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Original Article

Spectrum of rare *EGFR* mutations in Indonesian lung adenocarcinoma: Findings from an 8-year analysis of 4,778 cases highlighting the need for advanced targeted therapies

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Abstract

Lung cancer patients in Indonesia exhibit a high prevalence of epidermal growth factor receptor (*EGFR*) mutations, with a substantial proportion attributed to rare or uncommon variants. The clinical significance of rare *EGFR* mutations lies in their differential sensitivity to tyrosine kinase inhibitors (TKIs). While they are frequently resistant to first- and second- generation TKIs, they often respond to third-generation TKIs, necessitating tailored treatment options. The need for improving access to advanced targeted therapies in Indonesia also highlights the importance of conducting research on rare *EGFR* mutations. The aim of this study was to identify the spectrum and frequency of *EGFR* mutations in patients with lung adenocarcinoma in Indonesia. A cross-sectional observational study with total sampling was conducted from January 2016 to April 2024 to investigate *EGFR* mutation profiles in lung adenocarcinoma patients. Samples were acquired from patients with a confirmed anatomical pathology diagnosis from various healthcare centers across Indonesia. A total of 4,778 samples were analyzed using real-time quantitative polymerase chain reaction (RT-qPCR) on various specimen types to determine *EGFR* mutation prevalence and patterns. Associations between demographic data and *EGFR* mutation status were assessed. *EGFR* mutations were detected in 54.6% of samples, with common mutations (exon 19 deletions/insertions and point mutation L858R) comprising 76.2% of positive cases and rare mutations (exon 20 insertions, point mutation G719X, S768I, T790M, and L861Q) accounted for 20.3%. Significant

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associations were found between geographic origin, age, and sex with *EGFR* mutation status. This study confirms substantial genetic variability and geographical differences in *EGFR* mutations among Indonesian lung adenocarcinoma patients, emphasizing the urgent need for further research to prompt enhanced molecular diagnostics and targeted therapies in the region.

Keywords: Lung cancer, lung adenocarcinoma, genomics, rare mutation, *EGFR*

Introduction

Lung cancer, particularly non-small cell lung cancer (NSCLC), is a significant global health issue [1-3]. Indonesia, a developing nation and the world's largest archipelago with over 17,000 islands, is the fourth most populous country and the fifth largest in Asia [4]. Indonesia experiences particularly high incidence and mortality rates of lung cancer [3]. About 85% of lung cancers are NSCLC, and adenocarcinoma constitutes approximately 40–50% of all lung cancer cases [5-7], highlighting its significant contribution to the overall lung cancer burden.

NSCLC patients who are women, non-smokers, or light smokers predominantly exhibit mutations in the epidermal growth factor receptor (*EGFR*) gene, with a higher mutation rate in the Asian population (30–40%) compared to the Western population (10–15%) [8-12]. Previous studies in Indonesia, comprising approximately a combined 3,000 samples, showed an overall *EGFR* mutation rate of 34–67%, consistent with the data of a high mutation rate in the Asian population [13-17]. Mutations in *EGFR* gene are primarily located in the kinase domain, encompassing exons 18 to 21, leading to constitutive downstream EGFR signaling [18]. This results in cellular proliferation and tumorigenesis [19]. Different mutations within this domain have distinct structural impacts and functional consequences, particularly concerning kinase domain activation and drug binding [20]. Previous studies have shown that common or classical *EGFR* mutations, such as exon 19 deletions/insertions (ex19del/ins) and the L858R point mutation in exon 21, account for up to 90% of newly diagnosed *EGFR*-positive NSCLC and are predictive of response to the first-generation of *EGFR* tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib [21-23]. These mutations are also referred to as "common" *EGFR* mutations [19].

Uncommon or "rare" mutations within the kinase domain (such as exon 18 G719S/A/C, collectively known as G719X; exon 20 insertion (ex20ins), T790M, and S768I; exon 21 L861Q are also oncogenic, comprising 10–15% of all *EGFR* mutations [24-26]. Additionally, some patients exhibit complex mutations, involving either two rare mutations or a combination of common and rare mutations [27]. These rare *EGFR* mutations are clinically significant because they often have variable sensitivities to *EGFR* TKIs, with some showing resistance or reduced effectiveness to first- and second- generation TKIs (classical), while others can be effectively treated with third-generation TKIs such as osimertinib [19,28-30]. The T790M point mutation in exon 20 typically develops as an acquired resistance mutation to classical TKIs but remains sensitive to third-generation TKIs [22,26]. Reports of *de novo* T790M mutations exist, despite their rare occurrence [31]. *EGFR* ex20ins mutations represent another subset of *EGFR* mutations, accounting for approximately 10% of all NSCLCs, and are resistant to classical *EGFR* TKIs [32]. The scarcity of data on the prevalence and characteristics of rare *EGFR* mutations in Indonesian patients hinders the application of better treatment protocols for this subgroup [33]. Although there are studies on rare *EGFR* mutations in Indonesia, the number of cases examined is still limited, leaving a gap in the existing data [14,16]

Recent expert consensus recommendations in Asia encourage rare *EGFR* mutation detection owing to the higher prevalence of *EGFR* mutations in the Asian population [22,26,34]. Updated national guidelines for medical services and lung cancer management in Indonesia have recommended newer *EGFR* TKIs for advanced NSCLC treatment [33]. However, real-world accessibility remains limited [4]. Less than a dozen *EGFR* TKIs are available in Indonesia, but as of 2023, Indonesian national health insurance covers only a few targeted therapies, such as gefitinib, erlotinib, and afatinib, making them accessible to a broader patient population [4]. Advanced targeted therapies such as amivantamab, recently approved by the US Food and Drug Administration (FDA) for *EGFR* ex20ins [35], may take longer to become accessible in developing

countries such as Indonesia due to limited data, regulatory, logistical, and economic challenges. By providing comprehensive data on *EGFR* mutations in Indonesia, this study aims to lay the groundwork for broader access to targeted therapies, which is essential to improve outcomes in this understudied group of rare mutations.

Considering the limited research on rare *EGFR* mutations in Indonesia, the aim of this study was to identify the spectrum and frequency of *EGFR* mutations among patients with lung adenocarcinoma. This study highlights the urgent need for broader access to advanced targeted therapies by emphasizing the underreported rare mutations. This study may serve as a foundation for future research and healthcare policies, encouraging investment in the development and distribution of novel therapeutic options tailored to the genetic profiles of the Indonesian population.

Methods

Study design and setting

A cross-sectional observational study was conducted from January 2016 to April 2024 to analyze *EGFR* mutation profiles in patients with lung adenocarcinoma. A total of 4,778 samples were collected using total sampling. Molecular analyses were performed at the Department of Anatomical Pathology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia and the Department of Anatomical Pathology, Dr. Sardjito Hospital, Yogyakarta, Indonesia. The study variables included patient demographics (city of origin, age, sex), specimen types (trans-thoracic needle aspiration (TTNA) cytology, formalin-fixed paraffin-embedded (FFPE) blocks, blood or circulating tumor deoxyribonucleic acid (ctDNA), and pleural fluid), and *EGFR* mutation status, categorized into single common, single rare, and compound/complex mutations, as well as TKI-sensitive and TKI-resistant mutations.

