Human Cancer Biology

Clinical Cancer Research

Prevalence, Clinicopathologic Associations, and Molecular Spectrum of *ERBB2* (*HER2*) Tyrosine Kinase Mutations in Lung Adenocarcinomas

Maria E. Arcila[1], Jamie E. Chaft[2], Khedoudja Nafa[1], Sinchita Roy-Chowdhuri[1], Christopher Lau[1], Michael Zaidinski[1], Paul K. Paik[2], Maureen F. Zakowski[1], Mark G. Kris[2], and Marc Ladanyi[1]

Abstract

Purpose: Activating mutations in the tyrosine kinase domain of *HER2 (ERBB2)* have been described in a subset of lung adenocarcinomas (ADCs) and are mutually exclusive with *EGFR* and *KRAS* mutations. The prevalence, clinicopathologic characteristics, prognostic implications, and molecular heterogeneity of *HER2*-mutated lung ADCs are not well established in U.S. patients.

Experimental Design: Lung ADC samples ($N = 1,478$) were first screened for mutations in *EGFR* (exons 19 and 21) and *KRAS* (exon 2), and negative cases were then assessed for HER2 mutations (exons 19–20) using a sizing assay and mass spectrometry. Testing for additional recurrent point mutations in *EGFR, KRAS, BRAF, NRAS, PIK3CA, MEK1*, and *AKT* was conducted by mass spectrometry. *ALK* rearrangements and *HER2* amplification were assessed by FISH.

Results: We identified 25 cases with *HER2* mutations, representing 6% of *EGFR/KRAS/ALK*-negative specimens. Small insertions in exon 20 accounted for 96% (24/25) of the cases. Compared with insertions in *EGFR* exon 20, there was less variability, with 83% (20/24) being a 12 bp insertion causing duplication of amino acids YVMA at codon 775. Morphologically, 92% (23/25) were moderately or poorly differentiated ADC. *HER2* mutation was not associated with concurrent *HER2* amplification in 11 cases tested for both. *HER2* mutations were more frequent among never-smokers ($P < 0.0001$) but there were no associations with sex, race, or stage.

Conclusions: *HER2* mutations identify a distinct subset of lung ADCs. Given the high prevalence of lung cancer worldwide and the availability of standard and investigational therapies targeting *HER2*, routine clinical genotyping of lung ADC should include *HER2*. *Clin Cancer Res; 18(18); 4910–8. ©2012 AACR.*

Introduction

The human epidermal growth factor receptor 2 (HER2/ERBB2) is a receptor tyrosine kinase of the ERBB family which includes 3 additional members: EGFR (HER1/ERBB1), HER3 (ERBB3), and HER4 (ERBB4). Ligand binding to the extracellular domain of EGFR, HER3, and HER4, results in the formation of catalytically active homo- and heterodimers which, in turn, activate several downstream pathways involved in cellular proliferation, differentiation, migration, and apoptosis (1–3). Despite extensive structural homology with all other members of its family, both along the catalytic intracellular domain and the extracellular putative ligand-binding region, HER2 has no identified direct ligand. Instead, it functions as the preferred dimerization partner for all other ERBB family receptors (4–6). This observed superior ability for heterodimerization, coupled with a unique basal tyrosine kinase activity, confers to HER2 a pivotal role in signal transduction with corresponding significant roles in cancer development and progression when its function is deregulated.

Deregulation of the *HER2* gene, through protein overexpression and/or gene amplification, is found in many human cancers most notably breast, ovarian, gastric, and some biologically aggressive forms of uterine carcinomas (7–10). In most cases, overexpression correlates with poor prognosis and, in breast and gastric cancers, it can also predict benefit from HER2-targeted therapy. Trastuzumab, a humanized monoclonal antibody targeting the extracellular domain of HER2, has shown significant survival advantage in the treatment of HER2 overexpressing breast cancer (8, 11) when combined with cytotoxic chemotherapy.

Authors' Affiliations: [1]Department of Pathology, and [2]Thoracic Oncology Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Current address for C. Lau: National Human Genome Research Institute, NIH, Bethesda, MD.

Corresponding Author: Maria E. Arcila, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065. Phone: 212-639-7879; Fax: 212-639-6318; E-mail: arcilam@mskcc.org

doi: 10.1158/1078-0432.CCR-12-0912

Translational Relevance

The incidence, clinicopathologic characteristics, and prognostic implications of activating mutations in the tyrosine kinase domain of *HER2* in lung adenocarcinomas (ADCs) are not well established.

