Print ISSN: 2085-3297, Online ISSN: 2355-9179

The Indonesian BIOMEDICAL JOURNAL

Volume 15 Number 5
October 2023

Published by:

PERI
The Prodia Education & Research Institute

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Print ISSN: 2085-3297, Online ISSN: 2355-9179

The Indonesian BIOMEDICAL JOURNAL

Volume 15 Number 5, October 2023

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Print ISSN: 2085-3297, Online ISSN: 2355-9179

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The Indonesian Biomedical Journal
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Osimertinib as a Potential Targeted Therapy for Non-Small Cell Lung Carcinoma (NSCLC) Patients with EGFR Exon 20 T790M

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Received date: May 29, 2023; Revised date: Oct 1, 2023; Accepted date: Oct 9, 2023

Abstract

BACKGROUND: Emergence of drug resistance due to epidermal growth factor receptor (EGFR) Exon 20 T790M poses a challenge in the effective management of non-small cell lung carcinoma (NSCLC). Significant breakthrough in the management of NSCLC with a specific genetic alteration causes substantial condition improvement in patients whose cancer progressed after first-generation tyrosine kinase inhibitor treatment and who developed tumors with EGFR Exon 20 T790M mutation. The present study analyzed a cohort of NSCLC patients and investigated the incidence of the EGFR Exon 20 T790M status with Osimertinib therapy, along with its impact on survival rates.

METHODS: This was a retrospective cohort study on 22 NSCLC subjects who were genetically examined for EGFR status from plasmic cell free total nucleic acid. Subjects with EGFR Exon 20 T790M mutation were treated with/without Osimertinib. Demographic and clinical data were descriptively summarized, and the differences of each variable and correlation between survival rate and EGFR Exon 20 T790M were analyzed.

RESULTS: Subjects with (n=13) and without (n=9) EGFR Exon 20 T790M had survival rates of 10.77±2.45 and 4.78±1.48, respectively (p=0.000). Based on 1-year survival status of subjects with EGFR Exon 20 T790M, there were 3 Osimertinib-treated survivors and 2 Osimertinib-treated non-survivors. Eight subjects with EGFR Exon 20 T790M and without Osimertinib treatment did not survive (p=0.001).

CONCLUSION: Since the treatment of Osimertinib demonstrated a noteworthy survival rate among NSCLC subjects with EGFR Exon 20 T790M, thus Osimertinib could be suggested as a potential targeted therapy for NSCLC subjects with EGFR Exon 20 T790M.

KEYWORDS: non-small cell lung carcinoma, EGFR, Exon 20, T790M, osimertinib

Indones Biomed J. 2023; 15(5): 336-40

Introduction

The epidermal growth factor receptor (EGFR) mutation is an emerging and promising treatment target for non-small cell lung carcinoma (NSCLC).(1) This mutation is particularly prevalent in South-East Asian NSCLC patients, which is found in more than half of all NSCLC patients. Meanwhile it is also found in 10-20% of Caucasian patients.(2) NSCLC-associated EGFR mutation exhibits a higher incidence in women, non-smokers, and those with adenocarcinoma histology.(3) Fortunately, clinical outcomes for NSCLC patients have significantly improved since the introduction of tyrosine kinase inhibitors (TKIs) targeting EGFR.(4)

The initial generation of TKIs has shown remarkable results in prolonging patients' progression-free survival (PFS).(5,6) However, impact on overall survival (OS) may be limited due to disease progression. Recent findings have shed light on the differences in treatment response between the two most common types of EGFR mutations. Patients with Exon 19 deletion tend to have longer PFS on TKI treatment and show more extended OS compared to those with Exon 21 L858R point mutation.(7,8) Although there is an evidence suggesting that particular EGFR mutation had higher affinity with TKI (9), the true mechanism is still debated.

Despite the initial benefits observed with TKI therapy, a majority of NSCLC patients with active EGFR mutations develop resistance during treatment.(1) Since there has been a significant breakthrough in the management of NSCLC with a specific genetic alteration (10-12), there was substantial condition improvement in patients whose cancer progressed after first-generation TKI treatment and who developed tumors with EGFR Exon 20 T790M mutation.(13) The T790M mutation in NSCLC play role in reducing adenosine triphosphate (ATP)-competitive-kinase-inhibitor, so that the mutation can prevent the effect of inhibitor, thus EGFR-mediated signalling will not be defected.(14)

Based on above reasons, the present study was conducted to analyze a cohort of NSCLC patients and to investigate the incidence of the EGFR Exon 20 T790M status with Osimertinib therapy, along with its impact on survival rates. Understanding these factors will provide valuable insights in aiding the development of effective treatment strategies for NSCLC patients with EGFR mutations.

Methods

Subject and Data Collection

A retrospective cohort study was conducted on 22 NSCLC subjects within October 2019 and July 2022 in Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia, and its network centers. This study was approved by the Research Ethics Commission of the Faculty of Medicine, Universitas Hasanuddin (Approval No.: 270/UN4.6.4.5.31/PP36/2022). Demographic and clinical data including age, gender, body mass index, smoking status, Brinkman Index and family history were collected from subjects. Brinkman Index was calculated by multiplying average-cigarette-consumed-everyday with years-of-smoking, mild <200, moderate 200-600, severe >600.

EGFR Genetical Examination

All NSCLC subjects were genetically examined for EGFR status. From each NSCLC subject, 10 mL blood was collected. Blood sample was processed to collect plasmic cell free total nucleic acid (cfTNA) using MagMAX cell free total nucleic acid isolation kit (ThermoFisher, Waltham, MA, USA). DNA library was prepared Oncomine Lung cfDNA Assay (ThermoFisher). Then the sequencing was performed with Ion Torrent next-generation sequencing (NGS) (ThermoFisher). The NGS data was analyzed and interpreted with Ion Reporter and Oncomine Reporter.

Osimertinib Treatment and Survival Status

All NSCLC subjects were treated with Carboplatin and Paclitaxel in a cycle of 6-treatments and 21-days-interval. In the 6th month, EGFR genetical examinations were performed. Among the subjects with EGFR Exon 20 T790M, some received 80 mg Osimertinib for 1 dosage/daily (Tagrrisso, AstraZeneca, Cambridge, UK), while others continued with the second cycle of Carboplatin and Paclitaxel treatment. The subjects were observed until they deceased. The subject survival status was recorded for further analysis.

Statistical Analysis

Demographic and clinical data were descriptively summarized and statistically analyzed using Chi-Square test. Correlation between survival rate and EGFR Exon 20 T790M was analyzed with Log Rank (Mantel-Cox) test. One-year survival status and EGFR mutation in different exons were statistically analyzed using Chi-Square test as well. Significant values were determined at $p<0.05$. All

statistical analyses were performed using the Statistical Program for Social Sciences (SPSS) version 24.0 (IBM Corporation, Armonk, NY, USA).

Results

Based on the subjects' characteristic, there were 12 male and 10 female subjects with majority age of 45-65 years, non-smoking (Brinkman Index), and no family history of lung cancer (Table 1). Meanwhile, based on the EGFR mutation status, there were subjects with mutation of EGFR Exon 20 T790M and mutation of EGFR Exon 19 deletion (n=5) or EGFR Exon 21 L858R (n=8) (Table 2). There were also subjects without mutation of EGFR Exon 20 T790M but having mutation of EGFR Exon 19 deletion (n=5) or EGFR Exon 21 L858R (n=4).

Study subjects with (n=13) and without (n=9) EGFR Exon 20 T790M had a significnat mean difference of 7.77 months with *p*-value of 0.000 (Table 3). As shown in Kaplan-Meier survival curve (Figure 1), subjects without EGFR Exon 20 T790M (blue line) had survival time <10 months, while subjects with EGFR Exon 20 T790M (red line) had survival time >10 months with 3 positive censored tick marks. Based on subjetcs' 1-year survival status, there were more non-survivor with EGFR Exon 20 T790M (n=10) compared with the survivor (n=3), while all subjects without EGFR Exon 20 T790M did not survive (Table 4).

Among the subjects with EGFR Exon 20 T790M, there were subjects treated with (n=5) and without (n=8) Osimertinib. Based on 1-year survival status of subjects with EGFR Exon 20 T790M, there were 3 Osimertinib-treated survivors and 2 Osimertinib-treated non-survivors (Table 5). However, without any Osimertinib treatment, NSCLC subjects with EGFR Exon 20 T790M did not survive.

Table 1. The subject's characteristic based on EGFR Exon 20 T790M.

Variable	EGFR Exon 20 T790M [n (%)]		*p*- value[#]
	(+) (n=13)	(-) (n=9)	
Age			
<45 years old	-	2 (9.1)	0.204
45-65 years old	11 (50.0)	6 (27.3)	
>65 years old	2 (9.1)	1 (4.5)	
Gender			
Male	5 (22.7)	7 (31.8)	0.069
Female	8 (36.4)	2 (9.1)	
Body mass index			
Underweight	7 (31.8)	6 (27.3)	0.548
Normal	6 (27.3)	3 (13.6)	
Overweight	-	-	
Brinkman Index			
Non-smokers	7 (31.8)	2 (9.1)	0.003*
Mild	-	-	
Moderate	5 (22.7)	-	
Severe	1 (4.5)	7 (31.8)	
Family history			
Yes	4 (18.2)	2 (9.1)	0.658
No	9 (40.9)	7 (31.8)	

[#]Chi-Square test, *p<0.05.

Discussion

Lung cancer is a complex disease that often involves genetic alterations affecting the EGFR tyrosine kinase

Table 2. Correlation of EGFR Exon 20 T790M with other EGFR mutations.

Other EGFR Mutation	EGFR Exon 20 T790M [n (%)]		*p*- value[#]
	(+) (n=13)	(-) (n=9)	
EGFR Exon 19 deletion	5 (22.7)	5 (22.7)	0.429
EGFR Exon 21 L858R	8 (36.4)	4 (18.2)	

[#]Chi-Square test.

Table 3. Survival rate related to EGFR Exon 20 T790M.

Group	Survival Rate (Month) [Mean±SD]	Mean Difference	*p*- value[#]
EGFR Exon 20 T790M (+) (n=13)	10.77±2.45	7.77	0.000*
EGFR Exon 20 T790M (-) (n=9)	4.78±1.48		

[#]Log Rank (Mantel-Cox), *p<0.05.

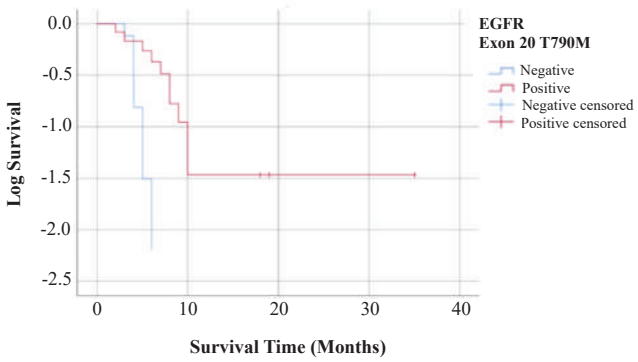

Figure 1. Kaplan-Meier survival curve of subjects with/without EGFR Exon 20 T790M.

signaling pathways, with approximately 75% of examined cases showing such changes.(15) Among these pathways, EGFR pathway is of particular interest with the most common mutations are in-frame deletions (85%–90%) of exon 19 deletion (45%–50%) and Exon 21 L858R mutation (40%–45%).(16) While targeted therapies like TKIs have shown promise in inhibiting tumor growth, the emergence of the EGFR Exon 20 T790M mutation has been identified as a common mechanism of resistance to these therapies. (17,18) The EGFR Exon 20 T790M competes with the TKI, reducing its efficacy and leading to treatment resistance.(14) Among the various post-TKI mutations, the EGFR Exon 20 T790M has the highest incidence.(1,17)

The EGFR Exon 20 T790M mutation is frequently observed in lung cancer patients who have previously undergone TKI therapy and developed resistance.(19) Interestingly, it tends to occur more often in individuals over the age of 40 and women.(20,21) In accordance, in this study, most subjects with EGFR Exon 20 T790M were in the age of 45-65 years old. Advanced age may be associated with a reduced function of tumor-suppressor genes (22), potentially contributing to the occurrence of the EGFR Exon 20 T790M.

In this study, smoking status, as assessed using the Brinkman index, non-smoker was correlated with the presence of the EGFR Exon 20 T790M. This result is in accordance with previous report, showing that the never smokers with EGFR Exon 20 T790M develop lung cancer more frequently than ever smokers. In addition, EGFR Exon 20 T790M was found more in female gender. (23) Similar data were also found in this study, most subjects with EGFR Exon 20 T790M were not smoking, and more female subjects (n=8) than male subjects (n=5) were detected with EGFR Exon 20 T790M. However, the underlying mechanism behind the higher occurrence of the EGFR Exon 20 T790M in non-smokers is not yet understood.

