-TKI treatment not due to acquired T790M mutation.

1.4 Null Hypothesis

1. The incidence of T790M mutation, as a mechanism of acquired resistance, causing disease progression in EGFR-mutant advanced NSCLC patients treated with first-line EGFR-TKI therapy at the University of Malaya Medical Centre is similar to that reported by other investigators.

2. There is no difference in the clinical characteristics of patients who acquired T790M mutation, causing resistance to first-line EGFR-TKI treatment, and the clinical characteristics of patients who develop resistance to first-line EGFR-TKI treatment not due to acquired T790M mutation.

2.0 LITERATURE REVIEW

This chapter will discuss the definitions and the terminology appropriate to the research variables being explored as well as to define the terms used for this research dissertation. This chapter will also elaborate in detail the background information related to the areas of interest in this proposal. The sections will also detail the issues pertaining to the methodology of the studies used in this proposal as well as the applicability in this context. The final sections of this literature review will explain in detail the various intricacies related to the workings of this study and how these were implemented.

2.1 The Burden of Lung Cancer

In 2014, cancer of the trachea, bronchus and lung accounted for 24.6% of all cancer mortality in males and 13% of all cancer deaths in women in Malaysia.[22] Cancer of the trachea, bronchus and lung is the leading cause of cancer deaths in males and the second most common cause of cancer death after breast cancer in females in Malaysia. According to the Malaysian National Cancer Registry 2007-2011, among the ten most common cancers in Malaysia, lung cancer is ranked third at 10.2%.[22]

Lung cancer is usually divided into 2 groups according to the type of cells[23]:

1. Non-small cell lung cancer (NSCLC). There are a few types which are:

- Adenocarcinoma (30-40%)
- Squamous cell carcinoma (30%)
- Large cell carcinoma (10%)

2. Small cell lung cancer (SCLC) (20%). This type of cancer is less common. However, the cancer cells grow at a very rapid rate and most of the time, will have spread to other parts of the body at diagnosis.

Other types of cancer found in the lungs are carcinoid and lymphoma. (5%)

2.2 Epidemiology of Lung Cancer in Malaysia

In Malaysia, lung cancer accounts for 13.4% of all cancers in males and 7% of all cancers in females.[22] Male to female ratio in terms of incidence of lung cancer is 1.9. There has been a change in the distribution of lung cancer cell types in Malaysia in recent years, similar to what has been observed worldwide, with adenocarcinoma overtaking squamous cell carcinoma as the most common histological subtype.

The Malaysian National Cancer Registry 2007-2011 shows that the age-standardized incidence of lung cancer for the Chinese is more than twice that of the Malays and Indians for both sexes.[22] The reason for this racial difference in predisposition to lung cancer is unknown. It is probably related to environmental risk factors such as diet, predisposing genetic factor or the way tobacco is metabolized in these three difference races.[24]

2.3 Staging of Lung Cancer

The most important prognostic indicator in lung cancer is the extent of disease and lymph node involvement. The American Joint Committee for Cancer (AJCC) [25] Staging and End Results Reporting has developed the TNM (tumor-node-metastasis) [26] staging system, which takes into account the degree of spread of primary tumour, the

extent of regional lymph node involvement and the presence or absence of distant metastases.

Recently, the 8th edition of the TNM staging system for lung cancer has been published[25] and is to be used for staging of new lung cancer cases diagnosed starting from January 2017. However, the patients in this study, who were diagnosed before this date, were staged using the 7th edition of the staging system. [27]

Traditionally, the TNM classification has been used for staging NSCLC. The T staging is determined by the size of the primary tumour in long axis, or direct extent of the tumour into adjacent structures such as mediastinum or chest wall. The 7th edition system has 5 size-based categories with cut-offs at 2, 3, 5 and 7 cm. Tumours measuring < 2 cm are classified as T1a, whereas those measuring 2-3 cm are classified as T1b. T2 disease is also subdivided into T2a (> 3 - 5 cm) and T2b (> 5 cm - 7cm). Tumours larger than 7 cm in diameter are classified as T3.

The N classification describes the degree of spread to regional lymph nodes. The international staging system for lung cancer defines the regional lymph nodes, the N component, as follows.[a,b,c] N0=no lymph node metastasis; N1 = metastasis to lymph nodes in the peribronchial or the ipsilateral hilar region or both, including direct extension; N2 = metastasis to ipsilateral mediastinal lymph nodes and subcarinal lymph nodes; and N3=metastasis to contralateral mediastinal lymph nodes, contralateral hilar lymph nodes, ipsilateral or contralateral scalene or supraclavicular lymph nodes.

The M staging defines the presence of metastases beyond the regional lymph nodes. In the 7th edition of the lung cancer staging classification, pleural or pericardial dissemination (effusions or nodules) are grouped into a new category (M1a). This category also includes additional nodules that are found in the contralateral lung. Extra-thoracic distant metastasis is sub-classified as M1b disease. According to TNM classification and A. Prof Frank Gaillard et al [28], advance lung disease is defined as any

patient in the stage IIIB and stage IV. Stage IIIB, i.e. any patient who has T1, 2 or 3, N3, M0 or T4, N0, 1, 2 or 3, M0 and the 5 year survival rate is 8%, whereas patients who are in stage IV may have any T, any N with M1 and the 5 year survival rate is 2%. In both stages, tumours are not resectable.