The current study represents the largest assessment for *HER2* mutations ($N = 1{,}478$) in predominantly Caucasian population and the most comprehensive concurrent analysis for other recurrent oncogene mutations. We show that mutations in *HER2* identify a distinct subset of lung ADCs with highest prevalence among never-smokers (5%) and which are mutually exclusive with other known driver oncogene mutations, making up 6% of lung ADCs lacking *EGFR*, *KRAS*, and *ALK* alterations. Given the high incidence of lung ADC, it is estimated that there are between 1,000 and 2,000 patients with *HER2*-mutated lung ADC diagnosed every year in the United States, and their identification would allow for assignment to one of many investigational agents targeting this pathway.

Similar findings have also been recently reported in a phase III study of patients with HER2-positive gastric carcinoma (12). In contrast, whereas overexpression and amplification of HER2 has been reported in up to 1 of 3 of non–small cell lung carcinomas (NSCLCs; refs. 13, 14), statistically significant differences in survival have not been observed (15), and trials exploring the advantage of treatment with trastuzumab have shown only modest or minimal clinical benefits (13, 16).

In 2004, activating mutations within the tyrosine kinase domain of the *HER2* gene were discovered in a small subset of NSCLCs (17–22). Their prevalence is reported to be up to 4% (20, 22), and both *in vitro* and *in vivo* studies confirm the oncogenic potential of these mutations (23–25). *In vitro* studies have shown that tumor cells harboring the most prevalent HER2 insertion (YVMA) not only exhibit ligand-independent tyrosine phosphorylation and stronger association with downstream signal transducers that mediate cell survival and proliferative processes, but also potently induce EGFR transphosphorylation, even in the presence of a kinase-dead EGFR (23). Tumor cells harboring *HER2* mutations are resistant to EGFR inhibitors but remain sensitive to both HER2 inhibitors and dual EGFR/HER2 inhibitors (25–27). The most commonly encountered mutations are in-frame insertions in exon 20, but point mutations along the tyrosine kinase domain have also been identified; all are mutually exclusive with common activating mutations in *EGFR* and *KRAS* (17, 20, 28). On the basis of published studies, the clinical and pathologic characteristics of patients with *HER2* mutations have been reported to be very similar to those with *EGFR* mutations, being more common in women, Asians, never-smokers, and in adenocarcinoma (compared with squamous carcinoma; ref. 20). To date, however, only a few studies focusing on *HER2* mutations have been published (17–22) and most were conducted predominantly in Asian patient populations. The incidence, clinicopathologic characteristics, and prognostic implications of *HER2* mutant lung cancer in the U.S. population remain to be more thoroughly defined.

In the current study, we aimed to (i) determine the frequency and spectrum of *HER2* mutations in a large cohort of U.S. patients with adenocarcinoma, (ii) assess the clinical and histopathologic characteristics of *HER2*-mutant tumors, (iii) confirm the mutually exclusive nature of mutations in other genes, including major and minor mutations in *EGFR*, *KRAS*, *BRAF*, *NRAS*, *PIK3CA*, *MEK1*, and *AKT* as well as *ALK* rearrangements, and (iv) compare the survival of patients with *HER2*-mutant tumors to those harboring other mutually exclusive mutations.

Materials and Methods

Patient selection and mutation analysis

Clinical cases of lung adenocarcinoma received for routine, reflex clinical *EGFR* and *KRAS* testing at Memorial Sloan-Kettering Cancer Center (New York, NY) between January 1, 2009, and December 31, 2010 were selected for the study. Hematoxylin and eosin–stained sections of formalin-fixed paraffin-embedded tissue were reviewed for each sample to identify and circle the areas of highest tumor density. Macrodissection was conducted on corresponding unstained sections to ensure at least 25% tumor content. Genomic DNA was extracted using the DNeasy Tissue kit (Qiagen) following the manufacturer's standard protocol. Clinical testing for *EGFR* mutations was carried out using fragment analysis for the detection of small indels in exons 19 and 20 and the L858R mutation in exon 21 using previously described methods refs. (29, 30). *KRAS* testing was conducted by a combination of standard sequencing and mass spectrometry genotyping (Sequenom) based on methods previously described (31). As a part of our standard panel of mutation analysis by mass spectrometry, all samples were also concurrently tested for other recurrent point mutations in *EGFR*, *KRAS*, *BRAF*, *NRAS*, *AKT*, *MAPK1*, and *PIK3CA* (Supplementary Table S1; refs. 32, 33). When sufficient tissue was available, samples that were *EGFR*/*KRAS* wild-type (WT) were tested for *ALK* rearrangements by FISH (Vysis *ALK* Break Apart FISH Probe Kit) using standard protocols (Supplementary Fig. S1).

