

Identification of Recurrent Insertions and Deletions in Exon 18 and 19 of Human Epidermal Growth Factor Receptor 2 as Potential Drivers in Non–Small-Cell Lung Cancer and Other Cancer Types

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abstract

PURPOSE Human epidermal growth factor receptor 2 (HER2) belongs to the same family as epidermal growth factor receptor (EGFR) and is known as an important cancer driver gene. Insertions and deletions (indels) are frequent driver mutations in both EGFR and HER2. The most common HER2 indels are the exon 20 insertions within the kinase domain, while others are rarely reported. Our study aimed to investigate other indels of HER2 that may act as driver mutations in Chinese patients with different cancer types.

METHODS In this retrospective study, patient samples were subjected to targeted sequencing covering HER2 and other cancer-related genes. Mutation profiles of patients harboring HER2 exon 18/19 indels were described. Identified HER2 exon 18/19 indels in our study were compared with external data from COSMIC. In silico and in vitro analyses were performed on selected indels of HER2 exon 18 and 19, respectively.

RESULTS A total of 25 indels in HER2 exon 18/19, 17 of which being recurrent, were identified in 20 of 53,591 patients with lung cancer (0.037%), two of 5,888 patients with colorectal cancer (0.034%), two of 3,774 patients with breast cancer (0.053%), and one of 14 patients with urothelial carcinoma of the renal pelvis (7.1%). Most patients harboring HER2 exon 18/19 indels were absent of known driver mutations. In lung cancer, mutation profiles were comparable between patients carrying HER2 exon 18/19 indels and the two established HER2 drivers (exon 20 insertions and S310 mutations). The in silico and in vitro analyses suggested an activated state conferred by HER2 exon 18/19 indels, which could be targeted by different tyrosine kinase inhibitors.

CONCLUSION Our study revealed a class of rare but unique indels in HER2 exon 18/19, which may act as driver mutations in several cancer types.

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INTRODUCTION

High-throughput genomic profiling with next-generation sequencing enabled identification of cancer driver genes.¹ A number of targeted therapies including tyrosine kinase inhibitors (TKIs) and monoclonal antibodies have been developed for patients with cancer with specific driver mutations. One of the well-known driver genes is the epidermal growth factor receptor (EGFR) gene, which plays an important role in the pathogenesis and progression of different cancer types.² TKIs such as gefitinib, erlotinib, and osimertinib are usually recommended for patients with non–small-cell lung cancer (NSCLC) with common sensitizing mutations of EGFR including exon 19 deletion and exon 21 L858R.^{3,4}

There are four members in the EGFR family including EGFR (also known as ERBB1), human epidermal

growth factor receptor 2 (HER2; ERBB2), HER3 (ERBB3), and HER4 (ERBB4).⁵ In addition to EGFR, HER2 has also been reported as a cancer driver gene. The presence of HER2 amplification and/or overexpression in breast cancer provides the therapeutic target for a variety of anti-HER2 therapies.⁶ Compared with HER2 gene amplification, mutations in HER2 are less common but it also emerged as molecular drivers in solid tumor.⁷ Previous studies reported that the prevalence of HER2 mutations ranged from 1.6% to 3.5%.^{8–11} Most mutations occurred within the extracellular domain and the kinase domain of HER2.¹¹ The exon 20 insertion p.A775_G776insYVMA in the kinase domain was frequently reported in lung cancer.¹² In Chinese population, exon 20 insertions of HER2 were identified in 2.4% of patients with NSCLC.¹³

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

In addition to gene amplification and exon 20 insertions, the role of other rare insertions and deletions (indels) in human epidermal growth factor receptor 2 (HER2) as cancer drivers remains undefined. Our study aimed to analyze HER2 indels outside exon 20 in more than 60,000 patients with non-small-cell lung cancer and other cancer types. Functional investigations were also performed in silico and in vitro.

Knowledge Generated

We identified a number of recurrent indels in exon 18/19 of HER2. The features of patient mutation profiles and the results of in silico and in vitro studies indicated that HER2 exon 18/19 indels may act as a novel class of cancer drivers.

Relevance

Patients with HER2 exon 18/19 indels were usually absent of other known cancer driver mutations therefore lack of specific treatment strategies. HER2 exon 18/19 indels showed response to different tyrosine kinase inhibitors in silico and in vitro. It provided novel insights for further clinical studies, which may bring treatment benefits to this patient subgroup.

Meanwhile, other mutations of HER2 are very rare, which was only reported in sporadic cases.^{14,15}

Insertions and deletions (indels) in the kinase domain have been identified in EGFR family members including EGFR and HER2 as important cancer driver mutations. The most common indels were EGFR exon 19 deletions in NSCLC.³ In addition, the exon 20 insertion of EGFR were reported in 12% of patients with NSCLC with EGFR mutations.¹⁶ Other rare forms of EGFR indels such as exon 18 deletions and exon 19 insertions were also reported in patients with NSCLC.¹⁷ In the EGFR gene, indels were usually identified in exon 18-20 at the kinase domain. In the HER2 gene, indels of the kinase domain are rarely reported except the exon 20 insertions. Herein, we focused on HER2 indels outside exon 20 and identified a number of recurrent indels in exon 18/19 of HER2 through next-generation sequencing. To our knowledge, this is the first study of HER2 exon 18/19 indels in solid tumors. The prevalence and mutation profiles of HER2 exon 18/19 indels in different cancer types were described. Further functional analysis was performed in vitro to investigate their oncogenic activity and sensitivity to different TKIs.