In this study, family history did not appear to have a significant association with the EGFR Exon 20 T790M, although there is a possibility of the mutation being inherited. (24) Additionally, the specific location of the mutation within the exon did not seem to be correlated. Although the EGFR Exon 20 T790M often coexists with other EGFR mutations, such as Exon 19 deletion and Exon 21 L858R mutation, the pathogenetic mechanism underlying this coexistence is still not fully understood.(25,26)

In this study, there was a significant difference in the survival rate of subjects with the EGFR Exon 20 T790M (10.77 months) than the one of subject without the mutation (4.78 months). These results were influenced by the Osimertinib treatment, since data of 1-year survival status showed that among 5 subjects treated with Osimertinib, 3 of them were survive (Table 5) with long survival time (Figure 1). These findings are consistent with similar studies which reported longer overall survival in subjects with EGFR Exon 20 T790M mutations who received EGFR-TKI treatment compared to those without the mutation and without EGFR-TKI treatment.(27)

There are some limitations of the current study. To overcome these limitations and gain a deeper understanding of the clinical outcomes and prognosis associated with the EGFR Exon 20 T790M, a multi-center study with a larger sample size is recommended. Such a study should also consider the type and duration of treatment provided, enabling a more robust analysis of the impact of the EGFR Exon 20 T790M on patient outcomes.

Table 4. One-year survival status related to EGFR Exon 20 T790M.

One-year Survival Status	EGFR Exon 20 T790M [n (%)]		*p*-value[#]
	(+) (n=13)	(-) (n=9)	
Survivor	3 (13.6)	-	0.121
Non-survivor	10 (45.5)	9 (40.9)	

[#]Chi-Square test.

Table 5. One-year survival status related to EGFR Exon 20 T790M with Osimertinib treatment.

One-year Survival Status	Osimertinib Therapy [n (%)]		*p*-value[#]
	(+) (n=5)	(-) (n=8)	
Survivor	3 (23.1)	-	0.001*
Non-survivor	2 (15.4)	8 (61.5)	

[#]Chi-Square test, *$p<0.05$.

Conclusion

Since treatment of Osimertinib demonstrated a noteworthy survival rate among NSCLC subjects with EGFR Exon 20 T790M, thus Osimertinib could be suggested as a potential targeted therapy for NSCLC subjects with EGFR Exon 20 T790M.

Authors Contribution

NZZ involved in concepting and planning the research. NZZ, HI, NAT, AS, NL, and HAP performed the data acquisition data analysis. FS aided in interpreting the results. NZZ and FS drafted and revised the final the manuscript. All authors took parts in giving critical revision of the manuscript.

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Character count:	18,570
Submission date:	17-Jan-2024 08:47AM (UTC+0700)
Submission ID:	2272240425

The Indonesian Biomedical Journal, Vol.15, No.5, October 2023, p.296-350 Print ISSN: 2085-3297, Online ISSN: 2355-9179

RESEARCH ARTICLE

Osimertinib as a Potential Targeted Therapy for Non-Small Cell Lung Carcinoma (NSCLC) Patients with EGFR Exon 20 T790M

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Received date: May 29, 2023; Revised date: Oct 1, 2023; Accepted date: Oct 9, 2023

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KEYWORDS: non-small cell lung carcinoma, EGFR, Exon 20, T790M, osimertinib

Indones Biomed J. 2023; 15(5): 336-40

InaBJ V15N5A9 - Osimertinib as a Potential Targeted Therapy for Non-Small Cell Lung Carcinoma (NSCLC) Patients with EGFR Exon 20 T790M

by Ferry Sandra

Submission date: 17-Jan-2024 08:47AM (UTC+0700)
Submission ID: 2272240425
File name: V15N5A9.pdf (1.26M)
Word count: 3538
Character count: 18570

The Indonesian Biomedical Journal, Vol.15, No.5, October 2023, p.296-350 Print ISSN: 2085-3297, Online ISSN: 2355-9179

RESEARCH ARTICLE

Osimertinib as a Potential Targeted Therapy for Non-Small Cell Lung Carcinoma (NSCLC) Patients with EGFR Exon 20 T790M

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Received date: May 29, 2023; Revised date: Oct 1, 2023; Accepted date: Oct 9, 2023

Abstract

BACKGROUND: Emergence of drug resistance due to epidermal growth factor receptor (EGFR) Exon 20 T790M poses a challenge in the effective management of non-small cell lung carcinoma (NSCLC). Significant breakthrough in the management of NSCLC with a specific genetic alteration causes substantial condition improvement in patients whose cancer progressed after first-generation tyrosine kinase inhibitor treatment and who developed tumors with EGFR Exon 20 T790M mutation. The present study analyzed a cohort of NSCLC patients and investigated the incidence of the EGFR Exon 20 T790M status with Osimertinib therapy, along with its impact on survival rates.

METHODS: This was a retrospective cohort study on 22 NSCLC subjects who were genetically examined for EGFR status from plasmic cell free total nucleic acid. Subjects with EGFR Exon 20 T790M mutation were treated with/without Osimertinib. Demographic and clinical data were descriptively summarized, and the differences of each variable and correlation between survival rate and EGFR Exon 20 T790M were analyzed.

RESULTS: Subjects with (n=13) and without (n=9) EGFR Exon 20 T790M had survival rates of 10.77±2.45 and 4.78±1.48, respectively (p=0.000). Based on 1-year survival status of subjects with EGFR Exon 20 T790M, there were 3 Osimertinib-treated survivors and 2 Osimertinib-treated non-survivors. Eight subjects with EGFR Exon 20 T790M and without Osimertinib treatment did not survive (p=0.001).

CONCLUSION: Since the treatment of Osimertinib demonstrated a noteworthy survival rate among NSCLC subjects with EGFR Exon 20 T790M, thus Osimertinib could be suggested as a potential targeted therapy for NSCLC subjects with EGFR Exon 20 T790M.

KEYWORDS: non-small cell lung carcinoma, EGFR, Exon 20, T790M, osimertinib

Indones Biomed J. 2023; 15(5): 336-40

Introduction

The epidermal growth factor receptor (EGFR) mutation is an emerging and promising treatment target for non-small cell lung carcinoma (NSCLC).(1) This mutation is particularly prevalent in South-East Asian NSCLC patients, which is found in more than half of all NSCLC patients. Meanwhile it is also found in 10-20% of Caucasian patients.(2) NSCLC-associated EGFR mutation exhibits a higher incidence in women, non-smokers, and those with adenocarcinoma histology.(3) Fortunately, clinical outcomes for NSCLC patients have significantly improved since the introduction of tyrosine kinase inhibitors (TKIs) targeting EGFR.(4)

The initial generation of TKIs has shown remarkable results in prolonging patients' progression-free survival (PFS).(5,6) However, impact on overall survival (OS) may be limited due to disease progression. Recent findings have shed light on the differences in treatment response between the two most common types of EGFR mutations. Patients with Exon 19 deletion tend to have longer PFS on TKI treatment and show more extended OS compared to those with Exon 21 L858R point mutation.(7,8) Although there is an evidence suggesting that particular EGFR mutation had higher affinity with TKI (9), the true mechanism is still debated.

Despite the initial benefits observed with TKI therapy, a majority of NSCLC patients with active EGFR mutations develop resistance during treatment.(1) Since there has been a significant breakthrough in the management of NSCLC with a specific genetic alteration (10-12), there was substantial condition improvement in patients whose cancer progressed after first-generation TKI treatment and who developed tumors with EGFR Exon 20 T790M mutation.(13) The T790M mutation in NSCLC play role in reducing adenosine triphosphate (ATP)-competitive-kinase-inhibitor, so that the mutation can prevent the effect of inhibitor, thus EGFR-mediated signalling will not be defected.(14)

Based on above reasons, the present study was conducted to analyze a cohort of NSCLC patients and to investigate the incidence of the EGFR Exon 20 T790M status with Osimertinib therapy, along with its impact on survival rates. Understanding these factors will provide valuable insights in aiding the development of effective treatment strategies for NSCLC patients with EGFR mutations.

Methods

Subject and Data Collection

A retrospective cohort study was conducted on 22 NSCLC subjects within October 2019 and July 2022 in Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia, and its network centers. This study was approved by the Research Ethics Commission of the Faculty of Medicine, Universitas Hasanuddin (Approval No.: 270/UN4.6.4.5.31/PP36/2022). Demographic and clinical data including age, gender, body mass index, smoking status, Brinkman Index and family history were collected from subjects. Brinkman Index was calculated by multiplying average-cigarette-consumed-everyday with years-of-smoking, mild <200, moderate 200-600, severe >600.

EGFR Genetical Examination

All NSCLC subjects were genetically examined for EGFR status. From each NSCLC subject, 10 mL blood was collected. Blood sample was processed to collect plasmic cell free total nucleic acid (cfTNA) using MagMAX cell free total nucleic acid isolation kit (ThermoFisher, Waltham, MA, USA). DNA library was prepared Oncomine Lung cfDNA Assay (ThermoFisher). Then the sequencing was performed with Ion Torrent next-generation sequencing (NGS) (ThermoFisher). The NGS data was analyzed and interpreted with Ion Reporter and Oncomine Reporter.

Osimertinib Treatment and Survival Status

All NSCLC subjects were treated with Carboplatin and Paclitaxel in a cycle of 6-treatments and 21-days-interval. In the 6th month, EGFR genetical examinations were performed. Among the subjects with EGFR Exon 20 T790M, some received 80 mg Osimertinib for 1 dosage/daily (Tagrrisso, AstraZeneca, Cambridge, UK), while others continued with the second cycle of Carboplatin and Paclitaxel treatment. The subjects were observed until they deceased. The subject survival status was recorded for further analysis.

Statistical Analysis

Demographic and clinical data were descriptively summarized and statistically analyzed using Chi-Square test. Correlation between survival rate and EGFR Exon 20 T790M was analyzed with Log Rank (Mantel-Cox) test. One-year survival status and EGFR mutation in different exons were statistically analyzed using Chi-Square test as well. Significant values were determined at $p<0.05$. All

statistical analyses were performed using the Statistical Program for Social Sciences (SPSS) version 24.0 (IBM Corporation, Armonk, NY, USA).

Results

Based on the subjects' characteristic, there were 12 male and 10 female subjects with majority age of 45-65 years, non-smoking (Brinkman Index), and no family history of lung cancer (Table 1). Meanwhile, based on the EGFR mutation status, there were subjects with mutation of EGFR Exon 20 T790M and mutation of EGFR Exon 19 deletion (n=5) or EGFR Exon 21 L858R (n=8) (Table 2). There were also subjects without mutation of EGFR Exon 20 T790M but having mutation of EGFR Exon 19 deletion (n=5) or EGFR Exon 21 L858R (n=4).

Study subjects with (n=13) and without (n=9) EGFR Exon 20 T790M had a significnat mean difference of 7.77 months with *p*-value of 0.000 (Table 3). As shown in Kaplan-Meier survival curve (Figure 1), subjects without EGFR Exon 20 T790M (blue line) had survival time <10 months, while subjects with EGFR Exon 20 T790M (red line) had survival time >10 months with 3 positive censored tick marks. Based on subjetcs' 1-year survival status, there were more non-survivor with EGFR Exon 20 T790M (n=10) compared with the survivor (n=3), while all subjects without EGFR Exon 20 T790M did not survive (Table 4).

Among the subjects with EGFR Exon 20 T790M, there were subjects treated with (n=5) and without (n=8) Osimertinib. Based on 1-year survival status of subjects with EGFR Exon 20 T790M, there were 3 Osimertinib-treated survivors and 2 Osimertinib-treated non-survivors (Table 5). However, without any Osimertinib treatment, NSCLC subjects with EGFR Exon 20 T790M did not survive.

Table 1. The subject's characteristic based on EGFR Exon 20 T790M.

Variable	EGFR Exon 20 T790M [n (%)]		*p*- value[#]
	(+) (n=13)	(-) (n=9)	
Age			
<45 years old	-	2 (9.1)	0.204
45-65 years old	11 (50.0)	6 (27.3)	
>65 years old	2 (9.1)	1 (4.5)	
Gender			
Male	5 (22.7)	7 (31.8)	0.069
Female	8 (36.4)	2 (9.1)	
Body mass index			
Underweight	7 (31.8)	6 (27.3)	0.548
Normal	6 (27.3)	3 (13.6)	
Overweight	-	-	
Brinkman Index			
Non-smokers	7 (31.8)	2 (9.1)	0.003*
Mild	-	-	
Moderate	5 (22.7)	-	
Severe	1 (4.5)	7 (31.8)	
Family history			
Yes	4 (18.2)	2 (9.1)	0.658
No	9 (40.9)	7 (31.8)	

[#]Chi-Square test, *p<0.05.

