ecancermedicalscience

Prevalence and treatment of human epidermal growth factor receptor 2-altered non-small cell lung cancer: a retrospective analysis and systematic literature review

Ning Yi Yap[1,2], Komathi Perumal[1,3] and Pathmanathan Rajadurai[1,2,4]

[1]Laboratory, Subang Jaya Medical Centre, Subang Jaya 47500, Selangor, Malaysia
[2]Jeffrey Cheah School of Medicine & Health Sciences, Monash University, Bandar Sunway, Petaling Jaya 47500, Selangor, Malaysia
[3]ePink Health Sdn. Bhd., Shah Alam 40150, Selangor, Malaysia
[4]Faculty of Medicine, Universiti Malaya, Kuala Lumpur 50603, Malaysia

Abstract

Human epidermal growth factor receptor 2 (HER2) is known for its oncogenic activities in diverse cancers, including non-small cell lung cancer (NSCLC). However, the prevalence of *HER2* alterations in Malaysian NSCLC patients remains unreported. This study examined the prevalence and characteristics of *HER2* mutations and amplification in a Malaysian cohort. Additionally, a systematic review was conducted to evaluate the global prevalence of *HER2* alterations in NSCLC, as well as the efficacy of HER2-targeted therapies observed in clinical trials. NSCLC tumour samples received from October 2019 to December 2022 for next-generation sequencing diagnostics were included in the retrospective analysis. In this patient cohort, *HER2* alteration was present in 5.8% of patients; 3.9% had *HER2* mutations, 1.5% had *HER2* amplifications and 0.4% were both *HER2*-mutated and amplified. *HER2* exon 20 insertions were the most common *HER2* variants, detected in 47/59 (79.7%) of *HER2*-mutated patients. Among cases with *HER2* exon 20 insertions, the Y772_A775dup variant was found in 34 patient samples. *HER2*-mutated patients were significantly younger than non-*HER2*-mutants (61 versus 64 years old; $p = 0.046$) and were inclined to be female and never-smokers, albeit not statistically significant. Patients with *HER2* amplification were more likely to have progressed post-tyrosine kinase inhibitor therapy ($p = 0.015$). The systematic review highlighted a global variation in the prevalence of *HER2* alterations in NSCLC, ranging from 0.3% to 9.1% for mutations and 0.2% to 19% for amplification. Finally, phase II clinical trials involving *HER2*-altered NSCLC patients demonstrated promising treatment outcomes with trastuzumab deruxtecan, trastuzumab emtansine, pyrotinib, pyrotinib + apatinib and trastuzumab + pertuzumab + docetaxel. In conclusion, the prevalence of *HER2* alteration among Malaysian NSCLC patients falls within the global range. A systematic review of clinical trials revealed promising treatment outcomes and Malaysian NSCLC patients with *HER2* alterations are anticipated to similarly benefit from *HER2*-targeted therapies.

Keywords: *HER2*, *Malaysia*, *next-generation sequencing*, *non-small cell lung cancer*, *Southeast Asia*

Research

Correspondence to: Pathmanathan Rajadurai
Email: drpathma@gmail.com

ecancer 2024, **18**:1734
https://doi.org/10.3332/ecancer.2024.1734

Published: 01/08/2024
Received: 28/05/2024

Publication costs for this article were supported by *ecancer* (UK Charity number 1176307).

Introduction

Human epidermal growth factor receptor 2 (HER2), also known as ErbB2, is a transmembrane glycoprotein receptor exhibiting intracellular tyrosine kinase activity [1]. It consists of extracellular, transmembrane and intracellular domains, and plays important roles in various cellular functions including adhesion, differentiation, growth, apoptosis and migration [2, 3]. HER2 has garnered considerable attention due to its role in tumorigenesis and potential as a therapeutic target [3, 4]. Its aberrations have been implicated in the development and progression of various cancers, including non-small cell lung cancer (NSCLC) [3].

Three types of *HER2* oncogene activating mechanisms have been described in cancers, and they include gene mutation, gene amplification and protein overexpression [3]. These mechanisms of *HER2* activation have significant implications on treatment strategies, and prognostic outcomes which may differ according to the cancer type. *HER2* amplification and overexpression are well-established predictive markers for response to HER2-targeted monoclonal antibodies such as trastuzumab, in patients with breast and gastric cancers [2, 5]. However, HER2 protein overexpression has not demonstrated reliability in identifying NSCLC patients who may benefit from HER2-targeted therapies [6, 7]. In contrast, *HER2* mutations have shown greater promise in selecting NSCLC patients who are likely to respond to HER2-targeted therapies [8].

Currently, standard chemotherapy or immunotherapy is administered to patients with *HER2*-mutant NSCLC, but their effectiveness as second- or later-line treatment is limited [9]. Nonselective tyrosine kinase inhibitors (TKIs) have shown limited benefit in NSCLC patients with *HER2* mutation, with objective response rates (ORRs) ranging from 0% to 19% [10]. Trastuzumab-based chemotherapy was not found to be superior to chemotherapy alone whereas selective HER2 TKIs (e.g., poziotinib and pyrotinib) showed better activity in pre-treated NSCLC patients with *HER2* mutation [10]. More favourable data were reported in phase II studies evaluating antibody-drug conjugates (ADC) ado-trastuzumab emtansine and trastuzumab deruxtecan in *HER2*-mutated NSCLC patients [11, 12]. These agents bring hope to the management of *HER2*-altered NSCLC.

Malaysia is a multi-ethnic Southeast Asian country, comprising an ethnic Malay majority, as well as significant Chinese, Indian and indigenous populations. Lung cancer survival is the worst among all cancers in Malaysia; the overall 5-year relative survival for lung, trachea and bronchus cancer among Malaysian patients was 11.0%, with a median survival time of 6.8 months [13]. The prevalence of *HER2* mutation and amplification among NSCLC patients is not well-reported in the Southeast Asia region, and has not been reported in Malaysia. Therefore, we have performed a retrospective study to elucidate the prevalence of *HER2* alterations and the characteristics of NSCLC patients with these alterations, based on diagnostic next-generation sequencing (NGS) performed at a tertiary private referral medical center in Malaysia. Additionally, a systematic literature review was conducted to offer a comprehensive overview of the existing evidence concerning the prevalence of *HER2* mutation/amplification in NSCLC, as well as the efficacy of HER2-targeted therapies observed in prospective clinical trials involving NSCLC patients with *HER2* alterations.

Methods

Determining prevalence of HER2 mutations and amplification among NSCLC patients in Malaysia.

Patient samples

Tumour samples from lung cancer patients from several medical centers in Malaysia were collected and sent to the Subang Jaya Medical Centre (SJMC) laboratory for NGS. We analyzed the NGS results of consecutive samples received from October 2019 to December 2022, to determine the prevalence of *HER2* mutations and amplification. Ethical committee approval for the analysis of the retrospective NGS data was granted by the SJMC ethics committee (Ref: 201907.3 and Ref: 202109.3).

NGS testing

DNA from samples received from October 2019 to 2020 was sequenced using the Ion AmpliSeq Colon and Lung Cancer Research Panel v2 (Thermo Fisher Scientific, Waltham, MA, USA), while ribonucleic acid (RNA) was sequenced using the Ion AmpliSeq RNA Fusion Lung Cancer Research Panel or the Oncomine Focus Assay (Thermo Fisher Scientific, Waltham, MA, USA). DNA and RNA from samples received from November 2020 to December 2022 were sequenced using the Oncomine Precision Assay GX (Thermo Fisher Scientific, Waltham, MA, USA). The NGS testing process followed the method previously described by Rajadurai *et al* [14]. *HER2* mutations and amplification were detected using the targeted NGS panels.

Table 1. Criteria for considering studies for systematic review, based on the population, intervention, comparator and PICOS structure.

	Inclusion criteria	
	Prevalence of *HER2* mutation/amplification in NSCLC	**Efficacy of *HER2*-targeted therapies in NSCLC**
Population	Patients with NSCLC and *HER2* mutations or amplification	
Intervention/Comparators	Any	Chemotherapy, immunotherapy, TKI, monoclonal antibody therapy or ADC
Outcomes	Prevalence of *HER2* mutations or amplification	ORR, DCR, median PFS and median OS
Study designs	Studies involving cohort of ≥ 200 NSCLC cases	Prospective studies with ≥10 NSCLC subjects published from 2018 to 2022

DCR: disease control rate; HER2: human epidermal growth factor receptor 2; NSCLC: non-small cell lung cancer; ORR: objective response rate; OS: overall survival; PFS: progression-free survival; TKI: tyrosine kinase inhibitor

Statistical analysis and data visualization

Statistical analysis was performed using the IBM SPSS Statistics Version 22.0 (IBM Corporation, Armonk, NY, USA). Patients' demographic and clinical characteristics were evaluated using Pearson's chi-square test or Fisher's test for categorical variables, while the Mann-Whitney test was employed for comparing the patients' age. The *HER2* mutation lollipop diagram was generated using the MutationMapper visualization tools available at the cBioPortal site (https://www.cbioportal.org/) [15].

Systematic review of HER2 mutations and amplification in NSCLC

The systematic literature review was conducted in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines [16].

Search strategy

A systematic literature review was conducted to obtain (a) the prevalence of *HER2* mutation/amplification in NSCLC, and (b) the efficacy of *HER2*-targeted therapies in NSCLC patients with *HER2* alterations. A comprehensive database search was performed in PubMed and Web of Science to identify relevant studies published up to 31 December 2022. The Medical Subject Headings and text word search terms used were ('*HER2*' or '*HER-2*' or '*ERBB2*' or '*ERBB-2*') AND ('lung cancer' or 'NSCLC'). The studies were screened by two reviewers (NYY and KP) based on the article titles, abstracts and contents. The general inclusion criteria used to evaluate records include articles or abstracts published in the English language, and studies in which NSCLC patients were included. Studies involving only *in vitro* or *in vivo* samples, and review-type articles were excluded. The article selection criteria based on the populations, interventions and comparators, outcomes and study design (PICOS) are shown in Table 1.

Specific inclusion and exclusion criteria were also defined according to the two subtopics of the systematic literature review, as follows: (a) For systematic review on the prevalence of *HER2* mutations and amplification, publications reporting the prevalence of *HER2* mutations and amplification from a cohort of ≥ 200 NSCLC cases were included. Analyses involving only epidermal growth factor receptor (*EGFR*), Kirsten rat sarcoma 2 viral oncogene homologue (*KRAS*), anaplastic lymphoma kinase (*ALK*) or ROS proto-oncogene 1 (*ROS1*) wild-type cases, analyses using liquid biopsy samples only and reports of HER2 protein overexpression were excluded. (b) For a systematic review of clinical trials evaluating the efficacy of treatment in NSCLC patients with *HER2* mutations or amplification, prospective studies with ≥10 NSCLC subjects that were published from 2018 to 2022 were included. Retrospective studies and case series were excluded.

Data extraction

Data were extracted from articles that met the defined inclusion and exclusion criteria by one independent reviewer, and verified by a second reviewer. Data extracted for two subtopics of the systematic literature review were as follows: (a) For systematic review on the prevalence of *HER2* mutations or amplification, data extracted were on the prevalence of *HER2* mutations and amplification. (b) For a systematic review on prospective clinical trials evaluating the efficacy of treatment in NSCLC patients with *HER2* mutations or amplification, data extracted were the ORR, disease control rate (DCR), median progression-free survival (PFS) and median overall survival (OS).

Results

Prevalence of HER2 mutations and amplification in Malaysian NSCLC patients

Patient cohort

The demographic features of 1,373 NSCLC patients whose tumour samples were analyzed at the SJMC laboratory are shown in Table 2. Approximately half of the patients were male (52.7%), and the median patient age was 64 years old (range 16–93 years old). Most of the patients were of Chinese descent (76%), and nearly half of the patients were never smokers (44.8%). Most tumours (84.6%) were adenocarcinomas, and 88.1% of patients had advanced stage NSCLC (stage III or IV disease). Most patients (77.7%) were TKI-naïve.