HER2 mutation analysis

Cases which were negative for the predominant activating *EGFR* (Exon 19 deletion and L858R) and *KRAS* (G12 and G13) mutations were selected for *HER2* testing, given the known mutually exclusive nature of these mutations. *HER2* molecular analysis was carried out by 2 methods: a sizing assay to detect small indels in exon 20 and a Sequenom assay panel to detect specific point mutations including L755S, D769H, V777L, and V777M. Testing by fragment analysis followed a protocol similar to *EGFR* testing with fluorescently labeled *HER2* primers (29, 30). Briefly, a 300-bp genomic DNA fragment encompassing the entire

coding region of exon 20 was amplified using the primers FW1: 5′-GTT TGG GGG TGT GTG GTC T -3′ and REV: 5′-Hex - CCT AGC CCC TTG TGG ACA TA -3′. PCR products were subjected to capillary electrophoresis on an ABI 3730 Genetic Analyzer (Applied Biosystems). Testing for recurrent point mutations was incorporated into the standard Sequenom testing panel with procedures as previously described. All positive cases were confirmed and further characterized by Sanger sequencing using the above primers without fluorescent label.

To confirm the mutually exclusivity of *HER2* exon 20 insertions with major *EGFR* and *KRAS* mutations, as well as other rarer mutations not well represented in our cohort, we also tested a separate set of adenocarcinomas with a known positive mutation profile and sufficient DNA. Also, to confirm that *HER2* mutations were confined to adenocarcinomas, we also tested a separate set of squamous and small cell carcinomas following similar protocols.

Analysis of *HER2* gene copy number alterations by FISH

Assessment of *HER2* gene copy number was conducted on the same formalin-fixed paraffin-embedded specimens used for DNA extraction. The Vysis PathVysion *HER2* DNA Probe Kit (Abbott Laboratories) was used following standard manufacturer's protocol. At least 40 cells were analyzed for each case by 2 reviewers and were classified according to published criteria (34, 35) as disomy, low polysomy ($\leq$4 copies of *HER2* in $\leq$40% of cells), high polysomy (>4 copies of HER2 in >40% of cells), or amplified (HER2/CEP17 ratio per cell $\geq$ 2, or homogeneously staining regions with $\geq$15 copies in $\geq$10% of the cells). Cases with a ratio between 1.8 and 2.2 were reviewed, and wider areas recounted to confirm their status as amplified or not amplified.

Statistical analysis

The association between *HER2* status and clinical and biologic characteristics was analyzed by Fisher exact test. Age differences were compared using the *t* test for independent samples. The 2-sided significance level was set at *P* less than 0.05.

Overall survival was calculated using the Kaplan–Meier method. Patients were followed from date of diagnosis of stage IIIb/IV disease to date of death or last follow-up. Survival data were obtained through medical records and the Social Security Death Index and were updated as of November 2011. Group comparisons were conducted using the log-rank test.

Results

Initial screening

A total of 1,478 ADCs were screened under the clinical assays and Sequenom mass spectrometry–based genotyping assays. Of these, 894 [60%, 95% confidence interval (CI), 58%–63%] were mutation positive (non-*HER2*) with a distribution as outlined in Supplementary Table S2. Among the remaining 584 "point mutation-negative" samples, 437 were tested by FISH for *ALK* rearrangements; 36 cases (8%) were positive (36 of 437; 95% CI, 6–11%).

HER2 mutations

Five hundred and sixty ADC samples that were negative for the predominant activating *EGFR* and *KRAS* mutations were tested for *HER2* insertions. This group included 80 cases with point mutations detected by the extended Sequenom panel, 26 cases with *ALK* rearrangement, and 454 samples with no mutations. Ninety-four cases (out of the above 584 "point mutation-negative" samples) could not be tested further due to insufficient DNA.

