The Oncologist®

Lung Cancer

Mutation Variants and Co-Mutations as Genomic Modifiers of Response to Afatinib in *HER2*-Mutant Lung Adenocarcinoma

Wenfeng Fang,[a,b,†] Shen Zhao,[a,b,†] Ying Liang,[a,b,†] Yunpeng Yang,[a,b,†] Lin Yang,[c,†] Xiaorong Dong,[d] Li Zhang,[e] Yong Tang,[f] Shoufeng Wang,[g] Yang Yang,[h] Xiaoyan Ma,[b] Minghui Wang,[i] Wenjing Wang,[j] Songhui Zhao,[j] Kai Wang,[j] Song Gao,[b] Li Zhang[a,b]

[a]Department of Medical Oncology and [b]State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-Sen University Cancer Center, Guangzhou, People's Republic of China; [c]Department of Thoracic Surgery, Shenzhen People's Hospital, 2nd Clinical Medical College of Jinan University, Shenzhen, People's Republic of China; [d]Cancer Center, Union Hospital and [e]Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People's Republic of China; [f]Department of Thoracic Surgery, General Hospital of Guangzhou Military Command of PLA, Guangzhou, People's Republic of China; [g]Department of Thoracic Surgery, Guangxi Medical University Affiliated Tumor Hospital, Nanning, People's Republic of China; [h]The First Affiliated Hospital of Guangxi University of Chinese Medicine, Nanning, People's Republic of China; [i]Department of Thoracic Surgery, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, People's Republic of China; [j]OrigiMed, Shanghai, People's Republic of China

[†]Contributed equally.

Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Lung cancer • ERBB2 • Mutation variant • Concomitant mutation • Intrinsic resistance

Abstract

Background. Human epidermal growth factor receptor 2 (*HER2*)-mutant lung cancer remains an orphan of specific targeted therapy. The variable responses to anti-HER2 therapies in these patients prompt us to examine impact of *HER2* variants and co-mutations on responses to anti-HER2 treatments in lung cancer.

Patients and Methods. Patients with stage IV/recurrent *HER2*-mutant lung cancers identified through next-generation sequencings were recruited from seven hospitals. The study comprised a cohort A to establish the patterns of *HER2* variants and co-mutations in lung cancer and a cohort B to assess associations between *HER2* variants, co-mutations, and clinical outcomes.

Results. The study included 118 patients (cohort A, $n = 86$; cohort B, $n = 32$). Thirty-one *HER2* variants and 35 co-mutations were detected. Predominant variants were *A775_G776insYVMA* (49/118, 42%), *G778_P780dup* (11/118, 9%), and *G776delinsVC* (9/118, 8%). *TP53* was the most common co-mutation (61/118, 52%). In cohort B, objective response rates with afatinib were 0% (0/14, 95% confidence interval [CI], 0%–26.8%), 40% (4/10, 14.7%–72.6%), and 13% (1/8, 0.7%–53.3%) in group 1 (*A775_G776insYVMA*, $n = 14$), group 2 (*G778_P780dup*, *G776delinsVC*, $n = 10$), and group 3 (missense mutation, $n = 8$), respectively ($p = .018$). Median progression-free survival in group 1 (1.2 months; 95% CI, 0–2.4) was shorter than those in group 2 (7.6 months, 4.9–10.4; hazard ratio [HR], 0.009; 95% CI, 0.001–0.079; $p < .001$) and group 3 (3.6 months, 2.6–4.5; HR, 0.184; 95% CI, 0.062–0.552; $p = .003$). *TP53* co-mutations (6.317; 95% CI, 2.180–18.302; $p = .001$) and *PI3K/AKT/mTOR* pathway activations (19.422; 95% CI, 4.098–92.039; $p < .001$) conferred additional resistance to afatinib.

Conclusion. *G778_P780dup* and *G776delinsVC* derived the greatest benefits from afatinib among *HER2* variants. Co-mutation patterns were additional response modifiers. Refining patient population based on patterns of *HER2* variants and co-mutations may help improve the efficacy of anti-HER2 treatment in lung cancer. ***The Oncologist*** 2020;25:e545–e554

Implications for Practice: Human epidermal growth factor receptor 2 (*HER2*)-mutant lung cancers are a group of heterogenous diseases with up to 31 different variants and 35 concomitant genomic aberrations. Different *HER2* variants exhibit divergent sensitivities to anti-HER2 treatments. Certain variants, *G778_P780dup* and *G776delinsVC*, derive sustained clinical benefits from afatinib, whereas the predominant variant, *A775_G776insYVMA*, is resistant to most anti-HER2 treatments. *TP53* is the most common co-mutation in *HER2*-mutant lung cancers. Co-mutations in *TP53* and the *PI3K/AKT/mTOR* pathway confer additional resistance to anti-HER2 treatments in lung cancer. The present data suggest that different *HER2* mutations in lung cancer, like its sibling epidermal growth factor receptor, should be analyzed independently in future studies.

Correspondence: Li Zhang, M.D., Department of Medical Oncology, Sun Yat-Sen University Cancer Center, 651 Dongfeng East Rd., Guangzhou, 510060, People's Republic of China. Telephone: 862087343458; e-mail: zhangli6@mail.sysu.edu.cn; zhangli@sysucc.org.cn; or Wenfeng Fang, M.D., Department of Medical Oncology, Sun Yat-Sen University Cancer Center, 651 Dongfeng East Rd., Guangzhou, 510060, People's Republic of China. Telephone: 862087343822; e-mail: fangwf@sysucc.org.cn Received July 22, 2019; accepted for publication October 14, 2019; published Online First on November 20, 2019. http://dx.doi.org/10.1634/theoncologist.2019-0547

Introduction

Human epidermal growth factor receptor 2 (*HER2*, *ERBB2*)–activating mutations were identified as oncogenic drivers and potential therapeutic targets in 2%–4% of lung cancers [1–4]. Unlike in breast cancer and gastric cancer, anti-HER2 treatments in HER2-positive/aberrant lung cancer all led to disappointing results [5–9]. After narrowing down their target population from HER2-aberrant to *HER2*-mutant, anti-HER2 therapies started to show efficacy in lung cancer [10–14]. However, treatment outcomes are still modest and variable. Poziotinib and TAK-788, two epidermal growth factor receptor (*EGFR*)/*HER2* exon 20 insertion inhibitors, failed to elicit response in patients with *HER2* mutations [15–17]. Ado-trastuzumab emtansine (T-DM1) showed a 44% partial response rate in *HER2*-mutant lung cancers in one study [13] but a 14.3% response rate in another [9]. Pyrotinib, one of the most promising new drugs for this population, showed a 31.7% response rate [14], which is still lower than expected for a targeted therapy.

Thus far, *HER2*-mutant lung cancer remains an orphan of any specific targeted therapy [18]. The limited efficacy and variable treatment outcomes of anti-HER2 therapies indicate the heterogeneity of these diseases [19]. To improve outcomes for these patients, deeper investigation into their heterogeneity and further refinement of the target population for anti-HER2 treatments are warranted.