2.4 Eastern Cooperative Oncology Group (ECOG) Performance Statuses

The Eastern Cooperative Oncology Group (ECOG) performance status at the time of disease progression are categorized as shown in Table 1.[29]

Table 1: Eastern Cooperative Oncology Group (ECOG) Performance Status[29]

ECOG PERFORMANCE STATUS	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair

2.5 Epidermal Growth Factor Receptor (EGFR) Mutation

The epidermal growth factor receptor (EGFR) is a 486-amino-acid receptor protein of 170 kDa with a single transmembrane sequence between 4 extracellular and 3 intracellular domains. EGFR belongs to the erbB family of closely related receptor tyrosine kinases, which include erbB1 (also known as EGFR), erbB2 (HER2), erbB3, and erbB4. Although their basic structures are similar, each one has distinct properties, including variations in tyrosine kinase activities. It has an extracellular ligand binding domain, a transmembrane portion, intracellular tyrosine kinase and regulatory domains. Upon binding of a specific ligand (e.g. epidermal growth factor), the normally functioning EGFR undergoes conformational change and phosphorylation of the intracellular domain.

At present, EGFR mutation is the strongest predictive biomarker for the efficacy of EGFR-TKIs.[30,31]

2.6 EGFR-tyrosine Kinase Inhibitor (EGFR-TKI)

A tyrosine kinase inhibitor (TKI) is a pharmaceutical agent that inhibits tyrosine kinases. Tyrosine kinases are enzymes responsible for the activation of many proteins by signal transduction cascades. The proteins are activated by adding a phosphate group to the protein (phosphorylation), a step that TKIs inhibit.

Table 2: Types of Generation EGFR-TKIs

Drug name	Generation of EGFR-TKI
Gefitinib, erlotinib	First-generation
Afatinib	Second-generation

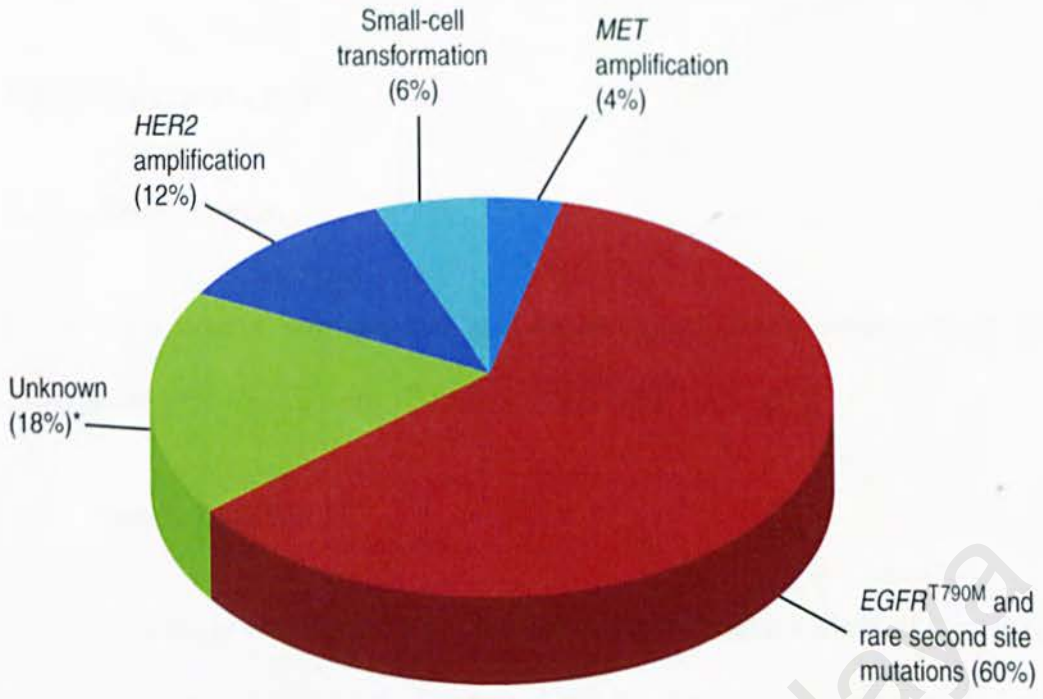
Table 2 shows the types of first- and second-generation EGFR-TKI available at University of Malaya Medical Centre.

The efficacy of EGFR-TKIs in the treatment of patients with advanced NSCLC harbouring activating EGFR mutations has been compared with standard platinum-doublet chemotherapy in several large phase III randomized controlled trials (WJTOG3405,[32] NEJ002,[33] IPASS,[34] OPTIMAL,[35]EURTAC,[36] ENSURE,[37] LUX Lung 3,[38] and LUX Lung 6[39]) which provide strong evidence that first-line treatment with EGFR-TKIs should be the treatment of choice for EGFR mutation-positive patients.