Among the 560 ADCs tested, we detected 26 *HER2* mutations in 25 cases (5%, 25/560; 95% CI, 3–7%). All mutations were mutually exclusive with point mutations in *EGFR*, *KRAS*, *BRAF*, *NRAS*, *PIK3CA*, *MEK1*, and *AKT* mutations as well as *ALK* rearrangements. An additional 53 *EGFR* and *KRAS* mutations were detected with Sequenom testing, therefore the *HER2* mutation rate among ADC-negative for both major and minor *EGFR* and *KRAS* mutations was 5% (26/507; 95% CI, 3%–7%). The incidence among the group negative for *EGFR*, *KRAS*, and *ALK* was 6% (20/335; 95% CI, 3–8%). No *HER2* mutations were identified among 104 squamous cell carcinomas and 6 small cell carcinomas tested.

Testing of a separate set of adenocarcinomas with a known positive mutation profile (n = 330, 80 *EGFR* ex 19 del, 79 L858R, 120 *KRAS* G12&G13, 7 *NRAS*, 3 *MAPK*, 2 *AKT*, 30 *BRAF*) confirmed the mutually exclusive nature of *HER2* exon 20 insertions with these mutations.

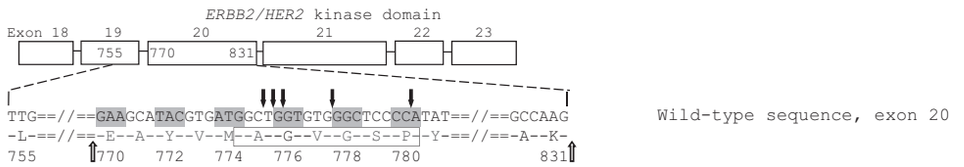
The vast majority of *HER2* mutations, 92% (24/26), were in-frame insertions in exon 20, which ranged from 3 to 12 bp, all nested in the most proximal region of the exon, between codons 775 and 881 (Fig. 1). The 12-bp insertion was the most common mutation (83%, 20 of 24) with all cases showing a duplication/insertion of 4 amino acids (YVMA) at codon 775. The 3 bp insertion was the second most common (8%, 2 of 24) and was characterized as a complex insertion-substitution G776 > VC by Sanger sequencing in the 2 identified cases. Two point mutations were also detected, L755S and G776C, corresponding to 8% (2 of 26) of all *HER2* mutations identified. The G776C mutation was found concurrently with the *HER2* V777_G778insCG (Fig. 3).

Clinical characteristics of patients with *HER2* mutations

Comparison of HER2 mutants with the HER2 wild-type group. The clinical characteristics of patients with *HER2* mutations are summarized in Table 1. After establishing the mutually exclusivity of *HER2* mutations with other driver gene mutations, we defined a *HER2* WT comparison group by combining all cases confirmed *HER2* negative by testing with all cases harboring a mutation in any other gene. This group of 1,359 cases is specified in Table 1 as the *HER2* WT group. Comparison with this group shows the patients with *HER2* mutations presented at a slightly younger age, with a median age of 64 years versus 66 years. The proportion of