METHODS

Patients and Samples

The study recruited patients diagnosed with malignant solid tumors in Tianjin Chest Hospital, The First Affiliated Hospital of Zhengzhou University, and The Sixth Affiliated Hospital of Sun Yat-sen University. Formalin-fixed paraffin-embedded (FFPE) tumor samples or plasma from the patients were sent to Burning Rock Biotech (Guangzhou, China) laboratory for genetic profiling. The panels of either 168 genes (Lung Plasma) or 520 genes (OncoScreen Plus) covered genomic regions of HER2 and other cancer driver genes. The demographic, clinical, and genetic profiling information of the patients were retrospectively collected from a deidentified database. The external data set was

obtained from the COSMIC (Catalog of Somatic Mutations in Cancer) database.¹⁸ The study was approved by the Institutional Review Board of Tianjin Chest Hospital. Written informed consent was obtained from each patient.

DNA Extraction

The QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) and the QIAamp DNA FFPE Tissue Kit (Qiagen, UK) were used to extract circulating cell-free DNA from plasma and tumor DNA from FFPE tumor samples, respectively, according to the manufacturer's instructions. The Qubit 2.0 fluorometer and the Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA) were used to measure DNA concentration.

Library Construction and Sequencing

The M220 Focused-ultrasonicator (Covaris, Woburn, MA) was used to shear DNA, followed by end repair, phosphorylation, and adaptor ligation. The Agencourt AMPure XP beads (Beckman Coulter, Brea, CA) were used to select DNA fragments with the range of 200-400 base pair. Then, hybridization with capture probe baits, hybrid selection with magnetic beads, and PCR amplification were performed. Target capture was performed with panels covering 168 or 520 cancer genes. DNA quality and fragment size were assessed using Bioanalyzer 2100 (Agilent, CA). The indexed samples were sequenced on Illumina NextSeq 500 paired-end system (Illumina, Inc, Hayward, CA).

Sequence Data Analysis

The paired-end reads were mapped to the human genome (hg19) by Burrows-Wheeler aligner v.0.7.10.¹⁹ Local alignment optimization, variant calling, and annotation were performed with the Genome Analysis Toolkit (GATK) v.3.2²⁰ and VarScan v.2.4.3.²¹ DNA translocation analysis was performed with Factera v.1.4.3.²² The variants were annotated with ANNOVAR (2016-02-01 release)²³ and SnpEff v.3.6.²⁴

In Silico and In Vitro Analyses

The in silico analysis was performed on the basis of crystal structures 2N2A covering HER2 exon 18 E698_P699insLL and 3PP0 covering HER2 exon 19 L755_E757delinsPQ, which were obtained from RCSB protein data bank.²⁵ Swiss-Model²⁶ and GROMACS²⁷ were used to construct protein model of HER2 mutants and wild-types. PubChem²⁸ was used to obtain molecular structures of four TKIs including neratinib, lapatinib, poziotinib, and afatinib. SwissDock²⁹ was used to simulate drug-protein interactions. The protein structures and drug-protein interaction were visualized using Chimera.³⁰

The in vitro analysis of HER2 exon 18 (E698_P699insLL) and exon 19 (L755_E757delinsPQ) indels were performed at Bio-science Co, Ltd (Hangzhou, China). Briefly, Ba/F3 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% fetal bovine serum (FBS) and 5 ng/mL recombinant mouse IL3. HER2 exon 18 (E698_P699insLL) and exon 19 (L755_E757delinsPQ mutation) mutants and wild-type HER2

were cloned into retroviral vector separately. In cell proliferation assay, a total of 3×10^4 transfected Ba/F3 cells were spread in a 6-well plate and grew in RPMI containing 10% FBS without IL3. The cell counting plate was used to count the total number of cells in each well every 24 hours. In drug inhibition assay, cells were treated with neratinib, lapatinib, poziotinib, and afatinib (Selleck Chemicals, Houston, TX) at concentrations of 0 nM, 0.01 nM, 0.1 nM, 1 nM, 10 nM, and 100 nM for 72 hours. The absorbance at 450 nm was measured to indicate relative growth percentage.

Statistical Analysis

The continuous variables were presented as mean or median. The categorical variables were presented as frequencies. Unpaired Wilcoxon signed-rank test was used to compare continuous variables, whereas two-sided Fisher's exact tests were used to compare categorical variables, as appropriate. $P < .05$ was considered statistically significant. All bioinformatics analyses were performed with R (v.3.5.3, the R Foundation for Statistical Computing, Vienna, Austria).

