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EPIDERMAL GROWTH FACTOR RECEPTOR MUTATIONS AND PROGNOSIS IN NON-SMALL CELL LUNG CANCER CASES IN BRUNEI DARUSSALAM.

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ABSTRACT

Introduction: This study characterizes Epidermal Growth Factor Receptor (EGFR) mutations in patients with non-small cell lung cancer (NSCLC) in Brunei Darussalam, and assesses the prognostic roles of EGFR mutations and survival benefit of EGFR tyrosine-kinase inhibitors (TKIs) therapy. **Materials and Methods:** Data from The Brunei Cancer Centre was used for retrospective analysis of clinico-pathological characteristics on NSCLC patients diagnosed from 2010 to 2017. The progression-free survival (PFS) of patients on EGFR TKI and overall survival (OS) of patients with EGFR-mutations and EGFR wild types were compared using survival analysis. **Results:** Result of NSCLC EGFR mutation analysis was evaluable in 71 cases, of which 40 cases were classified as EGFR wild type and 31 cases of EGFR mutations, indicating the prevalence of EGFR mutation of 43.7%, with most cases found in females (22, 71.0%) and non-smokers (20, 64.5%). Mean age of patients with NSCLC EGFR mutation was 63.3 years. Exon 21 point mutation (L858R) (15, 50.0%) was most prevalent, followed by Exon 19 deletion (12, 40.0%). Male gender (OR=0.24, P=0.022) and Stage IV disease (OR=0.24, P=0.040) tend to be significantly less associated with EGFR mutation. A total of 65.2% of patients who received EGFR TKI therapy have achieved an objective response rate. The median PFS for all patients treated with TKI was seven months. The median PFS of patients treated with first line TKI and initial chemotherapy were eight and six months (P=0.045) respectively. EGFR-mutated patients also showed improved OS compared to wild type (29 vs 17 months) although this did not achieved statistical significance (p=0.2). **Conclusion:** The observed improved median PFS and OS in our NSCLC EGFR-mutation patients on personalised EGFR TKI therapy supports the routine analysis for EGFR mutations in all patients diagnosed with NSCLC to identify individuals who may benefit from EGFR TKI therapy.

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INTRODUCTION

Lung cancer is by far the leading cause of cancer deaths worldwide with a mortality to incidence ratio of 0.85.[1] Data from the World Health Organization reported 9.6 million can-

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cancer deaths in 2018, with nearly one in five cases (19.0%) attributed to lung cancer.[2] Brunei is indifferent to its neighbouring South East Asian countries whereby the highest cancer mortality rate is due to lung cancer (19.3% in 2017).[3]

Historically, lung cancer has been classified into two major categories: Non Small Cell Lung Cancer (NSCLC) which accounts for approximately 85% of all cases and Small Cell Lung Cancer (SCLC).[4] Albeit being a common cancer, the prognosis of NSCLC patients remains poor when found at an advanced stage that is beyond curative resection.[5] In fact, the estimated five-year relative survival rate among patients of all races with lung cancer (18.6%) was lower compared to other solid cancers such as colorectal (64.5%), breast (89.6%) and prostate cancer (98.2%).[6] Based on local published data, the overall 5-year survival rate for patients with lung cancer was only 3.6% with a median survival time of 6.5 ± 0.9 months (95% CI: 4.7-8.4 months).[7]

The treatment paradigm for patients with advanced NSCLC has changed over the years. In this era of precision medicine, NSCLC is now being subdivided into molecular subtypes that may have implications on treatment with dedicated targeted and chemotherapeutic options. The subtypes are based on the presence or absence of activating mutations in Epidermal Growth Factor Receptor (EGFR), rearrangement in Anaplastic Lymphoma Kinase (ALK) or C-Ros Oncogene 1 (ROS-1) and others.[8]