In this study, we intended to establish the patterns of *HER2* variants and concomitant genomic alterations in lung cancer; identify potential modifiers of response to afatinib, an irreversible dual EGFR/HER2 kinase inhibitor [20, 21]; and explore mechanisms of intrinsic resistance in *HER2*-mutant lung adenocarcinoma.

Subjects, Materials, and Methods

Study Design and Population

This multicenter study involved seven hospitals in China. To establish the patterns of *HER2* variants and co-mutations in lung cancer, 2,035 consecutive patients with histologically confirmed stage IV or recurrent lung cancers who underwent next-generation sequencing (NGS)–based genomic testing (OrigiMed targeted NGS panels, OrigiMed, Shanghai, China) [22, 23] during routine clinical care from August 2016 to May 2018 were screened for *HER2* mutations (cohort A).

An independent cohort of patients with stage IV or recurrent *HER2*-mutant lung adenocarcinomas and afatinib treatment histories (cohort B) were identified and retrospectively analyzed for the associations between *HER2* variants, patterns of co-mutations, and clinical outcomes. For cohort B, eligible patients should have undergone tumor sampling before the start of afatinib (supplemental online information 1). All patients provided their written informed consent for treatment and for our use of their clinical data before enrolment. This study was approved by ethics committees of Sun Yat-Sen University Cancer Center and all participating sites. It was conducted according to the Declaration of Helsinki.

Genotyping and Three-Dimensional Modeling of HER2 Variants

Tumor samples were collected via surgical resection, computed tomography (CT)–guided biopsy, or bronchial biopsy. DNA was extracted from tumor samples and the matched blood samples for genomic testing (supplemental online information 1). *HER2* aberrations and concomitant genomic alterations were identified using targeted NGS panels for 22–450 cancer-related genes with a mean coverage depth of more than 800×. Genomic alterations assessed included single nucleotide variations, short and long insertions and deletions, copy number variations, and gene rearrangements in selected genes. For purpose of validation, the patterns of *HER2* variants and co-mutations observed in cohort A were compared with those observed in two public data sets, The Cancer Genome Atlas (TCGA) and Memorial Sloan Kettering integrated mutation profiling of actionable cancer targets (MSK-IMPACT).

For common *HER2* variants identified in the study, three-dimensional (3D) modeling in silico was performed to assess their drug-binding pockets. The 3D structural models were generated using the SWISS-MODEL server based on the crystal structure of human HER2 kinase domain (Protein Data Bank code 3PP0). Structural illustrations were prepared using PyMOL Molecular Graphic Systems (version 0.99, Schrödinger LLC, New York, NY, http://www.pymol.org/) [24].

Data Collection and Evaluation of Clinical Outcomes

For patients in cohort B, data on clinicopathological features and treatment histories were collected from medical records or via request forms (supplemental online information 1). Starting dose for afatinib was 40 mg or 30 mg once daily. Dose modifications based on tolerability were left to physicians' discretion. Follow-up included clinical examination and contrast-enhanced CT scans. Brain magnetic resonance imaging was routinely performed for patients with baseline brain metastases. Scan frequency intervals ranged between 4 and 6 weeks.

Clinical outcomes included progression-free survival (PFS), objective response rate (ORR), and disease control rate (DCR). PFS was measured from the date of afatinib initiation to the date of disease progression (PD) defined by RECIST 1.1 [25] or death. Patients without PD were censored on the date of last CT image. ORR was calculated as the total percentage of patients with a complete response or partial response. DCR was calculated as the total percentage of patients with complete response, partial response, or stable disease. Patients' CT images during the afatinib treatment were retrospectively collected to evaluate tumor responses according to RECIST version 1.1.

Statistical Analysis

Genotyping results and clinical outcomes on afatinib were analyzed in a double-blind manner. The distributions of *HER2* variants, co-mutations, and clinicopathological features were compared using a χ^2 test or Fisher's exact test. PFS curves were estimated using the Kaplan-Meier method. Differences between *HER2* variants and co-mutations were calculated

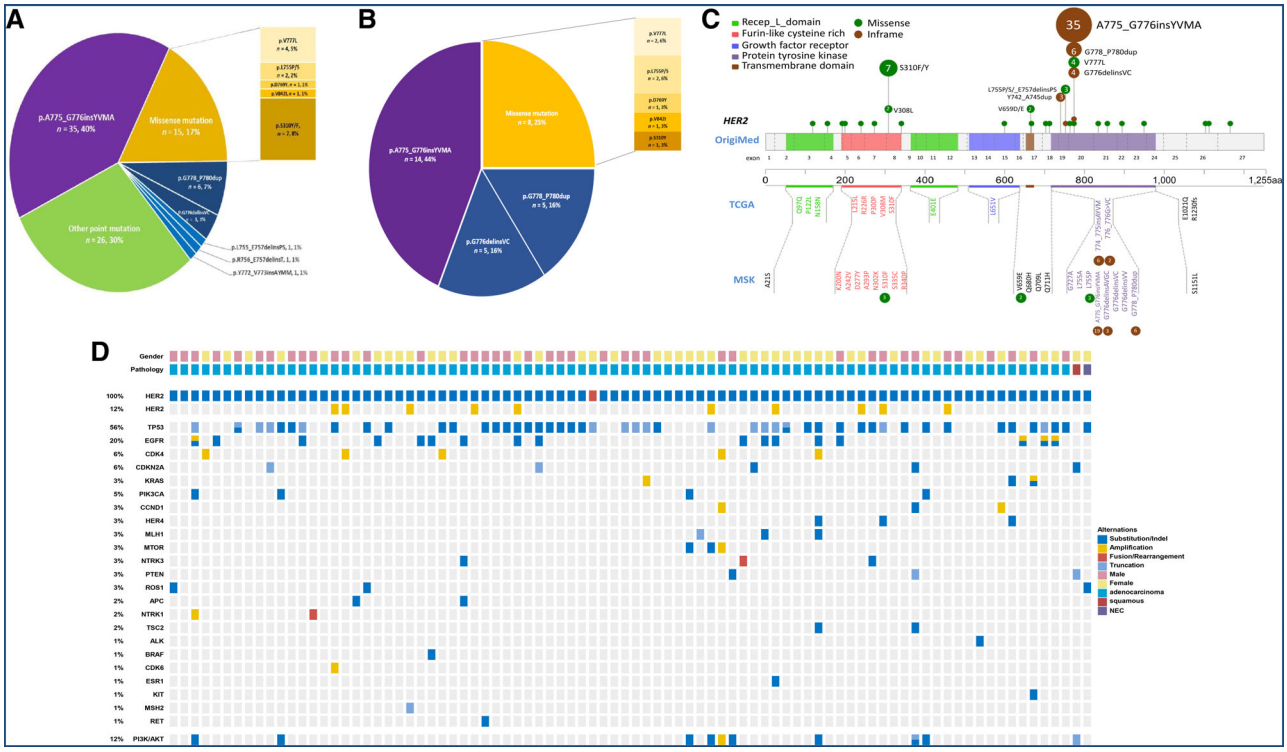

Figure 1. Mutational spectrum of *HER2*-mutant lung cancers observed in cohort A (*n* = 86) and cohort B (*n* = 32). **(A):** Distribution of specific *HER2* mutations in cohort A. **(B):** Distribution of specific *HER2* mutations in cohort B. **(C):** Spectrum of *HER2* mutations observed in this study versus those in TCGA and MSK-IMPACT. **(D):** Concomitant genomic alterations detected in patients with *HER2*-mutant lung cancers.
Abbreviations: HER2, human epidermal growth factor receptor 2; indel, insertion or deletion; MSK, Memorial Sloan Kettering integrated mutation profiling of actionable cancer targets; NEC, neuroendocrine carcinoma; TCGA, The Cancer Genome Atlas.

with the log-rank test. A multivariate Cox proportional hazards regression model was adopted to identify independent variables associated with PFS. Variables with $p < .10$ in the univariate Cox regression analysis were added in the multivariate analysis. All tests were two-sided. A value of $p < .05$ was deemed statistically significant unless stated otherwise. Analyses were conducted using the R software (version 3.5.1).