TABLE 1. Presence of HER2 Exon 18/19 Insertions and Deletions in the Study Cohort

Patient No.	Age (years)	Sex	HER2 exon	Indel Description	Counts	Cancer Type/Subtype	Stage
1	70	Female	18	p.V697_E698insVEL	1	Lung cancer/adenocarcinoma	IV
2	58	Female	18	p.E698_P699insLE	3	Lung cancer	IV
3	64	Male	18	p.E698_P699insLE		Lung cancer/adenocarcinoma	NA
4	88	Female	18	p.E698_P699insLE		Lung cancer/squamous cell carcinoma	III
5	56	Male	18	p.E698_P699insLL	2	Lung cancer/adenocarcinoma	III
6	36	Female	18	p.E698_P699insLL		Lung cancer/adenocarcinoma	III
7	70	Male	18	p.Q711_R1713delinsW	1	Lung cancer/adenocarcinoma	IV
8	43	Female	18	p.K722del	1	Colorectal cancer	IV
9	53	Male	19	p.L755_E757delinsPQ	5	Lung cancer/adenocarcinoma	IV
10	50	Male	19	p.L755_E757delinsPQ		Renal pelvis cancer	II
11	32	Female	19	p.L755_E757delinsPQ		Lung cancer	I
12	63	Female	19	p.L755_E757delinsPQ		Lung cancer/adenocarcinoma	IV
13	88	Female	19	p.L755_E757delinsPQ		Lung cancer/adenocarcinoma	IV
14	60	Male	19	p.L755_E757delinsPK	4	Lung cancer/adenocarcinoma	IV
15	52	Female	19	p.L755_E757delinsPK		Lung cancer/adenocarcinoma	IV
16	48	Male	19	p.L755_E757delinsPK		Lung cancer/adenocarcinoma	I
17	69	Female	19	p.L755_E757delinsPK		Lung cancer/adenocarcinoma	IV
18	NA	NA	19	p.L755_E757delinsPL	3	Lung cancer/adenocarcinoma	I
19	37	Female	19	p.L755_E757delinsPL		Lung cancer/adenocarcinoma	IV
20	58	Male	19	p.L755_E757delinsPL		Colorectal cancer	IV
21	52	Female	19	p.L755_E757delinsPS	1	Lung cancer/adenocarcinoma	II
22	55	Male	19	p.L755_E757delinsPT	1	Lung cancer/adenocarcinoma	IV
23	45	Female	19	p.V754_L755insP	1	Breast cancer	III
24	70	Female	19	p.L755_E759del	1	Breast cancer	III
25	79	Male	19	p.L755_T759delinsATKP	1	Lung cancer/adenocarcinoma	IV

Abbreviations: HER2, human epidermal growth factor receptor 2; NA, not available.

RESULTS

Distribution of HER2 Exon 18/19 Indels

A total of 25 indels in HER2 exon 18/19 were identified including 20 indels in 53,591 patients with lung cancer (0.037%), two indels in 5,888 patients with colorectal cancer (0.034%), two indels in 3,774 patients with breast cancer (0.053%), and one indel in 14 patients with urothelial carcinoma of the renal pelvis (7.1%). There were eight indels identified in HER2 exon 18, and 17 indels identified in HER2 exon 19. The most common location in exon 18 was p.E698_P699 (five of eight) including p.E698_E699insLE in three patients and p.E698_E699insLL in two patients. The most common location in exon 19 was p.L755_E757 (14 of 17) including p.L755_E757delinsPQ in five patients, p.L755_E757delinsPK in four patients, p.L755_E757delinsPL in three patients, p.L755_E757delinsPS in one patient, and p.L755_E757delinsPT in one patient. Overall, 17 indels were recurrent and eight indels were nonrecurrent. The detailed description of 25 patients harboring HER2 exon 18/19 indels were presented in Table 1.

We also evaluated the distribution of HER2 exon 18/19 indels on the basis of data from COSMIC, in which a total of 10 indels were reported. The cancer types reported by COSMIC were different from our study except for breast cancer. As shown in the COSMIC data set (Fig 1), HER2

exon 19 indels were identified in five of 10,510 patients with breast cancer (0.048%), of which four patients harbored p.L755_T759del and one patient harbored p.L755_T759delinsAQSQQ. In our study, the p.L755_T759del was also presented in a patient with breast cancer (Fig 1). Our study identified HER2 exon 18/19 indels in lung cancer, colorectal cancer, and urothelial carcinoma of the renal pelvis, whereas the COSMIC data set reported HER2 exon 18 indels in pancreatic cancer (1 of 2,475, 0.04%) and cervical cancer (4 of 1,755, 0.23%).