Tumours harbouring EGFR mutations are present in a distinct subgroup of NSCLC patients. The EGFR gene produces a 486 amino-acid receptor protein of 170kDa that is normally observed in some epithelial, mesenchymal and neurogenic tissues.[9,10] EGFR activating mutations, which can be found between Exons 18 to 21, result in a sustained stimulation of the growth signal transduction pathways.[9,10] This yields more aggressive tumour phenotypes and intensifies migration, thereby facilitating metastasis of tumour cells.[10]

Activating EGFR mutations in NSCLC cases represent a significant therapeutic target for Tyrosine Kinase Inhibitors (TKIs). Due to the similarity of TKIs with Adenosine Triphosphate, they bind to the EGFR protein kinase domain to prevent its autophosphorylation. This suppresses the excessively-stimulated signal transduction pathways, leading to decreased cell proliferation and increasing cell apoptosis.[11] The discovery of EGFR mutations therefore represents a predictive marker for a favourable response to EGFR TKI therapy.

Studies have consistently demonstrated an association between Asian ethnicity and EGFR mutations in NSCLC cases. The first 'mutMap' study of the incidence and coincidence of genetic mutations associated with NSCLC found a higher overall EGFR mutation frequency in Asian compared to Western patients (47.9% vs 19.2%).[12] The PIONEER study has also identified a high mutation frequency (51.4%) in stage IIIB or IV lung adenocarcinoma patients within seven Asian regions.[13] Interestingly, a recent study in Brunei also reported over-expression of EGFR protein (81.5% positive rate) in patients diagnosed with NSCLC.[14] However, the incidence of EGFR mutation in NSCLC cases in Brunei remains unknown. We conducted this study to determine the incidence of EGFR mutation, identify patient characteristics associated with EGFR mutations and investigate the treatment response to EGFR TKI therapy in patients diagnosed with NSCLC in Brunei Darussalam.

MATERIALS AND METHODS

Study Design and Patients

This was a retrospective cohort study con-

ducted at The Brunei Cancer Centre (TBCC), which is the national cancer centre of Brunei Darussalam and receives patient referrals throughout the country. All patients aged between 18 to 95 years diagnosed with NSCLC between 1st January 2010 to 15th March 2017 and with confirmed lung tissue sent for analysis of EGFR status (mutated or wild type) were included. Patients who had refused referral, unfit for systemic treatments and remained under palliative care in the primary referring hospitals were excluded from the study. This study was approved by the Medical and Health Research Ethics Committee (MHREC) of Ministry of Health in Brunei Darussalam, and Ethics Committee of PAPRSB Institute of Health Sciences (IHSREC).

Data Collection

A data collection Excel performa was designed to collect relevant patients' details. Patient-identifying information was excluded to secure patient confidentiality. Patients' demographic data and clinical characteristics such as age, gender, ethnicity, smoking status, date of diagnosis, subtype of NSCLC, EGFR mutation status, EGFR TKI treatment regimes, disease regression, sites of metastasis, recurrences and time of death were recorded.

EGFR Analysis and Classification

EGFR status and types of EGFR mutations were also recorded. Specimens were either obtained by fine needle aspiration cytology (FNAC) or core biopsy of the tumour and processed at the Department of Histopathology, Raja Isteri Pengiran Anak Saleha (RIPAS) Hospital. The Formalin-Fixed Paraffin-Embedded tumour blocks were then sent by courier to the National University Hospital (NUH) Singapore lab for EGFR mutation testing. The tests were carried out using the DNeasy Blood and Tissue Kit (Qiagen) for Polymerase Chain Reaction amplification of Exons 18 to 21 of the EGFR gene. Results were sent back and compiled by the Department of Histopathology, RIPAS Hospital and retrievable via the Hospital patients' electronic record system, Bru-HIMS.

Results of EGFR mutations analysis were classified as either: (a) *EGFR* mutation – NSCLC patients with *EGFR* mutated gene on Exons 18 to 21, (b) *EGFR* wild type – NSCLC patients with no known *EGFR* mutated gene detected, and (c) Not evaluable – NSCLC patients with unknown *EGFR* status as respective tissue samples were either not sent for *EGFR* mutation analysis, or had insufficient tissue samples for analysis.