A

Schematic organization of *ERBB2* kinase domain



B

Spectrum of *ERBB2/HER2* mutations

Mut size	Total Cases (n = 25)	Nucleotide sequence*	CDS mutation (inserted sequence)	Amino acid mutation
12-bp ins	19 (76%)	TTG==//==GAAGCATACGTGATGGC**ATACGTGATGGC**TGGTGTGGGCTCCCCATAT -L-==//==-E--A--Y--V--M--A**--Y--V--M--A**--G--V--G--S--P--Y	c.2324_2325ins12 (ATACGTGATGGC duplication*)	p.Ala775_Gly776insTyrValMetAla
12-bp ins	1 (4%)	TTG==//==GAAGCATACGTGATGGCT**TACGTGATGGCT**GGTGTGGGCTCCCCATAT -L-==//==-E--A--Y--V--M--A**--Y--V--M--A**--G--V--G--S--P--Y	c.2325_2326ins12 (TACGTGATGGCT duplication)	p.Ala775_Gly776insTyrValMetAla
3-bp ins	2 (8%)	TTG==//==GAAGCATACGTGATGGCTG**TGT**GTGTGGGCTCCCCATAT -L-==//==-E--A--Y--V--M--A**--V--C**--V--G--S--P--Y	c.2326_2327ins3 (TGT insertion)	p.Gly776>ValCys
9-bp ins	1 (4%)	TTG==//==GAAGCATACGTGATGGCTGGTGTGGGCTCCCC**TGGCTCCCC**ATAT -L-==//==-E--A--Y--V--M--A--G--V--G--S--P**--G--S--P**--Y	c.2339_2340ins9 (TGGTCTCCC insertion)	p.Pro780_Tyr781insGlySerPro
Subs+ 6-bp ins	1 (4%)	TTG==//==GAAGCATACGTGATGGCT**T**GTGTG**TGTGGG**GGCTCCCCATAT -L-==//==-E--A--Y--V--M--A**--C**--V**--C--G**--G--S--P--Y-	c.2326G>T and c.2331_2332ins6 (TGTGGG duplication)	p.Gly776Cys and p.Val777_Gly778insCysGly
Subs	1 (4%)	T**C**G==//==GAAGCATACGTGATGGCTGGTGTGGGCTCCCCATAT **-S-**==//==-E--A--Y--V--M--A--G--V--G--S--P--Y-	c.2264T>C (Substitution)	p.Leu755Ser

Figure 1. A, schematic organization of *ERBB2* kinase domain and detailed structure of the proximal region of Exon 20. Open black arrows flank the beginning and the end of exon 20. Solid black arrows mark the specific locations of the identified mutations. Mutation hotspot is demarcated by the box. B, spectrum of mutations: detailed description of all mutations identified. Insertion sequences are demarcated by the box; black cross marks the point mutations. In a forward sequence, this is indistinguishable from c. 2322_2323ins12 (GCATACGTGATG duplication), M774_A775insAYVM, due to the GC sequence at both ends of the insertion.

HER2-mutant patients presenting at age 64 or younger (64%) was greater than that of the *HER2* WT population (46%, $P = 0.04$). Significantly, more never-smokers harbored *HER2* mutations (5% vs. 1%, $P < 0.0001$), but there were no significant differences in sex ratios (female 2% vs. male 2%, $P = 0.83$) or in the stage at presentation. *HER2* mutations were not significantly more common among Asian patients (2/61, 3%) vs. Caucasian patients (23/1290, 2%; $P = 0.31$).

Comparison of HER2 mutants versus specific molecular subsets. When the *HER2* WT group is stratified into molecular subsets (Table 1), we find that the younger age association is lost with most groups but remains significant only when compared with the cohort with *KRAS* mutations ($P = 0.04$). Significant differences were identified in the smoking status of *HER2*-positive patients when compared with both *KRAS* and *BRAF* ($P < 0.0001$). Similar smoking differences were observed when *HER2*-mutated patients were compared with the *EGFR*/*KRAS*/*ALK* negative and the negative for all mutations assayed groups ($P < 0.0001$). In contrast, no significant differences in smoking were identified between the *HER2*-mutated and the *EGFR*-mutated groups. The stratification by molecular subtype also uncovered differences in the stage at presentation of the *HER2*-mutated group compared with both *ALK*-rearranged and *BRAF*-mutated cohorts, the latter 2 being associated with later stage (III–IV) presentation ($P = 0.003$ and 0.03, respectively).

Clinical outcomes

Because of the small sizes of stage- and mutation-specific cohorts, only overall survival was assessed. During follow-up, 468 patients presented with or developed advanced disease (stage IIIb or IV). This included 102 *EGFR*, 117 *KRAS*, 28 *ALK*, 10 *BRAF*, and 16 *HER2* patients. The median follow-up after the diagnosis of advanced disease for all patients was 19 months. The median overall survival by molecular cohort was: *HER2* 19 months, *EGFR* 30 months, *KRAS* 14 months,*ALK* 25 months, and *BRAF* 21 months. In this series, the overall survival of the *HER2* cohort did not differ significantly from the other molecularly defined cohorts (Fig. 2).