Mutation Profiles of Patients with HER2 Exon 18/19 Indels

In patients harboring HER2 exon 18/19 indels, actionable driver mutations were only observed in two of 25 patients (8%). One patient with lung cancer had EGFR L858R mutation and MET amplification, whereas another patient with lung cancer had MET amplification only. ALK, RET, ROS1, and BRAF fusion were not found, and neither was V600E. More than half of the patients with HER2 exon 18/19 indels (14 of 25, 56%) had concurrent mutations in TP53. In most patients (22 of 25, 88%), HER2 exon 18/19 indels were likely to be clonal mutations rather than subclonal mutations. As indicated by the allelic frequency (AF) ratio of HER2 exon 18/19 indels to max AF, 16 patients had high AF ratio (> 0.8) and six patients had moderate AF ratio (0.3-0.8; Fig 2). There was one patient with lung cancer

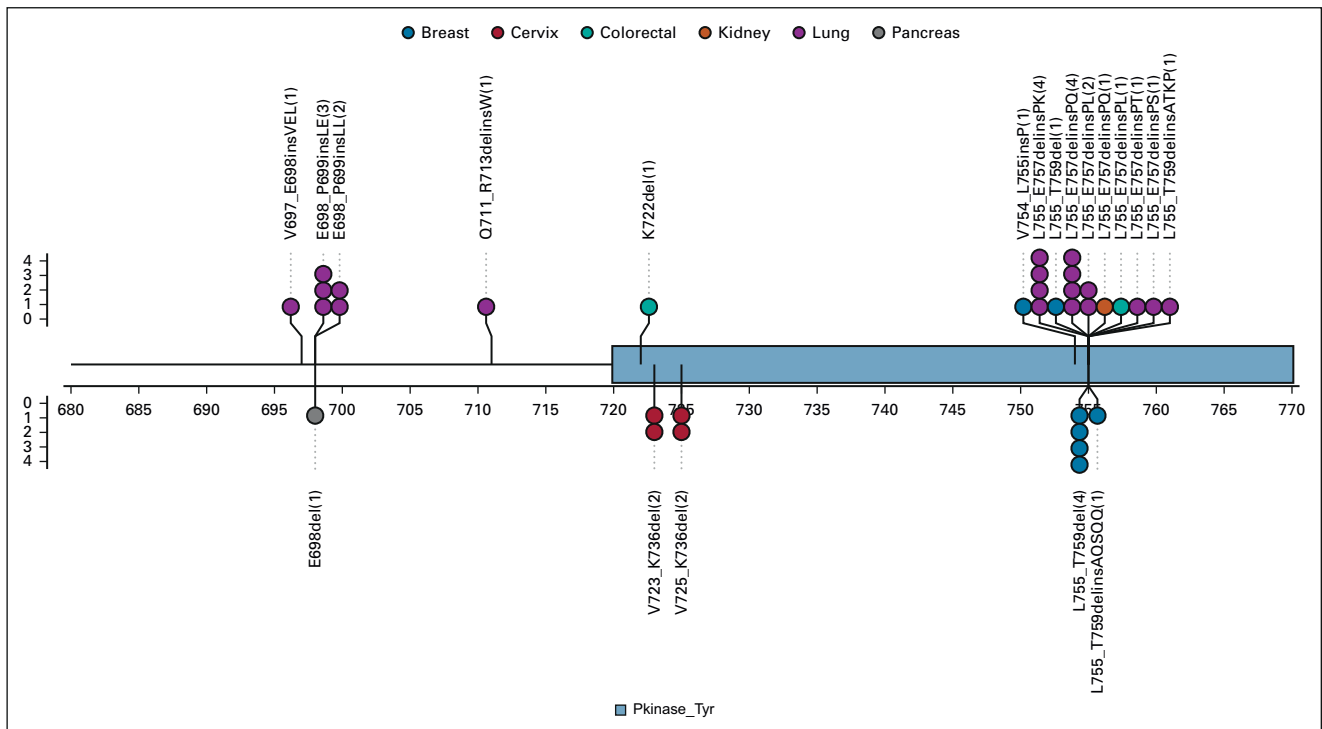


FIG 1. Presence of HER2 exon 18/19 indels in our study (top) and the COSMIC data set (bottom). HER2, human epidermal growth factor receptor 2; indels, insertions and deletions