Treatment Regime

All EGFR TKI therapy were started and monitored at TBCC. The starting dose of standard EGFR TKI therapy was Gefitinib 250mg once daily, Erlotinib 150mg once daily or Afatinib 40mg once daily. Dose reductions were carried out for some patients due to treatment-related toxicity. However, this was not reported as outcome for this study as it was not part of the scope of the current study.

Statistical Analyses

All data was analysed using IBM SPSS software version 23.0, and R software version 3.5.2. Analysis of association between patient characteristics and EGFR mutation status was performed using Simple and Multiple Logistic Regression only for patients with confirmed EGFR mutation. Kaplan-Meier curves and log-rank tests were used to determine the Progression-Free Survival (PFS) of patients on EGFR TKI and to compare the Overall Survival (OS) of mutated and wild type EGFR patients. PFS was defined as the time from the start of first EGFR TKI therapy to date of disease progression or the end of study for individuals who had no disease progression, while OS was defined as the time from first diagnosis to the date of death or last-follow up. Time of death was ascertained from death certificates issued at TBCC and in all hospitals in the 4 districts via Bru-HIMs. Lost to follow-up was

ascertained by checking the patients' last date of appointment and clinical notes entry from TBCC patients outpatient database and from Bru-HIMs. P-values <0.05 was considered statistically significant.

RESULTS

Out of 191 NSCLC cases recorded during the study period, EGFR mutation analysis were available for 71 cases, of which 40 were classified as EGFR wild type cases, 31 cases with EGFR mutations and 120 cases who were not evaluable (unknown EGFR status due to insufficient tissue for analysis or sample not send for analysis).

Table I shows the characteristics of the 31 patients whose tumours harboured EGFR mutations. The mean age of these 31 patients was 63.3 (SD 12.0) years. More than half were females (n=22, 71.0%) with a majority of Malay ethnicity (n=20, 64.5%). Majority (64.5%) were non-smokers and 64.5% presented with advanced Stage IV disease. Nineteen (61.3%) patients had metastases to more than 1 site and the most common sites of metastases were to bones (45.2%) and lung (32.3%).

Table I: Baseline characteristics of 31 patients with *EGFR* mutations

Variables	*n* (%)
Gender	
Male	9 (29.0)
Female	22 (71.0)
Age in years	
40-59	12 (38.7)
60-79	17 (54.8)
80-99	2 (6.5)
Ethnicity	
Malay	19 (61.3)
Others	12 (38.7)
Smoking Status	
Smoker/Ex-Smoker	10 (32.3)
Non-Smoker	20 (64.5)
Un-Known	1 (3.2)
Stage at First Diagnosis	
I	3 (9.7)
II	2 (6.4)
III	6 (19.4)
IV	20 (64.5)
Sites of Metastasis	
Bones	14 (45.2)
Lung	10 (32.3)
Pleura	9 (29.0)
Liver	7 (22.6)
Brain	6 (19.4)
Adrenal	4 (12.9)
Breast	1 (3.2)
Kidney	1 (3.2)
Pericardium	1 (3.2)
Psoas Muscle	1 (3.2)
Thyroid	1 (3.2)

Distribution of various types of EGFR mutation amongst mutated patients

Table II shows the mutation types in Exons 18 to 21, which were detected in 30 cases (one case excluded due to unavailable specific mutation data). Amongst these, 90.0% harboured a single Exon site EGFR mutation, while the remaining 10.0% had two Exons mutation.

Association of patient characteristics with EGFR mutation status

Patients' characteristics such as gender, smoking status and stage of disease at first diagnosis were significantly associated with EGFR mutation status on univariate analysis (Table III). However, only gender (p=0.022) and stage of disease at first diagnosis (p=0.040) were significantly associated with EGFR mutation status on multiple logistic regression analysis (Table IV). Male gender was 76% less likely to exhibit EGFR mutation compared to female (OR=0.24). Advanced stage IV NSCLC disease was also noted to be 76% less likely to have EGFR mutations compared to earlier stages (OR=0.24). There was no significant interaction between gender and stage variable (P=0.706).