Results

Patterns of HER2 Variants and Co-Mutations in Lung Cancer

Between August 2016 and May 2018, *HER2* mutations were identified in 86 (4.23%) out of 2,035 patients using the NGS assays (cohort A). This frequency of *HER2* mutation was comparable to previous reports [1, 2, 11] but was higher than those observed in TCGA (15/546, 2.75%) and MSK-IMPACT data sets (45/1275, 3.53%; supplemental online information 2). A separate cohort of 40 patients with stage IV or recurrent *HER2*-mutant lung adenocarcinomas and afatinib treatment histories were identified from May 2017 to May 2019. Among them, 32 patients were eligible and included in this study (cohort B; supplemental online information 1).

A total of 31 different *HER2* variants were detected in the 118 patients (Fig. 1A, B). The most frequent type of genomic alterations were exon 20 in-frame insertions in the kinase domain (*n* = 72, 61%), followed by missense mutations in the kinase domain (*n* = 27, 22%), extracellular domain (*n* = 17, 14%), and transmembrane domain (*n* = 2, 2%). *A775_G776insYVMA* was the most common *HER2* variant (*n* = 49, 42%), followed by *G778_P780dup* (*n* = 11, 9%), *G776delinsVC* (*n* = 9, 8%), *S310F/Y* (*n* = 8, 7%), and *V777L* (*n* = 7, 6%). The former three variants were all kinase domain exon 20 in-frame insertions, whereas *S310F/Y* and *V777L* were missense mutations in the extracellular domain and kinase domain, respectively. Comparable patterns of *HER2* variants were observed in TCGA and MSK-IMPACT (Fig. 1C).

Among the 24 concomitant aberrations detected in cohort A (Fig. 1D; supplemental online information 3), *TP53* aberrations were the most commonly detected co-mutations (*n* = 48, 56%). Ten patients (12%) carried concomitant aberrations in the PI3K/AKT/mTOR pathway (*PIK3CA*, *n* = 4; *PTEN*, *n* = 3; *mTOR*, *n* = 3; *TSC2*, *n* = 3), and ten patients (12%) had concomitant *HER2* amplifications. In cohort B, co-mutations in *TP53* and *PIK3CA* occurred in 13 (41%) and 5 (15%) patients, respectively (supplemental online information 4). Three patients (9%) had concomitant *HER2* amplifications. No co-occurring *KRAS* or *EGFR* mutations were detected. Genomic alterations of patients in cohort A and B are detailed in supplemental online information 3 and 4, respectively.

Overall Clinical Outcomes on Afatinib

Clinicopathological features of patients treated with afatinib are listed in Table 1. Most patients were women (*n* = 18,

Table 1. Baseline characteristics according to *HER2* mutation variants[a]

Characteristics	All, *n* (%)	No. of patients, *n* (%)			*p* value
		Group 1	Group 2	Group 3	
No. of patients (%)	32	14 (44)	10 (31)	8 (25)	
Median age, years (range)	55 (29–81)	54 (29–69)	52 (41–70)	58 (38–81)	.806
Gender					.656
Male	14 (44)	7 (50)	3 (30)	4 (50)	
Female	18 (56)	7 (50)	7 (70)	4 (50)	
ECOG performance status					.798
0–1	28 (88)	13 (93)	8 (80)	7 (88)	
≥2	4 (13)	1 (7)	2 (20)	1 (13)	
Smoking history					.562
Never smokers	25 (78)	10 (71)	9 (90)	6 (75)	
Former or current smokers	7 (22)	4 (29)	1 (10)	2 (25)	
Adenocarcinoma histology	32 (100)				
Stage at the initiation of afatinib					.757
Stage IV[b]	25 (78)	11 (79)	7 (70)	7 (88)	
Postoperation recurrent	7 (22)	3 (21)	3 (30)	1 (13)	
CNS metastasis					.593
Present	13 (39)	6 (43)	5 (50)	2 (25)	
Absent	19 (61)	8 (57)	5 (50)	6 (75)	
Extrathoracic metastasis (except for CNS)					.543
Present	18 (56)	9 (64)	4 (40)	5 (63)	
Absent	14 (44)	5 (36)	6 (60)	3 (38)	
Line of afatinib treatment					.983
First	9 (28)	4 (29)	3 (30)	2 (25)	
Second	6 (19)	3 (21)	2 (20)	1 (13)	
Third or more	17 (53)	7 (50)	5 (30)	5 (63)	
Prior therapy					.832
Pt-based chemotherapy	21 (66)	10 (71)	6 (60)	6 (75)	
Gefitinib, erlotinib, osimertinib	5 (16)	2 (15)	1 (10)	2 (25)	
HER2-targeted treatments[c]	4 (13)	3 (21)	0	1 (13)	
PD-1/PD-L1 inhibitor	6 (19)	2 (15)	1 (10)	3 (38)	
Co-mutations in *TP53*					.814
Present	13 (41)	5 (36)	5 (50)	3 (38)	
Absent	19 (59)	9 (64)	5 (50)	5 (63)	
Co-mutations in PI3K/Akt/mTOR pathway[d]					.653
Present	7 (22)	2 (14)	3 (30)	2 (25)	
Absent	25 (78)	12 (86)	7 (70)	6 (75)	
Best response to afatinib					.001
PR	5 (16)	0 (0)	4 (40)	1 (13)	
SD	17 (53)	5 (36)	6 (60)	6 (75)	
PD	10 (31)	9 (64)	0 (0)	1 (13)	
Overall response rate, %	16	0	40	13	.018
Disease control rate, %	69	36	100	88	.001
CT scan intervals					.316
4 weeks	23	11	6	6	
5 weeks	5	2	1	2	
6 weeks	4	1	3	0	
Median (95% CI)	4 (4–5)	4 (4–4.25)	4 (4–6)	4 (4–4.75)	

Abbreviations: CI, confidence interval; CNS, central nervous system; CT, computed tomography; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; PD, progression of disease; PR, partial response; Pt-based, platinum-based; SD, stable disease; TKI, tyrosine kinase inhibitor.