2.7 T790M Mutation

Despite the remarkable response of EGFR-mutant lung adenocarcinomas to first-line EGFR-TKIs, all these tumours progress, due to the development of various resistance mechanisms.[31] One of these resistant mechanisms is a secondary mutation located in exon 20 of the *EGFR* gene in which threonine is replaced by methionine in amino acid position 760 (T790M),[40] resulting in increased ATP affinity and steric hindrance as mentioned in section 1.1 above. T790M mutation, identified in 50-60% of patients, is by far the most common cause of acquired resistance to EGFR-TKIs compared to other resistant mechanisms such as cMET amplification (Figure 1).[41]

Figure 1: Frequency of Mechanisms of Acquired Resistance to EGFR-TKIs in EGFR-mutant Lung Cancer[41]



3.0 METHODOLOGY

3.1 Study Design

This was a retrospective and observational study conducted in University Malaya Medical Centre from 1st August 2015 to 31st July 2017.

3.2 Study Location

The study was conducted at the University of Malaya Medical Centre (UMMC), Lembah Pantai, 59100 Kuala Lumpur. UMMC is one of the largest medical centres under the aegis of the Ministry of Education, Malaysia and serves close to 2 million population of Kuala Lumpur and Petaling Jaya as a secondary and tertiary referral centre, besides being a tertiary referral centre for the whole of Malaysia. The sub-specialised, multi-department hospital provides comprehensive respiratory and oncology services, in addition to other major disciplines in medicine.

3.3 Study Population

The population in this study included all patients with EGFR-mutant advanced NSCLC who experienced disease progression while on first-line EGFR-TKI treatment at the Division of Respiratory Medicine, Department of Medicine, UMMC during the study period.

3.4 Sampling Method

This study used a convenience sampling method.

3.5 Sample Size & Power

The sample required to enable adequate powering of the study was calculated using the formula by Charan, J, & Biswas,T(2013) [43] for sample size estimation of a cross-sectional study with a qualitative outcome variable. The formula is as detailed below:

$$N = (Z_{1-\alpha/2})^2 \, p \, (1-p) / d^2$$

Where N= sample size

p= Expected proportion in population based on previous studies or pilot studies

$Z_{1-\alpha/2}$= value 1.96, for the conventional level of confidence of 95%

d = precision (in proportion of one), taken as 0.2 (giving a power of 80%) for this study

For this study, a previous study by Kuiper JL et al (2014) [14] was used to assist in determining the sample size calculation. In this previous study, an incidence of T790 mutation of 52% was discovered in the sample population. This formula yielded a sample of 24 people at a power of 80%. Assuming a possible non-completion rate of 20% (under the premise that inadequate data will be available from the case notes), an additional 5 people to make up for this 20% will mean a sample size of 29 people. At the current study population, the sample was more than adequately powered to detect the incidence of T790M mutation in the study population.

3.6 Study Instruments

A case report form (CRF) to document the patients' demographic and clinical data was specifically designed for this study.

3.6.1 Response Evaluation Criteria in Solid Tumours or RECIST 1.1[44]

Response evaluation criteria in solid tumours or RECIST 1.1[44] refers to a set of published rules used to assess tumour burden in order to provide an objective assessment of response to therapy. The criteria can be used with CT or MRI scanning results.

RECIST terminology characterises lesions as measurable versus non-measurable and target versus non-target lesions. Measurable lesions are the ones that can be assessed quantitatively. From among the measurable lesions, target lesions are selected. Once a lesion is identified as a target lesion, it is always referred to as one, even if it falls below the size limits for what is considered measurable at baseline.

Table 3: Definition of Response Assessment Response Assessment Using RECIST 1.1

Target Lesion Response	**Non-target Lesion Response**
Complete Response (CR) • All target lesions disappear Partial Response (PR) • 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD Progressive Disease (PD) • 20% increase from smallest sum of longest diameter recorded since treatment started (best response) Stable Disease (SD) • Neither PD nor PR	Complete Response (CR) • All non-target lesions gone • Tumour markers to normal levels Stable Disease (SD) • Persistence of >1 non-target lesion • Tumour marker level elevated Progressive Disease (PD) • Enlargement of non-target lesions

3.6.2 Progression-free Survival (PFS)

Progression-free survival (PFS) is the length of time from the initiation of a particular treatment to the detection of progressive disease or death. [45-46]

3.6.3 Treatment Beyond Progression

The ASPIRATION[47] trial has shown that when patients were permitted to continue EGFR-TKIs beyond RECIST progression, if the clinical benefit was obvious and the drug was well tolerated, many patients were able to maintain EGFR -TKI treatment for durable periods of time - some for years. This is because patients with slow and indolent low volume asymptomatic disease progression may continue their original EGFR-TKI and eventually switch treatment when the physician and patient feel that clinical symptoms are worsening.