Sample size and sampling method

To ensure statistical rigor, sample size calculations were conducted using a single-proportion formula for qualitative variables in a cross-sectional study [36]. The calculations were based on a 95% confidence interval and a 5% margin of error. Previous data indicated that 45% of lung adenocarcinoma patients exhibited *EGFR* mutations, with 15% classified as rare mutations and 85% as common mutations [6,8-10]. These estimates determined a minimum sample size of 100 for rare mutations and 363 for common mutations. To ensure adequate representation of both categories, a total of 363 samples were required. The study employed total sampling, including all eligible patients during the study period.

Eligibility criteria

Inclusion criteria included samples obtained from patients with a confirmed diagnosis of lung adenocarcinoma, as determined by anatomical pathology based on the World Health Organization (WHO) morphology and tumor classification by the International Agency for Research on Cancer (IARC) [37]. To ensure accurate *EGFR* mutation detection, tumor cellularity thresholds were applied as follows: only TTNA and FFPE specimens containing 50% or more tumor cell content, assessed microscopically by pathologist on hematoxylin-eosin stained sections, were included [38,39]. For blood/ctDNA, samples were included if they met pre-analytical quality controls, including a minimum peripheral blood volume of 4 mL for blood-derived ctDNA processing [40]. Sufficient DNA yield (≥5 ng/µL) is needed for blood/ctDNA and pleural fluids to be included in the analysis [40]. Exclusion criteria included samples with incomplete demographic information, such as missing data on city of origin, age, or sex. Samples were also excluded due to unclear histopathology or cytopathology records, DNA degradation in archival samples, or insufficient tumor cell/DNA content.

Samples collection

Histopathological samples were collected by pulmonologists from various healthcare facilities across Indonesia and subsequently sent to Dr. Sardjito Hospital, Yogyakarta, Indonesia. The sample types examined were TTNA cytology, FFPE blocks, blood/ctDNA samples, and pleural fluid. FFPE blocks were prepared from cell blocks or core needle biopsies when available. Each

patient contributed only one type of sample. The samples originated from patients across five major islands and several regions in Indonesia, including Java, Sumatra, the Riau Islands, Bangka Belitung, Borneo, Celebes, Papua, Bali, and Timor Island.

TTNA cytology procedures were performed under local anesthesia and guided by multi-slice computed tomography (MSCT) or ultrasound to accurately target lesions. Trained pulmonologists utilized fine needles (22G or 25G) to aspirate cellular material into a syringe, with core biopsy needles employed when more substantial tissue samples were required. The aspirated material was processed by preparing smears on glass slides, which were immediately air-dried for subsequent staining, and preserved as cell blocks for ancillary studies such as immunocytochemistry or molecular testing.

FFPE blocks were prepared from cell blocks derived from TTNA aspirates and core needle biopsy samples. The samples were fixed in 10% neutral buffered formalin for six to twelve hours, followed by processing through graded alcohol and xylene, and finally embedded in paraffin using tissue processors and paraffin embedding systems per protocol. This procedure was performed by trained pathology technicians to ensure consistent preparation.

For specimens other than blood/ctDNA or pleural fluid, the diagnosis of lung adenocarcinoma was reconfirmed at Dr. Sardjito Hospital, Yogyakarta, Indonesia, by two independent consultant pathologists specializing in respiratory pathology. In cases where only blood/ctDNA or pleural fluid specimens were available, confirmation was based on prior assessments conducted by pathologists at the originating healthcare centers. Molecular analyses of the collected samples were performed at Dr. Sardjito Hospital, Yogyakarta, Indonesia. Additionally, medical records were retrospectively reviewed to obtain demographic data, including city of origin, age, and sex, as well as information on specimen types.

DNA extraction

Total DNA was extracted from a single available specimen for each patient, which included TTNA cytology, FFPE blocks, blood/ctDNA, or pleural fluid samples. Extractions were performed manually in accordance with the manufacturer's protocols prior to *EGFR* mutation analysis. DNA extraction for TTNA cytology, FFPE blocks, and pleural fluid specimens utilized the Tissue Genomic DNA Mini Kit (GeneAll Clinic SV mini, Seoul, Korea), whereas blood/ctDNA samples were processed using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). To ensure contamination-free procedures, all steps were conducted within dedicated workstations with regular decontamination, and each sample was handled individually to prevent cross-contamination. To minimize DNA degradation, aliquoting was employed to avoid multiple freeze-thaw cycles. Extracted DNA was primarily used for *EGFR* mutation analysis, with any surplus stored at temperatures ranging from -20°C to -80°C to preserve integrity for potential future analyses. The quantity and quality of the extracted DNA were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA) before proceeding to *EGFR* mutation evaluation.

EGFR mutation evaluation with real-time quantitative polymerase chain reaction (RT-qPCR)

DNA samples were analyzed using the AmoyDx Human *EGFR* 29 Gene Mutations Fluorescence PCR Diagnostic Kit (Amoy Diagnostics, Xiamen, China), which detects T790M, L858R, L861Q, S768I, G719S, G719A, G719C, three insertions in exon 20, and 19 deletions/insertions in exon 19 (**Table 1**). The kit, following the manufacturer's protocol, is capable of detecting mutant DNA at a minimum threshold of 1%. Real-time quantitative polymerase chain reaction (RT-qPCR) was conducted using the Bioneer Exicycler 96 Real-Time Quantitative Thermal Block (Bioneer Corporation, Daejeon, Korea), adhering to the manufacturer's instructions. The cycle threshold (Ct) value, defined in the AmoyDx kit protocol as the point at which the fluorescence signal exceeds the background level, was used to determine *EGFR* mutation status. Samples were classified as positive (mutated) if the amplification curve crossed the specified Ct value. Samples with no amplification beyond the cutoff were classified as negative (wild-type).

Study variables

This study investigates the presence and types of *EGFR* mutation profiles in Indonesian patients with lung adenocarcinoma and explores associations with demographic characteristics. *EGFR* mutations were identified using the AmoyDx Human *EGFR* 29 Gene Mutations Fluorescence PCR Diagnostic Kit (Amoy Diagnostics, Xiamen, China) and categorized into three groups: common mutations (ex19del/ins, exon 21 L858R), rare mutations (exon 18 G719X, ex20ins, exon 20 S768I, exon 20 T790M, and exon 21 L861Q), and compound/complex mutations involving multiple subtypes (**Table 1**). Mutation frequencies were expressed as the number of samples (n) and percentages (%) for the overall cohort and among patients positive for *EGFR* mutations. Demographic data, including age, sex, and city of origin, were obtained from medical records. Age was stratified into two categories: below 60 years and 60 years or older, with the mean age reported as mean ± standard deviation (SD) or median (range). Sex was recorded as male or female. Cities of origin were grouped into major regions based on geographic location (e.g., Java Island, Sumatra Island, Riau Islands, Bangka Belitung Islands, Borneo Island, Celebes Island, Papua Island, Bali Island, Timor Island). Demographic variables were presented as the number of samples (n) and percentages (%). Data on specimen types, including TTNA cytology, FFPE blocks, blood/ctDNA, and pleural fluid, were retrieved from medical records, with distributions reported as the number of samples (n) and percentages (%). Descriptive statistics were employed for data analysis, presenting frequencies and percentages for categorical variables and mean±SD or median (range) for continuous variables.