Table 2. Clinical characteristics of Malaysian NSCLC patients whose tumour samples were analysed using NGS from October 2019 to December 2022.

Characteristics	Sample size (*N* = 1,373)
Median age, years (range)	64 (16–93)
Gender, *n* (%)	
Male	723 (52.7)
Ethnicity, *n* (%)	
Malay	191 (13.9)
Chinese	1044 (76)
Indians	54 (3.9)
Other	84 (6.1)
Smoking status, *n* (%)	
Never-smoker	615 (44.8)
Smoker	134 (9.8)
Ex-smoker	219 (16.0)
Unknown	405 (29.5)
Histology, *n* (%)	
Adenocarcinoma	1,161 (84.6)
Adenosquamous	29 (2.1)
Squamous cell carcinoma	121 (8.8)
Large cell carcinoma	8 (0.6)
Poorly differentiated NSCLC	42 (3.1)
NSCLC with neuroendocrine differentiation	5 (0.4)
Other	7 (0.5)

(Continued)

Table 2. Clinical characteristics of Malaysian NSCLC patients whose tumour samples were analysed using NGS from October 2019 to December 2022. ***(Continued)***

Characteristics	Sample size (*N* = 1,373)
Specimen site, *n* (%)	
Lung	922 (67.2)
Bronchial	56 (4.1)
Pleura	115 (8.4)
Chest wall	12 (0.9)
Lymph node	101 (7.4)
Bone	68 (5.0)
Liver	29 (2.1)
Brain	21 (1.5)
Other	49 (3.6)
Sample type, *n* (%)	
Biopsy	961 (70.0)
Fine needle aspirate	9 (0.7)
Fluid cytology	23 (1.7)
Resection	24 (1.7)
Unspecified	356 (25.9)
Stage, *n* (%)	
I	39 (2.8)
II	38 (2.8)
III	119 (8.7)
IV	1,090 (79.4)
Unspecified	87 (6.3)
Treatment status, *n* (%)	
TKI-naïve	1,067 (77.7)
Post-TKI progression	89 (6.5)
Unspecified	217 (15.8)

NGS: next-generation sequencing; NSCLC: non-small cell lung cancer; TKI: tyrosine kinase inhibitor

NGS and HER2 profile of NSCLC patient cohort

Among the NSCLC specimens analysed at our centre, nearly half (*n* = 627, 45.7%) showed *EGFR* alteration, followed by *KRAS* alteration (174, 12.7%) and *ALK* alteration (85, 6.2%). *HER2* alteration was present in 79 patients (5.8%); 54 (3.9%) of these were *HER2* mutations only, 20 (1.5%) were *HER2* amplification only and 5 (0.4%) were both *HER2*-mutated and amplified (Figure 1a). The *HER2* mutation variants reported in the NSCLC patients are shown in Figure 1b. *HER2* exon 20 insertions, found in the TKI domain, were the most common *HER2* variants among these NSCLC patients, with 47 out of 59 (79.7%) patients having this form of *HER2* alteration. The *HER2* exon 20 insertion Y772_A775dup was the most frequent *HER2* variant, found in 34 patient samples. Other *HER2* mutations are found in the extracellular ligand binding domain (S310S/Y, 7 patients) and transmembrane domain (V659E, 2 patients).

HER2 exon 20 insertions were mutually exclusive with *ALK*, *BRAF*, *EGFR*, *RET*, *ROS1* and *MET* genetic alterations. Two patients with *HER2* Y772_A775dup harboured *KRAS* alterations, one with *KRAS* amplification and the other *KRAS* K117N. *EGFR* sensitising mutations were detected in four patients with *HER2* S310S/Y variants; of these, two patients were post-TKI progression cases. In addition, 11 patients with *HER2* amplification had *EGFR* sensitising mutation; of these, 5 patients were post-TKI progression. *TP53* mutations were the most common co-mutations seen with *HER2* mutation (14 patients) and *HER2* amplification (9 patients).

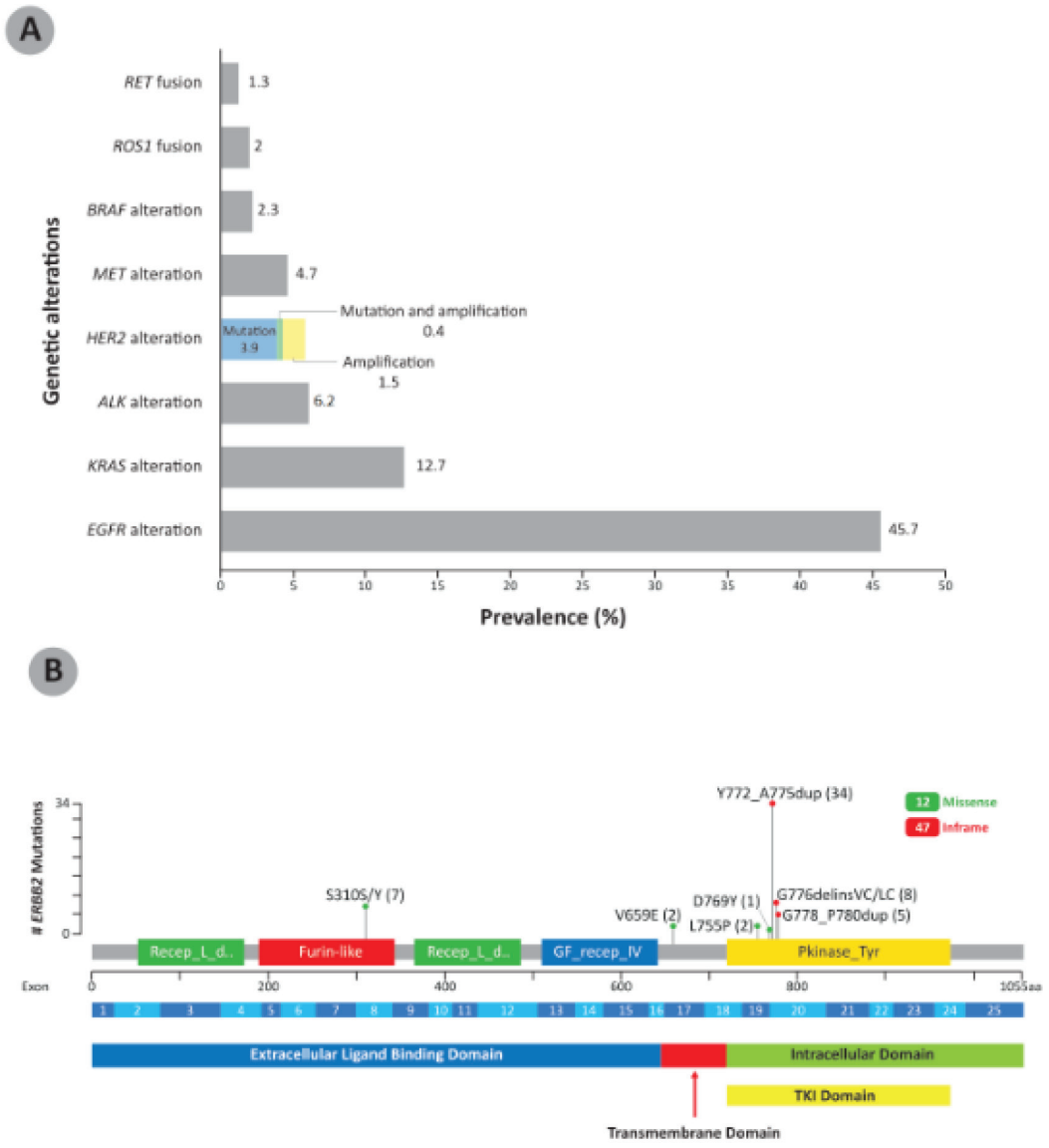

Figure 1. (a): Prevalence of genetic alterations in a cohort of NSCLC patients in Malaysia (*N* = 1,373). The breakdown of prevalence of *HER2* mutation, amplification, as well as mutation and amplification are shown in color. (b): *HER2* mutation variants reported in NSCLC patients in Malaysia (*n* = 59). The number of patients showing the specific mutations are indicated in brackets. *HER2* exon 20 insertions were the most common *HER2* variant in the NSCLC patients. BRAF: B-Raf proto-oncogene; MET: mesenchymal-epithelial transition; RET: RET proto-oncogene.

Characteristics of patients with HER2 mutations and amplification

Patients with *HER2* mutations were significantly younger than non-*HER2*-mutants (median age 61 versus 64 years old; $p = 0.046$), and were inclined to be female and never-smokers (not statistically significant; $p = 0.111$ and 0.204, respectively) (Table 3). On the other hand, patients with *HER2* amplification were inclined to be male, and ex- or current smokers (not statistically significant; $p = 0.157$ and $p = 0.159$, respectively). Patients with *HER2* amplification were more likely to have progressed post-TKI ($p = 0.015$). All five patients with *HER2* amplification who progressed post-TKI also had *EGFR* sensitising mutations.

Table 3. Characteristics of Malaysian NSCLC patients with *HER2* mutations and amplification compared with patients without the *HER2* alterations.

Characteristic	*HER2* mutations			*HER2* amplification		
	Without *HER2* mutation (*n* = 1,314)	With *HER2* mutations (*n* = 59)	*p*-value	Without *HER2* amplification (*n* = 1,348)	With *HER2* amplification (*n* = 25)	*p*-value
Median age, years (range)	64 (16–93)	61 (32–80)	0.046	64 (16–93)	63 (38–85)	0.892
Gender, *n* (%)						
Male	698 (53.1)	25 (42.4)	0.111	706 (52.4)	17 (68)	0.157
Female	616 (46.9)	34 (57.6)		642 (47.6)	8 (32)	
Ethnicity, *n* (%)						
Malay	184 (14)	7 (11.9)	0.525	188 (13.9)	3 (12)	0.959
Chinese	998 (76)	46 (78)		1,024 (76)	20 (80)	
Indian	50 (3.8)	4 (6.8)		53 (3.9)	1 (4)	
Other	82 (6.2)	2 (3.4)		83 (6.2)	1 (4)	
Smoking status, *n* (%)						
Never-smoker	582 (63.1)	33 (73.3)	0.204	609 (63.8)	6 (42.9)	0.159
Ex- or current smoker	341 (36.9)	12 (26.7)		345 (36.2)	8 (57.1)	
Stage, *n* (%)						
I-II	72 (5.9)	5 (4.2)	0.379	76 (6)	1 (4.8%)	1.000
III-IV	1158 (94.1)	51 (91.1)		1,189 (94)	20 (95.2)	
Treatment status, *n* (%)						
TKI-naïve	1,017 (92.1)	50 (96.2)	0.424	1,052 (92.6)	15 (75)	0.015
Post-TKI progression	87 (7.9)	2 (3.8)		84 (7.4)	5 (25)	
Histology, *n* (%)						
Adenocarcinoma	-	58 (98.3)	-	-	22 (88)	-
Adenosquamous		1 (1.7)			1 (4)	
SCC		-			2 (8)	

HER2: human epidermal growth factor receptor 2; SCC: squamous cell carcinoma; TKI: tyrosine kinase inhibitor

Systematic review of HER2 mutations and amplification in NSCLC

Study selection

The database search performed on PubMed and Web of Science yielded 5,828 unique records for screening. Of these, 295 records were retrieved for full text screening; most records were excluded due to non-relevance, unsuitable article type (review articles and case reports), non-English abstract or article, unsuitable studies (*in vitro* or *in vivo* studies, and immunohistochemistry (IHC) results) or clinical trials performed before 2018. Upon applying the PICOS criteria, 94 articles were included; of these, 76 articles reported the prevalence of *HER2* mutation and/or amplification, and 18 articles were prospective clinical trials evaluating the efficacy of treatment in NSCLC patients with *HER2* mutations or amplification (Figure 2).