Treatment response

Amongst 31 patients with EGFR mutation, 27 (87.1%) received EGFR TKI therapy of which

Table II: Distribution of mutation types on Exon 18 - 21 amongst 30 patients with confirmed EGFR mutation NSCLC.

Exon	Mutation Type	*n* (%)
18	E709K	1 (3.3)
19	L747-S752 deletion	1 (3.3)
	E746-A750 deletion	3 (10.0)
	K745-A750 deletion	8 (26.7)
	A743V	1 (3.3)
20	S768I	1 (3.3)
	F795S	1 (3.3)
	G779F	1 (3.3)
	D770-P772 duplication	1 (3.3)
21	L858R	13 (43.3)
	A871G	1 (3.3)
	P848L	1 (3.3)

(Footnote: One patient excluded in this analysis due to missing *EGFR* mutation type information).

22 (71.0%) had one line of TKI while five (16.1%) were given two lines. However, four (12.9%) patients did not receive any TKI therapies as they did not have advanced or relapsing disease. Gefitinib was the most common first line TKI prescribed to patients (29, 93.5%), followed by Afatinib (2, 6.5%).

Evaluation of treatment response revealed an objective response rate (complete or partial response) of 65.2% (15 cases), whereby 12 progressed at an average of 9.8 months later and two remained stable (no disease progression) as of the end of this study. Besides, 30.4% of EGFR-mutation cases (seven cases) were found to have stable disease upon receiving their first TKI treatment, but six cases progressed at an average of 10.8 months later. However, the best response to first line TKI therapy of four patients was not evaluable in this study.

Survival status in relation to EGFR mutation status

Survival analysis showed a median PFS of eight months for patients treated with first line EGFR TKI compared to six months for patients treated with initial chemotherapy (log rank test p=0.045).

A survival analysis was also done to determine the PFS of patients with the two most common EGFR mutations after receiving EGFR TKI; 10 cases of L858R mutation and eight cases of Exon 19 deletion. There was no difference in PFS between the two mutation types (median PFS of seven months for both groups, log-rank test p=0.142).

The Kaplan-Meier OS curves for both EGFR statuses shown in Figure 2 demonstrate non- significant OS times between these 2 groups (log rank test p=0.232). Till 15th

Table III: Association of patient characteristics with *EGFR* mutation status using Simple Logistic Regression, outcome as *EGFR* mutation (1=Mutated; 0=Wild).

Variable	Crude *OR*	(95% CI *OR*)	*Z* stat.	*P* value
Age (year)	1.03	(0.99; 1.07)	1.47	0.142
Gender				
Female	1.00	(0.07; 0.53)	-3.12	0.002*
Male	0.20			
Ethnicity				
Malay	1.00	(0.55; 4.01)	0.77	0.443
Others	1.47			
Smoking status				
Smoker	1.00	(1.28; 9.68)	2.40	0.016*
Non-Smoker	3.43			
Stage at first diagnosis				
Stage 1,2,3	1.00	(0.10; 0.98)	-1.96	0.050
Stage 4	0.32			

* Statistically significant with $p<0.05$

Table IV: Association of patient characteristics with *EGFR* mutation status using Multiple Logistic Regression, outcome as *EGFR* mutation (1=Mutated; 0=Wild).

Variable	Adjusted *OR*	(95% CI *OR*)	*Z* stat.	*P* value
Age (year)	1.03	(0.99; 1.09)	1.45	0.148
Gender				
Female	1.00	(0.06; 0.79)	-2.28	0.022*
Male	0.24			
Ethnicity				
Malay	1.00	(0.29; 3.51)	0.04	0.967
Others	1.03			
Smoking status				
Smoker	1.00	(0.63; 8.35)	1.26	0.207
Non-Smoker	2.27			
Stage at first diagnosis				
Stage 1,2,3	1.00	(0.05; 0.89)	-2.05	0.040*
Stage 4	0.24			

* Statistically significant with $p<0.05$

March 2017, the median OS time was 17 months and 29 months for EGFR wild type and EGFR mutation cases respectively.