Table 1. Epidermal growth factor receptor (*EGFR*) gene detected and categories for this study

EGFR mutation category	Exon and mutation type	Mutation detail
Common *EGFR* mutations - classical TKI sensitive	*EGFR* exon 19 deletions/insertions (ex19del/ins)	E746_A750del, E746_T751del, L747_E749del, L747_T751del, L747_S52 del, E746_T751>I, L747_P753>S, L746_T751>I/A, L746_S752>A/V/D, L747_A750>P, L747_P753>S/Q, L747_T751>S/P
	EGFR exon 21	L858R
Rare *EGFR* mutations - classical TKI sensitive	*EGFR* exon 18 (G719X)	G719A, G719S, G719C
	EGFR exon 20	S768I
	EGFR exon 21	L861Q
Rare *EGFR* mutations - classical TKI resistant	*EGFR* exon 20 insertions (ex20ins)	H773_V774insH, D770_N771insG, V769_D770insASV
	EGFR exon 20	T790M

TKI: tyrosine kinase inhibitor

Statistical analysis

Statistical analysis was conducted using Microsoft Excel v.2021 (Microsoft 365, Washington, USA) and SPSS 29.0 (IBM, New York, USA). Categorical data were presented as frequencies and percentages, while continuous data were initially assessed for normality using the Kolmogorov-Smirnov test. Normally distributed data were expressed as mean±SD; non-normally distributed data were reported as median (range). Chi-squared test was used to assess associations between demographic variables (city of origin, age category, sex) and *EGFR* mutation status, with a $p<0.050$ considered statistically significant. Pie charts were generated using Microsoft Excel v.2021 (Microsoft, Washington, USA) to visually illustrate the distribution of *EGFR* mutation types and subtypes.

Results

Patient demographics and specimen-tested characteristics

A total of 4,778 samples were initially collected for the study. Following the application of the eligibility criteria, 299 samples were excluded, resulting in 4,479 samples that were successfully included in the analysis. The demographic and specimen details are presented in **Table 2**. The samples were obtained from five major islands and various regions across Indonesia, with 71.6% from Java; 14.0% from Sumatra, the Riau Islands, and Bangka Belitung (representing western Indonesia); 3.4% from Borneo; and 11.0% from Celebes, Papua, Bali, and Timor (representing eastern Indonesia). The mean age of the patients was 59.15±11.67 years. Patients were categorized

into two age groups: <60 years (49.9%) and ≥60 years (50.1%). The cohort consisted predominantly of males (57.0%, n=2,553) and followed by females (43.0%, n=1,926). The types of DNA materials extracted for *EGFR* mutation analysis were predominantly TTNA cytology (88.9%), followed by FFPE blocks (9.5%), blood/ctDNA (1.5%), and pleural fluid (<1%).

Table 2. Demographic characteristics and specimen types of the included patients (n=4,479)

Characteristics	Frequency (percentage)
Region	
Java Island	3,207 (71.6)
Sumatra Island, Riau Islands, Bangka Belitung Islands	626 (14.0)
Borneo Island	152 (3.4)
Celebes Island, Papua Island, Bali Island, Timor Island	494 (11.0)
Age (years), mean ± standard deviation	59.15±11.67
<60	2,233 (49.9)
≥60	2,246 (50.1)
Sex	
Male	2,553 (57.0)
Female	1,926 (43.0)
Specimen types	
Trans-thoracic needle aspiration (TTNA) cytology	3,982 (88.9)
Formalin-fixed paraffin-embedded (FFPE) blocks	426 (9.5)
Blood/circulating tumor deoxyribonucleic acid (ctDNA)	69 (1.5)
Pleural fluid	2 (0.0)

EGFR mutation profiles and frequencies in lung adenocarcinoma patients

The overall *EGFR* mutation frequency was 54.6% (n=2,445/4,479), with 52.1% (n=2,333/4,479) exhibiting a single mutation and 2.5% (n=112/4,479) displaying complex mutations involving multiple subtypes (**Table 3**). The most prevalent mutations were exon 19 deletions/insertions (ex19del/ins) and exon 21 (L858R), accounting for 41.6% (n=1,863/4,479) of the entire cohort and 76.2% (n=1,863/2,445) of *EGFR* mutation-positive patients. Rare mutations, including exon 18 (G719X), exon 20 insertions (ex20ins), exon 20 (S768I), exon 20 (T790M), and exon 21 (L861Q), were identified in 11.1% (n=497/4,479) of the total cohort and 20.3% (n=497/2,445) of *EGFR* mutation-positive patients. A combination of common and rare mutations was detected in 1.9% (n=85/4,479) of the total cohort and 3.5% (n=85/2,445) of *EGFR* mutation-positive patients.

Among mutation-positive patients, 95.4% exhibited a single mutation (**Table 3**). The majority of these mutations were common TKI-sensitive mutations, with exon 19 deletions/insertions (ex19del/ins) being the most frequent (52.4%), followed by exon 21 L858R mutations (23.6%). Rare TKI-sensitive mutations included exon 18 G719X (6.9%), exon 21 L861Q (2.2%), and exon 20 S768I (1.7%). Classical TKI-resistant mutations were identified in exon 20 insertions (ex20ins) (4.2%) and T790M (4.3%). Complex mutations predominantly involved a combination of TKI-sensitive mutations and T790M, observed in 2.5% of mutation-positive patients (**Table 3**).

The distribution of *EGFR* mutations among mutation-positive patients (n=2,445) showed that the majority (76.01%) had a single common mutation in either ex19del/ins or L858R, followed by 19.3% with rare single mutations in G719X, ex20ins, S768I, T790M, or L861Q, and 4.6% with complex mutations (**Figure 1A**). Most mutations in the mutation-positive group were TKI-sensitive (87.7%) (**Figure 1B**). Among the single mutation group, the most common mutations were ex19del/ins (55.0%), L858R (24.8%), G719X (7.2%), T790M (4.5%), ex20ins (4.4%), L861Q (2.3%), and S768I (1.8%) (**Figure 1C**). In the rare mutations categories (n=585), 19.1% had complex mutations. The most prevalent rare mutations were G719X (28.9%), followed by T790M (18.1%), ex20ins (17.4%), L861Q (9.2%), and S768I (7.2%) (**Figure 1D**).