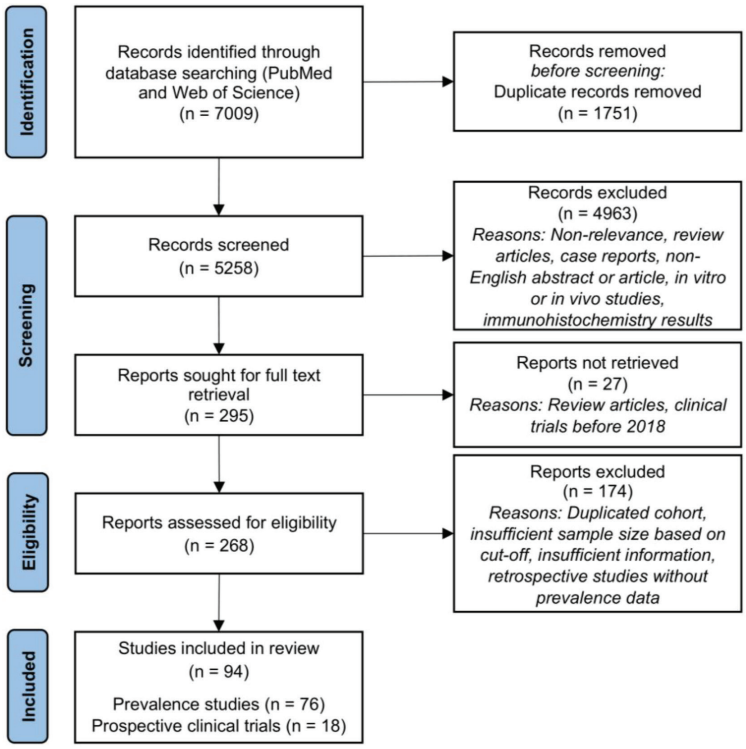

Figure 2. PRISMA diagram for inclusion of systematic review.

Prevalence of HER2 mutations and amplification

The prevalence of *HER2* mutations and amplification reported in global studies are shown in Table 4. *HER2* mutations in the studies were detected using various methods including Sanger sequencing, reverse transcription polymerase chain reaction (RT-PCR), NGS and matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) mass spectrometry (Supplementary Table S1 shows the full list of studies reporting the prevalence of *HER2* mutations and amplification). The prevalence of *HER2* mutations in NSCLC ranged from 0.3% to 9.1%. In some studies, the prevalence of *HER2* exon 20 insertions were specifically reported, ranging from 0.4% among African-American populations in North America, to 4% in North America and East Asia. *HER2* amplification was detected using fluorescent *in situ* hybridisation (FISH), silver *in situ* hybridisation (SISH), dual *in situ* hybridisation (DISH), multiplex ligation-dependent probe amplification (MLPA) or NGS. The prevalence of *HER2* amplification varied widely, from 0.2% reported in China, up to 19% reported in Japan [17, 18]. Two studies which reported relatively high prevalence of *HER2* amplification (14% and 19%) used the SISH or DISH method of detection [17, 19].

Efficacy of HER2-targeted therapies in NSCLC patients with HER2 mutations and amplification

Prospective clinical trials of various treatments for NSCLC patients with *HER2* mutations and amplification are shown in Table 5.

Two phase II trials of afatinib in NSCLC patients with *HER2* mutations were performed in post-progression patients; these trials revealed modest clinical benefits, i.e., ORR of 0%–7.7%, DCR of 53.9%–61.1%, median PFS of 2.8–4.0 months and median OS of 10–14 months. However, both studies did not compare the efficacy of afatinib in different *HER2* exon 20 insertion variants [20, 21].

Poziotinib achieved a higher ORR (27.8%–27.9%), DCR (70%–73%) and PFS (5.5 months) compared to afatinib in patients on subsequent lines of therapy [22, 23]. Poziotinib's DCR and PFS at subsequent lines of therapy were comparable to its use in the first line setting (ORR of 41%, DCR of 73% and PFS of 5.6 months) [22–24]. However, poziotinib did not receive United States Food and Drug Administration approval due to its modest efficacy, yet significant gastrointestinal and dermal toxicities [25].

Table 4. Prevalence of *HER2* mutations and amplification in NSCLC reported in global studies.

Region	Countries	Prevalence of *HER2* mutations	No. of studies	Prevalence of *HER2* exon 20 insertions only	No. of studies	Prevalence of *HER2* amplification	No. of studies
East Asia	China and Taiwan	1.9%–8.6%	18	1.6%–2.6%	8	0.2%–2.8%	8
	China (SCC only)	0.3%–9.1%	3	-	-	0.8%	1
	Japan and Korea	1.8%–4.9%	8	1.7%–4%	5	2.1%–19%	6
South Asia	India	-	-	1.5%	1	-	-
Southeast Asia	Singapore	3.1%	1	2.7%	1	-	-
North America	USA and Canada	1%–3.4%	10	3%–4% (0.4% among African-American)	4	0.4%–3%	5
South America	Brazil	4.9%	1	0.8%	1	-	-
Europe	Belgium, Finland, France, Germany, Greece, Italy, Spain and Switzerland	1.2%–3%	8	0.8%–1.7%	6	0.7%–9%	6
Australia	Australia	1%	1	-	-	-	-
Russia	Russia	-	-	-	-	6%	1

HER2: human epidermal growth factor receptor 2; SCC: squamous cell carcinoma

Treatment of lung cancer patients with trastuzumab emtansine at various lines of therapy yielded an overall ORR of 38.1%–51.0%, DCR of 52.4%–83.3% and PFS 2.8–5.0 months [8, 11, 26]. Li *et al* [8] reported comparable responses to trastuzumab emtansine among patients when stratified according to *HER2* status (mutation, amplification or combination of both). Although these trials recruited patients with central nervous system (CNS) metastasis, no subgroup analysis data were presented.

Pyrotinib has been investigated as a monotherapy, and in combination with apatinib for patients with *HER2* mutation or amplification [27–31]. The ORR, DCR and PFS were generally lower with pyrotinib monotherapy at 19.2%–30.0%, 74.4%–85.0% and 5.6–6.9 months, respectively, compared to 35.7%–51.5%, 93.9%–100% and 6.9–8.0 months seen in pyrotinib + apatinib [27–31]. In a subgroup analysis of pyrotinib monotherapy, the ORR were comparable between patients with and without brain metastases (25.0% versus 31.3%).

Treatment of post-progression patients with trastuzumab deruxtecan (at a dose of either 5.4 or 6.4 mg/kg) in DESTINY-Lung01 and DESTINY-Lung02 yielded an encouraging ORR of 42.9%–54.9% and DCR of 90.4%–92.9% [12, 32]. In addition, trastuzumab deruxtecan at 6.4 mg/kg also yielded a more prolonged PFS of 8.2 months and OS of 18.6 months [12]. Trastuzumab deruxtecan seemed to achieve better treatment outcomes in post-progression lung cancer patients, compared to other *HER2*-targeted therapies (pyrotinib, afatinib, poziotinib and trastuzumab emtansine) (Table 5). In the DESTINY-Lung01 trial, comparable responses were observed with trastuzumab deruxtecan between patients with CNS metastasis and those without [12]. Safety-wise, although the DESTINY-Lung02 trial demonstrated similar efficacy of trastuzumab deruxtecan at both 5.4 and 6.4 mg/kg, a lower incidence of toxicities was observed with the 5.4 mg/kg dosage.

In the Drug Rediscovery Protocol (DRUP) trial, trastuzumab + pertuzumab demonstrated limited activity in patients with heavily pre-treated *HER2*-positive NSCLC (ORR 8.3%; DCR 38.0%; PFS 4.0 months; OS 10.0 months) [33]. Comparatively, trastuzumab + pertuzumab + docetaxel achieved improved outcomes in the IFCT 1703-R2D2 trial (ORR 29.0%; DCR 87.0%; PFS 4.0 months; OS 10.0 months) [34]. However, the IFCT 1703-R2D2 trial only included patients with stage III disease, while the DRUP trial recruited patients with metastatic disease.

Table 5. Summary of phase II clinical trials of *HER2*-targeted therapies for NSCLC patients with *HER2* mutations and amplification.

Treatment[†]	Line of therapy	Phase (Trial name)	Sample size	Treatment outcome				References
				ORR (%)	DCR (%)	Median PFS (months)	Median OS (months)	
Afatinib	Subsequent line (post-progression)	II (NICHE)	13	7.7	53.8	4.0	14	[20]
		II	18	0	61.1	2.8	10.0	[21]
Poziotinib	First line	II (ZENITH20-4)	70	41.0	73.0	5.6	NA	[23]
	Subsequent line (post-progression)	II (ZENITH20)	90	27.8	70.0	5.5	NA	[24]
	Subsequent line (post-progression)	II	30	27.9	73.0	5.5	15	[22]
Pyrotinib	Subsequent line (post-progression)	II	60	30.0	85.0	6.9	14.4	[31]
	First line and subsequent line	II	78	19.2	74.4	5.6	10.5	[27]
Pyrotinib for *HER2* amplification only	First line and subsequent line	II	27	22.2	81.5	6.3	12.5	[28]
Pyrotinib + apatinib for *HER2* mutation and amplification	At least 2 prior lines	II (PATHER2)	33	51.5	93.9	6.9	14.8	[30]
Pyrotinib + apatinib	Subsequent line (post-progression)	II	14	35.7	100	8.0	12.9	[29]
Neratinib	NA	II (PUMA -NER-4201) & SUMMIT)	43	0–4.0	35.0–39.0	2.9–5.4	NA	[54]
Neratinib + temsirolimus or trastuzumab			95	8.0–14.0	28.0–49.0	4.0–4.1	NA	
Trastuzumab emtansine	Various lines	II	18	44.0	83.3	5.0	NA	[26]
	Subsequent line (post-progression)	II (JapicCTI-194620)	22	38.1	52.4	2.8	8.1	[11]
Trastuzumab emtansine for *HER2* mutation and amplification	Various lines	II	49	51.0	NA	5.0	NA	[8]
Trastuzumab deruxtecan (6.4 mg/kg)	Subsequent line (post-progression)	II (DESTINY-Lung01)	91	54.9	92.3	8.2	18.6	[12]
		II (DESTINY-Lung02)	28	42.9	92.9	NA	NA	[32]
Trastuzumab deruxtecan (5.4 mg/kg)			52	53.8	90.4			
Trastuzumab + pertuzumab	Subsequent line (post-progression)	II (DRUP)	24	8.3	38.0	4.0	10.0	[33]
Trastuzumab + pertuzumab + docetaxel	Subsequent line (post-progression)	II (IFCT 1703-R2D2)	45	29.0	87.0	6.8	17.6	[34]

[†]Studies recruited patients with *HER2* mutations unless stated otherwise (*HER2* amplification)
DCR: disease control rate; NA: not available; ORR: overall response rate; OS: overall survival; PFS: progression free survival

Finally, neratinib as monotherapy as well as in combination with temsirolimus or trastuzumab in NSCLC patients with *HER2* alteration produced inferior ORR (0%–14%) and DCR (28%–49%) compared with poziotinib, pyrotinib, trastuzumab emtansine and trastuzumab deruxtecan.

Discussion

This article aims to elucidate the prevalence of *HER2* mutations and amplification in NSCLC, as well as the clinical characteristics and mutational profiles of patients with these alterations, based on retrospective analysis of diagnostic NGS performed at a referral center in Malaysia. To the best of our knowledge, this article reports the first known statistics on *HER2* alterations among lung cancer patients in Malaysia. We also performed a systematic literature review to summarise the available evidence on the prevalence of *HER2* alteration in NSCLC and the treatment outcomes in these patients.

It is important to note that the frequency of *HER2* alterations may vary depending on the detection modalities used, target region of test assay, tumour heterogeneity, NSCLC subtype and sample type. Our systematic review on the prevalence of *HER2* mutations and amplification in NSCLC was analysed from a total of 76 articles; most articles described studies originated from East Asia, North America or Europe, with variations in the testing method used. The prevalence of *HER2* mutations reported may be higher in studies using NGS for testing, as more variants can be detected using this modality. In contrast, Sanger sequencing demonstrates lower sensitivity compared to NGS or RT-PCR. The assessment of *HER2* amplifications can be carried out utilising techniques such as FISH, SISH, DISH or NGS but currently, there is no standardised criteria for determining *HER2* amplification in NSCLC [1]. Finally, HER2 expression can be evaluated using IHC. The current testing recommendation is to include *HER2* mutation testing upfront as part of broad molecular profiling for NSCLC patients with advanced or metastatic disease, in particular, if approved therapies are available [1, 35].