DISCUSSIONS

Most of our EGFR mutation cases were of female gender (71.0%, p=0.002) and non-smokers (64.5%, p=0.016), as consistent to previously published data.[15] Although Asian female non-smokers are the specific epidemiological subgroup, EGFR mutations are not only restricted to these characteristics. In fact, 32.3% of our EGFR mutation cases were smokers, consistent with the PIONEER study (39.7%).[13] Therefore, it is of no surprise that clinicians often face challenges when attempting to identify potential patients for EGFR TKI therapy. These findings thus further support routine EGFR mutation screening for all pa-

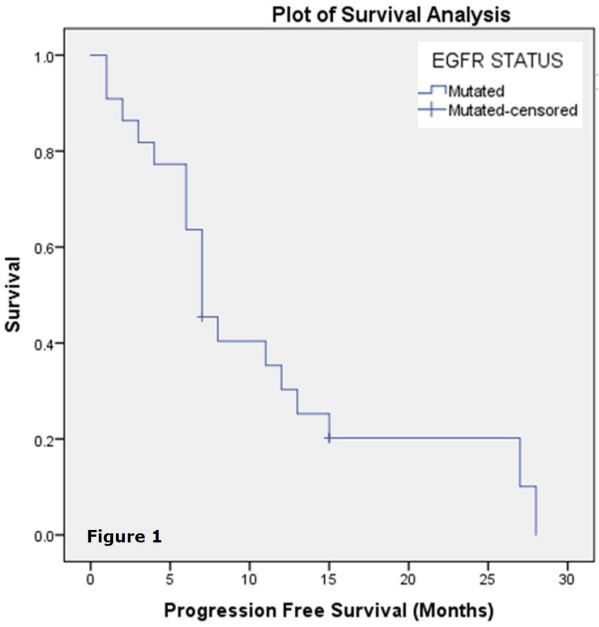

Figure 1: Kaplan Meier curve showing the Progression Free Survival for *EGFR* mutated patients treated with first *EGFR* TKI therapy.

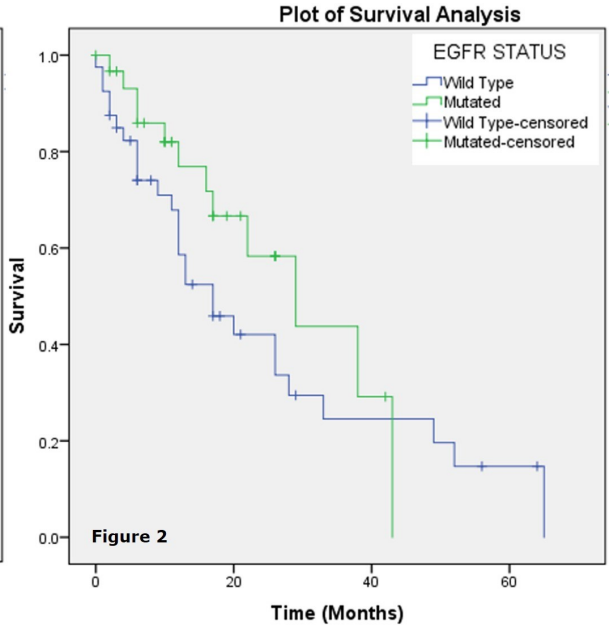

Figure 2: Kaplan Meier curve showing Overall Survival times between patients harboring *EGFR* mutation and *EGFR* wild type.

tients with NSCLC regardless of gender or smoking status.

Our results also demonstrated higher proportions of pleural and bone metastases in EGFR mutation cases compared to wild type (29.0% versus 17.5% and 45.2% versus 27.5% respectively). Consistently, other studies showed significantly higher rate of brain and pleural metastases in EGFR mutation cases (64.1% versus 35.9%, p=0.014 and 62.5% versus 43.6%, p=0.018 respectively).[16,17] These evidences allow us to infer that EGFR mutations may have contributed to disease progression and metastases in certain preferential sites.