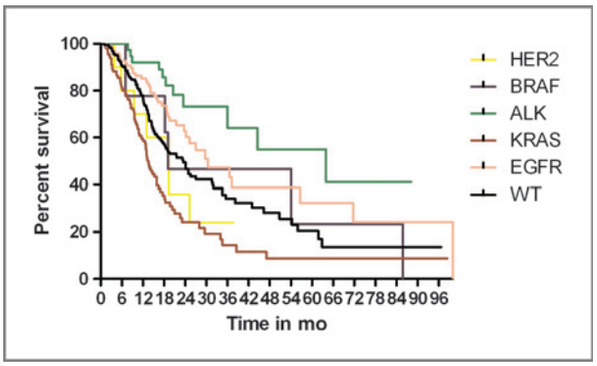


Figure 2. Kaplan–Meier curve for overall survival in patients with advanced stage (IIIB/IV) disease. The overall survival of the *HER2* cohort was not statistically different from the other molecularly defined cohorts.

Morphologic spectrum of *HER2* mutant lung adenocarcinomas

The vast majority of tumors harboring *HER2* mutations (92%, 23/25) were moderate (11) to poorly (12) differentiated and most (80%, 20/25) had high-grade morphology. Eighty-percent (80%, 20/25) were tumors of mixed phenotype with papillary, acinar, solid, and micropapillary, components as the most predominant patterns. Three cases had a mucinous component. A bronchoalveolar component was present in 6 tumors, but it was minimal in most cases (67%, 4/6). Only 3 tumors showed a pure phenotype (1 papillary, 1 micropapillary, and 1 solid) but all were small samples and may reflect limited sampling. Two cases were classified as poorly differentiated adenocarcinoma, not otherwise specified because of limited sampling, both with high-grade cytologic features (Fig. 3).

HER2 gene copy alterations by FISH

Analysis for *HER2* gene copy number alterations by FISH was conducted on 11 of the 25 *HER2* mutated cases based on tissue availability and in 39 *HER2* WT cases. None of the mutant cases showed *HER2* amplification. Instead, 2 cases (18%) showed high polysomy (>4 copies of both HER2 and CEP17) and 8 (73%) had low polysomy. Among the WT group, one case was amplified (3%, 1/39) with a HER2/CEP17 ratio of 5.9). In this group, 4 cases (10%) had high polysomy and 27 (69%) had low polysomy (Table 2). Of note, the *HER2*-amplified case was also found to harbor an *EGFR* exon 19 deletion.

Discussion

The management of lung adenocarcinomas has been transformed in the past decade by the identification of key genetic alterations that activate driver oncogenes. These alterations allow the assignment of patients to targeted treatments based on the specific molecular lesions detected in their tumors.

Mutations in the *HER2* gene identify a distinct subset of lung adenocarcinomas. Although less common than *EGFR* and *KRAS*, these mutations represent an additional target with already proven sensitivity to HER2 inhibitors in preclinical models (23–25, 36) and anecdotal clinical reports