In our retrospective analysis, *HER2* alteration was seen in 5.8% of Malaysian NSCLC patients. Of these, 3.9% had *HER2* mutations only and 1.5% had *HER2* amplifications only, and a small subset (0.4%) of our patient cohort were both *HER2*-mutated and amplified. Our prevalence findings fall within the range reported in global studies (0.3%–9.1% for *HER2* mutation and 0.2%–19% for *HER2* amplification) (Table 4). Specifically, the prevalence of *HER2* mutations (4.3%) was within the range reported by studies from East Asia (Table 4), with marginally lower overall prevalence from North America and Europe. The prevalence in this study was also slightly above Singapore (3.1%), the other Southeast Asian country with available published data. *HER2* exon 20 insertions were the most common *HER2* variants in our patient cohort. Similarly, available literature reported that most *HER2* mutations (90%) occur in the form of *HER2* exon 20 insertions, with Y772_A775dup (also referred to as A775_G776insYVMA, E770_A771insAYVM or A771_M774dup in scientific literature) being the most common subtype [3, 36]. Furthermore, in our patient cohort, *HER2* exon 20 insertions were mostly mutually exclusive to other driver mutations, with only the S310S/Y mutation found in the extracellular ligand binding domain co-occurring with *EGFR* sensitising mutations. This finding is also mirrored in another retrospective study, which found only eight patients (out of 12946 NSCLC patients) who had both *EGFR* and *HER2* mutations; of these eight patients, six patients had sensitising *EGFR* mutations and exon eight *HER2* mutation (S310F/Y) [37]. However, it is unclear whether if a concurrent *HER2* S310X mutation will affect response to EGFR TKIs.

In our patient cohort, those with *HER2* mutations tend to be younger than non-*HER2*-mutants (median age 61 versus 64 years old; $p = 0.046$) and were inclined to be female and never-smokers (not statistically significant; $p = 0.111$ and 0.204, respectively) (Table 3). *HER2* mutations have been reported to be significantly associated with never-smokers, patients of Asian origin and female patients [38, 39]. There may be a higher prevalence of *HER2* mutations in NSCLC patients from East Asia, although this could be attributed to the greater number of studies conducted in this region. *HER2* amplifications, on the other hand, have also been described as a potential mechanism of acquired resistance to EGFR TKI, as FISH analysis has revealed that *HER2* was amplified in 12% of tumours with acquired resistance, versus only 1% of untreated lung adenocarcinomas [40]. Other gene amplifications (e.g., *EGFR* and *MET*) are also known to act as resistance drivers against targeted therapy [41, 42]. These gene amplifications may occur de novo, or develop post-progression. In our patient cohort, all five patients with *HER2* amplification who progressed post-TKI also had *EGFR* sensitising co-mutations. These patients likely developed *HER2* amplification as acquired resistance to EGFR TKI. For these patients, therapies that target both *EGFR* and *HER2* may confer clinical benefit [43]. Future studies of the Malaysian NSCLC patient cohort with *HER2* alterations could benefit from analysis of treatment modalities and their impact on survival outcomes.

Our analysis of 18 prospective phase II clinical trials of various treatments for NSCLC patients with *HER2* alterations revealed promising treatment outcomes with trastuzumab deruxtecan, trastuzumab emtansine, pyrotinib, pyrotinib + apatinib and trastuzumab + pertuzumab + docetaxel. Both *HER2* mutation and amplification in lung cancer may be indicators of benefit with *HER2*-targeted therapy. *HER2* mutations particularly in the extracellular domain or kinase domain, as well as amplification, lead to *HER2* hyperactivation of downstream signalling cascades such as the PI3K and MAPK pathways [8]. Emerging therapeutic agents such as ADCs work by the selective binding of the monoclonal antibody component to the receptor's extracellular domain, and delivery of the cytotoxic payload to arrest malignant cell growth. Anti-HER2 ADCs have generally demonstrated clinical activity in lung cancers with *HER2*-activating mutations, irrespective of the level of protein expression [8].

A phase II trial investigating treatment with trastuzumab emtansine in NSCLC characterised by HER2 overexpression or mutation was stopped early due to limited efficacy [44]. The authors noted that IHC 3+ or IHC 2+/FISH-positive tumours showed limited response to the investigational agent in the study [44]. In the phase II DESTINY-Lung01 study, trastuzumab deruxtecan was also evaluated in HER2-overexpressed metastatic NSCLC (IHC 2+ and 3+) at two dose levels: 6.4 and 5.4 mg/kg. The ORR was 26.5% and 34.1%, DCR was 69.4% and 78.0%, PFS was 5.7 and 6.7 months and OS was 12.4 and 11.2 months at 6.4 and 5.4 mg/kg, respectively. Both trastuzumab deruxtecan doses showed consistent antitumor activity in heavily pre-treated patients with HER2-overexpressed NSCLC [45]. This is in contrast with trastuzumab emtansine, which demonstrated limited efficacy in HER2-overexpressed NSCLC; DCR were only 7% and 30% in IHC 2+ and 3+ cohorts, respectively [7, 44]. Trastuzumab deruxtecan has an 8:4 8:1 chemotherapy drug-to-antibody ratio, compared with trastuzumab emtansine's 3.5:1 chemotherapy drug-to-antibody ratio, which may explain the improved efficacy of trastuzumab deruxtecan [46]. Additionally, the membrane permeability of the cytotoxic payload of trastuzumab deruxtecan contributes to the bystander effect of inducing apoptosis in neighbouring tumour cells [12, 46]. Nonetheless, this higher drug-to-antibody ratio also leads to increased toxicities associated with trastuzumab deruxtecan treatment, in particular interstitial lung disease. Likewise, the DESTINY-Breast03 trial demonstrated that trastuzumab deruxtecan conferred better clinical benefit compared to trastuzumab emtansine in HER2-positive breast cancer [5]. The incidence of interstitial lung disease was reported to be higher in breast cancer patients treated with trastuzumab deruxtecan (15%) compared with trastuzumab emtansine (3%), although no grade 4/5 event was seen with either treatment [5].

The common *HER2* mutation, HER2 Y772_A775dup / A775_G776insYVMA, was identified to confer increased resistance to afatinib and chemotherapy treatments in patients with NSCLC [47–49]. However, it is unclear if this resistance extends to treatment with other HER2 TKIs and ADCs. NSCLC patients with *HER2* mutations have a higher incidence of brain metastases compared with patients with *EGFR* or *KRAS* mutations [50]. Moreover, *HER2* exon 20 YVMA insertion is also associated with a higher lifetime incidence of brain metastasis in advanced NSCLC, compared to non-YVMA cases [51]. This higher propensity for brain metastasis might contribute to the challenges faced in achieving effective responses to afatinib and chemotherapy treatments due to poor penetration of the blood-brain barrier. In the phase II trials for NSCLC patients with *HER2* mutations, sub-group analyses revealed comparable outcomes in patients with CNS metastasis who were treated with trastuzumab deruxtecan or pyrotinib. This finding is encouraging as it indicates that these treatment approaches could be effective in managing patients with CNS metastasis. *TP53* is a common co-mutation that may also affect treatment efficacy. Co-mutations in the TP53 pathway have been shown to confer additional resistance to afatinib therapy in lung cancer [52]. In breast cancer, *TP53*-mutated patients tended to have a worse prognosis with anti-HER2 TKI treatment compared to TP53-wild-type patients [53]. Given the frequent occurrence of *TP53* co-mutations in NSCLC patients, further investigation is warranted to better understand its implications for *HER2*-targeted therapies.

Conclusion

In conclusion, in this retrospective analysis of diagnostic NGS performed at a referral center in Malaysia, *HER2* alteration was present in 5.8% of Malaysian NSCLC patients. Of these, 3.9% had *HER2* mutation, 1.5% had *HER2* amplification and 0.4% had both *HER2* mutation and amplification. Most (79.7%) of Malaysian NSCLC patients with *HER2* mutation had *HER2* exon 20 insertions, with Y772_A775dup being the most frequent *HER2* mutation variant. These findings fall within the range reported in global studies; the prevalence of *HER2* mutations in NSCLC reported in global studies ranged from 0.3% to 9.1%, whereas the prevalence of *HER2* amplification ranged from 0.2% to 19%. A systematic review of prospective phase II clinical trials of various treatments for NSCLC patients with *HER2* alterations revealed promising treatment outcomes with trastuzumab deruxtecan, trastuzumab emtansine, pyrotinib, pyrotinib + apatinib and trastuzumab + pertuzumab + docetaxel. Malaysian NSCLC patients with *HER2* alteration are anticipated to similarly benefit from the abovementioned *HER2*-targeted therapies.

Acknowledgments

This study received grant support from AstraZeneca; however, the funder had no role in the study's design, analysis or manuscript drafting. Medical writing assistance for this article was provided by Mediconnexions Consulting Sdn Bhd. The authors also extend their gratitude to the SJMC laboratory staff for their valuable contributions to the NGS testing process.

Conflicts of interest

PR declares consultancies and receipt of speaker fees from AstraZeneca and Thermo Fisher, as well as research grants from AstraZeneca and Roche. NYY declares conference travel support from AstraZeneca. KP declares no conflict of interest regarding the publication of this article.

Funding

This study received grant support from AstraZeneca.

Author contributions

Conceptualisation, NYY and PR; *Supervision*, PR; *Funding Acquisition*, PR; *Data Curation*, NYY and KP; *Formal Analysis*, NYY; *Writing – Original Draft Preparation*, NYY and PR; *Writing – Review & Editing*, NYY, KP and PR.

Data availability

Data supporting the findings of this study are available from the corresponding author upon request.

References

1. Ren S, Wang J, and Ying J, *et al* (2022) **Consensus for HER2 alterations testing in non-small-cell lung cancer** *ESMO Open* **7**(1) 100395 https://doi.org/10.1016/j.esmoop.2022.100395 PMID: 35149428 PMCID: 8844658
2. Bang YJ, Van Cutsem E, and Feyereislova A, *et al* (2010) **Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial** *Lancet* **376**(9742) 687–697 https://doi.org/10.1016/S0140-6736(10)61121-X PMID: 20728210
3. Zeng J, Ma W, and Young RB, *et al* (2021) **Targeting HER2 genomic alterations in non-small cell lung cancer** *J Nat Cancer Center* **1**(2) 58–73 https://doi.org/10.1016/j.jncc.2021.04.001
4. Gutierrez C and Schiff R (2011) **HER2: biology, detection, and clinical implications** *Arch Pathol Lab Med* **135**(1) 55–62 https://doi.org/10.5858/2010-0454-RAR.1 PMID: 21204711 PMCID: 3242418
5. Hurvitz SA, Hegg R, and Chung WP, *et al* (2023) **Trastuzumab deruxtecan versus trastuzumab emtansine in patients with HER2-positive metastatic breast cancer: updated results from DESTINY-Breast03, a randomised, open-label, phase 3 trial** *Lancet* **401**(10371) 105–117 https://doi.org/10.1016/S0140-6736(22)02420-5
6. Gatzemeier U, Groth G, and Butts C, *et al* (2004) **Randomized phase II trial of gemcitabine-cisplatin with or without trastuzumab in HER2-positive non-small-cell lung cancer** *Ann Oncol* **15**(1) 19–27 https://doi.org/10.1093/annonc/mdh031