3.6.4. Duration of Treatment (DOT)

Duration of treatment (DOT) is defined as the length of treatment given to a patient until it is stopped because of symptomatic disease progression. [46]

3.7. Diagnosis of T790M Mutation

Before 1st January 2017, patients were investigated for acquired resistance mechanisms upon failure of first-line EGFR-TKI therapy by tissue re-biopsy using COBAS® Version 2 (v2) Real Time Polymerase Chain reaction (RT-PCR) method (Roche Molecular System Inc, Roche Molecular Diagnostics,4300 Hacienda Drive Pleasanton, CA 94588,USA).[48] The COBAS®v2 RT-PCR is able to detect G719X substitution mutations in exon 18, deletion mutations in exon 19, T790M and S768I substitution mutations and insertion mutations in exon 20, and L858R and L861Q

substitution mutations in exon 21.[48] This is the only method currently approved by the Food and Drug Administration (FDA) of the United States to detect sensitizing mutations and T790M mutation in tissue and also in blood samples. However, it is less sensitive for the detection of T790M mutation in the plasma.[49] All tissue re-biopsies were performed by trained interventional radiologists and the tissue samples were examined and assessed by a qualified pathologist at UMMC. Although members of the pathology and radiology team were not always the same persons, the techniques and standard operating procedures, especially in terms of the qualification of the ascertaining consultant/specialists were as accredited and thus deemed to be equivalent, without acting as a confounder for this study. Those patients who were not suitable for tissue re-biopsy underwent a liquid biopsy to detect T790M mutation in circulating cell-free DNA (cfDNA) originating from necrotic tumour cells sloughed from the tumour or from circulating tumour cells in the plasma. Peptide nucleic acid-locked nucleic acid (PNA-LNA) polymerase-chain reaction (PCR) clamp method (PANAGENE, South Korea) was used to detect cfDNA in plasma samples at the Subang Jaya Medical Centre. The PNA-LNA PCR clamp method has been determined to be adequately sensitive (sensitivity <2%) to detect EGFR mutations in cfDNA in plasma.[50]

After 1st of January 2017, blood samples were collected from patients and sent to a laboratory in Hong Kong, Sanomics Limited, where droplet digital PCR was used to detect mutant EGFR which included exon 19 deletion, exon 20 T790M mutation and exon 21 L858R. The analytical sensitivity of this droplet digital PCR has been found to be more than 99.9%. (http://www.sanomics.com/). For patients tested negative for T790M mutation by liquid biopsy, tumour re-biopsy was performed. However, both the PNA-LNA PCR clamp method and the droplet digital PCR method have not yet been approved for the detection of T790M mutation by the FDA.

3.8 Study Administration and Arrangements

A database of patients diagnosed with NSCLC harbouring sensitising EGFR mutations and treated with first-line EGFR-TKIs at UMMC was screened for patients who have developed disease progression during the study period from 1st August 2015 to 31st July 2017 and met the inclusion criteria for this study. The relevant demographic and clinical information were extracted from the patients' written and electronic medical records and entered into the CRF.

3.9 Validity, Reliability and Standardization of Methods

All the data collection was done by the author. The list of patients was counter-checked against University Malaya Medical Centre's Computerised Laboratory Records System to ensure that the results obtained for the T790 mutation were accurate as entered in the patients' case notes. This was an additional reliability counter-measure which increased the reliability of the collected data. The relevant case notes that fit the inclusion criteria were separated and then the relevant information extracted and entered into the CRFs. The CRFs were filled by both medical officers separately and at the end, both versions of the CRFs were counterchecked to ensure that all data had been accurately entered. These measures were designed to improve the validity and the reliability of the data collection.

3.10 Inclusion Criteria

1. Patients with advanced NSCLC harbouring activating (sensitising) EGFR mutations treated with first-line EGFR-TKIs.

2. Patients who developed resistance to EGFR-TKI therapy as defined by Jackman's Criteria for acquired resistance[8] which include the following:

a. Previously received treatment with single-agent (monotherapy) EGFR-TKI

b. Either of the following:

i. A tumour that harbours an EGFR mutation known to be associated with drug sensitivity;

ii. Objective clinical benefit from treatment with an EGFR-TKI as defined by either: (Documented partial/complete response (or) significant and durable (>6 months), clinical benefit (stable disease as defined by Response Evaluation Criteria in Solid Tumors (RECIST 1.1)[42] after initiation of first-line EGFR-TKI)

c. Systemic progression of disease (RECIST 1.1) while on continuous treatment with EGFR-TKI within the last 30 days

d. No intervening systemic therapy between cessation of EGFR-TKI and initiation of new therapy.

3.11 Exclusion Criteria

1. Patients with EGFR wild-type NSCLC
2. Patients who defaulted on treatment, i.e. not present at two subsequent follow-up visits.
3. Patients who were enrolled in other oncological clinical trials during the study period.

3.12 Research Ethics

Since this was a retrospective observational study using patient case records, it was not possible to obtain consent from each individual patient to allow the use of his/her clinical information. Ethical clearance for the study was provided by University

Malaya Medical Centre's Research Ethics Committee (2017828-5529). Approval of the study protocol is attached in the accompanying appendix.