[a]Type of *HER2* mutations: According to *HER2* mutant alleles, we divided the 32 patients into three subgroups. Group 1 consists of the most common 12-bp exon 20 insertion, A775_G776insYVMA; group 2 consists of non-YVMA exon 20 in-frame insertions including G776delinsVC (*n* = 5) and G778_P780dup (*n* = 5); and group 3 consists of *HER2* missense mutations.

[b]According to American Joint Commission on Cancer TNM staging (8th edition).

[c]Prior HER2-targeted treatments include trastuzumab (*n* = 3) and T-DM1 (*n* = 1).

[d]Concomitant mutations in PI3K-AKT-mTOR pathway detected in the 32 patients include *PIK3CA* (*n* = 5: p.H1047R [*n* = 2], p.H1047L [*n* = 1], p.E545K [*n* = 1], p.P539R [*n* = 1]), *PTEN* copy number loss (*n* = 1), and *mTOR* p.E2419K (*n* = 1).

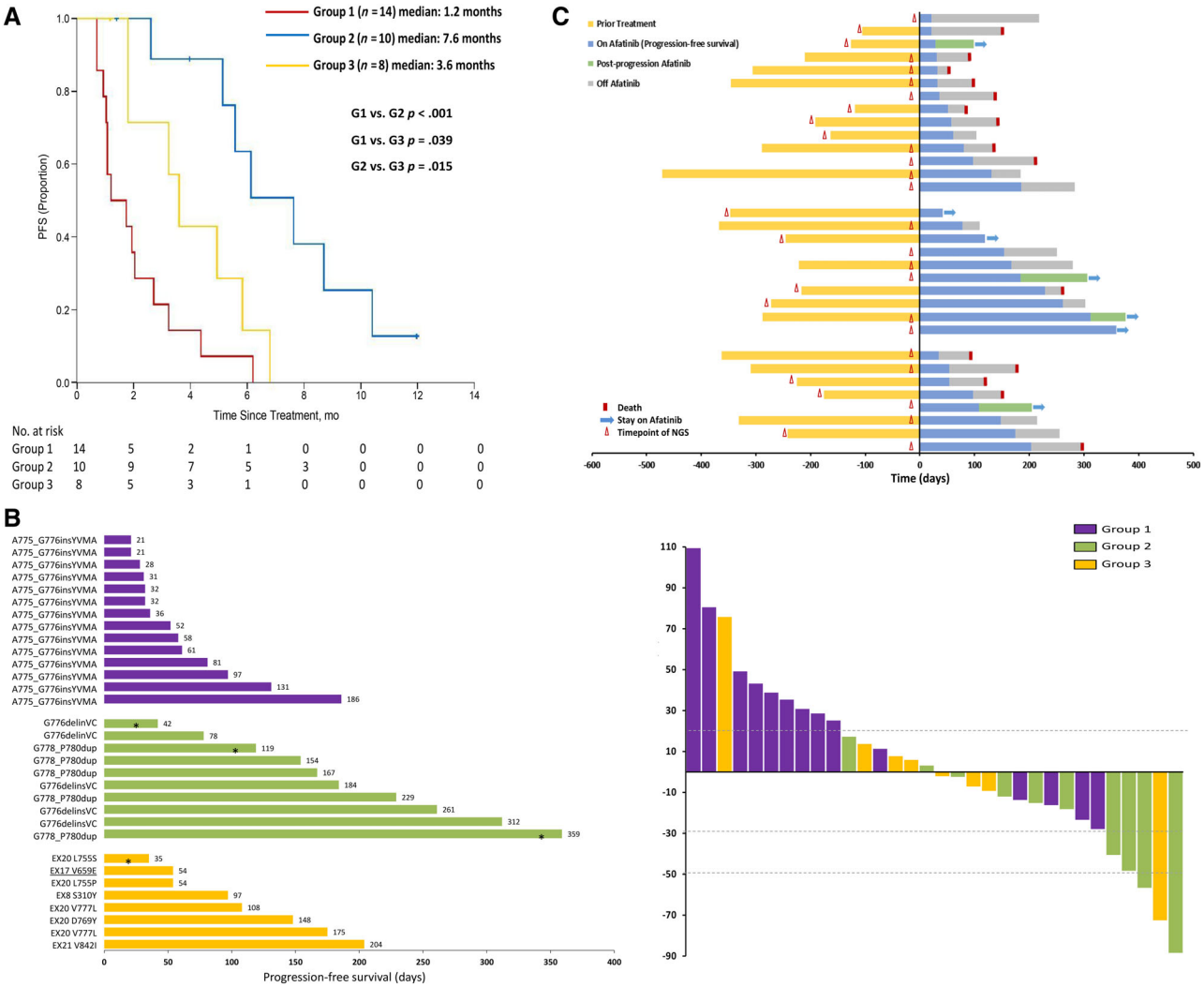

Figure 2. PFS and tumor responses to afatinib according to *HER2* mutation variants and treatment time of the 32 patients. **(A):** Kaplan-Meier curves for PFS in patients with *HER2* A775_G776insYVMA (group 1, *n* = 14), *HER2* G776delinsVC /G778_P780dup (group 2, *n* = 10), and *HER2* missense mutations (group 3, *n* = 8). **(B):** PFS and tumor shrinkage according to the type of *HER2* mutations. **(C):** Treatment time of the 32 patients. Prior treatment refers to the treatment from diagnosis of recurrent/stage IV diseases. *, patient censored. Abbreviations: G1, group 1; G2, group 2; G3, group 3; NGS, next-generation sequencing; PFS, progression-free survival.

56%) and never smokers (*n* = 26, 78%). Nine patients (28%) received afatinib as the first line, 6 (19%) as the second line, and 17 (53%) as third-line therapy or beyond (Fig. 2C). The median number of lines of prior systemic therapy was two (range, 0 to 5). Four patients (13%) had received HER2-targeted treatments before (trastuzumab, *n* = 3; T-DM1, *n* = 1).

The ORR and DCR with afatinib were 15.6% (95% confidence interval [CI], 5.9%–33.6%) and 68.8% (95% CI, 49.9%–83.3%), respectively. Confirmed partial responses were observed in five patients, two harboring *G778_P780dup*, two harboring *G776delinsVC*, and one carrying *V777L*. Ten patients (31.3%) had disease progression as the best response. Three patients carrying *A775_G776insYVMA* experienced disease progression within 30 days. By the time of data cutoff, 28 patients (87.5%) had experienced disease progression on afatinib. The median PFS for all patients and the responders was 3.2 months (95% CI, 2.0–4.5 months) and 7.6 months (95% CI, 3.8–11.5 months), respectively. The longest PFS (12.0 months) was observed in a patient with *G776delinsVC*. The overall survival data is immature for analysis with 15 deaths (46.9%) having occurred as of June 15, 2019.

Clinical Outcomes with Different *HER2* Mutation Variants

To examine the association between *HER2* variants and clinical outcomes on afatinib, we categorized *HER2* mutations in cohort B as: *HER2 YVMA* insertions (group 1: *A775_G776insYVMA*, *n* = 14, 44%); non-YVMA exon 20 insertions (group 2: *G776delinsVC*, *n* = 5; *G778_P780dup*, *n* = 5, 31%); or *HER2* missense mutations (group 3, *n* = 8, 25%). Table 1 lists the clinicopathological features of the three groups. Demographic characteristics and treatment histories were balanced across them. Genomic alterations of these patients are detailed in supplemental online information 4.