7. Peters S, Stahel R, and Bubendorf L, *et al* (2019) **Trastuzumab emtansine (T-DM1) in patients with previously treated HER2-overexpressing metastatic non-small cell lung cancer: efficacy, safety, and biomarkers** *Clin Cancer Res* **25**(1) 64–72 https://doi.org/10.1158/1078-0432.CCR-18-1590
8. Li BT, Michelini F, and Misale S, *et al* (2020) **HER2-mediated internalization of cytotoxic agents in ERBB2 amplified or mutant lung cancers** *Cancer Discov* **10**(5) 674–687 https://doi.org/10.1158/2159-8290.CD-20-0215 PMID: 32213539 PMCID: 7196485
9. Ettinger DS, Wood DE, and Aisner DL, *et al* (2023) **NCCN guidelines® insights: non-small cell lung cancer, version 2.2023** *J Natl Compr Canc Netw* **21**(4) 340–350 https://doi.org/10.6004/jnccn.2023.0020 PMID: 37015337
10. Riudavets M, Sullivan I, and Abdayem P, *et al* (2021) **Targeting HER2 in non-small-cell lung cancer (NSCLC): a glimpse of hope? An updated review on therapeutic strategies in NSCLC harbouring HER2 alterations** *ESMO Open* **6**(5) 100260 https://doi.org/10.1016/j.esmoop.2021.100260 PMID: 34479034 PMCID: 8414039
11. Iwama E, Zenke Y, and Sugawara S, *et al* (2022) **Trastuzumab emtansine for patients with non-small cell lung cancer positive for human epidermal growth factor receptor 2 exon-20 insertion mutations** *Eur J Cancer* **162** 99–106 https://doi.org/10.1016/j.ejca.2021.11.021
12. Li BT, Smit EF, and Goto Y, *et al* (2022) **Trastuzumab deruxtecan in HER2-mutant non-small-cell lung cancer** *N Engl J Med* **386**(3) 241–251 https://doi.org/10.1056/NEJMoa2112431 PMCID: 9066448
13. Malaysia MoH (2018) *Malaysian Study on Cancer Survival* (Putrajaya: Malaysia MoH)
14. Rajadurai P, Yap NY, and Mohamed Yousoof SB, *et al* (2023) **Mutational profiling of lung cancer using next generation sequencing: a Malaysian real-world clinical diagnostic experience** *J Mol Pathol* **4**(1) 31–43 https://doi.org/10.3390/jmp4010004
15. Gao J, Aksoy BA, and Dogrusoz U, *et al* (2013) **Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal** *Sci Signal* **6**(269) pl1 https://doi.org/10.1126/scisignal.2004088 PMID: 23550210 PMCID: 4160307
16. Page MJ, McKenzie JE, and Bossuyt PM, *et al* (2021) **The PRISMA 2020 statement: an updated guideline for reporting systematic reviews** *BMJ* **372** n71 https://doi.org/10.1136/bmj.n71 PMID: 33782057 PMCID: 8005924
17. Suzuki M, Shiraishi K, and Yoshida A, *et al* (2015) **HER2 gene mutations in non-small cell lung carcinomas: concurrence with Her2 gene amplification and Her2 protein expression and phosphorylation** *Lung Cancer* **87**(1) 14–22 https://doi.org/10.1016/j.lungcan.2014.10.014
18. Xue X, Asuquo I, and Hong L, *et al* (2020) **Catalog of lung cancer gene mutations among Chinese patients** *Front Oncol* **10** 1251 https://doi.org/10.3389/fonc.2020.01251 PMID: 32850378 PMCID: 7417348
19. Kim EK, Kim KA, and Lee CY, *et al* (2017) **The frequency and clinical impact of HER2 alterations in lung adenocarcinoma** *PLoS One* **12**(2) e0171280 https://doi.org/10.1371/journal.pone.0171280 PMID: 28146588 PMCID: 5287480
20. Dziadziuszko R, Smit EF, and Dafni U, *et al* (2019) **Afatinib in NSCLC with HER2 mutations: results of the prospective, open-label phase II NICHE trial of European thoracic oncology platform (ETOP)** *J Thorac Oncol* **14**(6) 1086–1094 https://doi.org/10.1016/j.jtho.2019.02.017 PMID: 30825613
21. Fan Y, Chen J, and Zhou C, *et al* (2020) **Afatinib in patients with advanced non-small cell lung cancer harboring HER2 mutations, previously treated with chemotherapy: a phase II trial** *Lung Cancer* **147** 209–213 https://doi.org/10.1016/j.lungcan.2020.07.017 PMID: 32738416
22. Elamin YY, Robichaux JP, and Carter BW, *et al* (2022) **Poziotinib for patients with HER2 exon 20 mutant non-small-cell lung cancer: results from a phase II trial** *J Clin Oncol* **40**(7) 702–709 https://doi.org/10.1200/JCO.21.01113 PMID: 8887948
23. Le X, Cornelissen R, and Garassino M, *et al* (2022) **Poziotinib in non-small-cell lung cancer harboring HER2 exon 20 insertion mutations after prior therapies: ZENITH20-2 trial** *J Clin Oncol* **40**(7) 710–718 https://doi.org/10.1200/JCO.21.01323 PMID: 8887939

24. Sun S, Prelaj A, and Baik C, *et al* (2022) **26MO efficacy and safety of poziotinib in treatment-naïve HER2 exon 20 insertion (ex20ins) mutated non-small cell lung cancer (NSCLC): ZENITH20-4** *Ann Oncol* **33** S13 https://doi.org/10.1016/j.annonc.2022.01.035

25. FDA Briefing Document (2022) **Poziotinib** [https://www.fda.gov/media/161676/download]

26. Li BT, Shen R, and Buonocore D, *et al* (2018) **Ado-trastuzumab emtansine for patients with HER2-mutant lung cancers: results from a phase II basket trial** *J Clin Oncol* **36**(24) 2532–2537 https://doi.org/10.1200/JCO.2018.77.9777 PMID: 29989854 PMCID: 6366814

27. Song Z, Li Y, and Chen S, *et al* (2022) **Efficacy and safety of pyrotinib in advanced lung adenocarcinoma with HER2 mutations: a multi-center, single-arm, phase II trial** *BMC Med* **20**(1) 42 https://doi.org/10.1186/s12916-022-02245-z PMID: 35101045 PMCID: 8805254

28. Song Z, Lv D, and Chen SQ, *et al* (2022) **Pyrotinib in patients with HER2-amplified advanced non-small cell lung cancer: a prospective, multicenter, single-arm trial** *Clin Cancer Res* **28**(3) 461–467 https://doi.org/10.1158/1078-0432.CCR-21-2936

29. Yang G, Xu H, and Xu F, *et al* (2021) **P86.02 Pyrotinib combined with apatinib for HER2-mutant non-small cell lung cancer: interim analysis from a phase II clinical study** *J Thorac Oncol* **16**(3) S672–S673

30. Yang G, Xu H, and Yang Y, *et al* (2022) **Pyrotinib combined with apatinib for targeting metastatic non-small cell lung cancer with HER2 alterations: a prospective, open-label, single-arm phase 2 study (PATHER2)** *BMC Med* **20**(1) 277 https://doi.org/10.1186/s12916-022-02470-6 PMID: 36031613 PMCID: 9422117

31. Zhou C, Li X, and Wang Q, *et al* (2020) **Pyrotinib in HER2-mutant advanced lung adenocarcinoma after platinum-based chemotherapy: a multicenter, open-label, single-arm, phase II study** *J Clin Oncol* **38**(24) 2753–2761 https://doi.org/10.1200/JCO.20.00297 PMID: 32614698

32. Goto K, Sang-We K, and Kubo T, *et al* (2022) **LBA55 trastuzumab deruxtecan (T-DXd) in patients (Pts) with HER2-mutant metastatic non-small cell lung cancer (NSCLC): interim results from the phase 2 DESTINY-Lung02 trial** *Ann Oncol* **33** Supp 7, S1422 https://doi.org/10.1016/j.annonc.2022.08.057

33. van Berge Henegouwen JM, Jebbink M, and Hoes LR, *et al* (2022) **Trastuzumab and pertuzumab combination therapy for advanced pre-treated HER2 exon 20-mutated non-small cell lung cancer** *Eur J Cancer* **171** 114–123 https://doi.org/10.1016/j.ejca.2022.05.009 PMID: 35716537

34. Mazieres J, Lafitte C, and Ricordel C, *et al* (2022) **Combination of trastuzumab, pertuzumab, and docetaxel in patients with advanced non–small-cell lung cancer harboring HER2 mutations: results from the IFCT-1703 R2D2 trial** *J Clin Oncol* **40**(7) 719–728 https://doi.org/10.1200/JCO.21.01455 PMID: 35073148

35. Rajadurai P, Cheah PL, and How SH, *et al* (2019) **Molecular testing for advanced non-small cell lung cancer in Malaysia: consensus statement from the College of Pathologists, Academy of Medicine Malaysia, the Malaysian Thoracic Society, and the Malaysian Oncological Society** *Lung Cancer* **136** 65–73 https://doi.org/10.1016/j.lungcan.2019.08.005 PMID: 31446227

36. Brazel D, Kroening G, and Nagasaka M (2022) **Non-small cell lung cancer with EGFR or HER2 exon 20 insertion mutations: diagnosis and treatment options** *BioDrugs* **36**(6) 717–729 https://doi.org/10.1007/s40259-022-00556-4 PMID: 36255589 PMCID: 9649507

37. Nagasaka M, Singh V, and Baca Y, *et al* (2022) **The effects of HER2 alterations in EGFR mutant non-small cell lung cancer** *Clin Lung Cancer* **23**(1) 52–59 https://doi.org/10.1016/j.cllc.2021.08.012

38. Shigematsu H, Takahashi T, and Nomura M, *et al* (2005) **Somatic mutations of the HER2 kinase domain in lung adenocarcinomas** *Cancer Res* **65**(5) 1642–1646 https://doi.org/10.1158/0008-5472.CAN-04-4235 PMID: 15753357

39. Sholl LM, Aisner DL, and Varella-Garcia M, *et al* (2015) **Multi-institutional oncogenic driver mutation analysis in lung adenocarcinoma: the lung cancer mutation consortium experience** *J Thorac Oncol* **10**(5) 768–777 https://doi.org/10.1097/JTO.0000000000000516 PMID: 25738220 PMCID: 4410843

40. Takezawa K, Pirazzoli V, and Arcila ME, *et al* (2012) **HER2 amplification: a potential mechanism of acquired resistance to EGFR inhibition in EGFR-mutant lung cancers that lack the second-site EGFRT790M mutation** *Cancer Discov* **2**(10) 922–933 https://doi.org/10.1158/2159-8290.CD-12-0108 PMID: 22956644 PMCID: 3473100

41. Coleman N, Hong L, and Zhang J, *et al* (2021) **Beyond epidermal growth factor receptor: MET amplification as a general resistance driver to targeted therapy in oncogene-driven non-small-cell lung cancer** *ESMO Open* **6**(6) 100319 https://doi.org/10.1016/j.esmoop.2021.100319 PMID: 34837746 PMCID: 8637467

42. Yang H, Wen L, and Zhao C, *et al* (2022) **EGFR amplification is a putative resistance mechanism for NSCLC-LM patients with TKI therapy and is associated with poor outcome** *Front Oncol* **12** 902664 https://doi.org/10.3389/fonc.2022.902664 PMID: 35978803 PMCID: 9376465

43. Gan J, Huang Y, and Liao J, *et al* (2021) **HER2 amplification in advanced NSCLC patients after progression on EGFR-TKI and clinical response to EGFR-TKI plus pyrotinib combination therapy** *Onco Targets Ther* **14** 5297–5307 https://doi.org/10.2147/OTT.S335217 PMID: 34824536 PMCID: 8609241

44. Hotta K, Aoe K, and Kozuki T, *et al* (2018) **A phase II study of trastuzumab emtansine in HER2-positive non-small cell lung cancer** *J Thorac Oncol* **13**(2) 273–279 https://doi.org/10.1016/j.jtho.2017.10.032 PMID: 29313813

45. Smit EF, Felip E, and Uprety D, *et al* (2022) **975P Trastuzumab deruxtecan in patients (pts) with HER2-overexpressing (HER2-OE) metastatic non-small cell lung cancer (NSCLC): results from the DESTINY-Lung01 trial** *Ann Oncol* **33** S994–S995 https://doi.org/10.1016/j.annonc.2022.07.1103