Discussion

Lung cancer is a complex disease that often involves genetic alterations affecting the EGFR tyrosine kinase

Table 2. Correlation of EGFR Exon 20 T790M with other EGFR mutations.

Other EGFR Mutation	EGFR Exon 20 T790M [n (%)]		*p*- value[#]
	(+) (n=13)	(-) (n=9)	
EGFR Exon 19 deletion	5 (22.7)	5 (22.7)	0.429
EGFR Exon 21 L858R	8 (36.4)	4 (18.2)	

[#]Chi-Square test.

Table 3. Survival rate related to EGFR Exon 20 T790M.

Group	Survival Rate (Month) [Mean±SD]	Mean Difference	*p*- value[#]
EGFR Exon 20 T790M (+) (n=13)	10.77±2.45	7.77	0.000*
EGFR Exon 20 T790M (-) (n=9)	4.78±1.48		

[#]Log Rank (Mantel-Cox), *p<0.05.

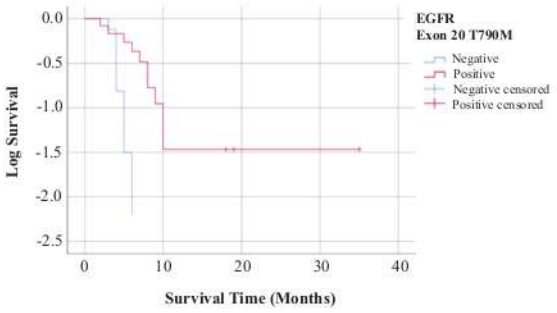

Figure 1. Kaplan-Meier survival curve of subjects with/without EGFR Exon 20 T790M.

signaling pathways, with approximately 75% of examined cases showing such changes.(15) Among these pathways, EGFR pathway is of particular interest with the most common mutations are in-frame deletions (85%–90%) of exon 19 deletion (45%–50%) and Exon 21 L858R mutation (40%–45%).(16) While targeted therapies like TKIs have shown promise in inhibiting tumor growth, the emergence of the EGFR Exon 20 T790M mutation has been identified as a common mechanism of resistance to these therapies. (17,18) The EGFR Exon 20 T790M competes with the TKI, reducing its efficacy and leading to treatment resistance.(14) Among the various post-TKI mutations, the EGFR Exon 20 T790M has the highest incidence.(1,17)

The EGFR Exon 20 T790M mutation is frequently observed in lung cancer patients who have previously undergone TKI therapy and developed resistance.(19) Interestingly, it tends to occur more often in individuals over the age of 40 and women.(20,21) In accordance, in this study, most subjects with EGFR Exon 20 T790M were in the age of 45-65 years old. Advanced age may be associated with a reduced function of tumor-suppressor genes (22), potentially contributing to the occurrence of the EGFR Exon 20 T790M.

In this study, smoking status, as assessed using the Brinkman index, non-smoker was correlated with the presence of the EGFR Exon 20 T790M. This result is in accordance with previous report, showing that the never smokers with EGFR Exon 20 T790M develop lung cancer more frequently than ever smokers. In addition, EGFR Exon 20 T790M was found more in female gender. (23) Similar data were also found in this study, most subjects with EGFR Exon 20 T790M were not smoking, and more female subjects (n=8) than male subjects (n=5) were detected with EGFR Exon 20 T790M. However, the underlying mechanism behind the higher occurrence of the EGFR Exon 20 T790M in non-smokers is not yet understood.

In this study, family history did not appear to have a significant association with the EGFR Exon 20 T790M, although there is a possibility of the mutation being inherited. (24) Additionally, the specific location of the mutation within the exon did not seem to be correlated. Although the EGFR Exon 20 T790M often coexists with other EGFR mutations, such as Exon 19 deletion and Exon 21 L858R mutation, the pathogenetic mechanism underlying this coexistence is still not fully understood.(25,26)

In this study, there was a significant difference in the survival rate of subjects with the EGFR Exon 20 T790M (10.77 months) than the one of subject without the mutation (4.78 months). These results were influenced by the Osimertinib treatment, since data of 1-year survival status showed that among 5 subjects treated with Osimertinib, 3 of them were survive (Table 5) with long survival time (Figure 1). These findings are consistent with similar studies which reported longer overall survival in subjects with EGFR Exon 20 T790M mutations who received EGFR-TKI treatment compared to those without the mutation and without EGFR-TKI treatment.(27)

There are some limitations of the current study. To overcome these limitations and gain a deeper understanding of the clinical outcomes and prognosis associated with the EGFR Exon 20 T790M, a multi-center study with a larger sample size is recommended. Such a study should also consider the type and duration of treatment provided, enabling a more robust analysis of the impact of the EGFR Exon 20 T790M on patient outcomes.

Table 4. One-year survival status related to EGFR Exon 20 T790M.

One-year Survival Status	EGFR Exon 20 T790M [n (%)]		*p*-value[#]
	(+) (n=13)	(-) (n=9)	
Survivor	3 (13.6)	-	0.121
Non-survivor	10 (45.5)	9 (40.9)	

[#]Chi-Square test.

Table 5. One-year survival status related to EGFR Exon 20 T790M with Osimertinib treatment.

One-year Survival Status	Osimertinib Therapy [n (%)]		*p*- value[#]
	(+) (n=5)	(-) (n=8)	
Survivor	3 (23.1)	-	0.001*
Non-survivor	2 (15.4)	8 (61.5)	

[#]Chi-Square test, *$p<0.05$.

Conclusion

Since treatment of Osimertinib demonstrated a noteworthy survival rate among NSCLC subjects with EGFR Exon 20 T790M, thus Osimertinib could be suggested as a potential targeted therapy for NSCLC subjects with EGFR Exon 20 T790M.

Authors Contribution

NZZ involved in concepting and planning the research. NZZ, HI, NAT, AS, NL, and HAP performed the data acquisition data analysis. FS aided in interpreting the results. NZZ and FS drafted and revised the final the manuscript. All authors took parts in giving critical revision of the manuscript.

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Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**T790M mutation on Non-Small Cell Lung Carcinoma (NSCLC): A Retrospective Cohort Study**".

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2.	Are the title and abstract of the manuscript appropriate?	Yes	
	Notes:		
3	Do the title and abstract reflect the study result/content?		NO
	Conclusion over estimate with result,		
4.	Is the significance of the study well explained at the Background?	Yes	
	Notes:		
5.	Are the research study methods technically correct, accurate, and complete enough to be reproduced/cited by other scientists?		No
	Notes: 1. need to be informed in greater detail about the molecular method that was used, how sensitively the mutation can be detected, and this information is very important in assessing how sensitively the method used distinguishes whether positive or negative results were obtained. 2. A very limited number of samples are available for analysis in order to determine		

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	whether or not the effects of therapy have an impact on patient survival. We suggest add more sample to sufficient conclude the results.		
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	Notes:		
7.	Are all figures and tables necessarily presented?		No
	Notes: 1. table number 4 not clear. Are exon 19 & 21 positive or negative no information. 2. Over estimate survival time 19,88 month (this study only 9 months)		
8.	Is there a logical flow of argument in the Discussion which elucidate all the presented/obtained data?	Yes	
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Reviewer 1

T790M mutation on Non-Small Cell Lung Carcinoma (NSCLC): A Retrospective Cohort Study

Abstract

Background: Mutations in the epidermal growth factor receptor (EGFR) tyrosine kinase receptor have become a significant treatment target in non-small cell lung carcinoma (NSCLC). However, the emergence of resistance due to T790M mutations poses a challenge in the effective management of this disease. ~~While much~~Though some information remains unknown, various studies have demonstrated that patients ~~positive for with~~the T790M-positive mutation exhibit improved progression-free survival (PFS), overall survival (OS), and better response to tyrosine kinase inhibitors (TKIs) treatment compared to T790M-negative patients. This ~~to~~ study aimed to describe the effects of T790M mutation status in lung cancer patients.

Methods: This was a~~A~~ retrospective cohort study on 22 NSCLC patients ~~aimed~~ to establish correlations by collecting medical record data from patients who were examined for the T790M mutation from blood circulating DNA (ctDNA) and analyzed by polymerase chain reaction (PCR) between October 2019 and July 2022 in XXX Hospital and its network hospital. Baseline data were descriptively summarized, and the differences in each variable between groups were calculated as well as the survival rate of osimertinib therapy and T790M mutation status.

Results: Our study found patients with the T790M mutation exhibited mean survival time of 19.88 months, compared to those without the mutation ($p = 0.005$) and significant association between patients harboring the T790M mutation and receiving osimertinib therapy ($p = 0.001$).

Conclusion: T790M positive mutation and osimertinib therapy demonstrated a noteworthy survival rate.

Comment [i1]: Title has to be revised to directly reflect the main findings of the study. A suggestion for title: "Non-Small Cell Lung Carcinoma (NSCLC) Subjects with T790M Positive Mutation Shows Better Survival Rate".

Comment [i2]: Methods section needs to be majorly revised, many information necessary to be described. So after the revision of the Methods section, please also revise the Methods in Abstract.

26 **Keywords**: non-small cell lung carcinoma, epidermal growth factor, T790M.

27

Introduction

Emerging as a promising treatment target for non-small cell lung carcinoma (NSCLC) is the mutation of epidermal growth factor receptor (EGFR).[1,2] This mutation is particularly prevalent in Asian populations, present in up to half of NSCLC patients, and is found in about 10% of Western populations.[3] NSCLC, associated with this molecular subtype of EGFR mutation, exhibits a higher incidence in women, non-smokers, and those with adenocarcinoma histology.[4,5] Fortunately, the clinical outcomes for NSCLC patients have significantly improved since the introduction of tyrosine kinase inhibitors (TKIs) targeting EGFR.[6,7]

The initial generation of TKIs has shown remarkable results in prolonging patients' progression-free survival (PFS).[8,9] However, the impact on overall survival (OS) may be limited due to disease progression.[10] Recent findings have shed light on the differences in treatment response between the two most common types of EGFR mutations. Patients with exon 19 deletions tend to have a longer PFS on TKI treatment and demonstrate a more extended OS compared to patients with the TKI point mutation 21L858R.[11] Although, there is evidence the mechanism involved.[12]

Despite the initial benefits observed with TKI therapy, a majority of NSCLC patients with active EGFR mutations develop resistance during treatment.[2] However, there has been a significant breakthrough in the management of metastatic NSCLC with a specific genetic alteration.[13–16] There was substantial improvement in their condition for patients whose cancer progressed after first-generation TKI treatment and who developed tumors with the T790M mutation.[17]

The aim of this study is to analyze a cohort of NSCLC patients and investigate the incidence of the T790M mutation status with osimertinib therapy, along with its impact on survival rates. Understanding these factors will provide valuable insights for pulmonology

and thoracic oncology experts, aiding in the development of effective treatment strategies for NSCLC patients with EGFR mutations.

Methods

Subject's and data collections

A retrospective cohort study was taken on 22 NSCLC patients with T790M mutation test (with targeted therapy EGFR-TKI and without) patients in XXX Hospital and its network, XXX, XXX, XXX. This study was approved by the Research Ethics Commission of XXX (No: XXX). Demographic and clinical data were collected by collecting medical record data from patients who were examined for the T790M mutation from blood circulating DNA (ctDNA) and analyzed and sequenced by polymerase chain reaction (PCR) between October 2019 and July 2022 in Clinical Laboratory of XXX Hospital.

Statistical analysis

Baseline data were descriptively summarized, and the differences in each variable between age, gender, body mass index, smoking status, family history, exon location, survival in one-year with T790M mutation were calculated using Chi-Square tests. Correlation between SR and T790M mutation status were analyzed by Log Rank (Mantel-Cox). Significant values were determined at $p<0.05$. The patient's SR were analyzed. Age, gender, and smoking status are also analyzed as control variables. All statistical analyses were performed using the Statistical Program for Social Sciences (SPSS) version 24.0 (IBM ~~SPSS 24, IL~~Corporation, Armonk, NY, USA).

Results

Based on the study findings, the majority of patients included in the analysis were males aged between 45 and 65 years, who were underweight, smokers, and did not have a family history of lung cancer (Table 1). Our study found that patients with the T790M

Comment [i3]: In the Methods section, there is no information about osimertinib therapy. Did all subjects receive it? If not all subjects, then provide information which and how many subject has received it.