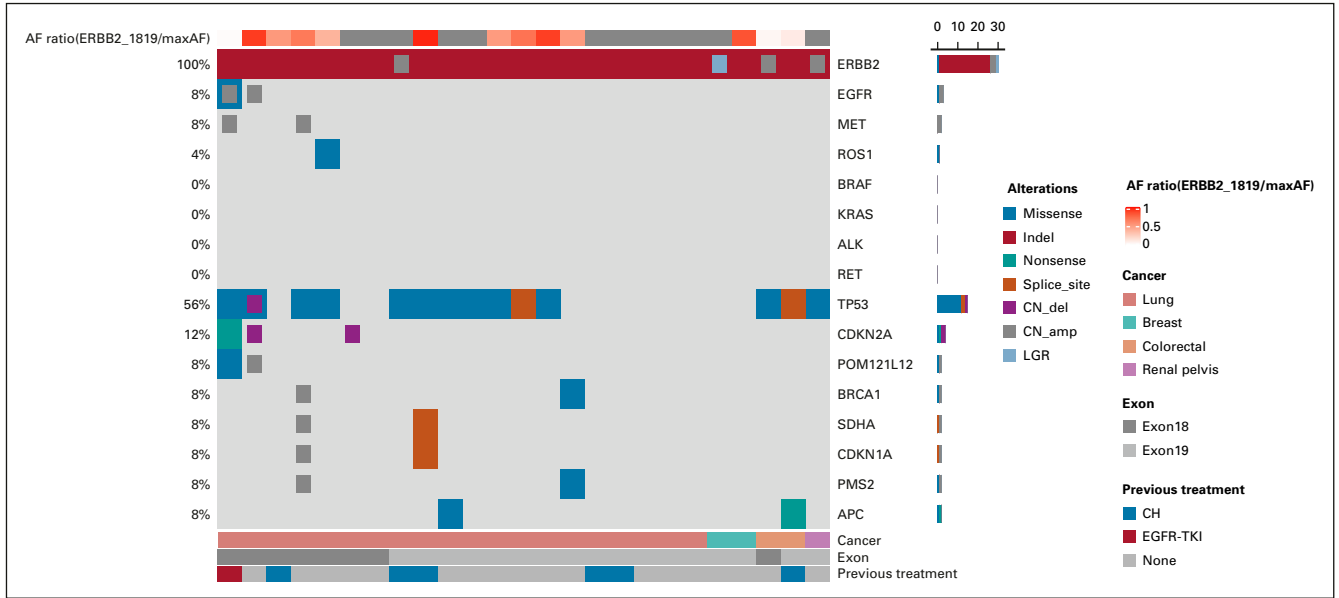


FIG 2. Oncoprint of 25 patients with HER2 exon 18/19 indels. AF, allelic frequency; HER2, human epidermal growth factor receptor 2; indels, insertions and deletions; TKI, tyrosine kinase inhibitor.

and two patients with colorectal cancer showed relatively low level of AF ratio. The detailed mutation profiles of each patient harboring HER2 exon 18/19 indels were indicated in [Figure 2](#).

Analysis of HER2 Exon 18/19 Indels in Patients With Lung Cancer

Among 20 patients with lung cancer with HER2 exon 18/19 indels, EGFR driver mutations were absent in most patients (19 of 20, 95%). The positive rate of EGFR driver mutation was significantly lower in patients with lung cancer with HER2 exon 18/19 indels than wild-types (5% v 47.4%, $P < .01$, [Fig 3](#)). The mutation rate of TP53 seemed higher in patients with lung cancer with HER2 exon 18/19 indels

than wild-types but the difference was not significant (66.7% v 45.7%, $P > .05$, [Fig 3](#)).

In the study, the two established driver mutations of HER2 known as exon 20 insertions and S310 mutations were identified in 1,224 patients with lung cancer. The molecular profiles between patients harboring HER2 exon 18/19 indels and the established HER2 mutations were compared ([Fig 4](#)). Both groups showed relatively low frequency of driver mutations in EGFR (5% v 7.6%, $P > .05$), MET (10% v 1.6%, $P > .05$), KRAS (0% v 1.4%, $P > .05$), and BRAF (0% v 0.1%, $P > .05$). The distribution of TP53 mutations was also comparable (55% v 37.3%, $P > .05$).