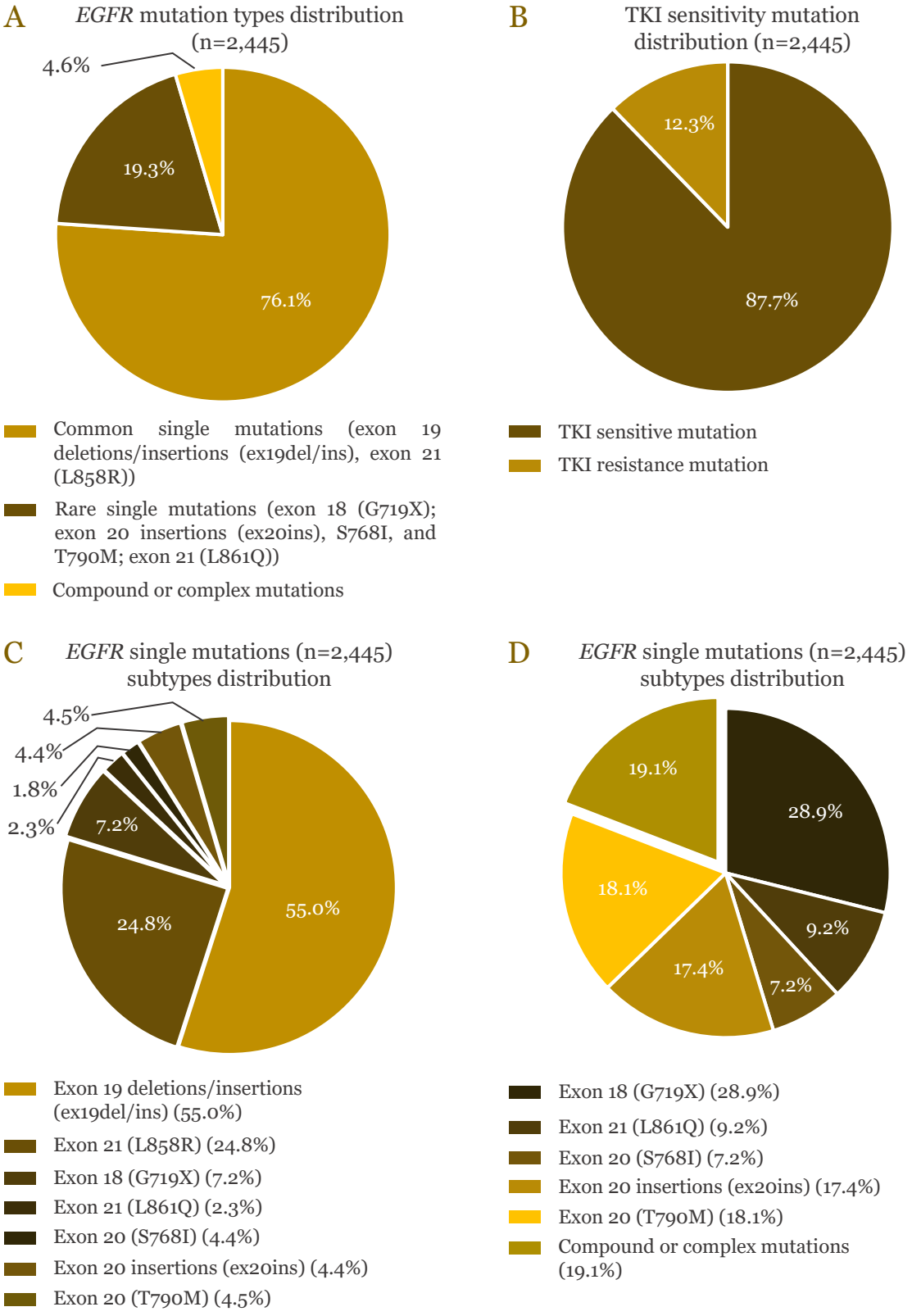

Figure 1. Pie charts illustrating the distribution of epidermal growth factor receptor (*EGFR*) mutation-positive cases in lung adenocarcinoma patients. (A) Distribution of common single mutations (exon 19 deletions/insertions (ex19del/ins) and exon 21 L858R) compared to rare single mutations (exon 18 G719X; exon 20 insertions (ex20ins), S768I, and T790M; exon 21 L861Q) and complex mutations; (B) proportion of classical tyrosine kinase inhibitor (TKI)-sensitive mutations versus TKI-resistant mutations, encompassing both single and complex mutations; (C) subtype distribution within single mutation samples; and (D) distribution of rare *EGFR* mutation subtypes, including rare complex mutations observed in lung adenocarcinoma.

Table 3. Distribution of epidermal growth factor receptor (*EGFR*) mutation profiles (types and subtypes) and frequencies in all lung adenocarcinoma patients (n=4,479) and *EGFR* mutation-positive patients (n=2,445)

EGFR mutation types and subtypes	Mutation frequency per all patients (n=4,479)	Mutation frequency per *EGFR* mutation-positive patients (n=2,445)
	n (%)	n (%)
Total *EGFR* positive mutation	2,445 (54.6)	2,445 (100)
Common mutations (Exon 19 deletions/insertions, Exon 21 L858R)	1,863 (41.6)	1,863 (76.2)
Rare mutations (Exon 18 G719X; Exon 20 insertions, S768I, and T790M; Exon 21 L861Q)	497 (11.1)	497 (20.3)
Mixture of common and rare mutations	85 (1.9)	85 (3.5)
EGFR single mutations	2,333 (52.1)	2,333 (95.4)
Common mutations; TKI-sensitive		
Exon 19 deletions/insertions (ex19del/ins)	1,282 (28.6)	1,282 (52.4)
Exon 21 (L858R)	578 (12.9)	578 (23.6)
Rare mutations; TKI-sensitive		
Exon 18 (G719X)	169 (3.8)	169 (6.9)
Exon 21 (L861Q)	54 (1.2)	54 (2.2)
Exon 20 (S768I)	42 (0.9)	42 (1.7)
Rare mutations; classical TKI-resistant		
Exon 20 insertions (ex20ins)	102 (2.3)	102 (4.2)
Exon 20 (T790M)	106 (2.4)	106 (4.3)
EGFR compound or complex mutations	112 (2.5)	112 (4.6)
Common TKI-sensitive (Exon 19 deletions/insertions and L858R)	3 (0.1)	3 (0.1)
Rare TKI-sensitive (G719X and L861Q; G719X and S768I; L861Q and S768I)	5 (0.1)	5 (0.2)
Common and rare TKI-sensitive	12 (0.3)	12 (0.5)
TKI-sensitive and resistant (T790M)	62 (1.4)	62 (2.5)
TKI-sensitive and resistant (Exon 20 insertions)	21 (0.5)	21 (0.8)
TKI-resistant (Exon 20 insertions and T790M)	9 (0.2)	9 (0.4)

TKI: tyrosine kinase inhibitor

Association of *EGFR* mutations with demographic characteristics and mutation frequencies in lung adenocarcinoma specimens

EGFR mutation status demonstrated significant associations with various demographic characteristics and specimen types (**Table 4**). Mutations were identified in 56.8% of samples from Java, 47.3% from Sumatra, Riau Islands, and Bangka Belitung, 48.0% from Borneo, and 51.2% from Celebes, Papua, Bali, and Timor ($p<0.001$). The age group was significantly correlated with mutation status, with mutations observed in 50.2% of patients under 60 years and 59.0% of those 60 years or older ($p<0.001$). The mutation rate was higher in females compared to males (62.5% vs 48.6%, $p<0.001$). Specimen types also showed variation in mutation frequency: blood/ctDNA samples exhibited the highest rate (73.9%), followed by TTNA cytology (54.8%), pleural fluid (50.0%), and FFPE blocks (49.8%). Common mutations associated with first-generation TKI-sensitive therapies (ex19del/ins and L858R) were more prevalent in females (51.1%) than males (48.9%). In contrast, rare TKI-sensitive mutations (G719X, L861Q, and S768I) and classical TKI-resistant mutations (T790M and ex20ins) were more frequently observed in males (57.0%) than females (43.0%).