3.13 Statistical Analysis

In the analysis of the demographic and clinical data of the patients, results for continuous variables were expressed as mean ± standard deviation (SD), median or range depending on normality of the variable distribution; while results for categorical variables were expressed as percentages. In univariate analysis of the demographic and clinical data, differences between groups were tested for significance with chi-square test with Yates' correction or Fisher's exact test whichever was appropriate for categorical variables; and Student's *t*-test for continuous variables as shown in Table 3 below. A two-sided p value of less than 0.05 was considered statistically significant. Kaplan-Meier survival curves were drawn for duration of treatment with first-line EGFR-TKI for patients who developed disease progression due to T790M mutation and for those who developed disease progression not due to T790M mutation. Duration of treatment for the two groups of patients was compared using the log-rank sum test. All statistical analyses were performed using SPSS (Statistical Package for Social Sciences) version 17 (IBM SPSS Modeler 17.0, UK)

Table 4: Variables and Statistical Tests Used to Make Comparisons

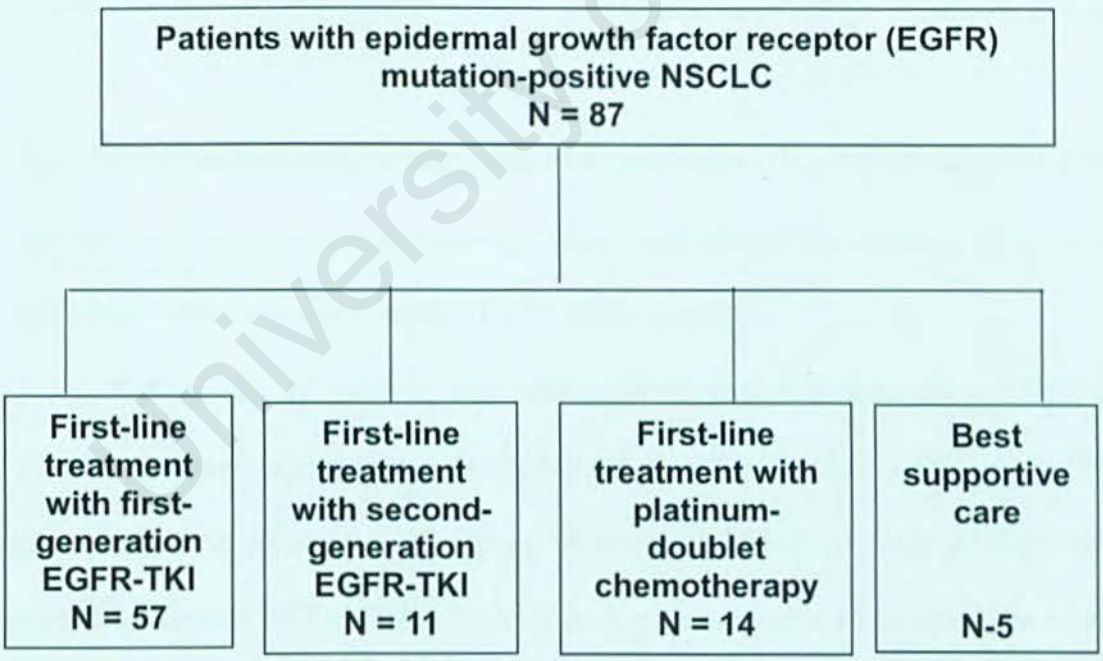
Variable	Type of Variable	Statistical Comparison Test Used
Duration of treatment	Continuous	Log-rank sum test of Kaplan-Meier survival curves
GFR mutation subtype	Categorical	Chi-Square test
ECOG performance status	Categorical	Chi-Square test
Best tumour response	Categorical	Chi-Square test
EGFR-TKI treatment beyond progression	Categorical	Chi-Square test
Ablative treatment in patients who received EGFR-TKI treatment beyond disease progression	Categorical	Chi-Square test

4.0 RESULTS

4.1 Study Flow

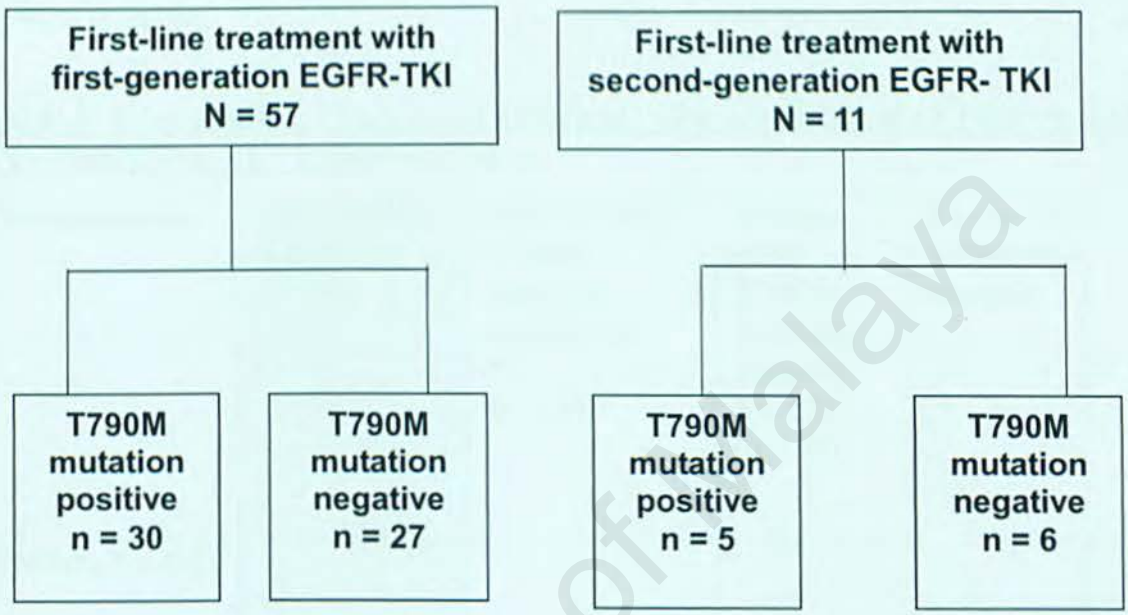
During the study period from 1st August 2015 to 31st July 2017, 87 patients with NSCLC harbouring sensitising EGFR mutations were treated at the Division of Respiratory Medicine, Department of Medicine, UMMC (Figure 2). Of these 87 patients, 57 received first-line treatment with first-generation EGFR-TKI (37 received gefitinib and 20 received erlotinib) while 11 received first-line treatment with second-generation EGFR-TKI, afatinib. Of the remaining 19 who were not treated with EGFR-TKI, 14 received standard platinum-doublet cytotoxic chemotherapy because of financial constraint and five patients received only best supportive care because of poor ECOG performance status in view of late diagnosis.