ORRs with afatinib were 0% (95% CI, 0%–26.8%), 40% (95% CI, 14.7%–72.6%), and 13% (95% CI, 0.7%–53.3%) in group 1, group 2, and group 3, respectively (*p* = .018). The proportion of patients achieving disease control was also

Table 2. Predictive factors for progression-free survival of afatinib treatment (n = 32)

Variable	Univariable analysis, p value	Multivariable analysis[a]	
		Hazard ratio (95% CI)	p value
Age (<60 vs. ≥60 years)	.765		
Gender (male vs. female)	.220		
ECOG performance status (0–1 vs. ≥2)	.832		
Smoking history (never vs. former)	.569		
Stage (postoperation vs. stage IV)	.349		
CNS metastasis (present vs. absent)	.644		
Extrathoracic metastasis other than CNS (present vs. absent)	.072	1.149 (0.431–3.065)	.782
Line of afatinib treatment (first vs. not first)	.295		
Prior gefitinib, erlotinib, osimertinib (present vs. absent)	.830		
Prior HER2-targeted treatments[b] (present vs. absent)	.179		
Co-mutations in *TP53* (present vs. absent)	.091	6.317 (2.180–18.302)	.001
Co-mutations in *PI3K-AKT-mTOR* pathway (present vs. absent)	.140	19.422 (4.098–92.039)	<.001
HER2 mutation variants[c]	<.001		<.001
Group 1 (*A775_G776insYVMA*)		1 (Reference)	
Group 2 (*G776delinsVC*, *G778_P780dup*)		0.009 (0.001–0.079)	<.00[d]
Group 3 (missense mutations)		0.184 (0.062–0.552)	.003[d]

Abbreviations: CI, confidence interval; CNS, central nervous system; ECOG, Eastern Cooperative Oncology Group; HER2, human epidermal growth factor 2.

[a]Covariates with p < .10 in the univariable analysis were added in the multivariable model. Status of PI3K-Akt-mTOR pathway mutation was also added because it was considered as a potentially relevant factor.

[b]Prior HER2-targeted treatments include trastuzumab (n = 3) and T-DM1 (n = 1).

[c]*HER2* mutation variants: According to *HER2* mutant alleles, we divided the 32 patients into three subgroups. Group 1 consists of the most common 12-bp exon 20 insertion, A775_G776insYVMA (n = 14); group 2 consists of other exon 20 in-frame insertions including G776delinsVC (n = 5) and G778_P780dup (n = 5); and group 3 consists of *HER2* missense mutations (n = 8).

[d]Comparison was conducted among three groups with Group 1 as the reference. Therefore, p < .017 was considered statistically significant.

significantly lower in group 1 (35.7%; 95% CI, 14.0%–64.4%; p = .001) than those in group 2 (100%; 95% CI, 65.6%–100%) and group 3 (87.5%; 95% CI, 46.7%–99.3%). Responses to afatinib in different *HER2* variants by mutation domain and mutation type were detailed in supplemental online information 5. The median PFS in group 1 (1.2 months; 95% CI, 0–2.4 months) was shorter than the median PFS in group 2 (7.6 months; 95% CI, 4.9–10.4; p < .001) and group 3 (3.6 months; 95% CI, 2.6–4.5 months; p = .039; Fig. 2A). Median PFS in group 2 was significantly longer than the median PFS in group 3 (p = .015; Fig. 2A). Between the two mutations in group 2, *G776delinsVC* and *G778_P780dup*, similar responses to afatinib were recorded, but numerically, *G776delinsVC* had longer median PFS (10.4 months; 95% CI, 3.9–16.7 months) than *G778_P780dup* (6.1 months; 95% CI, 3.7–8.6 months; p = .384). PFS and tumor shrinkage in patients with different *HER2* variants are detailed in Figure 2B. In multivariate analysis adjusting for extrathoracic metastasis and patterns of co-mutations (Table 2), the negative prognostic role of *HER2* mutations in group 1 was further established (hazard ratio $[HR]_{G2/G1}$, 0.009; 95% CI, 0.001–0.079; p < .001; $HR_{G3/G1}$, 0.184; 95% CI, 0.062–0.552; p = .003). Variants in group 2 (*G776delinsVC*, *G778_P780dup*) significantly correlated with the longest PFS among the three groups ($HR_{G2/G3}$, 0.050; 95% CI, 0.008–0.307; p = .001).

3D modeling in silico of *G776delinsVC* and *G778_P780dup* shows that neither the G776 VC insertion (red; Fig. 3A) nor the G778 GSP insertion (blue; Fig. 3B) led to marked changes in the structure of drug-binding pockets compared with the wild-type *HER2*. Meanwhile, 3D modeling of *A775_G776insYVMA* reveals that the YVMA insertion (magenta; Fig. 3C) contains two bulky side chains (Y776 and M778). This ball-and-stick model of the *HER2* YVMA insertion may induce steric hinderance of the drug-binding pocket and thus prevent its interaction with afatinib.

Clinical Outcomes According to Co-Mutation Patterns

To investigate whether the patterns of co-mutations affect tumor response to afatinib, we stratified clinical outcomes of patients in cohort B by the status of each co-mutation. *TP53* is the most commonly detected co-mutation in both cohorts (total n = 61, 52%). For cohort B patients, prior therapies (p = .518) and the line of afatinib treatment (p = .732) were balanced between those with and without *TP53* co-mutations. In univariate analysis, co-occurring *TP53* alterations were enriched in patients with shorter PFS (2.6 vs. 4.4 months; p = .091). This negative impact became significant after adjusting for *HER2* mutation variants (HR, 4.121; 95% CI, 1.588–10.697, p = .004; Fig. 4A) and in multivariate analysis (HR, 6.317; 95% CI, 2.180–18.302; p = .001; Table 2). Impacts of other co-mutations could not be accounted for because of the small sample size.

Next, we expanded the analysis to genomic aberrations at the pathway level. Seven patients (22%) in cohort B carried