46. Nader-Marta *et al* (2023) **Antibody–drug conjugates: the evolving field of targeted chemotherapy for breast cancer treatment** https://doi.org/10.1177/17588359231183679

47. Yang G, Xu H, and Hu J, *et al* (2022) **Specific HER2 exon 20 Gly776 deletion-insertions in non-small cell lung cancer: structural analysis and sensitivity to HER2-targeted tyrosine kinase inhibitors** *Front Pharmacol* **13** 806737 https://doi.org/10.3389/fphar.2022.806737 PMID: 35330827 PMCID: 8940162

48. Fang W, Zhao S, and Liang Y, *et al* (2020) **Mutation variants and co-mutations as genomic modifiers of response to afatinib in HER2-mutant lung adenocarcinoma** *Oncologist* **25**(3) e545–e554 https://doi.org/10.1634/theoncologist.2019-0547 PMID: 32162827 PMCID: 7066719

49. Liu Z, Wu L, and Cao J, *et al* (2018) **Clinical characterization of ERBB2 exon 20 insertions and heterogeneity of outcomes responding to afatinib in Chinese lung cancer patients** *Onco Targets Ther* **11** 7323–7331 https://doi.org/10.2147/OTT.S173391 PMID: 30425522 PMCID: 6205822

50. Offin M, Feldman D, and Ni A, *et al* (2019) **Frequency and outcomes of brain metastases in patients with HER2-mutant lung cancers** *Cancer* **125**(24) 4380–4387 https://doi.org/10.1002/cncr.32461 PMID: 31469421 PMCID: 6891113

51. Yang S, Wang Y, and Zhao C, *et al* (2021) **Exon 20 YVMA insertion is associated with high incidence of brain metastasis and inferior outcome of chemotherapy in advanced non-small cell lung cancer patients with HER2 kinase domain mutations** *Transl Lung Cancer Res* **10**(2) 753–765 https://doi.org/10.21037/tlcr-20-559 PMID: 33718019 PMCID: 7947396

52. Yuan B, Zhao J, and Zhou C, *et al* (2020) **Co-occurring alterations of ERBB2 exon 20 insertion in non-small cell lung cancer (NSCLC) and the potential indicator of response to afatinib** *Front Oncol* **10** 729 https://doi.org/10.3389/fonc.2020.00729 PMID: 32477948 PMCID: 7236802

53. Liu B, Yi Z, and Guan Y, *et al* (2022) **Molecular landscape of TP53 mutations in breast cancer and their utility for predicting the response to HER-targeted therapy in HER2 amplification-positive and HER2 mutation-positive amplification-negative patients** *Cancer Med* **11**(14) 2767–2778 https://doi.org/10.1002/cam4.4652 PMID: 35393784 PMCID: 9302303

54. Li B, Gandhi L, and Besse B, *et al* (2021) **FP14.15 neratinib-based combination therapy in *HER2*-mutant lung adenocarcinomas: findings from two international phase 2 studies** *J Thorac Oncol* **16**(3) S234 https://doi.org/10.1016/j.jtho.2021.01.158

Supplementary materials

Supplementary Table S1 provides the list of studies reporting the prevalence of *HER2* mutations and amplification.

Supplementary Table S1. Studies reporting the prevalence of *HER2* mutations and amplification.

Region	Histology type	Sample size	*HER2* mutations		*HER2* amplification		References
			Prevalence	Detection method†	Prevalence	Detection method	
Japan and Taiwan USA and Australia	NSCLC (Only found in adenocarcinoma)	269 and 145 157 and 100	3% and 1.4% (4% adenocarcinoma) 0 and 1% (0.4% adenocarcinoma)	PCR ex 18–24	-		[1]
China	NSCLC	49 adenocarcinoma 153 SCC 10 adenosquamous carcinoma	1 (2%) adenocarcinoma	PCR ex 19–20	-		[2]
China	Adenocarcinoma	981	27 (2.8%)	PCR ex 18–21	-		[3]
China	SCC	310	1 (0.3%)	PCR ex 18–21	-		[4]
China	Adenosquamous Adenocarcinoma	76 646	1.3% 2.6%	PCR ex 20 only	-		[5]
China	Adenocarcinoma SCC Adenosquamous LCC	1,356 310 57 19	2.5% 0.3% 1.8% 0	PCR All mutation	-		[6]
China	NSCLC	859	2.4%	PCR ex 20 ins and NGS	-		[7]
China	NSCLC	1,200	4.3%	NGS	2.4%	NGS	[8]
China	NSCLC	640	1.9%	RT-PCR	-		[9]
China	NSCLC	884	2.1%	NGS	2.1%	NGS	[10]
China	NSCLC	1,929	2.5%	N/A ex 18–21	0.2%	N/A	[11]
China	NSCLC	3,440	8.6%	NGS	0.9%	NGS	[12]
China	NSCLC	20,656	5.94% all mutations 1.4% ex 20 ins	N/A	-		[13]
China	SCC	488 (tissue and plasma)	9.06%	NGS	0.8%	NGS	[14]
China	NSCLC	1,087 tissue, 368 blood, 68 pleural effusion	5.6%	NGS	2.8%	NGS	[15]

(Continued)

Supplementary Table S1. Studies reporting the prevalence of *HER2* mutations and amplification. ***(Continued)***

Region	Histology type	Sample size	*HER2* mutations		*HER2* amplification		References
			Prevalence	Detection method†	Prevalence	Detection method	
China	NSCLC	21,745	3.1% activating mut	NGS	-		[16]
China	NSCLC	18,205	1.56% ex 20 ins	NGS ex 20 ins	-		[17]
China	Adenocarcinoma	8,247	2.5% ex 20 ins	NGS ex 20 ins	-		[18]
China	NSCLC	1,875	1.9% HER2 ins	PCR ex 20 ins	-		[19]
China	NSCLC	781	1.92%	RT-PCR or NGS All mut	-		[20]
China	NSCLC	1,497	2.9%	RT-PCR or NGS All mut	-		[21]
China	NSCLC	249	6.8%	N/A	1.6%	N/A	[22]
China	NSCLC	7,520	2.3% ex 20 ins	NGS ex 20 ins	-		[23]
China	NSCLC	1,270	1.7% ex 20 ins	NGS	-		[24]
Taiwan	Adenocarcinoma	888	4.5%	RT-PCR ex 18–21	-		[25]
Taiwan	NSCLC	1,001	1.7% HER2 mutation	MALDI-TOF	-		[26]
Japan	Adenocarcinoma	411	7 (1.7%)	PCR ex 20 ins	-		[27]
Japan	Adenocarcinoma	243	2.7% mut	PCR ex 20 ins	2.1%–3.7%	FISH or DISH	[28]
Japan	NSCLC	1,275 all 1,055 adenocarcinoma	3.6% all 4.3% adenocarcinoma	PCR ex 20 ins	19%	DISH	[29]
Japan	NSCLC	206	4.9%	NGS	-		
Japan	NSCLC	504	2.6%	PCR (ex 18–21)	-		[30]
Japan	NSCLC	3,441	4% ex 20 ins	NGS ex 20 ins	-		[31]
Japan	NSCLC (Only found in adenocarcinoma)	223	1.8% all, 2.6% adenocarcinoma	PCR ex 19–20	-		[32]
Japan	NSCLC	349	1.7% all	PCR ex 18–24	-		[33]
Japan	NSCLC	313	2.6% all 3.3% adenocarcinoma 1.3% SCC	PCR ex 19–20	-		[34]
Korea	NSCLC	271 adenocarcinoma	2.6%	NGS all mut	-		[35]
Korea	NSCLC	969	4.2%	NGS all	2.9%	SISH	[36]
Korea	NSCLC	1,108	2% ex 20 ins	NGS ex 20 ins	1.4%	NGS	[37]
Singapore	Adenocarcinoma	1,252	3.1% all 2.7% ex 20 ins	NGS	-		[38]
India	NSCLC	204	1.5%	PCR ex 20 ins	-		[39]

(Continued)

Supplementary Table S1. Studies reporting the prevalence of *HER2* mutations and amplification. ***(Continued)***

Region	Histology type	Sample size	*HER2* mutations		*HER2* amplification		References
			Prevalence	Detection method[†]	Prevalence	Detection method	
USA	NSCLC	344	4%	PCR ex 20 ins	-		[40]
USA	NSCLC	733 adenocarcinoma	3%	MALDI-TOF, PCR	-		[41]
USA (African American)	NSCLC	260	0.4%	NGS ins 20	-		[42]
China USA	NSCLC	490 2,200	3.5% 2.7%	NGS all	2.4% 1.5%	NGS	[43]
USA	NSCLC	1,674	3%	NGS	-		[44]
USA	NSCLC	6,832	3.4%	NGS all	3%	NGS	[45]
USA	NSCLC	1,006	1.3%	NGS	-		[46]
USA	Adenocarcinoma	920	3%	NGS (ex 20 ins)	-		[47]
USA	Adenocarcinoma	302	1%	NGS	-		[48]
USA	NSCLC	43,706	2.3% 1.5% Ex 20	NGS	2%		[49]
USA	NSCLC	570	5.1% ex 19 and 20	PCR, RT-PCR and NGS	0.4%	FISH	[50]
USA	NSCLC	12,946	1.5%	NGS all	1.1%	NGS	[51]
Canada	NSCLC	1,395	2.2% Ex 20 (1.6%) Transmembrane (0.3%)	NGS all	-		[52]
France	NSCLC	284	3%	N/A	-		[53]
France	NSCLC	11,723	0.8%	PCR ex 20	-		[54]
France	Adenocarcinoma	Caucasian 1,940 African 240 Asian 39	8.1% Asian 3.3% African 1.0% Caucasian	PCR ex 20 ins	-		[55]
France	Non-squamous NSCLC	2,921	2%	Not specified	-		[56]
France Switzerland Spain	Adenocarcinoma	3,800	1.7%	PCR (ex 20 ins)	-		[57]
Belgium	NSCLC	234	0.9%	NGS ex 20 ins	-		[58]
Finland	NSCLC	425	2%	NGS	-		[59]
Germany	NSCLC	398	1%	NGS ex 20	-		[60]
Germany	NSCLC	4,500	2.3% 1.5% ex 20 ins	NGS ex 20 ins and non-ex 20 ins	0.7% Five patients both mut and amp	NGS	[61]

(Continued)

Research

Supplementary Table S1. Studies reporting the prevalence of *HER2* mutations and amplification. *(Continued)*

Region	Histology type	Sample size	*HER2* mutations		*HER2* amplification		References
			Prevalence	Detection method†	Prevalence	Detection method	
Greece	NSCLC	502	1.8% all 2% adenocarcinoma	NGS all	1%	RT-PCR	[62]
Switzerland	Adenocarcinoma	469	1.9%	NGS all	-		[63]
Italy	Adenocarcinoma	537	3%	RT-PCR or NGS	1.9%	FISH	[64]
Netherlands	NSCLC	3,342	1.2%	NGS cohort ex 18–20	-		[65]
International cBioportal	NSCLC	1,934	4.3%	NGS all	-		[66]
Brazil	NSCLC	513	4.9% alterations	NGS all	-		[67]
Brazil	NSCLC	722	0.8%	NGS (ex 20 ins only)	-		[68]
Germany	NSCLC	378	-	-	2%	FISH	[69]
Germany	NSCLC	526	-	-	3.2% NSCLC, 6.3% in adenocarcinoma	FISH	[70]
France	NSCLC	250	-	-	9%	FISH	[71]
Japan	NSCLC	270	-	-	3% NSCLC	FISH	[72]
Korea	Adenocarcinoma	321	-	-	14.3%	SISH	[73]
China	NSCLC	7,643	-	-	2.8%	NGS	[74]
Russia	NSCLC	218	-	-	6% NSCLC	FISH	[75]
Poland	NSCLC	239	-	-	1%	MLPA	[76]