Figure 2: Disposition of Patients



All patients with disease progression and who fulfilled Jackman's Criteria underwent repeat biopsy: either liquid or tissue re-biopsy to detect T790M mutation.

Figure 3: Detection of T790M Mutation on Disease Progression While on First-line EGFR-TKI Treatment



Most of the patients were treated with first-generation TKIs which included gefitinib and erlotinib because of the relatively fewer side effects and toxicity of these drugs compared to the second-generation EGFR-TKI, afatinib.

Among the 57 patients who were treated with first-generation EGFR-TKIs, T790M mutation was detected by re-biopsy in 30 patients (52.6%) while T790M mutation was not detected in 27 patients. Among the 11 patients who were treated with second-generation EGFR-TKI, T790M mutation was detected by re-biopsy in 6 patients (54.5%) ($p=0.915$). The overall incidence of T790M mutation was 35 out of 68 patients (51.5%).

4.2 Descriptive Analysis

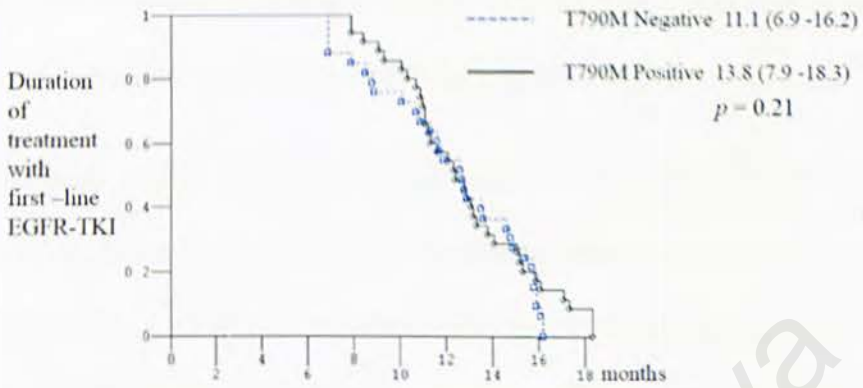
Table 5 shows the comparison of the demographic, clinical and treatment characteristics of patients who developed acquired resistance to first-line EFGR-TKI treatment due to T790M mutation with those who developed resistance not due to T790M mutation.

Table 5: Demographic, Clinical and Treatment Characteristics of 68 Patients with First-line EGFR-TKI Treatment Failure

Characteristics	Total number of patients (n = 68)	Patients with T790M mutation positive, No. (%) (n = 35)	Patients with T790M mutation negative, No. (%) (n = 33)	P value of univariate analysis
Gender, No. (%)				0.592
Male	28 (41.2)	16 (57.1)	12 (42.9)	
Female	40 (58.8)	19 (47.5)	21 (52.5)	
Smoking history, No. (%)				0.484
Never smoke	51 (80.0)	28 (54.9)	23 (45.1)	
Previous or current smoker	17 (20.0)	7 (41.2)	10 (58.8)	
EGFR mutation subtype, No. (%)				0.617
Exon 19 deletion	41(60.3)	20 (48.8)	21(51.2)	
Exon 21 L858R point mutation	24 (35.3)	14 (58.3)	10 (41.6)	
Others	3 (4.4)	1 (33.3)	2(66.7)	

Best tumour response to first-line EGFR-TKI treatment, No. (%)				0.507
Partial response	57 (83.8)	28 (49.1)	29(50.9)	
Stable disease	10 (14.7)	6 (60.0)	4 (40)	
Progressive disease	1 (1.5)	1 (100)	0 (0.0)	
Duration of treatment, in months median (IQR)		13.8 (7.9-18.3)	11.1(6.9-16.2)	0.21
ECOG performance status at time of disease progression, No. (%)				0.028
0 -1	30 (44.1)	10 (33.3)	20 (67.7)	
2-4	38 (55.9)	25 (65.8)	13 (34.2)	
First-line EGFR-TKI treatment beyond disease progression, No. (%)				0.674
YES	50 (73.5)	27(54)	23 (46.0)	
NO	18 (26.5)	8 (44.4)	10 (55.6)	

Figure 4:Kaplan-Meier curves of duration of treatment with first-line EGFR-TKI for patients who developed disease progression due to and not due to T790M mutation.