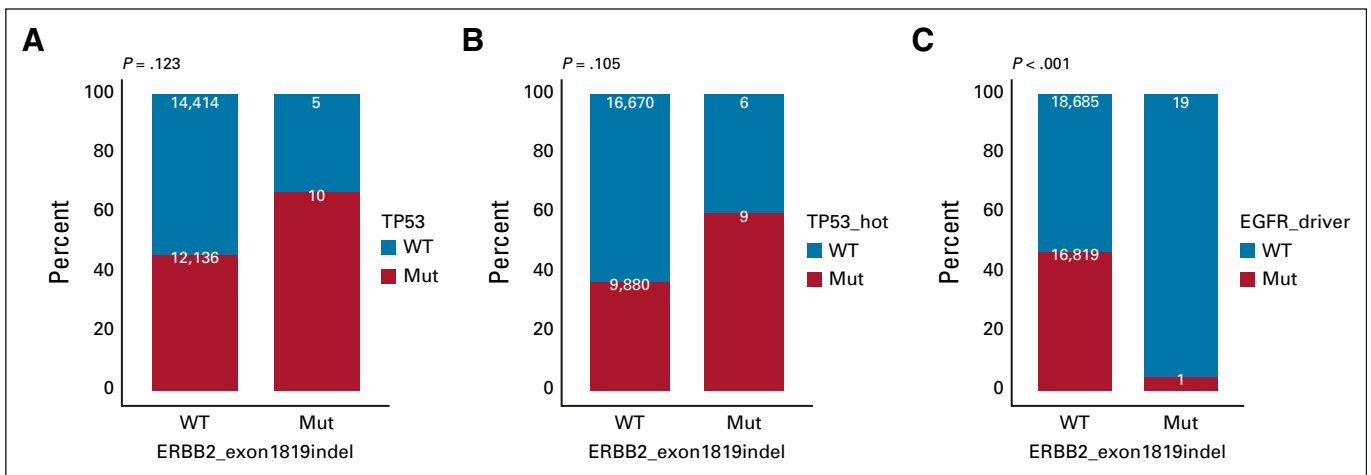


FIG 3. Presence of (A) TP53 mutations, (B) TP53 hotspot mutations and (C) EGFR driver mutations between patients harboring HER2 exon 18/19 indels and WT. In the study, TP53 hotspot mutations include mutations in exon 5-8 of TP53. EGFR driver mutations include L858R, T790M, G719, E709K, S768I, L861Q, L792H, G796R, C797S, 19del, and 20ins. EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; indels, insertions and deletions; mut, mutation; WT, wild-type.

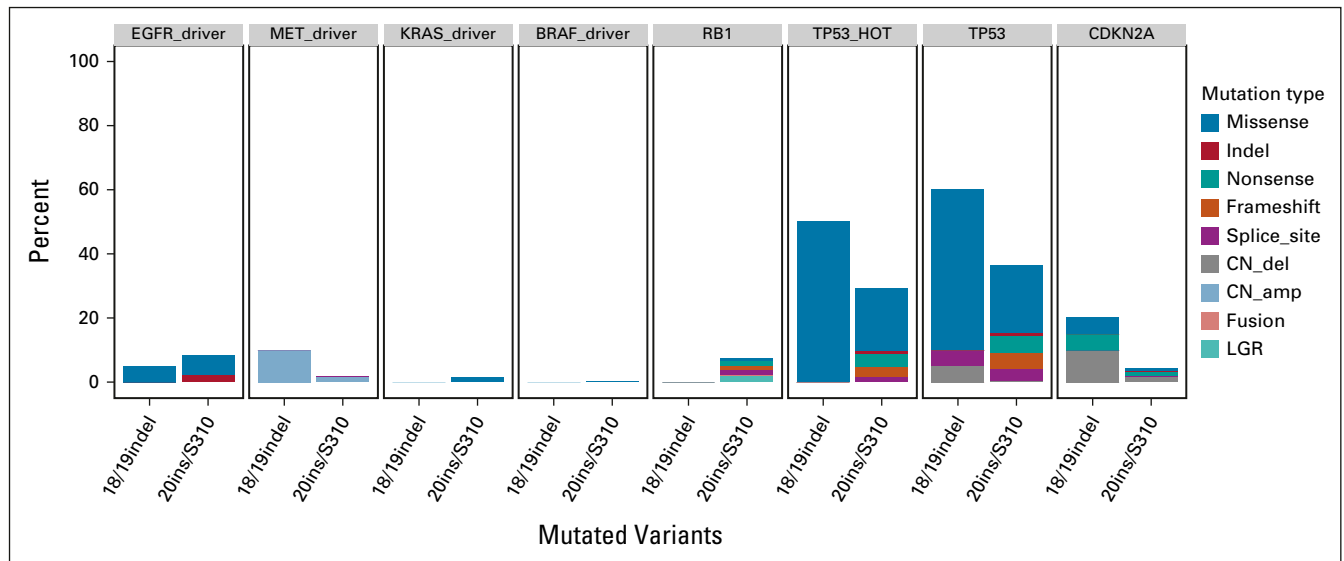


FIG 4. Presence of EGFR driver mutations, MET driver mutations, KRAS driver mutations, BRAF driver mutations, RB1 mutations, TP53 hotspot mutations, TP53 mutations, and CDKN2A mutation between patients harboring HER2 exon 18/19 indels and HER2 20ins/S310 mutation. In the study, EGFR driver mutations include L858R, T790M, G719, E709K, S768I, L861Q, L792H, G796R, C797S, 19del, and 20ins. MET driver mutations include MET splice and MET exon 14 skipping. KRAS driver mutations include G12, G13, G61, and A146 missense mutations. BRAF driver mutations include BRAF V600E, G469, G466, G464, Y472, N581, D594, L597, and K601 missense mutations. TP53 hotspot mutations include mutations in exon 5-8 of TP53. EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; indels, insertions and deletions.

In Silico and In Vitro Analyses of HER2 Exon 18/19 Indels

Two frequent indels in our study occurred in exon 18 (E698_P699insLL) and exon 19 (L755_E757delinsPQ) of HER2 were selected for further in silico and in vitro analyses. The E698_P699insLL in exon 18 is located in the short helical cytoplasmic juxtamembrane region A of HER2. In E698_P699insLL mutant, the distance between C-termini of transmembrane domains in HER2 dimer was farther than that in wild-type HER2 ($P < .05$, Data Supplement), which indicated that the mutant protein might be in a more activated status.³¹ The L755_E757delinsPQ in exon 19 is located in the kinase domain of HER2. The in silico analysis identified a number of amino acids in the L755_E757delinsPQ mutant and wild-type HER2 that could interact with TKIs including neratinib, lapatinib, poziotinib, and afatinib (Data Supplement). The L755_E757delinsPQ mutant showed better binding abilities to different TKIs than wild-types as measured by the change in Gibbs free energy (ΔG , Data Supplement, $P < .05$).