Table 4. Association between epidermal growth factor receptor (*EGFR*) mutation status, demographic characteristics, and mutation frequency across specimen types in lung adenocarcinoma patients

Variables	Total (n)	*EGFR* mutations		*p*-value*	Common mutations[a]	Rare mutations[b]	
		Wild-type	Mutation		TKI-sensitive	TKI-sensitive	TKI-resistant
		n (%)	n (%)		n (%)	n (%)	n (%)
Overall (n)	4,479	2,034	2,445		1,875	270	300
Major islands of Indonesia				<0.001			
Java Island	3,207	1,384 (43.2)	1,823 (56.8)		1,424 (75.9)	192 (71.1)	207 (69.0)

Variables	Total (n)	*EGFR* mutations		*p*-value*	Common mutations[a]	Rare mutations[b]	
		Wild-type	Mutation		TKI-sensitive	TKI-sensitive	TKI-resistant
		n (%)	n (%)		n (%)	n (%)	n (%)
Sumatra Island, Riau Islands, Bangka Belitung Islands	626	330 (52.7)	296 (47.3)		205 (10.9)	46 (17.0)	45 (15.0)
Borneo Island	152	79 (52.0)	73 (48.0)		61 (3.3)	5 (1.9)	7 (2.3)
Celebes Island, Papua Island, Bali Island, Timor Island	494	241 (48.8)	253 (51.2)		185 (9.9)	27 (10.0)	41 (13.7)
Age (years)				<0.001			
<60	2,233	1,113 (49.8)	1,120 (50.2)		865 (46.1)	112 (41.5)	143 (47.7)
≥60	2,246	921 (41.0)	1,325 (59.0)		1,010 (53.9)	158 (58.5)	157 (52.3)
Sex				<0.001			
Male	2,553	1,312 (51.4)	1,241 (48.6)		916 (48.9)	154 (57.0)	171 (57.0)
Female	1,926	722 (37.5)	1,204 (62.5)		959 (51.1)	116 (43.0)	129 (43.0)
Specimen types							
TTNA cytology	3,982	1,801 (45.2)	2,181 (54.8)		1,677 (89.4)	237 (87.8)	267 (89.0)
FFPE blocks	426	214 (50.2)	212 (49.8)		169 (9.0)	27 (10.0)	16 (5.3)
Blood/ctDNA	69	18 (26.1)	51 (73.9)		28 (1.5)	6 (2.2)	17 (5.7)
Pleural fluid	2	1 (50.0)	1 (50.0)		1 (0.1)	0 (0.0)	0 (0.0)

ctDNA: circulating tumor deoxyribonucleic acid; *EGFR*: epidermal growth factor receptor; FFPE: formalin-fixed paraffin-embedded; TKI: tyrosine kinase inhibitor; TTNA: trans-thoracic needle aspiration
[a]Common mutations refer to exon 19 deletions/insertions and exon 21 (L858R), both of which are sensitive to TKI therapy
[b]Rare mutations include single or complex mutations involving exon 18 (G719X), exon 20 (S768I), and exon 21 (L861Q), excluding common mutations, exon 20 insertions, or exon 20 (T790M) in the TKI-sensitive category. The classical TKI-resistant category encompasses samples positive for exon 20 insertions and/or exon 20 (T790M)
*Analyzed using Chi-squared test

Discussion

This study successfully compiled and analyzed one of the largest cohorts of lung adenocarcinoma samples in Indonesia (n=4,479). The overall *EGFR* mutation rate in this population (54.3%) falls within the range observed in previous studies conducted with smaller sample sizes in Indonesia (35–64%, combined total of 2,975 samples) [13-17,41]. This prevalence is also consistent with the *EGFR* mutation rates reported across Asia (30–60% in unselected populations, with the lowest in India and the highest in Vietnam) [8,9,42-44]. A higher mutation rate was observed in female compared to male patients (62.5% vs 48.6%, $p<0.001$), aligning with previous studies [8-12]. The majority of *EGFR* mutations were detected as single mutations (95.4%), with only a small proportion (4.6%) harboring complex mutations, representing 2.5% of all samples. This detection rate is notably lower than that reported in earlier studies in Indonesia, where complex mutations were found in up to 20% of *EGFR*-mutated patients, or 10% of all NSCLC cases [14,16]. In comparison, complex mutation rates in other countries have ranged from 0.95% to 15% of all NSCLC cases [28,45,46]. Most complex mutation variants involved both TKI-sensitive and TKI-resistant mutations, such as T790M and ex20ins, which are linked to poor clinical outcomes [45,47,48]. The lower detection rate of complex mutations in this study may be attributed to differences in testing methods (with next generation sequencing (NGS) detecting more mutations than commercial kits), as well as variations in patient populations (e.g., staging at the time of testing), which may introduce bias in comparisons [8,46].

EGFR mutations in NSCLC exhibit significant genetic heterogeneity, encompassing more than 200 distinct mutations [49]. The majority (85–90%) of these mutations are classified as common or "classical," specifically ex19del/ins and L858R, while the remaining 10–15% consist of rare mutations, including ex20ins, G719X, L861Q, S768I, and T790M [19,49,50]. In this study, 76.2% of *EGFR*-mutated patients harbored common mutations, 20.3% had rare mutations, and the remaining patients exhibited a combination of both. Further analysis revealed a higher prevalence of rare *EGFR* mutations among male patients, which aligned with previous studies reporting that rare *EGFR* mutations were more common in males and smokers [14,17,51-56]. Although this study did not characterize smoking status, it is noteworthy that a significant

proportion of Indonesian males are smokers; 54.4–67.0% of males and only 2.7% of females in Indonesia are daily smokers, with males being 30 times more likely to smoke than females, positioning Indonesia as the third highest in the world for smoking prevalence [3,57]. Smoking is associated with an increased somatic mutation burden and heightened apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like (APOBEC) activity, which may contribute to the higher frequency of complex and rare *EGFR* mutations in lung cancer [56]. Consequently, the high smoking prevalence in Indonesia could potentially influence *EGFR* mutation rates, though further research is required to substantiate this hypothesis. While passive smoking remains a concern, previous studies have suggested that it does not correlate with *EGFR* mutations in lung cancer patients who have never smoked [58,59].

Previous studies in Indonesia have reported varying prevalences of rare *EGFR* mutations. A study involving 1,874 cytological specimens from newly diagnosed NSCLC patients identified rare mutations in up to 29.0% of *EGFR*-mutated patients or 12.9% of all patients [14]. Similarly, a study of 627 tissue biopsy and 80 ctDNA samples reported that approximately 13% of NSCLC samples harbored rare *EGFR* mutations [16]. Both studies were conducted on Java Island and did not specify the geographic origin of all samples [14,16]. In contrast to these earlier studies, which did not fully specify sample geographic origin or included multiple NSCLC subtypes, our study specifically focused on lung adenocarcinoma and encompassed a larger sample size than previous published Indonesian reports combined [13-17]. While focusing exclusively on lung adenocarcinoma may have contributed to the higher prevalence of rare mutations observed, compared to broader NSCLC studies [60], this study's extensive sample size enhances its novelty and underscores its potential to impact future research in Indonesia.

Interestingly, the rate of ex20ins, a well-established TKI-resistant rare mutation, observed in this study is notably higher than in recent Indonesian studies [14,16]. Specifically, ex20ins was identified in 2.3% of all lung adenocarcinoma samples and 4.2% of *EGFR*-mutated samples in this cohort, compared to less than 1% in both all NSCLC and *EGFR*-mutated patients in previous studies [14,16]. Globally, ex20ins is recognized as the third most common *EGFR* mutation in lung cancer, with reported frequencies reaching up to 10%, and a 4% rate being commonly documented [10,32,61,62]. These findings highlight the importance of this mutation, which has not been reported at such a high frequency in the Indonesian population.