Kaplan-Meier curves of duration of treatment with first-line EGFR-TKI for patients who developed disease progression due to and not due to T790M mutation.

Of the 68 patients who developed disease progression, 42 (61.7%) underwent tissue re-biopsy and 15 (22.1%) underwent liquid biopsy as the first investigation to determine the presence of T790M mutation. For the latter group, 11 (16.2%) subsequently also needed to undergo tumour tissue re-biopsy because of a negative liquid biopsy for T790M. T790M mutation was detected in 35 of the 68 patients (51.5%). In short, half of our patients (51.5%) had T790M mutation identified as the resistance mechanism accounting for first-line EGFR-TKI treatment failure while T790M was not detected in the other half (48.5%).

The duration of treatment with first-line EGFR-TKI for the T790M-positive group was 13.8 months whereas it was shorter at 11.1 months in the T790M-negative group. This difference of 2.7 months,however,is not statistically significant as shown in Figure4.

Although a higher proportion of patients, whose best response to first-line EGFR-TKI treatment was stable disease, were found to be T790M-positive on disease progression [6 of 10 (60%)], this was not significantly different from the proportion of patients, whose best response to first-line EGFR-TKI treatment was partial response, who were tested positive for T790M [28 of 57 (49.1%)] (p=0.507).

A significantly higher proportion of patients who developed resistance not due to T790M mutation had a better ECOG performance status (0 or 1) at the time of disease progression [20 of 33 (60.6%)] compared to that of those who acquired T790M mutation as the resistance mechanism to first-line EGFR-TKI treatment [10 of 35 (28.6%)] (p=0.028).

27 out of 35 patients (77.1%) who acquired T790M mutation continued to receive first-line EGFR-TKI treatment beyond RECIST-defined disease progression while 23 out of 33 patients (46%) in the T790M mutation-negative group continued to receive first-line EGFR-TKI treatment beyond disease progression (p=0.674).

5.0 DISCUSSION

5.1 Interpretation and Findings

This study shows that acquired T790M mutation in exon 20 of the EGFR gene has been identified as the cause of resistance to first-line EGFR-TKI treatment in 51.5% of the 68 patients with advanced NSCLC harbouring sensitising EGFR mutations which is similar to the frequency of 50-60% reported by others[45,51]. Kuiper et al[14] reported an acquired T790M mutation incidence of 52% while Oxnard et al[45] and Hata et al[51] reported similar results in American and Japanese patients with EGFR-mutant NSCLC treated with EGFR-TKIs, respectively.

Ke et al [52] has shown acquired T790M mutation is more likely to occur in patients harbouring exon 19 deletion mutation than in those with exon 21 L858R mutation (50.4% versus 36.5%). Higher frequencies of T790M mutation in patients initially harbouring exon 19 deletion mutation are also reported by Nosaki et al[53] [Del19 vs L858 R, 219 (55.4%) vs 149 (37.7%)] and Matsuo et al.[46][Del19 vs L858 R, 26 (63%) vs 12 (38%)]. However, another published study has found a similar T790M positivity rate in patients with exon 19 deletion mutation and in patients with exon 21 L858R mutation. [14,51,54,55]

No studies except for the study by Nosaki et al[53] have reported a statistically significant association. Unlike our study, Nosaki et al[53] observed some minor mutations and included them in their statistical tests for association.

We did not find a higher frequency of T790M mutation among our patients with initial exon 19 mutation. In fact, a slightly higher proportion of patients with initial exon 21 mutation developed resistance due to T790M mutation (58.3%) compared to that of patients with initial exon 19 mutation (48.7%). However, the difference was not statistically significant.

Exon 19 deletion mutation was a more common sensitising mutation (60.3%) than exon 21 L858R mutation (35.3%) in our patients. Exon 19 deletion mutation was also found to be more prevalent than exon 21 L858R mutation as a sensitising EGFR mutation in the AURA extension,[56] [Del19 (71%) vs L858 R (25%)], AURA 2[57] [Del19 (65%) vs L858 R (32%)], and AURA 3[58] [Del19 (62%) vs L858 R (32%)]- studies on patients pre-treated with an EGFR-TKI and acquired T790M mutation.Patients with acquired T790M mutation, irrespective of the initial EGFR activating mutation subtypes, have longer overall survival period which suggests that resistance acquired through the T790M mutation follows a more indolent course than clinical resistance not due to this mutation.[59]

In keeping with the more indolent nature of progressive disease due to T790M mutation, the median duration of first-line EGFR-TKI treatment before disease progression in our patients who developed resistance due to T790M mutation was 13.8 months. This was 2.7 months longer than the median duration of 11.1 months in our patients in whom T790M was not identified as the cause of resistance to first-line EGFR-TKI treatment. However, the difference was not statistically significant probably due to the small sample size.

In keeping with the more indolent nature of progressive disease due to T790M mutation, 77.1% of our 35 patients who acquired T790M mutation continued to derive clinical benefit when their first-line EGFR-TKI treatment was continued beyond RECIST-defined radiological disease progression while only 23 of our 33 patients (46%) in the T790M mutation-negative group were continued on first-line EGFR-TKI treatment beyond radiological disease progression. However, the difference was not statistically significant.