The proliferation assay in Ba/F3 cells indicated that the two indels of HER2 exon 18/19 showed survival advantage compared with wild-type HER2 after IL3 withdrawal (Fig 5A). The two selected indels also showed response to several HER2 TKIs including neratinib, lapatinib, poziotinib, and afatinib. As indicated by the drug response curve (Data Supplement), inhibitory effects on cell viability were varied among different TKIs and mutations. In our study, lapatinib, poziotinib, and afatinib showed comparable inhibitory abilities between the two HER2 indels, which

were stronger than wild-type HER2. Meanwhile, different responses to neratinib were observed between the two HER2 indels and a stronger inhibitory effect was observed in wild-type HER2 (Fig 5B).

DISCUSSION

HER2 mutations have been recognized as oncogenic drivers in various cancer types.⁷ In addition to HER2 exon 20 insertions, other indels within the HER2 kinase domain and other functional domains were rarely reported.⁷ Our study observed the presence of HER2 exon 18/19 indels in four cancer types. The highest rate of HER2 exon 18/19 indels was observed in urothelial carcinoma of the renal pelvis (7.1%), but the sample size of this cancer type in our study was very small ($n = 14$). In other cancer types, relatively low rates of HER2 exon 18/19 indels were consistently observed in our study (0.034%-0.053%) and the external data set (0.04%-0.23%). The cancer types involving HER2 exon 18/19 indels were largely different between our study and the external data set. Breast cancer is the only cancer type that was reported both by our study and the external data set. In the COSMIC data set, there were more than 20,000 patients with lung cancer and more than 7,000 patients with colorectal cancer. However, no indels in HER2 exon 18/19 were reported in these two common cancer types. The different distribution of HER2 exon 18/19 indels between our study and COSMIC is intriguing, which might be associated with the genetic background of different populations. There were 18 of 25 indels in our study and nine of 10 indels in the COSMIC data set located in the kinase domain of HER2. Although cancer

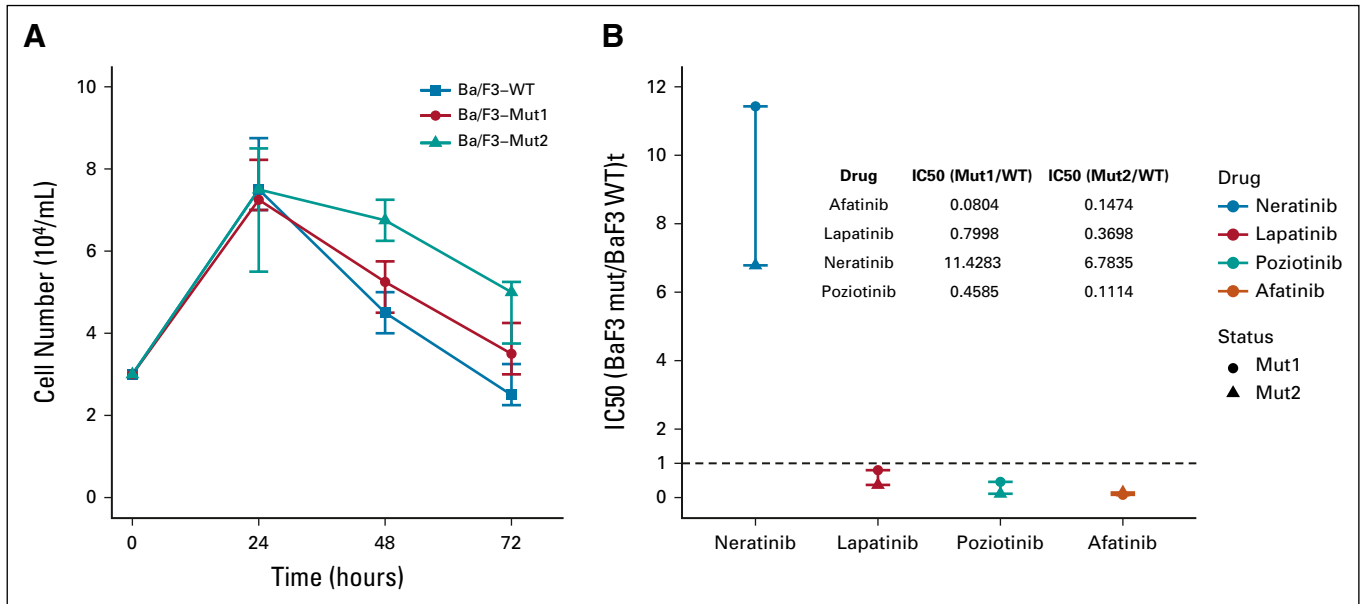


FIG 5. Proliferation of HER2 exon 18/19 indels and wild-type in (A) Ba/F3 cells and (B) comparison of TKI inhibition on HER2 exon 18/19 indels with WT. Mut1: L755_E757delinsPQ, Mut2: E698_P699insLL. HER2, human epidermal growth factor receptor 2; indels, insertions and deletions; mut, mutation; TKI, tyrosine kinase inhibitor; WT, wild-type.