Comment [i4]: Eventhough this is a retrospective study, however author has to be able to provide clear information about how to collect the blood serum and what methods used for T790 mutation test.

Please also describe how to classify the subjects as T790-positive and T790-negative.

Comment [i5]: Demographic and clinical data including…? Add the parameters here.

Comment [i6]: Need to be briefly explained in the separate sub-section. Any use of tools, kit, and materials should also be mentioned completed with the information of the manufacturer company name, city, and country of location.

Comment [i7]: Does SR stands for survival rates? It has not been mentioned before, hence it has to be defined. And you have to be consistent whether to use the term 'overall survival' or 'survival rate' for this.

Comment [i8]: Use the term 'subjects' instead of 'patients' in the Results section.

mutation exhibited mean survival time of 19.88 months, compared to those without the mutation. The presence of the T790M mutation was found to be correlated with survival rate (Table 2 & Figure 1), a. Although there was~~are~~ no correlation between T790M mutation with one-year survival rate and exon location (Table 3 & Table 4). Our study identified a significant association between patients ~~harboring~~ with the T790M mutation and receiving osimertinib therapy (p = 0.001). Significant association was observed between the administration of osimertinib therapy and improved one-year survival rates in these patients (Table 5).

Table 1. Patient's characteristic and T790M mutation

Variable	T790M mutation N(%)		Total	p-value
	Positive (+)	Negative (-)		
Age				
<45 years old	0 (0.0)	2 (9.1)	2 (9.1)	
45-65 years old	11 (50.0)	6 (27.2)	17 (77.3)	0.204
>65 years old	2 (9.1)	1 (4.6)	3 (13.6)	
Gender				
Male	5 (22.7)	7 (31.8)	12 (54.5)	0.069
Female	8 (36.4)	2 (9.1)	10 (45.5)	
Body mass indexe				
Underweight	7 (31.8)	6 (27.3)	13 (59.1)	
Normal	6 (27.3)	3(13.6)	9 (40.9)	0.548
Overweight	0 (0.0)	0 (0.0)	0 (0.0)	
Smoking status				
Yes	6 (27.3)	7 (31.8)	13 (59.1)	0.170
No	7 (31.8)	2 (9.1)	9 (40.9)	
Brinkman Index				
Non-smokers	7 (31,8)	2 (9,1)	9 (40.9)	
Moderate	5 (22.7)	0 (0.0)	5 (22.7)	0.003*
Severe	1 (4.6)	7 (31.8)	8 (36.4)	
Family history				
Yes	4 (18.2)	2 (9.1)	6 (27.3)	0.658
No	9 (40.9)	7 (31.8)	16 (72.7)	

Chi-Square test, *significant if p<0.05.

Table 2. Survival rate related to T790M mutation status

Group	Survival rate (months)±SD	Mean difference	p-value
T790M (+)	19.88±3.36	9.81	0.005*
T790M (-)	5.74±0.49		

—Log Rank (Mantel-Cox), SD standard of deviation, MD mean difference, *significant if p<0.05.

Comment [i9]: n for each should be mentioned.

Comment [i10]: Has to be mentioned in the Methods before along with other parameter. And for Brinkman Index should be briefly explained what this index referring to and how to classify it.

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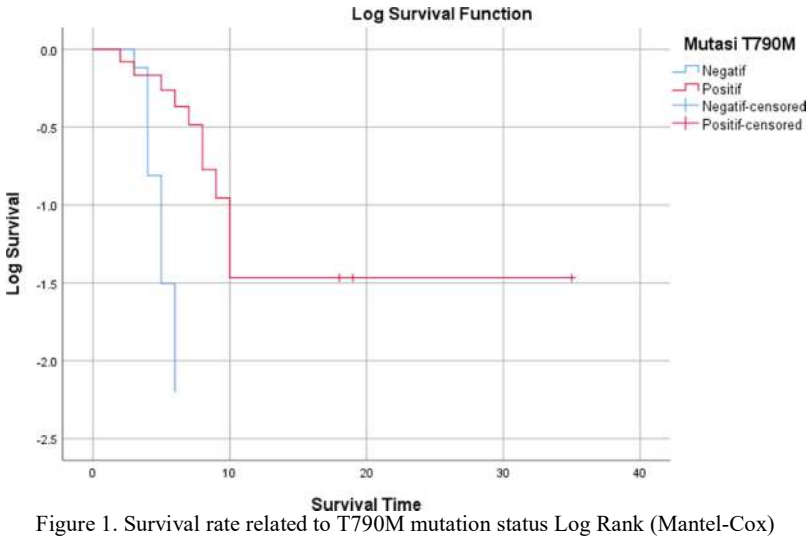

Figure 1. Survival rate related to T790M mutation status Log Rank (Mantel-Cox)

Table 3. One-year survival rate in T790M mutation

Survival status	T790M mutation N(%)		Total	p-value
	Positive (+)	Negative (-)		
Survivor	3 (10.5)	0 (0.0)	3 (13.6)	0.121
Non-survivor	10 (42.1)	9 (47.4	19 (86.4)	

Chi-square, *significant if p<0.05.

Table 4. Exon-related T790M mutation

Exon	T790M mutation N(%)		Total	p-value
	Positive (+)	Negative (-)		
Exon 19	5 (22.7)	5 (22.7)	10 (45.5)	0.429
Exon 21	8 (36.3)	4 (18.2)	12 (54.5)	

Chi-square, *significant if p<0.05.

Comment [i11]: Table 3 and Table 4 can actually be combined/merged into one table.

Table 5. One-year survival rate in NSCLC patients with and without T790M mutation with osimertinib therapy

Mutation of T790M status	Survival status	Therapy		Total	p-value
		Osimertinib	Without osimertinib		
Positive (+)	Survivor	3 (13.6)	0 (0.0)	3 (13.6)	0.001*
	Non-survivor	2 (9.1)	8 (36.6)	10 (45.5)	
Negative (-)	Survivor	0 (0.0)	0 (0.0)	0 (0.0)	
	Non-survivor	0 (0.0)	9 (40.9)	9 (40.9)	

Chi-square, *significant

Discussion

Lung cancer is a complex disease that often involves genetic alterations affecting the tyrosine kinase signaling pathways, with approximately 75% of examined cases showing such changes.[18] Among these pathways, the epidermal growth factor receptor (EGFR) pathway is of particular interest, with around 65% of lung cancer in tyrosine kinase pathways cases exhibiting EGFR mutations.[19] While targeted therapies like tyrosine kinase inhibitors

(TKIs) have shown promise in inhibiting tumor growth, the emergence of the T790M mutation has been identified as a common mechanism of resistance to these therapies.[20,21] The T790M mutation competes with the TKI, reducing its efficacy and leading to treatment resistance. Among the various post-TKI mutations, the T790M mutation has the highest incidence.[22]

The T790M mutation is frequently observed in lung cancer patients who have previously undergone TKI therapy and developed resistance.[23] Interestingly, it tends to occur more often in individuals over the age of 40 and men. Advanced age may be associated with a reduced ability of genes to suppress oncogenes, potentially contributing to the occurrence of the T790M mutation. Additionally, epidemiological data suggest that men are more likely to have familial lung cancer. However, the exact reasons for this gender disparity remain unclear and warrant further investigation.[24]

Smoking status, as assessed using the Brinkman index, has been correlated with the presence of the T790M mutation. Prolonged smoking can trigger changes in cellular differentiation patterns, which may influence the development of this mutation.[25] However, the underlying mechanism behind the higher occurrence of the T790M mutation in non-smokers is not yet understood.[26] Furthermore, body mass index (BMI) has not shown a relationship with the T790M mutation.[27] On the other hand, family history does not appear to have a significant association with the T790M mutation, although there is a possibility of the mutation being inherited.[28,29] Additionally, the specific location of the mutation within the exon does not seem to be correlated. Although the T790M mutation often coexists with other EGFR mutations, such as exon 19 deletion and exon 21 L858R mutation, the pathogenetic mechanism underlying this coexistence is still not fully understood.[30,31]

Comment [i12]: In your study results, there is significant correlation of Brinkman Index and the T790 mutation, please add more discussion about the mechanism.

Reference No.26 was published on 2018, you should look for more recent literatures to provide information about this.

The study's results highlight a significant difference in the one-year survival rates of lung cancer patients with the T790M mutation who received osimertinib compared to those

without the mutation. Patients undergoing EGFR-TKI therapy exhibited an average survival time of 19.88 months, with a mean difference of 9.81 months compared to patients without the T790M mutation, who had an average survival time of 5.74 months. These findings are consistent with similar studies which reported longer overall survival in patients with T790M mutations who received EGFR-TKI therapy compared to those without the mutation.[32,33] Importantly, these improvements in survival have been observed across different age groups and regardless of previous therapies received.[34,35]

Comment [i13]: Why? Explain a little about the possible correlation that allow subjects with EGFR-TKI therapy to have better OS in subjects with T790 mutation.

However, it is important to acknowledge the limitations of the current study. One significant limitation is the need for more comprehensive data on the specific type of EGFR-TKI administered to each patient. To overcome these limitations and gain a deeper understanding of the clinical outcomes and prognosis associated with the T790M mutation, a multi-center study with a larger sample size is recommended. Such a study should also consider the type and duration of treatment provided, enabling a more robust analysis of the impact of the T790M mutation on patient outcomes. By addressing these limitations, researchers can further enhance our knowledge of the T790M mutation and its implications for the management of lung cancer. Further research is necessary~~warranted~~ to explore optimal treatment strategies and improve patient outcomes in this subgroup.

Conclusion

T790M positive mutation and osimertinib therapy demonstrated a noteworthy survival rate. These findings contribute to the potential benefits of targeted therapies for NSCLC patients harboring T790M mutation. ~~Further research is warranted to explore optimal treatment strategies and improve patient outcomes in this subgroup.~~

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Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2023140 Editor Decision Round 1 - Resubmit for Review

Ferry Sandra <ferry@trisakti.ac.id> Mon, Jul 24, 2023 at 7:45 PM
To: Secretariat of InaBJ <secretariatinabj@gmail.com>

Dear Secretariat of The Indonesian Biomedical Journal,

Please find the revision of the manuscript M2023140 that was previously titled "T790M mutation on Non-Small Cell Lung Carcinoma (NSCLC): A Retrospective Cohort Study" as well as the response letter addressing the reviewer's and editor's question. We have revised the manuscript accordingly. Thank you for your kind assistance.

Regards,
Ferry Sandra
[Quoted text hidden]
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Ferry Sandra, D.D.S., Ph.D.
Head of Medical Research Center
Universitas Trisakti

2 attachments

Round 1 Revision from Author.docx
95K

Round 1 Response Form from Author.xlsx
14K

Non-Small Cell Lung Carcinoma (NSCLC Subjects with T790M Positive Mutation Shows Better Survival Rate

Abstract

Background: Mutations in the epidermal growth factor receptor (*EGFR*) tyrosine kinase receptor have become a significant treatment target in non-small cell lung carcinoma (NSCLC). However, the emergence of resistance due to T790M mutations poses a challenge in the effective management of this disease. Though some information remains unknown, various studies have demonstrated that patients with T790M-positive mutation exhibit improved progression-free survival (PFS), overall survival (OS), and better response to tyrosine kinase inhibitors (TKIs) treatment compared to T790M-negative patients. This study aimed to describe the effects of T790M mutation status in lung cancer patients.

Methods: This was a retrospective cohort study on 22 NSCLC subjects to establish correlations by collecting data from subjects who were examined for the T790M mutation from plasma circulating tumor DNA (ctDNA) with next-generation sequencing analysis (NGS) and sequenced by Ion GeneStudio S5 with *EGFR* T790M (ThermoFisher, CA, United States) between October 2019 and July 2022 in Dr. Wahidin Sudirohusodo Hospital and its network hospital. Baseline data were descriptively summarized, and the differences in each variable between groups were calculated as well as the survival rate of osimertinib therapy and T790M mutation status.

Results: Our study found subjects with the T790M mutation exhibited mean survival time of 19.88 months, compared to those without the mutation ($p = 0.005$) and significant association between subjects with the T790M mutation and receiving osimertinib therapy ($p = 0.001$).

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Keywords: non-small cell lung carcinoma, epidermal growth factor, T790M.