The increased prevalence of ex20ins mutations carries significant therapeutic implications. Amivantamab, a bispecific antibody targeting both EGFR and mesenchymal-epithelial transition (MET) receptors, has been approved by the FDA for the treatment of NSCLC patients with ex20ins mutations [35,63,64]. This novel therapy effectively addresses resistance associated with ex20ins mutations, which are typically refractory to classical *EGFR* TKIs. The higher incidence of ex20ins mutations in the Indonesian population underscores the potential benefit of incorporating amivantamab into national treatment guidelines, potentially improving clinical outcomes for Indonesian patients [33].

This study included 70% of patients from Java Island and 30% from other regions across Indonesia, with *EGFR* mutation status significantly associated with sample origin ($p<0.001$). A higher proportion of mutations was observed in Java and eastern regions (Celebes, Papua, Bali, Timor Islands) compared to western regions (Sumatra, Riau Islands, Borneo) ($p<0.001$). Indonesia's diverse ethnicities, which include over 633 ethnic groups and more than 1,000 distinct ethnicities [65], may influence *EGFR* mutation prevalence [42,66], as indicated by previous studies on genetic variations, such as polymorphisms in EDAR370A and ABCC11G180A genes [67]. Similarly, in India, an ethnically diverse country, regional variations in *EGFR* mutation rates have been observed, with rates ranging from 16% to 43% between South and North India [68]. These variations highlighted the influence of environmental, geographical, ethnic, and lifestyle factors on genetic alterations [69]. Additionally, a study has shown that exposure to air pollution may promote the development of lung adenocarcinoma in preexisting *EGFR*-mutated lung cells [70]. While geographic information was included in earlier Indonesian studies, no analysis of *EGFR* mutation distribution by region was conducted [14]. The lack of detailed ethnic data in this study limits further exploration of these variables.

A significant proportion of *EGFR* mutations (73.9%) were detected in blood/ctDNA samples, suggesting the potential of liquid biopsies for mutation detection in NSCLC, particularly when

tissue biopsy is not feasible [71-73]. However, it is important to note that only 1.5% of the total samples were derived from liquid biopsies, as cytology specimens were predominantly used for molecular testing in this study. While liquid biopsy offers a non-invasive and convenient option, it is highly specific but less sensitive in detecting *EGFR* mutations [74-77]. Liquid biopsies are particularly valuable for identifying secondary mutations, such as T790M, which often emerge after TKI treatment and contribute to therapy resistance in advanced NSCLC [75,76]. Incorporating liquid biopsy into clinical practice could enhance patient monitoring of minimal residual disease and facilitate timely adjustments to treatment strategies [78-80]. Ongoing studies, including those in Indonesia, continue to provide promising insights into the utility of liquid biopsies for *EGFR* mutation detection and disease monitoring in NSCLC [41,72,76,80-82].

This study provides valuable insights but has several limitations. The reliance on RT-qPCR instead of advanced molecular diagnostic techniques such as NGS may have led to underestimation or false negatives for *EGFR* single or complex mutations, particularly for low-abundance variants [46]. Technical limitations of RT-qPCR, including primer specificity and amplification efficiency, also need consideration. The absence of detailed ethnic data restricts a full understanding of genetic diversity and its impact on mutation rates across Indonesia. Future research incorporating more advanced molecular techniques, larger populations, and comprehensive demographic and clinicopathological data is needed to improve data quality. Nevertheless, this study highlights the need for targeted treatments, emphasizing the widespread and diverse *EGFR* mutations, including the high frequency of TKI-resistant ex20ins mutations. Enhanced molecular diagnostics and a broader range of TKIs could significantly improve clinical outcomes for lung cancer patients in Indonesia.

Conclusion

Mutations in the *EGFR* gene were identified in 54.6% of lung adenocarcinoma samples from Indonesia, with common mutations (exon 19 deletions/insertions and point mutation L858R) accounting for 76.2% and rare mutations (exon 20 insertions, point mutations G719X, S768I, T790M, L861Q) representing 20.3%. In this population, demographic characteristics, such as age and sex, were significantly associated with *EGFR* mutation status. This study highlights the age, sex, and geographical variability of *EGFR* mutations in a large cohort of lung adenocarcinoma patients, underscoring the need for personalized treatment strategies and advanced molecular diagnostics in Indonesia.

Ethics approval

The protocol of this study received approval from the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia (Approval number: KE/FK/1182/EC/2024). All research involving human participants and/or human tissue samples was conducted in full compliance with relevant ethical guidelines and regulations. Written informed consent was obtained from all participants prior to their inclusion in the study.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

This study used artificial intelligence (AI) tools and methodologies in the following capacities of which AI-based language model ChatGPT was employed in the language refinement (improving grammar, sentence structure, and readability of the manuscript). We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

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References

1. Zou K, Sun P, Huang H, *et al.* Etiology of lung cancer: Evidence from epidemiologic studies. J Natl Cancer Cent 2022;2(4):216-225.
2. Meyer ML, Peters S, Mok TS, *et al.* Lung cancer research and treatment: Global perspectives and strategic calls to action. Ann Oncol 2024;35(12):1088-1104.
3. Bray F, Laversanne M, Sung H, *et al.* Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024;74(3):229-263.
4. Asmara OD, Tenda ED, Singh G, *et al.* Lung cancer in Indonesia. J Thorac Oncol 2023;18(9):1134-1145.
5. Huang CY, Hsieh MJ, Wu WJ, *et al.* Association of endothelial nitric oxide synthase (*eNOS*) polymorphisms with *EGFR*-mutated lung adenocarcinoma in Taiwan. J Cancer 2018;9(14):2518-2524.
6. Dela Cruz CS, Tanoue LT, Matthay RA. Lung cancer: Epidemiology, etiology, and prevention. Clin Chest Med 2011;32(4):605-644.
7. Osmani L, Askin F, Gabrielson E, *et al.* Current WHO guidelines and the critical role of immunohistochemical markers in the subclassification of non-small cell lung carcinoma (NSCLC): Moving from targeted therapy to immunotherapy. Semin Cancer Biol 2018;52:103-109.
8. Melosky B, Kambartel K, Häntschel M, *et al.* Worldwide prevalence of epidermal growth factor receptor mutations in non-small cell lung cancer: A meta-analysis. Mol Diagn Ther 2022;26(1):7-18.
9. Zhang YL, Yuan JQ, Wang KF, *et al.* The prevalence of EGFR mutation in patients with non-small cell lung cancer: A systematic review and meta-analysis. Oncotarget 2016;7(48):78985-78993.
10. Ettinger DS, Wood DE, Aisner DL, *et al.* Non–small cell lung cancer, version 3.2022, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 2022;20(5):497-530.
11. Yatabe Y. Molecular pathology of non-small cell carcinoma. Histopathology 2024;84(1):50-66.
12. Midha A, Dearden S, McCormack R. EGFR mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: A systematic review and global map by ethnicity (mutMapII). Am J Cancer Res 2015;5(9):2892.
13. Sari L, Purwanto P. Mutasi EGFR pada non-small cell lung cancer di Rumah Sakit Kanker "Dharmais." Indones J Cancer 2016;10(4):131-136.
14. Syahruddin E, Wulandari L, Muktiati NS, *et al.* Uncommon EGFR mutations in cytological specimens of 1,874 newly diagnosed Indonesian lung cancer patients. Lung Cancer 2018;9:25-34.
15. Raharjo BN, Sugiharto S. Prevalensi mutasi epidermal growth factor receptor (EGFR) pada adenokarsinoma paru di Rumah Sakit MRCCC Siloam Jakarta. Tarumanagara Med J 2022;4(2):235-242.
16. Hanafi AR, Hanif MA, Pangaribuan MTG, *et al.* Genomic features of lung cancer patients in Indonesia's national cancer center. BMC Pulm Med 2024;24(1):1-11.
17. Masykura N, Zaini J, Syahruddin E, *et al.* Impact of smoking on frequency and spectrum of K-RAS and EGFR mutations in treatment naive Indonesian lung cancer patients. Lung Cancer 2019;10:57-66.
18. Shigematsu H, Gazdar AF. Somatic mutations of epidermal growth factor receptor signaling pathway in lung cancers. Int J Cancer 2006;118(2):257-262.
19. Borgeaud M, Parikh K, Banna GL, *et al.* Unveiling the landscape of uncommon EGFR mutations in NSCLC – A systematic review. J Thorac Oncol 2024;19(7):973-983.