types and mutations were different, the locations of indels reported in our study and the COSMIC data were similar. In HER2 exon 18, indels mainly occurred at the residue 698 position outside the kinase domain and the 722-725 position within the kinase domain. In HER2 exon 19, indels mainly occurred at the 755 position in the kinase domain. Therefore, these loci could very likely be hotspots of HER2 indels in exon 18/19.

Most patients harboring HER2 exon 18/19 indels in our study are treatment-naïve and showed high levels of normalized AF of HER2 exon 18/19 indels. Therefore, we believe that the majority of HER2 exon 18/19 indels identified in our study are de novo clonal mutations rather than acquired subclonal mutations. HER2 mutations usually do not coexist with common driver mutations in patients with cancer.⁸ Indeed, we only observed two of 25 patients with HER2 exon 18/19 indels who had concurrent actionable cancer driver mutations. In our study, the majority of patients had lung cancer. As one of the most common driver gene, EGFR driver mutations were presented in nearly half of the patients with lung cancer with wild-type HER2. However, the rate of EGFR driver mutation in patients with HER2 exon 18/19 indels was significantly lower. In addition, the frequencies of established driver mutations in genes such as EGFR, KRAS, BRAF, and MET were comparable between patients with HER2 exon 18/19 indels and patients with common HER2 mutations including exon 20 insertions and S310 mutations. The featured mutation profiles of HER2 exon 18/19 indels presented in treatment-naïve patients indicated that it might be a novel class of cancer driver.

To date, clinical studies for patients with HER2 exon 18/19 indels are lacking. Some studies evaluated different TKIs for patients with HER2 exon 20 insertion but the therapeutic effects are limited to relatively small samples and the results are varied in different studies.³² The study from the European EUHER2 cohort reported the use of neratinib, lapatinib, and afatinib in 29 patients with NSCLC with HER2 exon 20 insertion.³³ The overall response rate was 7.4%, and the disease control rate was 55.5%.³³ A single-arm phase II trial in patients with advanced NSCLC harboring HER2 exon 20 mutations reported that afatinib did not show the expected potential for disease control in NSCLC, although more than half of the patients achieved disease control during the study.³⁴ In 26 patients with NSCLC from the SUMMIT basket trial, only one patient with L755S missense mutation achieved partial response, whereas no responses were observed in other patients with HER2 exon 20 insertions.³⁵ A novel TKI named pozitotinib showed improved clinical activity on NSCLC with HER2 exon 20 mutations in vitro and in vivo.³⁶ It also showed treatment effects on patients with NSCLC with HER2 exon 20 mutations and is now being further evaluated in ongoing trials.³⁷ In our study, only one patient with HER2 exon 18/19 indels received TKI treatment at baseline, given the co-occurrence of EGFR L858R mutation. Treatment and follow-up information of patients after the identification of HER2 exon 18/19 indels were largely unavailable because of the retrospective design, which is a limitation of the study.

Understanding the mechanisms underlying TKI treatment response could contribute to more precise treatment

decisions. A study used structural modeling and molecular dynamics simulations on common HER2 exon 20 insertions including Y772_A775dup and G778_P780dup.³⁸ Their sensitivity to TKIs such as afatinib could be explained by the kinase conformational landscape featured by the length of the α C- β 4 loop and residues at HER2 776 and 778 positions.³⁸ In this study, we selected two frequent indels in exon 18 and exon 19 of HER2 for *in silico* and *in vitro* analyses. More activated status of exon 18 indel and better binding abilities of exon 19 indel to different TKIs were observed *in silico*. Further *in vitro* analysis observed that these two indels showed survival advantages during proliferation assay and responses to several HER2 TKIs. In this study, only two recurrent indels in HER2 exon 18/19 were analyzed *in silico* and *in vitro*. It remains undefined

whether other indels in HER2 exon 18/19 could have different interactions and sensitivities with different TKIs. For example, insertions in EGFR exon 20 are generally resistant to TKIs except a few insertions such as A763_Y764insFQEA.³⁹ Therefore, it is worthwhile to perform more comprehensive analysis on indels of HER2 exon 18/19 in future studies.

In conclusion, HER2 exon 18/19 indels were presented in a small subset of patients with different cancer types. The features of mutation profiles indicated that HER2 exon 18/19 indels may act as a novel class of cancer drivers. Although it showed response to different TKIs *in vitro*, further studies are warranted to identify appropriate treatment options for patients with cancer with HER2 exon 18/19 indels.

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Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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No potential conflicts of interest were reported.

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