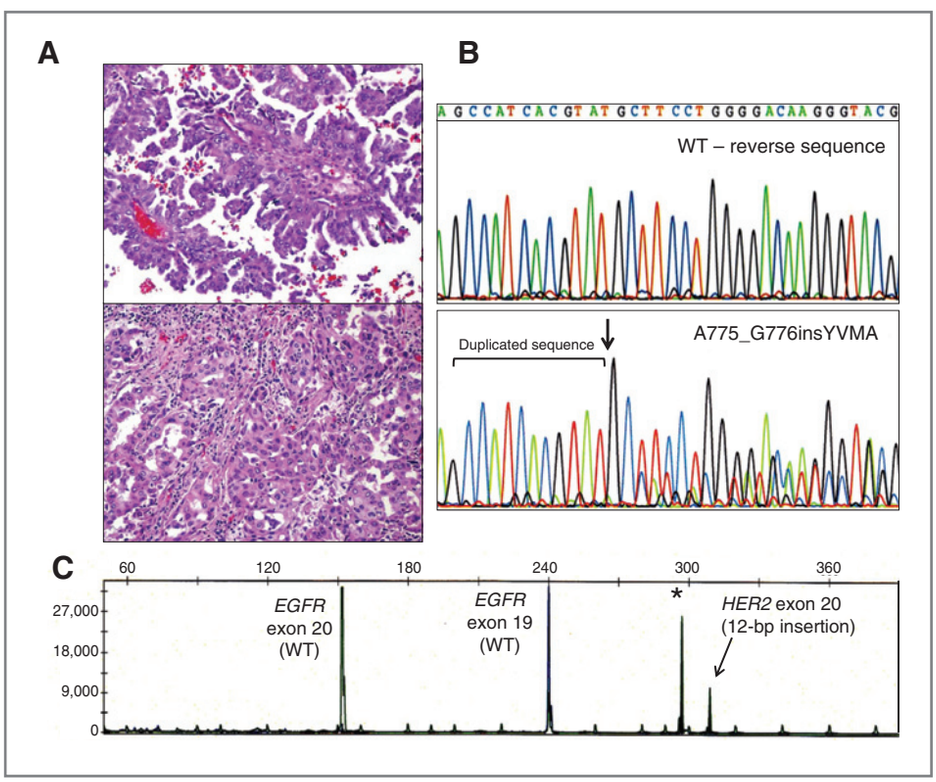


Figure 3. Representative case harboring the most common *ERBB2* mutation, A775_G776insYVMA. A, morphologically, this tumor showed a mixed phenotype with papillary, micropapillary, and solid components. B, bottom tracing, standard sequencing (reverse) shows further characterization of the mutation as A775_G776insYVMA (2324_2325ins12 [ATACGTGATGGC]). The arrow marks the beginning of the insertion sequence. Top tracing, the reverse WT sequence for comparison. C, ABI tracing of the sizing assay shows a heterozygous 12 bp insertion (arrow); asterisk marks the adjacent wild-type peak. This case was concurrently tested for indels in exon 19 and 20 of *EGFR* using a multiplex assay and illustrates the mutually exclusive nature of these mutations.

Table 1. Comparison of clinical characteristics of *HER2* mutant patients versus other molecularly defined subsets

	HER2 **(*n* = 25)**	**HER2 WT (*n* = 1359)**	***EGFR*** **(*n* = 359)**	***KRAS*** **(*n* = 495)**	**EML4-ALK (n = 35)**	***BRAF*** **(n = 25)**
Gender						
Female/male	17/8	862/497	260/99	326/169	19/16	17/8
Median age, y						
Range	64 (51–84)	66 (32–90)	66(32–90)	67 (38–86)	56 (39–78)	70 (54–79)
Pts <64/≥66 y	16/9	588/771($P = 0.04$)	158/201	205/290 ($P = 0.04$)	21/14	12/13
Smoking status						
Never/smoker	17/8	340/1,019 ($P < 0.0001$)	192/167	30/465 ($P < 0.0001$)	21/14	3/22 ($P = 0.0001$)
Stage						
I–II/III–IV	15/10	631/728	147/212	246/249	7/28 ($P = 0.0025$)	11/14 ($P = 0.03$)
Ethnicity						
Asian/Caucasian	2/23	59/1,267				

NOTE: *P* values in parentheses based on comparison with *HER;* only significant values are annotated.

(37, 38). Both trastuzumab and lapatinib are available and newer agents, such as dacomitinib and afatinib, are in clinical trials specifically for this indication (36, 39, 40). Given the high prevalence of lung adenocarcinomas, the targeting of these mutations could benefit thousands of patients each year in the United States and elsewhere.

The clinicopathologic characteristics and prognostic implications of *HER2*-mutated lung adenocarcinoma remain poorly defined. Previous studies report attributes that parallel those seen with *EGFR* mutations, including associations with female sex, Asian ethnicity, never-smoker status, and adenocarcinoma subtype. Most studies, however, have been conducted in Asia. Only 2 studies have included Caucasian patients, a single study of 157 U.S. patients in whom no mutations were detected (20), and a study of 402 European patients in which a mutation rate of 2% was identified (17). To our knowledge, our study represents the largest assessment for *HER2* mutations in a predominantly Caucasian population and the most comprehensive analysis for other mutations in the same cohort.