20. Yoshikawa S, Kukimoto-Niino M, Parker L, *et al.* Structural basis for the altered drug sensitivities of non-small cell lung cancer-associated mutants of human epidermal growth factor receptor. Oncogene 2012;32(1):27-38.
21. Jackman DM, Yeap BY, Sequist LV, *et al.* Exon 19 deletion mutations of epidermal growth factor receptor are associated with prolonged survival in non-small cell lung cancer patients treated with gefitinib or erlotinib. Clin Cancer Res 2006;12(13):3908-3914.
22. Levantini E, Maroni G, Del Re M, *et al.* EGFR signaling pathway as therapeutic target in human cancers. Semin Cancer Biol 2022;85:253-275.
23. Li AR, Chitale D, Riely GJ, *et al.* EGFR mutations in lung adenocarcinomas: Clinical testing experience and relationship to EGFR gene copy number and immunohistochemical expression. J Mol Diagn 2008;10(3):242-248.
24. Ou SHI, Lin HM, Hong JL, *et al.* Real-world response and outcomes in patients with NSCLC with EGFR exon 20 insertion mutations. JTO Clin Res Rep 2023;4(10):100558.
25. Pretelli G, Spagnolo CC, Ciappina G, *et al.* Overview on therapeutic options in uncommon EGFR mutant non-small cell lung cancer (NSCLC): New lights for an unmet medical need. Int J Mol Sci 2023;24(10):8878.
26. Ricciuti B, Baglivo S, Paglialunga L, *et al.* Osimertinib in patients with advanced epidermal growth factor receptor T790M mutation-positive non-small cell lung cancer: Rationale, evidence and place in therapy. Ther Adv Med Oncol 2017;9(6):387-403.
27. Hsieh MH, Fang YF, Chang WC, *et al.* Complex mutation patterns of epidermal growth factor receptor gene associated with variable responses to gefitinib treatment in patients with non-small cell lung cancer. Lung Cancer 2006;53(3):311-322.
28. Harrison PT, Vyse S, Huang PH. Rare epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer. Semin Cancer Biol 2020;61:167-179.
29. Bar J, Peled N, Schokrpur S, *et al.* Uncommon EGFR mutations: International case series on efficacy of osimertinib in real-life practice in first-line setting (UNICORN). J Thorac Oncol 2023;18(2):169-180.
30. Okuma Y, Kubota K, Shimokawa M, *et al.* First-line osimertinib for previously untreated patients with NSCLC and uncommon EGFR mutations: The UNICORN phase 2 nonrandomized clinical trial. JAMA Oncol 2024;10(1):43-51.
31. Holt ME, Misch A, Rao SK, *et al.* De novo EGFR T790M mutations in a community-based oncology practice. J Clin Oncol 2022;40 Suppl 16:3146-3146.
32. Vyse S, Huang PH. Targeting EGFR exon 20 insertion mutations in non-small cell lung cancer. Signal Transduct Target Ther 2019;4(1):5.
33. Kementerian Kesehatan Republik Indonesia. Keputusan Menteri Kesehatan Republik Indonesia nomor HK.01.07/MENKES/1438/2023 tentang pedoman nasional pelayanan kedokteran tata laksana kanker paru. Jakarta: Kemeterian Kesehatan Republik Indonesia; 2023.
34. Mitsudomi T, Tan D, Yang JCH, *et al.* Expert consensus recommendations on biomarker testing in metastatic and nonmetastatic NSCLC in Asia. J Thorac Oncol 2023;18(4):436-446.
35. Chon K, Larkins E, Chatterjee S, *et al.* FDA-approval summary: Amivantamab for the treatment of patients with non-small cell lung cancer with EGFR exon 20 insertion mutations. Clin Cancer Res 2023;29(17):3262-3266.
36. Charan J, Biswas T. How to calculate sample size for different study designs in medical research?. Indian J Psychol Med 2013;35(2):121.
37. WHO Classification of Tumours Editorial Board. Thoracic tumours. Lyon: International Agency for Research on Cancer; 2021.
38. Lindeman NI, Cagle PT, Beasley MB, *et al.* Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Thorac Oncol 2013;8(7):823-859.
39. Lindeman NI, Cagle PT, Aisner DL, *et al.* Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: Guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. J Mol Diagn 2018;20(2):129-159.
40. Wan R, Wang Z, Lee JJ, *et al.* Comprehensive analysis of the discordance of EGFR mutation status between tumor tissues and matched circulating tumor DNA in advanced non-small cell lung cancer. J Thorac Oncol 2017;12(9):1376-1387.
41. Taufik H, Soeroso NN, Tarigan SP, *et al.* Proportion of mutation of epidermal growth factor receptor (EGFR) genes from tissue biopsy and liquid biopsy ctDNA in lung adenocarcinoma. J Respir Indo 2020;40(3):150-155.