Introduction

Epidermal growth factor receptor (*EGFR*) mutation is an emerging and promising treatment target for non-small cell lung carcinoma (NSCLC).[1,2] This mutation is particularly prevalent in Asian populations, present in up to half of NSCLC patients, and is found in about 10% of Western populations.[3] NSCLC, associated with *EGFR* mutation, exhibits a higher incidence in women, non-smokers, and those with adenocarcinoma histology.[4,5] Fortunately, the clinical outcomes for NSCLC patients have significantly improved since the introduction of tyrosine kinase inhibitors (TKIs) targeting EGFR.[6–10]

The initial generation of TKIs has shown remarkable results in prolonging patients' progression-free survival (PFS).[11,12] However, the impact on overall survival (OS) may be limited due to disease progression.[13] Recent findings have shed light on the differences in treatment response between the two most common types of *EGFR* mutations. Patients with exon 19 deletion tend to have a longer PFS on TKI treatment and show a more extended OS compared to those with exon 21 L858R point mutation.[14] Although, there is evidence the mechanism involved in TKIs therapy on *EGFR* mutation is thigter covalent binding but the true mechanism is still debated.[15,16]

Despite the initial benefits observed with TKI therapy, a majority of NSCLC patients with active *EGFR* mutations develop resistance during treatment.[2] However, there has been a significant breakthrough in the management of metastatic NSCLC with a specific genetic alteration.[17–20] There was substantial condition improvement in patients whose cancer progressed after first-generation TKI treatment and who developed tumors with T790M mutation.[21] T790M mutation in NSCLC play role as a genomic stability marker and reduce adenosine triphosphate (ATP)-competitive kinase to prevent EGFR-mediated signalling.[22] This mutation is responsible for better prognosis in NSCLC patients.

The present study was conducted to analyze a cohort of NSCLC patients and investigate the incidence of the T790M mutation status with osimertinib therapy, along with

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Methods

Subject's and data collections

A retrospective cohort study was taken on 22 NSCLC subjects (with osimertinib therapy n=5, without n=17) with T790M mutation test subjects in Dr. Wahidin Sudirohusodo Hospital and its network, Makassar, South Sulawesi, Indonesia. This study was approved by the Research Ethics Commission of of the Faculty of Medicine, Hasanuddin University (No: 270/UN4.6.4.5.31/PP36/2022). Demographic and clinical data (age, gender, body mass index, smoking status, Brinkman Index, family history and exon location) were collected by collecting medical record data from subjects who were examined for the T790M mutation from plasma (6 mL of plasma) circulating tumor DNA (ctDNA) with next-generation sequencing analysis (NGS) and sequenced by Ion GeneStudio S5 with *EGFR* T790M (ThermoFisher, CA, United States) between October 2019 and July 2022 in Prodia Laboratorium and Clinic. The subjects observed until they pass away.

Statistical analysis

Baseline data were descriptively summarized, and the differences in each variable between age, gender, body mass index, smoking status, Brinkman Index, family history, exon location, survival in one-year with T790M mutation were calculated using Chi-Square tests. Correlation between survival rate (SR) and T790M mutation status were analyzed by Log Rank (Mantel-Cox). Significant values were determined at $p<0.05$. The subject's SR were analyzed. Age, gender, and smoking status are also analyzed as control variables. All

74 statistical analyses were performed using the Statistical Program for Social Sciences (SPSS)
75 version 24.0 (IBM Corporation, Armonk, NY, USA).

76 **Results**

77 Based on the study findings, the majority of subjects included in the analysis were
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Smoking status				
Yes	6 (27.3)	7 (31.8)	13 (59.1)	0.170
No	7 (31.8)	2 (9.1)	9 (40.9)	
Brinkman Index				
Non-smokers	7 (31,8)	2 (9,1)	9 (40.9)	
Moderate	5 (22.7)	0 (0.0)	5 (22.7)	0.003*
Severe	1 (4.6)	7 (31.8)	8 (36.4)	
Family history				
Yes	4 (18.2)	2 (9.1)	6 (27.3)	0.658
No	9 (40.9)	7 (31.8)	16 (72.7)	

88 Chi-Square test, *p<0.05, Brinkman index was calculated by multiply average
89 cigar consumed everyday with years of smoking, mild <200, moderate 200-600,
90 severe >600,

Table 2. Survival rate related to T790M mutation status

Group	Survival rate (months)±SD	Mean difference	p-value
T790M (+)	19.88±3.36	9.81	0.005*
T790M (-)	5.74±0.49		

Log Rank (Mantel-Cox), SD standard of deviation, MD mean difference, *p<0.05

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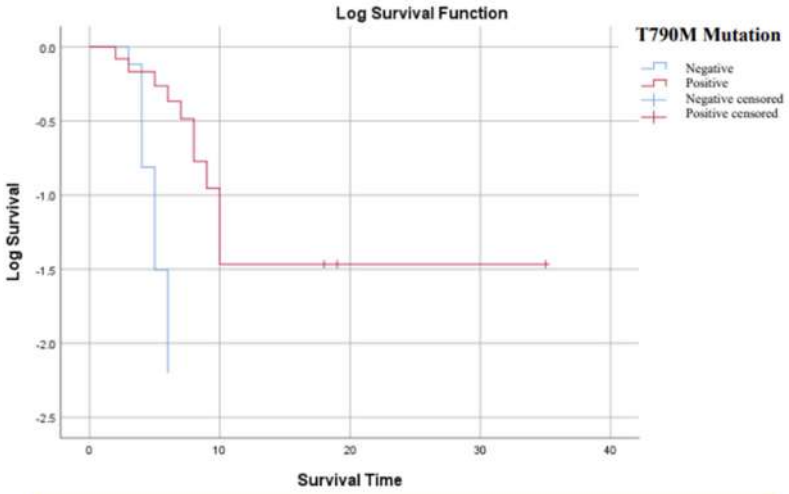

Figure 1. Survival rate related to T790M mutation status Log Rank (Mantel-Cox)

Table 3. One-year survival rate and exon location in T790M mutation

Variable	T790M mutation N(%) Positive (+) (n=13)	Negative (-) (n=9)	Total	p-value
Survival Status				
Survivor	3 (10.5)	0 (0.0)	3 (13.6)	0.121
Non-survivor	10 (42.1)	9 (47.4	19 (86.4)	
Exon location				
Exon 19	5 (22.7)	5 (22.7)	10 (45.5)	0.429
Exon 21	8 (36.3)	4 (18.2)	12 (54.5)	

Chi-square, *p<0.05

Table 4. One-year survival rate in NSCLC subjects with osimertinib therapy

One-year survival rate	Therapy Osimertinib (n=5)	Without osimertinib (n=17)	Total	p-value
Survivor	3 (13.6)	0 (0.0)	3 (13.6)	0.001*
Non-survivor	2 (9.1)	17 (77.3)	10 (86.4)	

Chi-square, *p<0.05

Discussion

Lung cancer is a complex disease that often involves genetic alterations affecting the tyrosine kinase signaling pathways, with approximately 75% of examined cases showing

such changes.[23] Among these pathways, the epidermal growth factor receptor (EGFR) pathway is of particular interest, with around 65% of lung cancer in tyrosine kinase pathways cases exhibiting EGFR mutations.[24] While targeted therapies like tyrosine kinase inhibitors (TKIs) have shown promise in inhibiting tumor growth, the emergence of the T790M mutation has been identified as a common mechanism of resistance to these therapies.[25,26] The T790M mutation competes with the TKI, reducing its efficacy and leading to treatment resistance. Among the various post-TKI mutations, the T790M mutation has the highest incidence.[27]

The T790M mutation is frequently observed in lung cancer patients who have previously undergone TKI therapy and developed resistance.[28] Interestingly, it tends to occur more often in individuals over the age of 40 and men. Advanced age may be associated with a reduced ability of genes to suppress oncogenes, potentially contributing to the occurrence of the T790M mutation. Additionally, epidemiological data suggest that men are more likely to have familial lung cancer. However, the exact reasons for this gender disparity remain unclear and warrant further investigation.[29]

Smoking status, as assessed using the Brinkman index, has been correlated with the presence of the T790M mutation. Prolonged smoking can trigger changes in cellular differentiation patterns, which may influence the development of this mutation.[30] However, the underlying mechanism behind the higher occurrence of the T790M mutation in non-smokers is not yet understood.[31] T790M mutation and smoking are inversely correlated. Non-smoker have higher risk of this mutation. This is correlated with previous study that conclude higher prevalence in non-smoker than higher Brinkman index population.[10] Furthermore, body mass index (BMI) has not shown a relationship with the T790M mutation.[32] On the other hand, family history does not appear to have a significant association with the T790M mutation, although there is a possibility of the mutation being inherited.[33,34]

Additionally, the specific location of the mutation within the exon does not seem to be correlated. Although the T790M mutation often coexists with other EGFR mutations, such as exon 19 deletion and exon 21 L858R mutation, the pathogenetic mechanism underlying this coexistence is still not fully understood.[35,36]

The study's results highlight a significant difference in the one-year survival rates of lung cancer subjects with the T790M mutation who received osimertinib compared to those without the mutation. Subjects undergoing EGFR-TKI therapy exhibited an average survival time of 19.88 months, with a mean difference of 9.81 months compared to subjects without the T790M mutation, who had an average survival time of 5.74 months. These findings are consistent with similar studies which reported longer overall survival in subjects with T790M mutations who received EGFR-TKI therapy compared to those without the mutation.[37,38] Two mechanism that proposed EGFR-TKI affect T790M positive are covalent binding EGFR-TKI therapy with T790M-positive are firmer and EGFR-TKI therapy also reduce ATP-competitive kinase to prevent EGFR-mediated signalling. This two mechanism gave better prognosis to subject T790M mutation.[2,22] Importantly, these improvements in survival have been observed across different age groups and regardless of previous therapies received.[39,40]

However, it is important to acknowledge the limitations of the current study. One significant limitation is the need for more comprehensive data on the specific type of EGFR-TKI administered to each patient. To overcome these limitations and gain a deeper understanding of the clinical outcomes and prognosis associated with the T790M mutation, a multi-center study with a larger sample size is recommended. Such a study should also consider the type and duration of treatment provided, enabling a more robust analysis of the impact of the T790M mutation on patient outcomes. By addressing these limitations, researchers can further enhance our knowledge of the T790M mutation and its implications

for the management of lung cancer. Further research is necessary to explore optimal treatment strategies and improve outcome in this subgroup.

Conclusion

T790M positive mutation demonstrated a noteworthy survival rate among NSCLC subjects. Osimertinib therapy may have potential beneficial effect on T790M mutation NSCLC therapy. These findings contribute to the potential benefits of targeted therapies for NSCLC subjects with T790M mutation.

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Response Form for Reviewer's Comments

Corresponding Author : Ferry Sandra
Manuscript Code : M2023140
Manuscript Title : T790M mutation on Non-Small Cell Lung Carcinoma (NSCLC): A Retrospective Cohort Study

No. (Reviewer and comments number code)	Comments (Comments/question from reviewer or editor)	Author's Response (Please write your response regarding the comment here)	Line Number (Please write the line number of the said revision)
R1 #1	Conclusion in the Abstract section is overclaimed. Please rewrite.	we re-conclude the conclusion	24
R1 #2	Please explain in a more detailed manner how molecular methods (for example: PCR) in this study were carried out. Briefly mention the protocol of each method.	we add the methods	63-7
R1 #3	A very limited number of subjects were included in this study to determine the effects of Osimertinib therapy on patient's survival. More subjects should be recruited to obtain representative results.	this case is very rare, we can't add more subject's	57-8
R1 #4	Do you overestimate the survival time? The survival time 19.88 months, but you only conducted the study for only 9 months.	9 months for sampling, the cohort is keep going in until subjects pass away	67
R1 #5	Only 3 survivors included in this study. More subjects should be recruited to obtain representative results.	this case is very rare, we can't add more subject's	100
R1 #6	Calculation of survival rate related to Osimertinib therapy should be performed only in subjects with positive T790M mutation, because subjects with negative T790M mutation did not receive Osimertinib. Please recalculate the survival rate.	we recalculate the one year survival rate with osimertinib therapy	100
R2 #1	Title has to be revised to directly reflect the main findings of the study. A suggestion for title: "Non-Small Cell Lung Carcinoma (NSCLC) Subjects with T790M Positive Mutation Shows Better Survival Rate".	we admit the new title	1
R2 #2	Methods section needs to be majorly revised, many information necessary to be described. So after the revision of the Methods section, please also revise the Methods in Abstract.	we revised the methods section	57-67
R2 #3	In the Methods section, there is no information about osimertinib therapy. Did all subjects receive it? If not all subjects, then provide information which and how many subject has received it.	not all subjects received osimertinib therapy	58