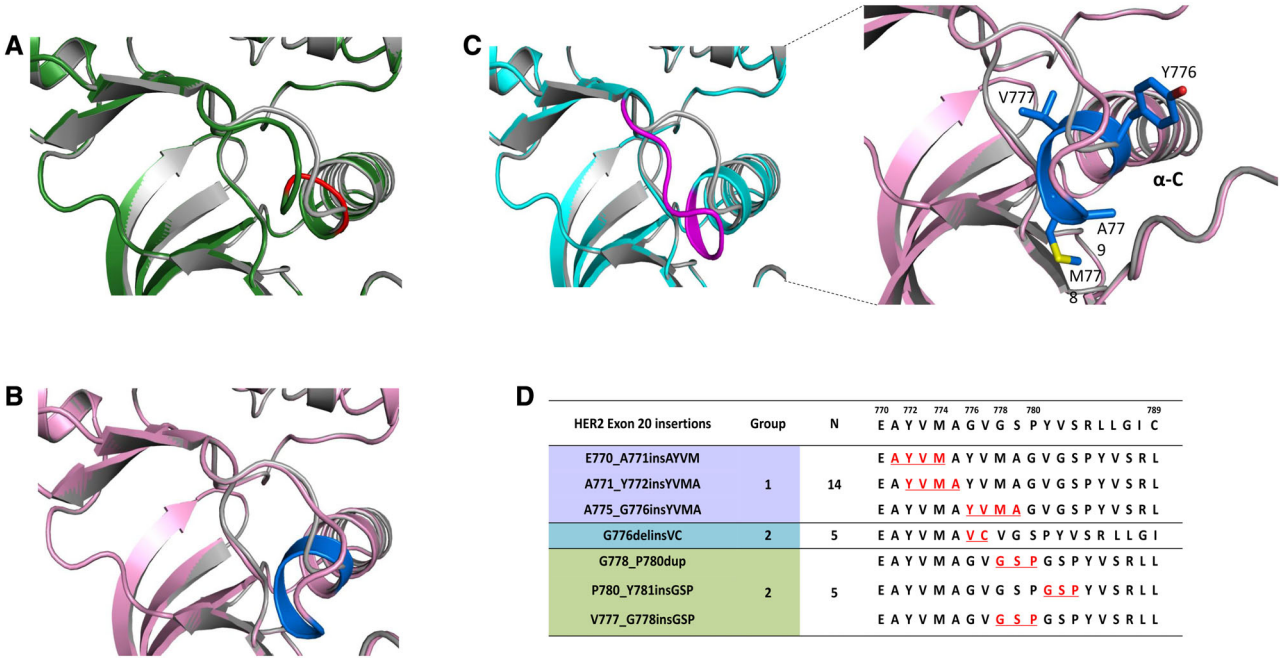

Figure 3. Three-dimensional (3D) modeling and the full amino acid sequences of three *HER2* exon 20 insertions. **(A):** 3D modeling of *G776delinsVC* versus wild type. The VC insertion is colored red. **(B):** 3D modeling of *G778_P780dup* versus wild type. The duplicated G778-P780 insertion is colored blue. **(C):** 3D modeling of *A775_G776insYVMA* versus wild type. The YVMA insertion is colored magenta. **(D):** Three types of *HER2* exon 20 insertions with full amino acid sequences (insertions are underlined).

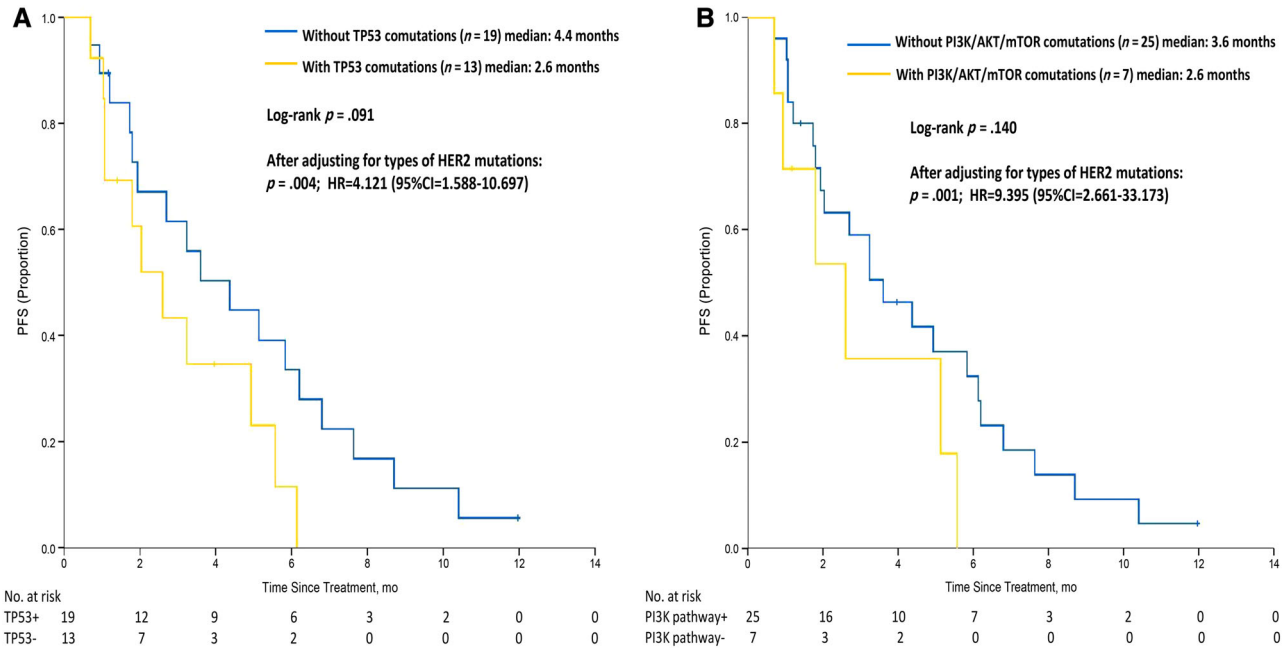

Figure 4. PFS according to status of co-mutations in *TP53* and the PI3K/AKT/mTOR pathway. **(A):** Kaplan-Meier curves for PFS in patients without *TP53* co-mutations (*n* = 19) versus those with *TP53* co-mutations (*n* = 13). **(B):** Kaplan-Meier curves for PFS in patients without co-mutations in the *PI3K/AKT/mTOR* pathway (*n* = 25) versus those with such co-mutations (*n* = 7).
Abbreviations: CI, confidence interval; HR, hazard ratio; PFS, progression-free survival.

co-mutations that could activate the *PI3K/AKT/mTOR* pathway (*PIK3CA*, *n* = 5 [p.H1047R, *n* = 2; p.H1047L, *n* = 1; p.E545K, *n* = 1; p.P539R, *n* = 1]; *PTEN* copy number loss, *n* = 1; *mTOR* p.E2419K, *n* = 1). These patients tended to have shorter PFS in univariate analysis (2.6 vs. 3.6 months; *p* = .140). After adjusting for *HER2* variants, co-mutations in the *PI3K/AKT/mTOR* pathway significantly correlated with worse clinical outcomes in *HER2*-mutant adenocarcinoma treated with afatinib (HR, 9.395; 95% CI, 2.661–33.173; *p* = .001; Fig. 4B). Multivariate analysis yielded similar results (HR, 19.422; 95% CI, 4.098–92.039; *p* < .001; Table 2).

Discussion

To our knowledge, we present the first study to examine the clinical impact of mutation variants and co-mutation patterns in *HER2*-mutant adenocarcinoma. A total of 118 patients with *HER2*-mutant lung cancers were included in this study, making it the largest analysis dedicated to *HER2*-mutant lung cancers to date.

We identified specific *HER2* variants and co-mutations as potential genomic modifiers of response to anti-HER2 therapies in lung adenocarcinoma. The best clinical outcomes of afatinib were recorded in patients carrying two non-YVMA exon 20 insertions, *G776delinsVC* or *G778_P780dup*, whereas little response was observed in *A775_G776insYVMA*, the predominant *HER2* variant in lung cancer. *TP53* co-mutations and the *PI3K/AKT/mTOR* pathway activations confer additional resistance to afatinib beyond *HER2* variants.