It is our standard practice to continue treating our patients with first-line EGFR-TKI even after disease progression was shown on CT scan, as long as the patients are not symptomatic of the disease progression. This practice is endorsed by the NCCN[20] guidelines and supported by the findings of the ASPIRATION[47] study. Yap et al[60] elaborate that continuing TKIs beyond disease progression is becoming increasingly commonplace in patients with indolent, small volume asymptomatic growth, who may potentially continue to derive ongoing clinical benefit and to avoid a 'withdrawal tumour flare'. In previous studies, Maruyama et al[61] retrospectively analyzed 60 patients who had treatment failure after achieving disease control with gefitinib. These patients were treated with or without continuing gefitinib. Continuing the drug was associated with a better survival based on multivariate analyses (HR 0.51, 95%CI: 0.26–0.98, p = 0.042). Faehling et al[62] also reported retrospectively that NSCLC patients who were treated with erlotinib beyond disease progression responded well, thus showing treatment with TKI after disease progression could lead to longer overall survival.

In this study, we found that at the time of disease progression, the proportion of patients with better ECOG performance status of 0 or 1 was significantly higher in those who developed resistance not due to T790M mutation (67.7%) compared to those who acquired T790M mutation as the resistance mechanism to first-line EGFR-TKI treatment (32.3%).

Hata et al[54] reported that the proportion of patients who had better ECOG performance at the time of disease progression was significantly higher in those who developed resistance not due to T790M mutation (61%) compared to those who acquired T790M mutation (39%).

A few studies shared a similar result profile when it came to best tumour response as this study did. Takeda et al[63] showed that 42 patients had a partial response

(PR) to TKI therapy, 14 achieved stable disease (SD) and 6 had complete response (CR). CR and PR patients showed better PFS and OS with these groups experiencing rapid tumor regression (the median time to treatment response (TTR) was $\leq$ 4.2 weeks). The best tumour response did not seem to be influenced by the pattern of tumour shrinkage.

Matsuo et al.[46] shows similar proportion whereby the median PFS among who fail first line EGFR-TKI treatment was longer in the T790M mutation-positive group (13.6 months, 95% CI: 9.2–15.8) than in the negative group (7 months, 95% CI: 3.7–8.5). Matsuo et al.[46] shown statistically significant in term of PFS ($p = 0.037$) however there was no significant difference in overall survival between patients with T790M mutation (45.2 months; 95% CI, 31.4–51.1) and those without (40.1 months; 95% CI, 21.7–45.8) ($p = 0.278$).

5.2 Strength of the Study

As far as we are aware, this study is the first study in Malaysia to determine the frequency of acquired T790M mutation as a resistance mechanism to first-line EGFR-TKI treatment in patients with advanced EGFR-mutant NSCLC. This study is the first to characterise the demographic and clinical characteristics of patients who develop resistance to first-line EGFR-TKI treatment due to T790M mutation and those not due to T790M mutation.

5.3 Study Limitations

1. This study was retrospective with its attendant limitations including incomplete data.

2. Due to the limited period of the study, the study population was understandably small. Financial constraints could have affected the number of patients with EGFR-mutant NSCLC who could afford EGFR-TKI treatment and the study results may not be

representative of all EGFR-mutant NSCLC patients treated with first-line EGFR-TKIs. A single centre study could also have introduced bias to the results which may not be generalised to all patients with this disease in Malaysia.

3. As explained in the methodology section, the methods of detecting T790M mutation were not uniform for all patients and were dependent on the availability of the tests at different time points especially when the technology of detecting T790M was rapidly evolving. The methods used, with different sensitivities in detecting T790M mutation, are potential confounders in the study. However, this is to be expected in the real-world.

4. This study only focused on T790M as a resistance mechanism. There are, however, many mechanisms of resistance to EGFR-TKI treatment that were not addressed in this study.

5.4 Recommendations

However, these limitations played only a small part in establishing the importance of this study as a baseline-setter in evidence-building in this scientific area. The true magnitude of the relationships established in this study needs to be investigated under robustly designed conditions in order to test their actual causality hypothesis, which in turn will lay the foundation for further interventional trials that seek to address and correct the shortcomings of current clinical practice. One immediate recommendation that the author makes from this study is to call for the establishment of a comprehensive prospective cohort of NCSLC patients across the country in order to identify the mechanisms of resistance to EGFR-TKI treatment in this group of patients. As resistance mechanisms are not restricted to only acquired T790M mutation, such studies should explore the use of tissue re-biopsy as well as liquid biopsy in establishing the other mechanisms of resistance

6. CONCLUSIONS

In conclusion, this study has identified T790M mutation as the acquired resistance mechanism causing first-line EGFR-TKI treatment failure in 51.4% of patients with advanced EGFR-mutant NSCLC. The duration of first-line EGFR-TKI treatment appears to be longer in those patients who acquired T790M mutation compared to those who did not. Otherwise, there was no significant difference in the clinical and treatment characteristics between patients with and without acquired T790M mutation as the cause of resistance to first-line EGFR-TKI treatment. At the time of disease progression, a significantly higher proportion of patients who did not acquire T790M mutation had a better ECOG performance status, compared to patients with acquired T790M mutation.

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