Numerous studies have affirmed that EGFR TKI therapy yields superior outcomes when given to NSCLC patients with EGFR mutation. In fact, three landmark trials in 2004 showed significant responses (65-90.0%) of EGFR mutation cases with Gefitinib or Erlotinib.[10] Consistently, 65.2% of our patients showed partial response and 30.4% had stable disease upon receiving first line TKI. Results of our study therefore further support the benefits of EGFR TKI therapy in NSCLC patients with EGFR mutation in Brunei Darussalam.

Additionally, seven phase II prospective studies on first generation TKIs in NSCLC patients with EGFR mutation have demonstrated a PFS ranging from 7.7 to 14 months.[18] However, the median PFS of our study (seven months) was lower than previously reported by other authors. This may be due to our small study sample size of EGFR mutation NSCLC patients and certain unidentified biological factors within the Bruneian community that could render EGFR TKI therapies less effective. Therefore, this warrants further studies to explore the resistance mechanisms to EGFR TKI in our patients.

Evidences also suggest that certain mutations (Exon 19 deletions) have better survival outcomes compared to patients with other mutations.[19] Jackman et al. reported significantly improved PFS (24 versus 10 months, p=0.040) and OS (38 versus 17 months, p=0.039) for patients with Exon 19 deletions compared to patients with L858R mutations.[19] However, our study showed no difference in PFS for both groups (log rank test p=0.142). This again may be due to our small sample size (10 cases of L858R mutation vs eight cases of Exon 19 deletion), thus minor changes in disease prognosis can cause large fluctuations in the overall PFS.

Acquired resistance to EGFR TKIs is an inevitable and major problem in the clinical settings. Point mutation in Exon 20 that substitutes Threonine to Methionine (T790M) has accounted for resistance in greater than 50.0% of initially EGFR TKI-sensitive NSCLCs.[20] Therefore, it is crucial to detect secondary resistance mechanisms to the initial TKIs offered as studies showed a response rate of 61.0% in patients with acquired T790M mutation when treated with Osimertinib, a third generation EGFR TKI.[21] Currently, we do not have data on acquired T790M mutation in our cohort but this can be further explored in future studies.

Furthermore, we found non-significant OS times between EGFR mutation and EGFR wild type cases (log rank test p=0.232). Similar studies conducted by researches in Taiwan and Korea have also reported similar results (p=0.450 and p=0.390 respectively).[19] However, one particular study has contradicted EGFR mutation as a significant factor in influencing the survival time although a significantly higher OS was found in EGFR mutation cases (P=0.014).[19] This may be due to factors such as co-morbidities, distant metastases and the stage of cancer which could increase mortality rate.

Limitations

Our study unavoidably had selection bias as not all lung cancer patients were referred to TBCC nor sent for EGFR mutation analysis. Retrospective nature of our study design may also lead to information bias. A small sample size is another limitation. Furthermore, we were unable to include response rate of all EGFR mutations towards EGFR TKI therapy due to the insufficient number to draw appropriate conclusions. This could be the topic of ongoing studies locally. A follow-up study could also evaluate the rate of re-biopsy of tumour specimen to detect T790M mutation for patients who have progressed on initial EGFR TKI therapy.

CONCLUSIONS

This study characterizes the phenotypes of patients harbouring NSCLC EGFR mutations in Brunei Darussalam. The prevalence of EGFR mutations was found to be high (43.7%), thus further supporting EGFR mutation screening in all patients with NSCLC. Although our results were comparable to other studies conducted in Asian patients, the PFS rate on EGFR TKI observed locally was inferior to published studies thus warrants further exploration. Nevertheless, our study confirms the role of EGFR mutation as a predictive marker to identify patients who will potentially benefit from a personalized treatment approach with EGFR TKI therapy.

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DISCLOSURE

All authors have made substantial contribution to the manuscript. The authors do not declare any financial or conflicts of interest in preparing this manuscript.

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