42. Shi Y, Au JSK, Thongprasert S, *et al.* A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). J Thorac Oncol 2014;9(2):154-162.
43. Noronha V, Budukh A, Chaturvedi P, *et al.* Uniqueness of lung cancer in Southeast Asia. Lancet Reg Health Southeast Asia 2024;27:100430.
44. Lam DCL, Liam CK, Andarini S, *et al.* Lung cancer screening in Asia: An expert consensus report. J Thorac Oncol 2023;18(10):1303-1322.
45. Castañeda-González JP, Chaves JJ, Parra-Medina R. Multiple mutations in the EGFR gene in lung cancer: A systematic review. Transl Lung Cancer Res 2022;11(10):2148-2163.
46. Mao L, Zhao W, Li X, *et al.* Mutation spectrum of EGFR from 21,324 Chinese patients with non-small cell lung cancer (NSCLC) successfully tested by multiple methods in a CAP-accredited laboratory. Pathol Oncol Res 2021;27:602726.
47. Gao X, Zhao Y, Bao Y, *et al.* Poor prognosis with coexistence of EGFR T790M mutation and common EGFR-activating mutation in non-small cell lung cancer. Cancer Manag Res 2019;11:9621-9630.
48. Lindeman NI, Cagle PT, Aisner DL, *et al.* Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: Guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. Arch Pathol Lab Med 2018;142(3):321-346.
49. Castellanos E, Feld E, Horn L. Driven by mutations: The predictive value of mutation subtype in EGFR-mutated non-small cell lung cancer. J Thorac Oncol 2017;12(4):612-623.
50. John T, Taylor A, Wang H, *et al.* Uncommon EGFR mutations in non-small-cell lung cancer: A systematic literature review of prevalence and clinical outcomes. Cancer Epidemiol 2022;76:102080.
51. Wu JY, Yu CJ, Chang YC, *et al.* Effectiveness of tyrosine kinase inhibitors on "uncommon" epidermal growth factor receptor mutations of unknown clinical significance in non-small cell lung cancer. Clin Cancer Res 2011;17(11):3812-3821.
52. Kauffmann-Guerrero D, Huber RM, Reu S, *et al.* NSCLC patients harbouring rare or complex EGFR mutations are more often smokers and might not benefit from first-line tyrosine kinase inhibitor therapy. Respiration 2018;95(3):169-176.
53. Lohinai Z, Hoda MA, Fabian K, *et al.* Distinct epidemiology and clinical consequence of classic versus rare EGFR mutations in lung adenocarcinoma. J Thorac Oncol 2015;10(5):738-746.
54. Takamochi K, Oh S, Suzuki K. Differences in EGFR and KRAS mutation spectra in lung adenocarcinoma of never and heavy smokers. Oncol Lett 2013;6(5):1207-1212.
55. Wang Q, Mou J, Yang X, *et al.* EGFR mutations in patients with lung adenocarcinoma in southwest China: Are G719S/A and L861Q more likely detected in tumors derived from smokers?. Lung Cancer 2013;4:27-33.
56. Ko HW, Shie S Sen, Wang CW, *et al.* Association of smoking status with non-small cell lung cancer patients harboring uncommon epidermal growth factor receptor mutation. Front Immunol 2022;13:1011092.
57. Worth Health Organization RO for SEA. Global adult tobacco survey: Indonesia report 2011. New Delhi: World Health Organization; 2012.
58. Couraud S, Debieuvre D, Moreau L, *et al.* No impact of passive smoke on the somatic profile of lung cancers in never-smokers. Eur Respir J 2015;45(5):1415-1425.
59. Taga M, Mechanic LE, Hagiwara N, *et al.* EGFR somatic mutations in lung tumors: Radon exposure and passive-smoking in former- and never-smoking U.S. Women. Cancer Epidemiol Biomarkers Prev 2012;21(6):988.
60. Yoon HY, Ryu JS, Sim YS, *et al.* Clinical significance of EGFR mutation types in lung adenocarcinoma: A multi-centre Korean study. PLoS One 2020;15(2):e0228925.
61. Wang F, Li C, Wu Q, *et al.* EGFR exon 20 insertion mutations in non-small cell lung cancer. Transl Cancer Res 2020;9(4):2982-2991.
62. Van Sanden S, Murton M, Bobrowska A, *et al.* Prevalence of epidermal growth factor receptor exon 20 insertion mutations in non-small-cell lung cancer in europe: A pragmatic literature review and meta-analysis. Target Oncol 2022;17(2):153-166.
63. Zhou C, Tang KJ, Cho BC, *et al.* Amivantamab plus chemotherapy in NSCLC with EGFR exon 20 insertions . N Engl J Med 2023;389(22):2039-2051.
64. Park K, Haura EB, Leighl NB, *et al.* Amivantamab in egfr exon 20 insertion- mutated non-small-cell lung cancer progressing on platinum chemotherapy: Initial results from the chrysalis phase i study. J Clin Oncol 2021;39(30):3391-3402.
65. Ananta A, Arifin EN, Hasbullah MS, *et al.* ISEAS working paper on a new classification of Indonesia's ethnic groups (based on the 2010 population census). Singapore: Institute of Southeast Asian Studies; 2014.

66. Lopez-Chavez A, Thomas A, Evbuomwan MO, *et al.* EGFR mutations in Latinos from the United States and Latin America. J Glob Oncol 2016;2(5):259-267.
67. Soraas L, Stebbing J. Geographic variation in EGFR mutation frequency in lung adenocarcinoma may be explained by interethnic genetic variation. J Thorac Oncol 2018;13(3):454-458.
68. Kumari N, Kumari N, Singh S, *et al.* Epidermal growth factor receptor mutation frequency in squamous cell carcinoma and its diagnostic performance in cytological samples: A molecular and immunohistochemical study. World J Oncol 2019;10(3):142-150.
69. Mitsudomi T. Molecular epidemiology of lung cancer and geographic variations with special reference to EGFR mutations. Transl Lung Cancer Res 2014;3(4):205-211.
70. Hill W, Lim EL, Weeden CE, *et al.* Lung adenocarcinoma promotion by air pollutants. Nature 2023;616(7955):159.
71. Goldman JW, Noor ZS, Remon J, *et al.* Are liquid biopsies a surrogate for tissue EGFR testing?. Ann Oncol 2018;29 Suppl 1:i38-i46.
72. Kosasih AS, Hanafi ARR, Sukartini N, *et al.* The role of liquid biopsy for detecting epidermal growth factor receptor (EGFR) mutations in patients with advanced non-small cell lung cancer (NSCLC) – A preliminary study in Indonesian population. Indones J Cancer 2023;17(3):203-210.
73. Kwapisz D. The first liquid biopsy test approved. Is it a new era of mutation testing for non-small cell lung cancer?. Ann Transl Med 2017;5(3):46-46.
74. Wang N, Zhang X, Wang F, *et al.* The diagnostic accuracy of liquid biopsy in EGFR-mutated NSCLC: A systematic review and meta-analysis of 40 Studies. SLAS Technol 2021;26(1):42-54.
75. Cai J, Wang W, Zhang W. A meta-analysis of liquid biopsy versus tumor histology for detecting EGFR mutations in non-small cell lung cancer. Transl Oncol 2024;47:102022.
76. Bonanno L, Dal Maso A, Pavan A, *et al.* Liquid biopsy and non-small cell lung cancer: Are we looking at the tip of the iceberg?. Br J Cancer 2022;127(3):383-393.
77. Koc A, Goksel T, Komurcuoglu B, *et al.* Liquid biopsy for EGFR mutations in non-small cell lung cancer cases by RT-PCR. Eur Respir J 2018;52 Suppl 62:PA1757.
78. Pellini B, Chaudhuri AA. Circulating tumor DNA minimal residual disease detection of non-small-cell lung cancer treated with curative intent. J Clin Oncol 2022;40(6):567.
79. García-Pardo M, Makarem M, Li JJN, *et al.* Integrating circulating-free DNA (cfDNA) analysis into clinical practice: Opportunities and challenges. Br J Cancer 2022;127(4):592-602.
80. Phallen J, Leal A, Woodward BD, *et al.* Early noninvasive detection of response to targeted therapy in non-small cell lung cancer. Cancer Res 2019;79(6):1204-1213.
81. Syahruddin E, Fachrucha F, Zaini J, *et al.* Liquid biopsy and tissue biopsy for epidermal growth factor receptor (EGFR) mutation analysis in lung adenocarcinoma. Indones J Cancer Control 2024;3(1):9-13.
82. Pan Y, Zhang JT, Gao X, *et al.* Dynamic circulating tumor DNA during chemoradiotherapy predicts clinical outcomes for locally advanced non-small cell lung cancer patients. Cancer Cell 2023;41(10):1763-1773.e4.