†Note: Detection method specified as PCR utilised PCR for amplification of nucleic acid and subsequent sequencing using conventional techniques, e.g. Sanger sequencing

amp: amplified; DISH: dual *in situ* hybridisation; ex: exon; FISH: fluorescent *in situ* hybridisation; HER2: human epidermal growth factor receptor 2; ins: insertion; LCC: large cell carcinoma; MALDI-TOF: matrix-assisted laser desorption ionisation-time of flight; MLPA: multiplex ligation-dependent probe amplification; mut: mutation; N/A: not available; NGS: next-generation sequencing; NSCLC: non-small cell lung cancer; PCR: polymerase chain reaction; RT-PCR: reverse transcription polymerase chain reaction; SCC: squamous cell carcinoma; SISH: silver *in situ* hybridisation; USA: United States of America

References

1. Shigematsu H, Takahashi T, and Nomura M, *et al* (2005) **Somatic mutations of the HER2 kinase domain in lung adenocarcinomas** *Cancer Res* **65**(5) 1642–1646 https://doi.org/10.1158/0008-5472.CAN-04-4235 PMID: 15753357
2. Feng S, Ling H, and Guo H, *et al* (2014) **Infrequent ERBB2 mutations in Chinese patients with non-small cell lung cancer** *J Thorac Dis* **6**(5) 503–506 PMID: 24822110 PMCID: 4015026
3. Hu H, Pan Y, and Li Y, *et al* (2014) **Oncogenic mutations are associated with histological subtypes but do not have an independent prognostic value in lung adenocarcinoma** *Onco Targets Ther* **7** 1423–1437 https://doi.org/10.2147/OTT.S58900 PMID: 25152623 PMCID: 4140237

4. Pan Y, Wang R, and Ye T, *et al* (2014) **Comprehensive analysis of oncogenic mutations in lung squamous cell carcinoma with minor glandular component** *Chest* **145**(3) 473–479 https://doi.org/10.1378/chest.12-2679

5. Wang R, Pan Y, and Li C, *et al* (2014) **Analysis of major known driver mutations and prognosis in resected adenosquamous lung carcinomas** *J Thorac Oncol* **9**(6) 760–768 https://doi.org/10.1097/JTO.0b013e3182a406d1 PMID: 24481316

6. Wang R, Zhang Y, and Pan Y, *et al* (2015) **Comprehensive investigation of oncogenic driver mutations in Chinese non-small cell lung cancer patients** *Oncotarget* **6**(33) 34300–34308 https://doi.org/10.18632/oncotarget.5549 PMID: 26486077 PMCID: 4741453

7. Song Z, Yu X, and Shi Z, *et al* (2016) **HER2 mutations in Chinese patients with non-small cell lung cancer** *Oncotarget* **7**(47) 78152–78158 https://doi.org/10.18632/oncotarget.11313 PMID: 27825109 PMCID: 5363651

8. Wen S, Dai L, and Wang L, *et al* (2019) **Genomic signature of driver genes identified by target next-generation sequencing in Chinese non-small cell lung cancer** *Oncologist* **24**(11) e1070–e1081 https://doi.org/10.1634/theoncologist.2018-0572 PMID: 30902917 PMCID: 6853120

9. Yang S, Song Z, and Cheng G (2019) **Genomic alterations and survival in young patients aged under 40 years with completely resected non-small cell lung cancer** *Ann Transl Med* **7**(7) 140 https://doi.org/10.21037/atm.2019.03.39 PMID: 31157261 PMCID: 6511547

10. Li D, Ding L, and Ran W, *et al* (2020) **Status of 10 targeted genes of non-small cell lung cancer in eastern China: a study of 884 patients based on NGS in a single institution** *Thorac Cancer* **11**(9) 2580–2589 https://doi.org/10.1111/1759-7714.13577 PMID: 32729257 PMCID: 7471050

11. Xue X, Asuquo I, and Hong L, *et al* (2020) **Catalog of lung cancer gene mutations among chinese patients** *Front Oncol* **10** 1251 https://doi.org/10.3389/fonc.2020.01251 PMID: 32850378 PMCID: 7417348

12. Sun S, Du W, and Sun Q, *et al* (2021) **Driver gene alterations profiling of Chinese non-small cell lung cancer and the effects of co-occurring alterations on immunotherapy** *Cancer Med* **10**(20) 7360–7372 https://doi.org/10.1002/cam4.4178 PMID: 34599863 PMCID: 8525092

13. Wang D, Yuan H, and Zhu H (2021) **The characteristics of ERBB2 exon 20 insertion in a large cohort of Chinese NSCLC patients** *Cancer Res* **81**(13_Suppl)

14. Chen Y, Kong W, and Yu Z, *et al* (2022) **Genetic alteration and PD-L1 expression profiles of Chinese patients with lung squamous cell carcinoma** *Pathol Res Pract* **231** 153761 https://doi.org/10.1016/j.prp.2022.153761 PMID: 35151031

15. Li H, Li X, and Lan S, *et al* (2022) **ERBB2 mutation landscape in non-small cell lung cancer patients in Northeast China** *Tumori J* **109**(3) 276–281 https://doi.org/10.1177/03008916221101426

16. Fan Xu, Qingshan Li, and Wenxin LI, *et al* (2022) **Molecular characteristics of ERBB2-activating mutations in Chinese patients with NSCLC** *J Clin Oncol* **40**(16) 8546 https://doi.org/10.1200/JCO.2022.40.16_suppl.8546

17. Yang L, Huang T, and Qu Y, *et al* (2022) **Real-world frequency of non-small cell lung cancer with ERBB2 exon 20 insertion (Exon20ins) mutations by site of insertion** *J Clin Oncol* **40**(16) e15026 https://doi.org/10.1200/JCO.2022.40.16_suppl.e15026

18. Tian P, Zeng H, and Ji L, *et al* (2021) **Lung adenocarcinoma with ERBB2 exon 20 insertions: comutations and immunogenomic features related to chemoimmunotherapy** *Lung Cancer* **160** 50–58 https://doi.org/10.1016/j.lungcan.2021.07.014 PMID: 34403912

19. Bu S, Wang R, and Pan Y, *et al* (2017) **Clinicopathologic characteristics of patients with HER2 insertions in non-small cell lung cancer** *Ann Surg Oncol* **24**(1) 291–297 https://doi.org/10.1245/s10434-015-5044-8

20. Xu C, Wang W, and Zhuang W, *et al* (2017) **P1.02-046 mutational subtypes and prognosis of non-small-cell lung cancer harboring HER2 mutations** *J Thorac Oncol* **12**(11) S1940 https://doi.org/10.1016/j.jtho.2017.09.777

21. Zhou J, Ding N, and Xu X, *et al* (2020) **Clinical outcomes of patients with HER2-mutant advanced lung cancer: chemotherapies versus HER2-directed therapies** *Ther Adv Med Oncol* **12** 1758835920936090 https://doi.org/10.1177/1758835920936090 PMID: 32647540 PMCID: 7325548

22. Diao WY, Ding CL, and Yuan BY, *et al* (2021) **Clinical characteristics and prognosis of HER2 gene phenotype in patients with non-small cell lung cancer** *Int J Gen Med* **14** 9153–9161 https://doi.org/10.2147/IJGM.S328908 PMID: 34880654 PMCID: 8646112

23. Liu Z, Wu L, and Cao J, *et al* (2018) **Clinical characterization of ERBB2 exon 20 insertions and heterogeneity of outcomes to afatinib in Chinese lung cancers** *J Thorac Oncol* **13**(12) S1046–S1047 https://doi.org/10.1016/j.jtho.2018.10.017

24. Chen K, Pan G, and Cheng G, *et al* (2021) **Immune microenvironment features and efficacy of PD-1/PD-L1 blockade in non-small cell lung cancer patients with EGFR or HER2 exon 20 insertions** *Thorac Cancer* **12**(2) 218–226 https://doi.org/10.1111/1759-7714.13748 PMCID: 7812071

25. Gow CH, Chang HT, and Lim CK, *et al* (2017) **Comparable clinical outcomes in patients with HER2-mutant and EGFR-mutant lung adenocarcinomas** *Genes Chromosomes Cancer* **56**(5) 373–381 https://doi.org/10.1002/gcc.22442 PMID: 28063177

26. Chu CH, Huang YH, and Lee PH, *et al* (2022) **Various impacts of driver mutations on the PD-L1 expression of NSCLC** *PLoS One* **17**(8) e0273207 https://doi.org/10.1371/journal.pone.0273207 PMID: 35980949 PMCID: 9387808

27. Serizawa M, Koh Y, and Kenmotsu H, *et al* (2014) **Assessment of mutational profile of Japanese lung adenocarcinoma patients by multitarget assays** *Cancer* **120**(10) 1471–1481 https://doi.org/10.1002/cncr.28604 PMID: 24700479

28. Yoshizawa A, Sumiyoshi S, and Sonobe M, *et al* (2014) **HER2 status in lung adenocarcinoma: a comparison of immunohistochemistry, fluorescence *in situ* hybridization (FISH), dual-ISH, and gene mutations** *Lung Cancer* **85**(3) 373–378 https://doi.org/10.1016/j.lungcan.2014.06.007 PMID: 25047676

29. Suzuki M, Shiraishi K, and Yoshida A, *et al* (2015) **HER2 gene mutations in non-small cell lung carcinomas: concurrence with HER2 gene amplification and HER2 protein expression and phosphorylation** *Lung Cancer* **87**(1) 14–22 https://doi.org/10.1016/j.lungcan.2014.10.014

30. Tomizawa K, Suda K, and Onozato R, *et al* (2011) **Prognostic and predictive implications of HER2/ERBB2/neu gene mutations in lung cancers** *Lung Cancer* **74**(1) 139–144 https://doi.org/10.1016/j.lungcan.2011.01.014 PMID: 21353324

31. Udagawa H, Matsumoto S, and Ohe Y, *et al* (2019) **Clinical outcome of non-small cell lung cancer with EGFR/HER2 exon 20 insertions identified in the LC-SCRUM-Japan** *J Thorac Oncol* **14**(10) S224 https://doi.org/10.1016/j.jtho.2019.08.443

32. Sonobe M, Manabe T, and Wada H, *et al* (2006) **Lung adenocarcinoma harboring mutations in the ERBB2 kinase domain** *J Mol Diagn* **8**(3) 351–356 https://doi.org/10.2353/jmoldx.2006.050132 PMID: 16825508 PMCID: 1867605

33. Yokoyama T, Kondo M, and Goto Y, *et al* (2006) **EGFR point mutation in non-small cell lung cancer is occasionally accompanied by a second mutation or amplification** *Cancer Sci* **97**(8) 753–759 https://doi.org/10.1111/j.1349-7006.2006.00233.x PMID: 16863509 PMCID: 11158750

34. Takahashi T, Sonobe M, and Kobayashi M, *et al* (2010) **Clinicopathologic features of non-small-cell lung cancer with EML4-ALK fusion gene** *Ann Surg Oncol* **17**(3) 889–897 https://doi.org/10.1245/s10434-009-0808-7 PMID: 20183914

35. Li S, Choi YL, and Gong Z *et al* (2016) **Comprehensive characterization of oncogenic drivers in Asian lung adenocarcinoma** *J Thorac Oncol* **11**(12) 2129–2140 https://doi.org/10.1016/j.jtho.2016.08.142 PMID: 27615396

36. Lee Y, Lee B, and Choi YL, *et al* (2022) **Clinicopathologic and molecular characteristics in ERBB2 (HER2)-altered non-small cell lung cancer** *Virchows Archiv* **481**(SUPPL 1) S151–S152

37. Lee K, Jung HA, and Sun JM, *et al* (2020) **Clinical characteristics and outcomes of non-small cell lung cancer patients with HER2 alterations in Korea** *Cancer Res Treat* **52**(1) 292–300 https://doi.org/10.4143/crt.2019.186 PMCID: 6962476

38. Tan AC, Saw SPL, and Chen J, *et al* (2022) **Clinical and genomic features of HER2 exon 20 insertion mutations and characterization of HER2 expression by immunohistochemistry in East Asian non-small-cell lung cancer** *JCO Precis Oncol* **6** e2200278 https://doi.org/10.1200/PO.22.00278 PMID: 36240473