R2 #4	Even though this is a retrospective study, however author has to be able to provide clear information about how to collect the blood serum and what methods used for T790 mutation test. Please also describe how to classify the subjects as T790-positive and T790-negative.	we revised the methods section	57-67
R2 #5	Demographic and clinical data including…? Add the parameters here.	we add the parameters	61-2
R2 #6	Need to be briefly explained in the separate sub-section. Any use of tools, kit, and materials should also be mentioned completed with the information of the manufacturer company name, city, and country of location.	we add the methods	63-6
R2 #7	Does SR stands for survival rates? It has not been mentioned before, hence it has to be defined. And you have to be consistent whether to use the term 'overall survival' or 'survival rate' for this.		72
R2 #8	Use the term 'subjects' instead of 'patients' in the Results section.		
R2 #9	The total number of subjects (n) with positive (n=13)and negative (n=9) mutation should be mentioned in the table heading.		87, 97
R2 #10	Has to be mentioned in the Methods before along with other parameter. And for Brinkman Index should be briefly explained what this index referring to and how to classify it.		
R2 #11	Table 3 and Table 4 can actually be combined/merged into one table.	we merged the table	
R2 #12	In your study results, there is significant correlation of Brinkman Index and the T790 mutation, please add more discussion about the mechanism. Reference No.26 was published on 2018, you should look for more recent literatures to provide information about this.		
R2 #13	Why? Explain a little about the possible correlation that allow subjects with EGFR-TKI therapy to have better OS in subjects with T790 mutation.	we reexplain the discussion about egfr tki	139-144
SE #1	Incomplete sentence. What mechanism do you refer to in this sentence? Please revise this sentence.	we complete the sentence	39-40
SE #2	Please add more explanations about T790M mutation and its role in NSCLC in the Introduction section.		46-8
SE #3	Some annotations in Figure 1 is still using bahasa Indonesia. Please use English instead.	we remake the diagram	94

SE #4	Please cite the following articles as your references: https://doi.org/10.21705/mcbs.v1i2.10 https://doi.org/10.21705/mcbs.v6i2.246 https://doi.org/10.21705/mcbs.v6i2.232	We add these references	181-8

Note : R1 = Reviewer 1
R2 = Reviewer 2
SE = Section Editor

UNIVERSITAS TRISAKTI

Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2023140 Editor Decision Round 2 - Revisions Required

Secretariat of InaBJ <secretariatinabj@gmail.com> Fri, Aug 11, 2023 at 11:21 AM
To: ferry@trisakti.ac.id

Dear Dr. Ferry Sandra,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**Non-Small Cell Lung Carcinoma (NSCLC) Subjects with T790M Positive Mutation Shows Better Survival Rate**".

Our decision is: **Revisions Required.**

Find the file attached to see detailed comments from reviewers. Please make sure you read all the comments and revise the manuscript based on the suggestions given.

Revise this manuscript thoroughly before **August 25, 2023**. Mark/highlighted the revised part of the manuscript, so that the editor will notice the changes.

When you are done, you can upload it in: https://inabj.org/index.php/ibj/author/submissionReview/2431, or simply send us an email of your revised manuscript and response letter.

Please let us know when you have received this email. If you have any questions, do not hesitate to contact us. Thank you for your attention. We wish you a nice day.

Best Regards,
--
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3 attachments

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Manuscript Review Form

Reviewer	:	Reviewer 1
Manuscript #	:	M2023140
Manuscript Title	:	Non-Small Cell Lung Carcinoma (NSCLC) Subjects with T790M Positive Mutation Shows Better Survival Rate

No.	Manuscript Components	Yes	No
1.	Does this manuscript present new ideas or results that have not been previously published?		
	Notes:		
2.	Are the title and abstract of the manuscript appropriate?		
	Notes:		
3	Do the title and abstract reflect the study result/content?		
4.	Is the significance of the study well explained at the Background?		
	Notes:		
5.	Are the research study methods technically correct, accurate, and complete enough to be reproduced/cited by other scientists?		
6.	Are the results, ideas, and data presented in this manuscript important enough for publication?		
	Notes:		

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7.	Are all figures and tables necessarily presented?		
	Notes:		
8.	Is there a logical flow of argument in the Discussion which elucidate all the presented/obtained data?		
	Notes:		
9.	Are the conclusions and interpretations valid and supported by the data?		
10.	Is the manuscript clear, comprehensible, and written in a good English structure?		
	Notes:		

Specific Reviewer's Comments and Suggestions:

(These comments may be in addition to or in lieu of reviewer comments inserted into the text of the manuscript. Use as many lines as needed.)

All corrections and clarifications have been accepted.

Reviewer's Recommendation (Please tick only one option)	√
Accept Submission (No significant alterations suggested)	√
Revisions Required (Suggest changes to the manuscript as specified in this review)	
Resubmit for Review (Major revisions should be made and suggestions as specified in this review must be addressed. Revised manuscript should be resubmitted to the reviewer for further review)	
Decline Submission (Do not encourage a rewrite, manuscript is totally rejected)	

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Further Reviewer's Comments Regarding Disposition of the Manuscript:
All corrections and clarifications have been accepted.

Date and Sign:
August 11, 2023

Reviewer 1

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Manuscript Review Form

Reviewer	:	Reviewer 2
Manuscript #	:	M2023140
Manuscript Title	:	Non-Small Cell Lung Carcinoma (NSCLC) Subjects with T790M Positive Mutation Shows Better Survival Rate

No.	Manuscript Components	Yes	No
1.	Does this manuscript present new ideas or results that have not been previously published?		
	Notes:		
2.	Are the title and abstract of the manuscript appropriate?		
	Notes:		
3	Do the title and abstract reflect the study result/content?		
4.	Is the significance of the study well explained at the Background?		
	Notes:		
5.	Are the research study methods technically correct, accurate, and complete enough to be reproduced/cited by other scientists?		
6.	Are the results, ideas, and data presented in this manuscript important enough for publication?		
	Notes:		

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7.	Are all figures and tables necessarily presented?		
	Notes:		
8.	Is there a logical flow of argument in the Discussion which elucidate all the presented/obtained data?		
	Notes:		
9.	Are the conclusions and interpretations valid and supported by the data?		
10.	Is the manuscript clear, comprehensible, and written in a good English structure?		
	Notes:		

Specific Reviewer's Comments and Suggestions:

(These comments may be in addition to or in lieu of reviewer comments inserted into the text of the manuscript. Use as many lines as needed.)

Reviewer's Recommendation (Please tick only one option)	√
Accept Submission (No significant alterations suggested)	
Revisions Required (Suggest changes to the manuscript as specified in this review)	√
Resubmit for Review (Major revisions should be made and suggestions as specified in this review must be addressed. Revised manuscript should be resubmitted to the reviewer for further review)	
Decline Submission (Do not encourage a rewrite, manuscript is totally rejected)	

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Further Reviewer's Comments Regarding Disposition of the Manuscript:

There are some issues that still need to be confirmed/answered by the author(s).

Date and Sign:
August 11, 2023

Reviewer 2

Non-Small Cell Lung Carcinoma (NSCLC) Subjects with T790M Positive Mutation ~~Shows~~ Exhibits Better Survival Rate

Abstract

Background: Mutations in the epidermal growth factor receptor (*EGFR*) tyrosine kinase receptor have become a significant treatment target in non-small cell lung carcinoma (NSCLC). However, the emergence of resistance due to T790M mutations poses a challenge in the effective management of this disease. Though some information remains unknown, various studies have demonstrated that patients with T790M-positive mutation exhibit improved progression-free survival (PFS), overall survival (OS), and better response to tyrosine kinase inhibitors (TKIs) treatment compared to T790M-negative patients. This study aimed to describe the effects of T790M mutation status in lung cancer patients.

Methods: This was a retrospective cohort study on 22 NSCLC subjects with/without osimertinib therapy. ~~to establish correlations by collecting data from subjects who were~~ Secondary data from subjects underwent the examined for the T790M mutation from plasma circulating tumor DNA (ctDNA) with next-generation sequencing analysis (NGS) and sequenced by Ion GeneStudio S5 with *EGFR* T790M (ThermoFisher, CA, United States) were collected.~~between October 2019 and July 2022 in Dr. Wahidin Sudirohusodo Hospital and its network hospital~~. Baseline data were descriptively summarized, and the differences of~~in~~ each variable between groups were calculated, as well as the correlation between survival rate of osimertinib therapy and T790M mutation status.

Results: ~~Our study found~~The result of this study showed that subjects with the T790M mutation exhibited mean survival time of 19.88 months, compared to those without the mutation ($p = 0.005$) and significant association between subjects with the T790M mutation and receiving osimertinib therapy ($p = 0.001$).

Conclusion: T790M positive mutation demonstrated a noteworthy survival rate.

Keywords: non-small cell lung carcinoma, epidermal growth factor, T790M.

Introduction

Epidermal growth factor receptor (*EGFR*) mutation is an emerging and promising treatment target for non-small cell lung carcinoma (NSCLC).[1,2] This mutation is particularly prevalent in Asian populations, present in up to half of NSCLC patients, and is found in about 10% of Western populations.[3] NSCLC, associated with *EGFR* mutation, exhibits a higher incidence in women, non-smokers, and those with adenocarcinoma histology.[4,5] Fortunately, the clinical outcomes for NSCLC patients have significantly improved since the introduction of tyrosine kinase inhibitors (TKIs) targeting EGFR.[6–10]

The initial generation of TKIs has shown remarkable results in prolonging patients' progression-free survival (PFS).[11,12] However, the impact on overall survival (OS) may be limited due to disease progression.[13] Recent findings have shed light on the differences in treatment response between the two most common types of *EGFR* mutations. Patients with exon 19 deletion tend to have a longer PFS on TKI treatment and show a more extended OS compared to those with exon 21 L858R point mutation.[14] Although, there is evidence the mechanism involved in TKIs therapy on *EGFR* mutation is thigter covalent binding but the true mechanism is still debated.[15,16]

Despite the initial benefits observed with TKI therapy, a majority of NSCLC patients with active *EGFR* mutations develop resistance during treatment.[2] However, there has been a significant breakthrough in the management of metastatic NSCLC with a specific genetic alteration.[17–20] There was substantial condition improvement in patients whose cancer progressed after first-generation TKI treatment and who developed tumors with T790M mutation.[21] T790M mutation in NSCLC play role as a genomic stability marker and reduce adenosine triphosphate (ATP)-competitive kinase to prevent EGFR-mediated signalling.[22] This mutation is responsible for better prognosis in NSCLC patients.

The present study was conducted to analyze a cohort of NSCLC patients and investigate the incidence of the T790M mutation status with osimertinib therapy, along with its impact on survival rates. Understanding these factors will provide valuable insights for pulmonology and thoracic oncology experts, aiding in the development of effective treatment strategies for NSCLC patients with EGFR mutations.

Methods

Subject's and data collections

A retrospective cohort study was taken on 22 NSCLC subjects (with osimertinib therapy n=5, without n=17) with T790M mutation test subjects in Dr. Wahidin Sudirohusodo Hospital and its network, Makassar, South Sulawesi, Indonesia. This study was approved by the Research Ethics Commission of of the Faculty of Medicine, Hasanuddin University (No: 270/UN4.6.4.5.31/PP36/2022). Demographic and clinical data (age, gender, body mass index, smoking status, Brinkman Index, family history and exon location) were collected by collecting medical record data from subjects who were examined for the T790M mutation from plasma (6 mL of plasma) circulating tumor DNA (ctDNA) with next-generation sequencing analysis (NGS) and sequenced by Ion GeneStudio S5 with *EGFR* T790M (ThermoFisher, CA, United States) between October 2019 and July 2022 in Prodia Laboratorium and Clinic. The subjects observed until they pass away.

Comment [NMD1]: Eventhough you have stated the laboratory where you conduct the analysis, however the methods used is still needed to be briefly explained in the separate sub-section.

Statistical analysis

Baseline data were descriptively summarized, and the differences in each variable between age, gender, body mass index, smoking status, Brinkman Index, family history, exon location, survival in one-year with T790M mutation were calculated using Chi-Square tests. Correlation between survival rate (SR) and T790M mutation status were analyzed by Log Rank (Mantel-Cox). Significant values were determined at $p<0.05$. The subject's SR were

analyzed. Age, gender, and smoking status are also analyzed as control variables. All statistical analyses were performed using the Statistical Program for Social Sciences (SPSS) version 24.0 (IBM Corporation, Armonk, NY, USA).

Results

Comment [NMD2]: Author may needs to add more narration and explanation regarding the data in the Results section.

Based on the study findings, the majority of subjects included in the analysis were males aged between 45 and 65 years, who were underweight, smokers, and did not have a family history of lung cancer (Table 1). Our study found that subjects with the T790M mutation exhibited mean survival time of 19.88 months, compared to those without the mutation. The presence of the T790M mutation was found to be correlated with SR (Table 2 & Figure 1), although there was no correlation between T790M mutation with one-year survival rate and exon location (Table 3). A~~Our study identified a~~ significant association between subjects with the T790M mutation and receiving osimertinib therapy was found (p = 0.001). Significant association was observed between the administration of osimertinib therapy and improved one-year survival rates in these subjects (Table 4).