Thus far, there is no standard targeted therapy for *HER2*-mutant lung cancers. Studies investigating anti-HER2 strategies either reported disappointing results or did not contain a large enough sample size to provide conclusive evidence [7, 12, 15, 16, 19, 26–28]. Despite the overall limited efficacy of afatinib in *HER2*-mutant lung cancers (ORR, 15.6%; median PFS, 3.2 months; 95% CI, 2.0–4.5), sustained clinical benefits were observed in patients carrying *G776delinsVC* (ORR, 40%; median PFS, 10.4 months; 95% CI, 3.9–16.7) and *G778_P780dup* (ORR, 40%; median PFS, 6.1 months; 95% CI, 3.7–8.6). Different *HER2* missense mutations also exhibited divergent sensitivities to afatinib. Although no statistical comparison was performed because of the small sample size, our data identified *V777L* as a potentially sensitive variant and *L755P/S* as a resistant one.

Consistent with our findings in afatinib, clinical trials of dacomitinib and neratinib also indicated that different *HER2* variants responded differently to individual HER2-targeted agents [7, 12]. Responses to dacomitinib were only recorded in *G778_P780dup* and *M774delinsWLV* [7], whereas neratinib showed a higher potency in kinase domain missense mutations [12]. Neither of them was effective for *A775_G776insYVMA*, the predominant *HER2* variant in lung cancer. Notably, responses to afatinib in *HER2 YVMA* insertions have been described in some studies [27, 29, 30], whereas there also are reports of rapid disease progression after the treatment of afatinib in patients with identical mutations [19, 26]. In our study, two out of 14 patients carrying *A775_G776insYVMA* stayed on afatinib for more than 6 months. But generally, compared with other variants, *HER2 YVMA* insertion correlated with significantly worse clinical outcomes on afatinib (ORR, 0%; median PFS, 1.2 months; 95% CI, 0–2.4). In accordance with our results, Nagano et al. reported higher half maximal inhibitory concentration of afatinib against *A775_G776insYVMA* compared with *G776delinsVC* and *V777L* in preclinical models [31].

Taken together, our data support the notion that *HER2*-mutant lung cancers represent a heterogenous group of diseases with variable sensitivities to anti-HER2 treatments. Different *HER2* variants should be investigated independently or at least appropriately grouped. The activity of specific anti-HER2 agent may be confined to specific *HER2* mutations, which could be masked by a lack of activity in other mutations when *HER2*-mutant lung cancers are evaluated as a whole group. Given the low occurrence rate of *HER2* mutation in lung cancer, multicenter participation and predefined subgroup analysis of specific *HER2* variants may be worth consideration in future studies.

Activation of the *PI3K/AKT/mTOR* pathway was reported as a potential resistance mechanism in HER2-postive breast cancer and gastric cancer [32–34], but never in lung cancer. In our study, three patients who experienced disease progression within 30 days of treatment carried *A775_G776insYVMA* plus concomitant *PIK3CA* E545K, *PIK3CA* H1047R, and *TP53* R273C, respectively. Multivariate analyses identified co-mutations in *TP53* and the *PI3K/AKT/mTOR* pathway as independent negative prognostic factors in *HER2*-mutant lung adenocarcinomas treated with afatinib. Collectively, these data suggest that *TP53* co-mutations and *PI3K/AKT/mTOR* pathway activations play a role in the primary resistance to HER2-targeted therapies in lung adenocarcinoma. The status of these co-mutations should be considered when defining the target population for anti-HER2 treatments, because patients carrying these genomic aberrations may not benefit from the anti-HER2 monotherapy.

Limitations of this study include its retrospective nature and the small sample size. Cautions should be taken in data interpretation and extrapolation. The limited number of patients in group 3 prevent us giving conclusive information on the drug sensitivity of specific missense mutations. Notably, missense mutations in transmembrane domain (*V689/G660*) had been reported as sensitive mutations to afatinib [35]. However, our study only identified one patient with *V659E* treated with afatinib, who had stable disease for 54 days under afatinib treatment. Findings regarding *G776delinsVC* and *G778_P780dup* should be validated in studies with larger sample sizes. Future studies are warranted to verify and expand on our findings.

Conclusion

Our data suggest *G778_P780dup* and *G776delinsVC*, two non-YVMA exon 20 insertions, derived the greatest benefits from afatinib compared with other variants. *TP53* co-mutations and *PI3K/AKT/mTOR* pathway activations confer additional resistance to afatinib beyond *HER2* variants. Our study highlights the heterogeneity of *HER2*-mutant lung cancers. Refining patient population based on patterns of *HER2* variants and co-mutations may help identify effective anti-HER2 treatments in future studies.

Acknowledgments

We thank Boehringer Ingelheim for their assistance in the collection of clinical data. We thank OrigiMed, Beijing Genomics Institute, Guangzhou Burning Rock Medical Examination Institute, Guangzhou Mygene Medical Institute, Geneseeq, Genetron Health, 3D Medicines Co., Ltd., and Beijing Geneplus Medical Examination laboratories for conducting genomic testing.

This study was funded by National Key R&D Program of China (2016YFC0905500, 2016YFC0905503), Science and Technology Program of Guangdong (2017B020227001), Chinese National Natural Science Foundation (81772476), and National Natural Science Funds of China (81602011). The funding organizations had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

The data set supporting the conclusions of this article are available in the Research Data Deposit public platform (www.researchdata.org.cn) under the accession number RDDA2018000915.

Author Contributions

Conception/design: Wenfeng Fang, Shen Zhao, Ying Liang, Yunpeng Yang, Song Gao, Li Zhang (senior author)

Provision of study material or patients: Wenfeng Fang, Shen Zhao, Ying Liang, Yunpeng Yang, Lin Yang, Xiaorong Dong, Li Zhang (7th author), Yong Tang, Shoufeng Wang, Yang Yang, Xiaoyan Ma, Minghui Wang, Kai Wang

Collection and/or assembly of data: Wenfeng Fang, Shen Zhao, Ying Liang, Yunpeng Yang, Lin Yang, Xiaoyan Ma, Wenjing Wang, Songhui Zhao, Kai Wang, Song Gao, Li Zhang (senior author)

Data analysis and interpretation: Wenfeng Fang, Shen Zhao, Ying Liang, Yunpeng Yang, Xiaoyan Ma, Wenjing Wang, Songhui Zhao, Song Gao, Li Zhang (senior author)

Manuscript writing: All authors

Final approval of manuscript: All authors

Disclosures

Wenjing Wang: OrigiMed (E); **Songhui Zhao:** OrigiMed (E); **Kai Wang:** OrigiMed (E). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

References

1. Stephens P, Hunter C, Bignell G et al. Lung cancer: Intragenic ERBB2 kinase mutations in tumours. Nature 2004;431:525–526.

2. Arcila ME, Chaft JE, Nafa K et al. Prevalence, clinicopathologic associations, and molecular spectrum of ERBB2 (HER2) tyrosine kinase mutations in lung adenocarcinomas. Clin Cancer Res 2012; 18:4910–4918.