39. Bhaumik S, Ahmad F, and Das BR (2016) **Somatic mutation analysis of KRAS, BRAF, HER2 and PTEN in EGFR mutation-negative non-small cell lung carcinoma: determination of frequency, distribution pattern and identification of novel deletion in HER2 gene from Indian patients** *Med Oncol* **33**(10) 117 https://doi.org/10.1007/s12032-016-0828-7 PMID: 27637917

40. Cardarella S, Ortiz TM, and Joshi VA, *et al* (2012) **The introduction of systematic genomic testing for patients with non-small-cell lung cancer** *J Thorac Oncol* **7**(12) 1767–1774 https://doi.org/10.1097/JTO.0b013e3182745bcb PMID: 23154547 PMCID: 3500523

41. Kris MG, Johnson BE, and Berry LD, *et al* (2014) **Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs** *J Am Med Assoc* **311**(19) 1998–2006 https://doi.org/10.1001/jama.2014.3741

42. Araujo LH, Lammers PE, and Matthews-Smith V, *et al* (2015) **Somatic mutation spectrum of non-small-cell lung cancer in African Americans: a pooled analysis** *J Thorac Oncol* **10**(10) 1430–1436 https://doi.org/10.1097/JTO.0000000000000650 PMID: 26301800 PMCID: 4618391

43. Xu C, Zhang Y, and Liu D, *et al* (2018) **P1.01-99 detecting HER2 alterations by next generation sequencing (NGS) in patients with advanced NSCLC from the United States and China** *J Thorac Oncol* **13**(10) S502 https://doi.org/10.1016/j.jtho.2018.08.655

44. Sacher AG, Dahlberg SE, and Heng J, *et al* (2016) **Association between younger age and targetable genomic alterations and prognosis in non-small-cell lung cancer** *JAMA Oncol* **2**(3) 313–320 https://doi.org/10.1001/jamaoncol.2015.4482 PMID: 26720421 PMCID: 4819418

45. Suh JH, Johnson A, and Albacker L, *et al* (2016) **Comprehensive genomic profiling facilitates implementation of the National Comprehensive Cancer Network guidelines for lung cancer biomarker testing and identifies patients who may benefit from enrollment in mechanism-driven clinical trials** *Oncologist* **21**(6) 684–691 https://doi.org/10.1634/theoncologist.2016-0030 PMID: 27151654 PMCID: 4912374

46. Illei PB, Belchis D, and Tseng LH, *et al* (2017) **Clinical mutational profiling of 1006 lung cancers by next generation sequencing** *Oncotarget* **8**(57) 96684–96696 https://doi.org/10.18632/oncotarget.18042 PMID: 29228562 PMCID: 5722514

47. Pillai RN, Behera M, and Berry LD, *et al* (2017) **HER2 mutations in lung adenocarcinomas: a report from the lung cancer mutation consortium** *Cancer* **123**(21) 4099–4105 https://doi.org/10.1002/cncr.30869 PMID: 28743157 PMCID: 5650517

48. Miller TE, Yang M, and Bajor D, *et al* (2018) **Clinical utility of reflex testing using focused next-generation sequencing for management of patients with advanced lung adenocarcinoma** *J Clin Pathol* **71**(12) 1108–1115 https://doi.org/10.1136/jclinpath-2018-205396 PMID: 30228211 PMCID: 6900927

49. Ou SHI, Madison R, and Robichaux JP, *et al* (2019) **Characterization of 648 non-small cell lung cancer (NSCLC) cases with 28 unique HER2 exon 20 insertions** *J Clin Oncol* **37**(15) 9063 https://doi.org/10.1200/JCO.2019.37.15_suppl.9063

50. Patil T, Mushtaq R, and Marsh S, *et al* (2020) **Clinicopathologic characteristics, treatment outcomes, and acquired resistance patterns of atypical EGFR mutations and HER2 alterations in Stage IV non-small-cell lung cancer** *Clin Lung Cancer* **21**(3) E191–E204 https://doi.org/10.1016/j.cllc.2019.11.008

51. Nagasaka M, Singh V, and Baca Y, *et al* (2022) **The effects of HER2 alterations in EGFR mutant non-small cell lung cancer** *Clin Lung Cancer* **23**(1) 52–59 https://doi.org/10.1016/j.cllc.2021.08.012

52. Kuang S, Fung AS, and Perdrizet KA, *et al* (2022) **Upfront next generation sequencing in non-small cell lung cancer** *Curr Oncol* **29**(7) 4428–4437 https://doi.org/10.3390/curroncol29070352 PMID: 35877212 PMCID: 9319994

53. Couraud S, Souquet PJ, and Paris C, *et al* (2015) **BioCAST/IFCT-1002: epidemiological and molecular features of lung cancer in never-smokers** *Eur Respir J* **45**(5) 1403–1414 https://doi.org/10.1183/09031936.00097214 PMID: 25657019

54. Barlesi F, Souquet J, and Paris C, *et al* (2016) **Routine molecular profiling of patients with advanced non-small-cell lung cancer: results of a 1-year nationwide programme of the French Cooperative Thoracic Intergroup (IFCT)** *Lancet* **387**(10026) 1415–1426 https://doi.org/10.1016/S0140-6736(16)00004-0 PMID: 26777916

55. Saffroy R, Morère JF, and Bosselut N, *et al* (2017) **Impact of country of birth on genetic testing of metastatic lung adenocarcinomas in France: African women exhibit a mutational spectrum more similar to Asians than to Caucasians** *Oncotarget* **8**(31) 50792–50803 https://doi.org/10.18632/oncotarget.15132 PMID: 28881604 PMCID: 5584205

56. Lamy T, Cabarrou B, and Planchard D, *et al* (2021) **Biomarker testing in older patients treated for an advanced or metastatic non-squamous non-small-cell lung cancer: the French ESME real-life multicenter cohort experience** *Cancers (Basel)* **14**(1) 92 https://doi.org/10.3390/cancers14010092

57. Mazières J, Peters S, and Lepage B, *et al* (2013) **Lung cancer that harbors an HER2 mutation: epidemiologic characteristics and therapeutic perspectives** *J Clin Oncol* **31**(16) 1997–U307 https://doi.org/10.1200/JCO.2012.45.6095 PMID: 23610105

58. D'Haene N, Le Mercier M, and De Neve N, *et al* (2015) **Clinical application of targeted next-generation sequencing for lung cancer patients: a Belgian experience** *Cancer Res* **75**(22) A1–39 https://doi.org/10.1158/1538-7445.TRANSCAGEN-A1-39

59. Mäki-Nevala S, Sarhadi VK, and Rönty M, *et al* (2016) **Hot spot mutations in Finnish non-small cell lung cancers** *Lung Cancer* **99** 102–110 https://doi.org/10.1016/j.lungcan.2016.06.024 PMID: 27565922

60. Tafe LJ, Pierce KJ, and Peterson JD, *et al* (2016) **Clinical genotyping of non-small cell lung cancers using targeted next-generation sequencing: utility of identifying rare and co-mutations in oncogenic driver genes** *Neoplasia* **18**(9) 577–583 https://doi.org/10.1016/j.neo.2016.07.010 PMID: 27659017 PMCID: 5031899

61. Volckmar AL, Christopoulos P, and Kirchner M, *et al* (2021) **Targeting rare and non-canonical driver variants in NSCLC - an uncharted clinical field** *Lung Cancer* **154** 131–141 https://doi.org/10.1016/j.lungcan.2021.02.022 PMID: 33667718

62. Tsoulos N, Papadopoulou E, and Metaxa-Mariatou V, *et al* (2017) **Tumor molecular profiling of NSCLC patients using next generation sequencing** *Oncol Rep* **38**(6) 3419–3429 PMID: 29130105 PMCID: 5783588

63. Grosse A, Grosse C, and Rechsteiner M, *et al* (2019) **Analysis of the frequency of oncogenic driver mutations and correlation with clinicopathological characteristics in patients with lung adenocarcinoma from Northeastern Switzerland** *Diagn Pathol* **14(1)** 18 https://doi.org/10.1186/s13000-019-0789-1

64. Dall'Olio FG, Conci N, and Rossi G, *et al* (2020) **Comparison of sequential testing and next generation sequencing in advanced lung adenocarcinoma patients - a single centre experience** *Lung Cancer* **149** 5–9 https://doi.org/10.1016/j.lungcan.2020.08.008 PMID: 32932213

65. Steeghs EMP, Groen HJM, and Schuuring E, *et al* (2022) **Mutation-tailored treatment selection in non-small cell lung cancer patients in daily clinical practice** *Lung Cancer* **167** 87–97 https://doi.org/10.1016/j.lungcan.2022.04.001 PMID: 35461050

66. Wei X, Gao X, and Zhang X, *et al* (2019) **P2.14-49 Molecular characteristics of HER2 mutations in non-small cell lung cancer** *J Thorac Oncol* **14**(10) S848–S849 https://doi.org/10.1016/j.jtho.2019.08.1834

67. Mascarenhas E, Gelatti AC, and Araújo LH, *et al* (2021) **Comprehensive genomic profiling of Brazilian non-small cell lung cancer patients (GBOT 0118/LACOG0418)** *Thorac Cancer* **12**(5) 580–587 https://doi.org/10.1111/1759-7714.13777 PMCID: 7919136

68. de Oliveira Cavagna R, Zaniolo BG, and de Paula FE, *et al* (2022) **ERBB2 exon 20 insertions are rare in Brazilian non-small cell lung cancer** *Thorac Cancer* **13**(23) 3402–3407 https://doi.org/10.1111/1759-7714.14605 PMID: 36251951 PMCID: 9715798

69. Heinmöller P, Gross C, and Beyser K, *et al* (2003) **HER2 status in non-small cell lung cancer: results from patient screening for enrollment to a phase II study of herceptin** *Clin Cancer Res* **9**(14) 5238–5244 PMID: 14614004

70. Grob TJ, Kannengiesser I, and Tsourlakis MC, *et al* (2012) **Heterogeneity of ERBB2 amplification in adenocarcinoma, squamous cell carcinoma and large cell undifferentiated carcinoma of the lung** *Modern Pathol* **25**(12) 1566–1573 https://doi.org/10.1038/modpathol.2012.125

71. Tagliamento M, Auclin E, and Valent A, *et al* (2022) **1090P HER2 copy number variation in non-small cell lung cancer (NSCLC)** *Ann Oncol* **33**(7) S1050 https://doi.org/10.1016/j.annonc.2022.07.1215

72. Inoue Y, Matsuura S, and Kurabe N, *et al* (2015) **Clinicopathological and survival analysis of japanese patients with resected non-small-cell lung cancer harboring NKX2-1, SETDB1, MET, HER2, SOX2, FGFR1, or PIK3CA gene amplification** *J Thorac Oncol* **10**(11) 1590–1600 https://doi.org/10.1097/JTO.0000000000000685 PMID: 26536195

73. Kim EK, Kim KA, and Lee CY, *et al* (2017) **The frequency and clinical impact of HER2 alterations in lung adenocarcinoma** *Plos One* **12**(2) e0171280 https://doi.org/10.1371/journal.pone.0171280 PMID: 28146588 PMCID: 5287480

74. Zhou C, Ai X, and Gu D, *et al* (2021) **P53.07 clinical and genomic insights into of Chinese lung cancer patients with HER2 amplification** *J Thorac Oncol* **16**(10) S1128 https://doi.org/10.1016/j.jtho.2021.08.556

75. Kobyakov DS, Avdalyan AM, and Klimachev VV, *et al* (2015) **Non-small cell lung cancer: HER2 oncogene status** *Arkh Patol* **77**(2) 3–9 https://doi.org/10.17116/patol20157723-9 PMID: 26027392

76. Lewandowska MA, Czubak K, and Klonowska K, *et al* (2015) **The use of a two-tiered testing strategy for the simultaneous detection of small EGFR mutations and EGFR amplification in lung cancer** *Plos One* **10**(2) e0117983 https://doi.org/10.1371/journal.pone.0117983 PMID: 25719557 PMCID: 4342230