Table 1. Subject's characteristic and T790M mutation

Variable	T790M mutation N(%)		Total	p-value
	Positive (+) (n=13)	Negative (-) (n=9)		
Age				
<45 years old	0 (0.0)	2 (9.1)	2 (9.1)	
45-65 years old	11 (50.0)	6 (27.2)	17 (77.3)	0.204
>65 years old	2 (9.1)	1 (4.6)	3 (13.6)	
Gender				
Male	5 (22.7)	7 (31.8)	12 (54.5)	0.069
Female	8 (36.4)	2 (9.1)	10 (45.5)	
Body mass index				
Underweight	7 (31.8)	6 (27.3)	13 (59.1)	
Normal	6 (27.3)	3(13.6)	9 (40.9)	0.548
Overweight	0 (0.0)	0 (0.0)	0 (0.0)	
Smoking status				
Yes	6 (27.3)	7 (31.8)	13 (59.1)	0.170
No	7 (31.8)	2 (9.1)	9 (40.9)	
Brinkman Index				
Non-smokers	7 (31,8)	2 (9,1)	9 (40.9)	
Moderate	5 (22.7)	0 (0.0)	5 (22.7)	0.003*
Severe	1 (4.6)	7 (31.8)	8 (36.4)	
Family history				
Yes	4 (18.2)	2 (9.1)	6 (27.3)	0.658
No	9 (40.9)	7 (31.8)	16 (72.7)	

Chi-Square test, *p<0.05, Brinkman index was calculated by multiply average cigar consumed everyday with years of smoking, mild <200, moderate 200-600, severe >600,

Table 2. Survival rate related to T790M mutation status (n=22).

Group	Survival rate (months)±SD	Mean difference	p-value
T790M (+)	19.88±3.36	9.81	0.005*
T790M (-)	5.74±0.49		

Log Rank (Mantel-Cox), SD standard of deviation, MD mean difference, *p<0.05

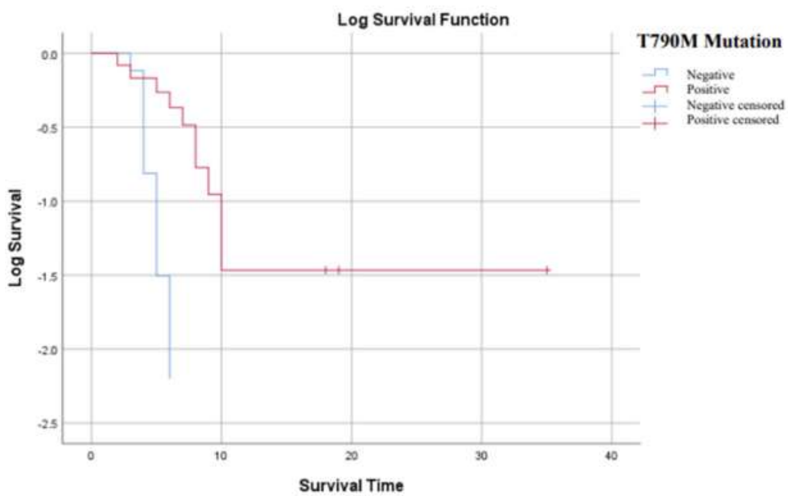

Figure 1. Survival rate related to T790M mutation status Log Rank (Mantel-Cox)

Table 3. One-year survival rate and exon location in T790M mutation

Variable	T790M mutation N(%) Positive (+) (n=13)	Negative (-) (n=9)	Total	p-value
Survival Status				
- Survivor	3 (10.5)	0 (0.0)	3 (13.6)	0.121
- Non-survivor	10 (42.1)	9 (47.4)	19 (86.4)	
Exon location				
- Exon 19	5 (22.7)	5 (22.7)	10 (45.5)	0.429
- Exon 21	8 (36.3)	4 (18.2)	12 (54.5)	

Chi-square, *p<0.05

Table 4. One-year survival rate in NSCLC subjects with osimertinib therapy

One-year survival rate	Therapy: Osimertinib (n=5)	Without osimertinib (n=17)	Total	p-value
Survivor	3 (13.6)	0 (0.0)	3 (13.6)	0.001*
Non-survivor	2 (9.1)	17 (77.3)	10 (86.4)	

Chi-square, *p<0.05

Discussion

Lung cancer is a complex disease that often involves genetic alterations affecting the tyrosine kinase signaling pathways, with approximately 75% of examined cases showing

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Comment [NMD3]: Why did you combine the survival rate between the T790M-positive and T790-negative?

Meanwhile in the narration of results section you mentioned that a significant association between subjects with the T790M mutation and receiving osimertinib therapy was found (p = 0.001). How can you come to this conclusion when the comparation between the negative and the positive was not provided?

such changes.[23] Among these pathways, the epidermal growth factor receptor (EGFR) pathway is of particular interest, with around 65% of lung cancer in tyrosine kinase pathways cases exhibiting EGFR mutations.[24] While targeted therapies like tyrosine kinase inhibitors (TKIs) have shown promise in inhibiting tumor growth, the emergence of the T790M mutation has been identified as a common mechanism of resistance to these therapies.[25,26] The T790M mutation competes with the TKI, reducing its efficacy and leading to treatment resistance. Among the various post-TKI mutations, the T790M mutation has the highest incidence.[27]

The T790M mutation is frequently observed in lung cancer patients who have previously undergone TKI therapy and developed resistance.[28] Interestingly, it tends to occur more often in individuals over the age of 40 and men. Advanced age may be associated with a reduced ability of genes to suppress oncogenes, potentially contributing to the occurrence of the T790M mutation. Additionally, epidemiological data suggest that men are more likely to have familial lung cancer. However, the exact reasons for this gender disparity remain unclear and warrant further investigation.[29]

Smoking status, as assessed using the Brinkman index, has been correlated with the presence of the T790M mutation. Prolonged smoking can trigger changes in cellular differentiation patterns, which may influence the development of this mutation.[30] However, the underlying mechanism behind the higher occurrence of the T790M mutation in non-smokers is not yet understood.[31] T790M mutation and smoking are inversely correlated. Non-smoker have higher risk of this mutation. This is correlated with previous study that conclude higher prevalence in non-smoker than higher Brinkman index population.[10] Furthermore, body mass index (BMI) has not shown a relationship with the T790M mutation.[32] On the other hand, family history does not appear to have a significant association with the T790M mutation, although there is a possibility of the mutation being inherited.[33,34]

Additionally, the specific location of the mutation within the exon does not seem to be correlated. Although the T790M mutation often coexists with other EGFR mutations, such as exon 19 deletion and exon 21 L858R mutation, the pathogenetic mechanism underlying this coexistence is still not fully understood.[35,36]

The study's results highlight a significant difference in the one-year survival rates of lung cancer subjects with the T790M mutation who received osimertinib compared to those without the mutation. Subjects undergoing EGFR-TKI therapy exhibited an average survival time of 19.88 months, with a mean difference of 9.81 months compared to subjects without the T790M mutation, who had an average survival time of 5.74 months. These findings are consistent with similar studies which reported longer overall survival in subjects with T790M mutations who received EGFR-TKI therapy compared to those without the mutation.[37,38] Two mechanism that proposed EGFR-TKI affect T790M positive are covalent binding EGFR-TKI therapy with T790M-positive are firmer and EGFR-TKI therapy also reduce ATP-competitive kinase to prevent EGFR-mediated signalling. This two mechanism gave better prognosis to subject T790M mutation.[2,22] Importantly, these improvements in survival have been observed across different age groups and regardless of previous therapies received.[39,40]

However, it is important to acknowledge the limitations of the current study. One significant limitation is the need for more comprehensive data on the specific type of EGFR-TKI administered to each patient. To overcome these limitations and gain a deeper understanding of the clinical outcomes and prognosis associated with the T790M mutation, a multi-center study with a larger sample size is recommended. Such a study should also consider the type and duration of treatment provided, enabling a more robust analysis of the impact of the T790M mutation on patient outcomes. By addressing these limitations, researchers can further enhance our knowledge of the T790M mutation and its implications

for the management of lung cancer. Further research is necessary to explore optimal treatment strategies and improve outcome in this subgroup.

Conclusion

T790M positive mutation demonstrated a noteworthy survival rate among NSCLC subjects. Osimertinib therapy may have potential beneficial effect on T790M mutation NSCLC therapy. These findings contribute to the potential benefits of targeted therapies for NSCLC subjects with T790M mutation.

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[InaBJ] M2023140 Editor Decision Round 2 - Revisions Required

Ferry Sandra <ferry@trisakti.ac.id> Sun, Oct 1, 2023 at 5:17 PM
To: Secretariat of InaBJ <secretariatinabj@gmail.com>

Dear Secretariat of The Indonesian Biomedical Journal,

Good day. I apologize for the delay, but I've completed the revision of manuscript M2023140. I've made several changes to the document and addressed all the reviewers' comments as requested.

Thank you.

Regards,
Ferry Sandra
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Ferry Sandra, D.D.S., Ph.D.
Head of Medical Research Center
Universitas Trisakti

Round 2 Revision from Author.docx
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Osimertinib as a Potential Targeted Therapy for Non-Small Cell Lung Carcinoma (NSCLC) Patients with EGFR Exon 20 T790M

Abstract

Background: Emergence of drug resistance due to epidermal growth factor receptor (EGFR) exon 20 T790M poses a challenge in the effective management of non-small cell lung carcinoma (NSCLC). Significant breakthrough in the management of NSCLC with a specific genetic alteration causes substantial condition improvement in patients whose cancer progressed after first-generation tyrosine kinase inhibitor treatment and who developed tumors with EGFR Exon 20 T790M mutation. The present study analyzed a cohort of NSCLC patients and investigated the incidence of the EGFR Exon 20 T790M status with osimertinib therapy, along with its impact on survival rates.

Methods: This was a retrospective cohort study on 22 NSCLC subjects who were genetically examined for EGFR status from plasmic cell free total nucleic acid. Subjects with EGFR Exon 20 T790M mutation were treated with/without osimertinib. Demographic and clinical data were descriptively summarized, and the differences of each variable and correlation between survival rate and EGFR Exon 20 T790M were analyzed.

Results: Subjects with (n=13) and without (n=9) EGFR Exon 20 T790M had survival rates of 10.77±2.45 and 4.78±1.48, respectively (p=0.000). Based on 1-year survival status of subjects with EGFR Exon 20 T790M, there were 3 osimertinib-treated survivors and 2 osimertinib-treated non-survivors. Eight subjects with EGFR Exon 20 T790M and without osimertinib treatment did not survive (p=0.001).

Conclusion: Since treatment of osimertinib demonstrated a noteworthy survival rate among NSCLC subjects with EGFR Exon 20 T790M, thus osimertinib could be suggested as a potential targeted therapy for NSCLC subjects with EGFR Exon 20 T790M.

26 **Keywords**: non-small cell lung carcinoma, EGFR, Exon 20, T790M, osimertinib

Introduction

Epidermal growth factor receptor (EGFR) mutation is an emerging and promising treatment target for non-small cell lung carcinoma (NSCLC).(1) This mutation is particularly prevalent in South-East Asian patients, found in more than half of all NSCLC patients, while found in 10-20% of Caucasian patients.(2) NSCLC associated with EGFR mutation exhibits a higher incidence in women, non-smokers, and those with adenocarcinoma histology.(3) Fortunately, the clinical outcomes for NSCLC patients have significantly improved since the introduction of tyrosine kinase inhibitors (TKIs) targeting EGFR.(4)

The initial generation of TKIs has shown remarkable results in prolonging patients' progression-free survival (PFS).(5,6) However, the impact on overall survival (OS) may be limited due to disease progression. Recent findings have shed light on the differences in treatment response between the two most common types of EGFR mutations. Patients with exon 19 deletion tend to have a longer PFS on TKI treatment and show a more extended OS compared to those with exon 21 L858R point mutation.(7,8) Although there is an evidence suggesting that particular EGFR mutation had higher affinity with TKI (9), the true mechanism is still debated.