3. Perera S, Li D, Shimamura T et al. HER2 YVMA drives rapid development of adenosquamous lung tumors in mice that are sensitive to BIBW2992 and rapamycin combination therapy. Proc Natl Acad Sci USA 2009;106:474–479.

4. Shimamura T, Ji H, Minami Y et al. Non-small-cell lung cancer and Ba/F3 transformed cells harboring the ERBB2 G776insV_G/C mutation are sensitive to the dual-specific epidermal growth factor receptor and ERBB2 inhibitor HKI-272. Cancer Res 2006;66:6487–6491.

5. Liu S, Li S, Hai J et al. Targeting HER2 aberrations in non-small cell lung cancer with osimertinib. Clin Cancer Res 2018;24:2594–2604.

6. Mar N, Vredenburgh JJ, Wasser JS. Targeting HER2 in the treatment of non-small cell lung cancer. Lung Cancer 2015;87:220–225.

7. Kris MG, Camidge DR, Giaccone G et al. Targeting HER2 aberrations as actionable drivers in lung cancers: Phase II trial of the pan-HER tyrosine kinase inhibitor dacomitinib in patients with HER2-mutant or amplified tumors. Ann Oncol 2015;26: 1421–1427.

8. Peters S, Stahel R, Bubendorf L et al. Trastuzumab emtansine (T-DM1) in patients with previously treated HER2-overexpressing metastatic non-small cell lung cancer: Efficacy, safety and biomarkers. Clin Cancer Res 2019;25: 64–72.

9. Hotta K, Aoe K, Kozuki T et al. A phase II study of trastuzumab emtansine in HER2-positive non-small cell lung cancer. J Thorac Oncol 2018; 13:273–279.

10. Li BT, Ross DS, Aisner DL et al. HER2 amplification and HER2 mutation are distinct molecular targets in lung cancers. J Thorac Oncol 2016;11: 414–419.

11. Mazieres J, Peters S, Lepage B et al. Lung cancer that harbors an HER2 mutation: Epidemiologic characteristics and therapeutic perspectives. J Clin Oncol 2013;31:1997–2003.

12. Hyman DM, Piha-Paul SA, Won H et al. HER kinase inhibition in patients with HER2- and HER3-mutant cancers. Nature 2018;554:189–194.

13. Li B, Shen R, Buonocore D. Ado-trastuzumab emtansine for patients with HER2-mutant lung cancers: Results from a phase II basket trial. J Clin Oncol 2018;36:2532–2537.

14. Gao G, Li X, Wang Q et al. Single-arm, phase II study of pyrotinib in advanced non-small cell lung cancer (NSCLC) patients with HER2 exon 20 mutation. J Clin Oncol 2019;37 (suppl 15):9089A.

15. Kim TM, Lee KW, Oh DY et al. Phase 1 studies of poziotinib, an irreversible pan-HER tyrosine kinase inhibitor in patients with advanced solid tumors. Cancer Res Treat 2018;50:835–842.

16. Robichaux JP, Elamin YY, Tan Z et al. Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer. Nat Med 2018;24: 638–646.

17. Doebele RC, Riely GJ, Spira A et al. First report of safety, PK, and preliminary antitumor activity of the oral EGFR/HER2 exon 20 inhibitor TAK-788 (AP32788) in non-small cell lung cancer (NSCLC). J Clin Oncol 2018;36(suppl 15): 9015A.

18. Cappuzzo F, Landi L. HER2 deregulation in lung cancer: Right time to adopt an orphan? Clin Cancer Res 2018;24:2470–2472.

19. Chuang JC, Stehr H, Liang Y et al. ERBB2-mutated metastatic non-small cell lung cancer: Response and resistance to targeted therapies. J Thorac Oncol 2017;12:833–842.

20. Yap TA, Vidal L, Adam J et al. Phase I trial of the irreversible EGFR and HER2 kinase inhibitor BIBW 2992 in patients with advanced solid tumors. J Clin Oncol 2010;28:3965–3972.

21. Yang JCH, Shih JY, Su WC et al. Afatinib for patients with lung adenocarcinoma and epidermal growth factor receptor mutations (LUX-Lung 2): A phase 2 trial. Lancet Oncol 2012;13: 539–548.

22. Chen L, Chen C, Chen D et al. A novel approach to detect large indels from targeted sequencing data in clinical cancer setting. J Clin Oncol 2017;35(suppl 15):e13002.

23. Liu W, Mu S, Yao J et al. Analytical and clinical validation of a next-generation sequencing-based circulating tumor DNA (ctDNA) assay assures its clinical application. Ann Oncol 2017;28(suppl 5): 1266PA.

24. Waterhouse A, Bertoni M, Bienert S. SWISS-MODEL: Homology modelling of protein structures and complexes. Nucleic Acids Res 2018;46: W296–W303.

25. Eisenhauer EA, Therasse P, Bogaerts J et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–247.

26. Costa DB, Jorge SE, Moran JP et al. Pulse afatinib for ERBB2 exon 20 insertion-mutated lung adenocarcinomas. J Thorac Oncol 2016;11: 918–923.

27. De Greve J, Teugels E, Geers C et al. Clinical activity of afatinib (BIBW 2992) in patients with lung adenocarcinoma with mutations in the kinase domain of HER2/neu. Lung Cancer 2012; 76:123–127.

28. Mazieres J, Barlesi F, Filleron T et al. Lung cancer patients with HER2 mutations treated with chemotherapy and HER2-targeted drugs: Results from the European EUHER2 cohort. Ann Oncol 2016;27:281–286.

29. Lai WV, Lebas L, Barnes TA et al. Afatinib in patients with metastatic or recurrent HER2-mutant lung cancers: A retrospective international multicentre study. Eur J Cancer 2019;109: 28–35.

30. Peters S, Curioni-Fontecedro A, Nechushtan H et al. Activity of afatinib in heavily pretreated patients with HER2 mutation-positive advanced NSCLC: Findings from a global named patient use program. J Thorac Oncol 2018;13:1897–1905.

31. Nagano M, Kohsaka S, Ueno T et al. High-throughput functional evaluation of variants of

unknown significance in ERBB2. Clin Cancer Res 2018;24:5112–5122.

32. Hanker AB, Pfefferle AD, Balko JM et al. Mutant PIK3CA accelerates HER2-driven transgenic mammary tumors and induces resistance to combinations of anti-HER2 therapies. Proc Natl Acad Sci USA 2013;110:14372–14377.

33. Berns K, Horlings HM, Hennessy BT et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 2007;12:395–402.

34. Liu J, Pan C, Guo L et al. A new mechanism of trastuzumab resistance in gastric cancer: MACC1 promotes the Warburg effect via activation of the PI3K/AKT signaling pathway. J Hematol Oncol 2016; 9:76.

35. Ou SI, Schrock AB, Bocharov EV et al. HER2 transmembrane domain (TMD) mutations (V659/G660) that stabilize homo- and heterodimerization are rare oncogenic drivers in lung adenocarcinoma that respond to afatinib. J Thorac Oncol 2017;12:446–457.

See http://www.TheOncologist.com for supplemental material available online.