Despite the initial benefits observed with TKI therapy, a majority of NSCLC patients with active EGFR mutations develop resistance during treatment.(1) Since there has been a significant breakthrough in the management of NSCLC with a specific genetic alteration (10-12), there was substantial condition improvement in patients whose cancer progressed after first-generation TKI treatment and who developed tumors with EGFR exon 20 T790M mutation.(13) The T790M mutation in NSCLC play role in reducing adenosine triphosphate (ATP)-competitive-kinase-inhibitor, so that the mutation can prevent the effect of inhibitor, thus EGFR-mediated signalling will not be defected.(14)

The present study was conducted to analyze a cohort of NSCLC patients and to investigate the incidence of the EGFR exon 20 T790M status with osimertinib therapy, along with its impact on survival rates. Understanding these factors will provide valuable insights in aiding the development of effective treatment strategies for NSCLC patients with EGFR mutations.

Methods

Subject and Data Collection

A retrospective cohort study was conducted on 22 NSCLC subjects within October 2019 and July 2022 in Dr. Wahidin Sudirohusodo Hospital and its network, Makassar, South Sulawesi, Indonesia. This study was approved by the Research Ethics Commission of the Faculty of Medicine, Hasanuddin University (No: 270/UN4.6.4.5.31/PP36/2022). Demographic and clinical data including age, gender, body mass index, smoking status, Brinkman Index and family history were collected. Brinkman index was calculated by multiplying average-cigarette-consumed-everyday with years-of-smoking, mild <200, moderate 200-600, severe >600.

EGFR Genetical Examination

All NSCLC subjects were genetically examined for EGFR status. From each NSCLC subject, 10 mL blood was collected. Blood sample was processed to collect plasmic cell free total nucleic acid (cfTNA) using MagMAX cell free total nucleic acid isolation kit (ThermoFisher, Waltham, MA, USA). DNA library was prepared Oncomine Lung cfDNA Assay (ThermoFisher). Then the sequencing was performed with Ion Torrent next-generation sequencing (NGS) (ThermoFisher). The NGS data was analyzed and interpreted with Ion Reporter and Oncomine Reporter.

Osimertinib Treatment and Survival Status

All NSCLC subjects were treated with carboplatin and paclitaxel in a cycle of 6-treatments and 21-days-interval. In the 6^{th} month, EGFR genetical examinations were performed. Among the subjects with EGFR Exon 20 T790M, some received 80 mg osimertinib 1 dosage/daily (Tagrrisso, AstraZeneca, Cambridge, UK), while others continued with the second cycle of carboplatin and paclitaxel treatment. The subjects were observed until they deceased. The subject survival status was recorded for further analysis.

Statistical analysis

Demographic and clinical data were descriptively summarized and statistically analyzed using Chi-Square test. Correlation between survival rate and EGFR Exon 20 T790M was analyzed with Log Rank (Mantel-Cox) test. One-year survival status and EGFR mutation in different exons were statistically analyzed using Chi-Square test as well. Significant values were determined at $p<0.05$. All statistical analyses were performed using the Statistical Program for Social Sciences (SPSS) version 24.0 (IBM Corporation, Armonk, NY, USA).

Results

Based on the subject characteristic, there were 12 male and 10 female subjects with majority age of 45-65 years, non-smoking (Brinkman Index), and no family history of lung cancer (Table 1). Meanwhile, based on EGFR mutation status, there were subjects with mutation of EGFR Exon 20 T790M and mutation of EGFR Exon 19 deletion (n=5) or EGFR Exon 21 L858R (n=8) (Table 2). There were also subjects without mutation of EGFR Exon

20 T790M but having mutation of EGFR Exon 19 deletion (n=5) or EGFR Exon 21 L858R (n=4).

Subjects with (n=13) and without (n=9) EGFR Exon 20 T790M had mean difference of 7.77 months with *p*-value of 0.000 (Table 3). As shown in Kaplan-Meier survival curve (Figure 1), subjects without EGFR Exon 20 T790M (blue line) had survival time <10 months, while subjects with EGFR Exon 20 T790M (red line) had survival time >10 months with 3 positive censored tick marks. Based on 1-year survival status, there were more non-survivor with EGFR Exon 20 T790M (n=10) compared with the survivor (n=3), while all subjects without EGFR Exon 20 T790M did not survive (Table 4).

Among the subjects with EGFR Exon 20 T790M, there were subjects treated with (n=5) / without (n=8) osimertinib. Based on 1-year survival status of subjects with EGFR Exon 20 T790M, there were 3 osimertinib-treated survivors and 2 osimertinib-treated non-survivors (Table 5). However, without osimertinib treatment, subjects with EGFR Exon 20 T790M did not survive.

Table 1. Subject's characteristic based on EGFR Exon 20 T790M

Variable	EGFR Exon 20 T790M (n (%))		#p
	(+) (n=13)	(-) (n=9)	
Age			
<45 years old	-	2 (9.1)	
45-65 years old	11 (50.0)	6 (27.3)	0.204
>65 years old	2 (9.1)	1 (4.5)	
Gender			
Male	5 (22.7)	7 (31.8)	0.069
Female	8 (36.4)	2 (9.1)	
Body mass index			
Underweight	7 (31.8)	6 (27.3)	
Normal	6 (27.3)	3 (13.6)	0.548
Overweight	-	-	
Brinkman Index			
Non-smokers	7 (31.8)	2 (9.1)	
Mild	-	-	0.003*
Moderate	5 (22.7)	-	
Severe	1 (4.5)	7 (31.8)	
Family history			
Yes	4 (18.2)	2 (9.1)	0.658
No	9 (40.9)	7 (31.8)	

#Chi-Square test, *p<0.05

Table 2. Correlation of EGFR Exon 20 T790M with other EGFR mutations

Other EGFR Mutation	EGFR Exon 20 T790M (n (%)) (+) (n=13)	(-) (n=9)	#p
EGFR Exon 19 deletion	5 (22.7)	5 (22.7)	0.429
EGFR Exon 21 L858R	8 (36.4)	4 (18.2)	

#Chi-square

Table 3. Survival rate related to EGFR Exon 20 T790M

Group	Survival Rate (Month) Mean±SD	Mean Difference	#p
EGFR Exon 20 T790M (+) (n=13)	10.77±2.45	7.77	0.000*
EGFR Exon 20 T790M (-) (n=9)	4.78±1.48		

#Log Rank (Mantel-Cox), *p<0.05, SD: standard deviation

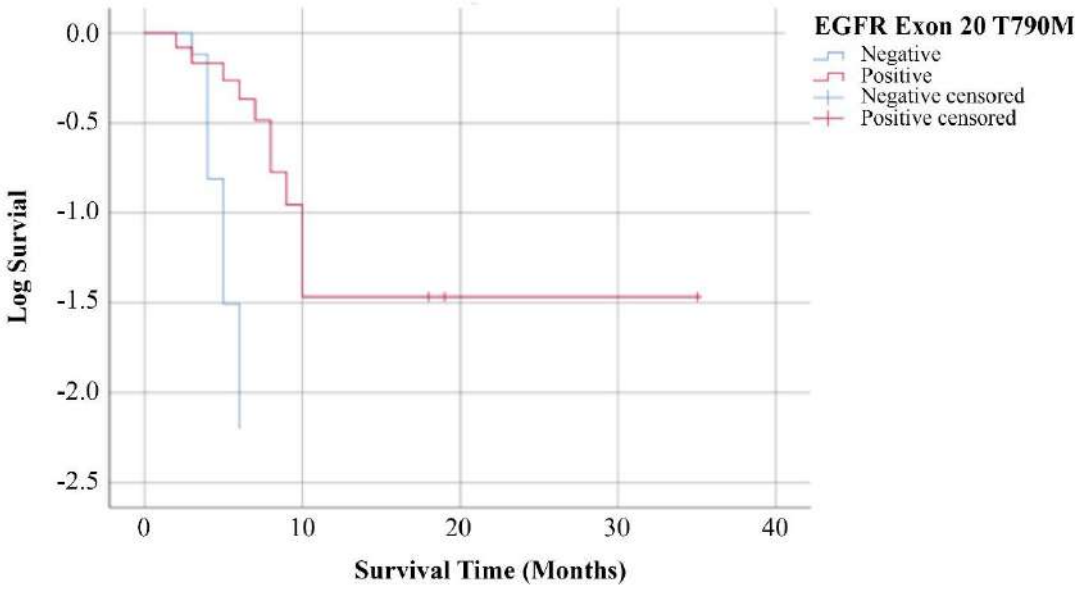

Figure 1. Kaplan-Meier survival curve of subjects with/without EGFR Exon 20 T790M

Table 4. One-year survival status related to EGFR Exon 20 T790M

One-year Survival Status	EGFR Exon 20 T790M (n (%)) (+) (n=13)	(-) (n=9)	#p
Survivor	3 (13.6)	-	0.121
Non-survivor	10 (45.5)	9 (40.9)	

#Chi-square

Table 5. One-year survival status related to EGFR Exon 20 T790M with osimertinib treatment

One-year survival status	Osimertinib Therapy (+) (n=5)	(-) (n=8)	#p
Survivor	3 (23.1)	-	0.001*
Non-survivor	2 (15.4)	8 (61.5)	

#Chi-square, *p<0.05

Discussion

Lung cancer is a complex disease that often involves genetic alterations affecting the EGFR tyrosine kinase signaling pathways, with approximately 75% of examined cases showing such changes.(15) Among these pathways, EGFR pathway is of particular interest with the most common mutations are in-frame deletions (85%–90%) of exon 19 deletion (45%–50%) and exon 21 L858R mutation (40%–45%).(16) While targeted therapies like TKIs have shown promise in inhibiting tumor growth, the emergence of the EGFR Exon 20 T790M mutation has been identified as a common mechanism of resistance to these therapies. (17,18) The EGFR Exon 20 T790M competes with the TKI, reducing its efficacy and leading to treatment resistance.(14) Among the various post-TKI mutations, the EGFR Exon 20 T790M has the highest incidence.(1,17)

The EGFR Exon 20 T790M mutation is frequently observed in lung cancer patients who have previously undergone TKI therapy and developed resistance.(19) Interestingly, it tends to occur more often in individuals over the age of 40 and women.(20,21) In accordance, in this study, most subjects with EGFR Exon 20 T790M were in the age of 45-65 years old. Advanced age may be associated with a reduced function of tumor-suppressor genes (22), potentially contributing to the occurrence of the EGFR Exon 20 T790M.

In this study, smoking status, as assessed using the Brinkman index, non-smoker was correlated with the presence of the EGFR Exon 20 T790M. This result is in accordance with previous report, showing that the never smokers with EGFR Exon 20 T790M develop lung cancer more frequently than ever smokers. In addition, EGFR Exon 20 T790M was found more in female gender.(23) Similar data were also found in this study, most subjects with EGFR Exon 20 T790M were not smoking, and more female subjects (n=8) than male subjects (n=5) were detected with EGFR Exon 20 T790M. However, the underlying

mechanism behind the higher occurrence of the EGFR Exon 20 T790M in non-smokers is not yet understood.

In this study, family history did not appear to have a significant association with the EGFR Exon 20 T790M, although there is a possibility of the mutation being inherited.(24) Additionally, the specific location of the mutation within the exon did not seem to be correlated. Although the EGFR Exon 20 T790M often coexists with other EGFR mutations, such as exon 19 deletion and exon 21 L858R mutation, the pathogenetic mechanism underlying this coexistence is still not fully understood.(25,26)

In this study, there was a significant difference in the survival rate of subjects with the EGFR Exon 20 T790M (10.77 months) than the one of subject without the mutation (4.78 months). These results were influenced by the osimertinib treatment, since data of 1-year survival status showed that among 5 subjects treated with osimertinib, 3 of them were survive (Table 5) with long survival time (Figure 1). These findings are consistent with similar studies which reported longer overall survival in subjects with EGFR Exon 20 T790M mutations who received EGFR-TKI treatment compared to those without the mutation and without EGFR-TKI treatment (27).

There are some limitations of the current study. To overcome these limitations and gain a deeper understanding of the clinical outcomes and prognosis associated with the EGFR Exon 20 T790M, a multi-center study with a larger sample size is recommended. Such a study should also consider the type and duration of treatment provided, enabling a more robust analysis of the impact of the EGFR Exon 20 T790M on patient outcomes.

Conclusion

Since treatment of osimertinib demonstrated a noteworthy survival rate among NSCLC subjects with EGFR Exon 20 T790M, thus osimertinib could be suggested as a potential targeted therapy for NSCLC subjects with EGFR Exon 20 T790M.

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[InaBJ] M2023140 Editor Decision - Manuscript Accepted

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Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**Osimertinib as a Potential Targeted Therapy for Non-Small Cell Lung Carcinoma (NSCLC) Patients with EGFR Exon 20 T790M**."

Our decision is to: **Accept Manuscript.**

Your manuscript will be sent to our publisher for typesetting and you should receive the proofreading in due course.

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