As *HER2* mutations have been previously shown to be mutually exclusive with major mutations in *EGFR* and *KRAS*, we selected this negative group as our target for testing, allowing us to enrich for mutant cases. In this subset, we identified 25 *HER2*-positive cases, all mutually exclusive with other recurrent point mutations in *EGFR*, *KRAS*, *BRAF*, *NRAS*, *PIK3CA*, *MEK1* and *AKT*, as well as *ALK* rearrangements. Additional testing of a separate set of cases known to be positive for major *EGFR* and *KRAS* further confirmed the mutually exclusive nature of these mutations. The incidence of *HER2* mutations was 5% and 6% among the *EGFR*/*KRAS*-negative and the *EGFR*/*KRAS*/*ALK*-negative groups, respectively. On the basis of these mutually exclusive relationships, the proportion of tumors negative for *EGFR*, *KRAS*, and *ALK*, and the prevalence of *HER2* mutations in the latter group, we estimate the overall prevalence in the entire group (1,478 patients) to be approximately 2%, which is similar to that observed by Buttita and colleagues (17), in their smaller study of European patients.

Among patients with *HER2* mutations, insertions in exon 20 represented the vast majority of the alterations detected (96%, 24/25 patients). As a group, these mutations resemble activating mutations found within exon 20 of *EGFR* (non-T790M) which have been associated with primary resistance to both first and second generation tyrosine kinase inhibitors (41). In both cases, insertions are in-frame, ranging from 3 to 12 base pairs and confined to a short stretch within the most proximal region of the exon (between the 7th and the 12th codon of the exon). Compared with *EGFR*, *HER2* insertions are less heterogeneous, with over 80% of cases showing the A775_G776insYVMA insertion/duplication (Fig. 4). Two of the mutations

Table 2. HER2 copy number alterations in *HER2* mutant tumors

HER2 **copy number alterations**	**HER2 mutant (*n* = 11)**	***HER2*** **WT (*n* = 39)**	***P***
Amplified	0	1 (3%)	NS
High polysomy	2 (18%)	4 (10%)	NS ($P = 0.29$)
Low polysomy	8 (73%)	27(69%)	NS
Disomy (or near disomy)	1 (9%)	7 (18%)	NS

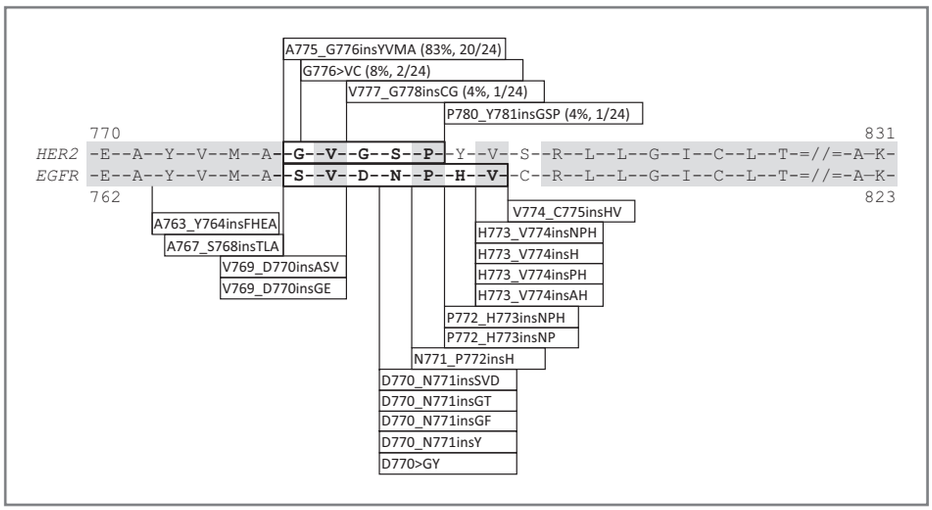


Figure 4. Positions of the *HER2* exon 20 insertions identified in the present study and comparison with the spectrum of *EGFR* exon 20 insertion mutations identified at our institution over a 3-year period. Insertions in *HER2* show significantly less heterogeneity compared with EGFR with over 80% of HER2 alterations being represented by the A775_G776insYVMA.

detected in our series have not been reported in previous studies (V777_G778insCG and G776C). The significance of the G776C mutation found concurrently with a 6-bp insertion is unknown.

Having confirmed the mutually exclusive nature of *HER2* mutations with other driver oncogenes, we then compared the clinical characteristics of *HER2*-positive patients with the WT group (cases confirmed *HER2*-negative by testing plus cases with mutations in another gene). In agreement with other groups (17, 20, 42), we found a significant association with never-smoker status ($P < 0.0001$). In contrast, we did not identify a significant difference by sex or race, although the low number of Asian patients limited the power of this analysis. Patients with *HER2* mutations presented at a slightly younger age compared with the overall *HER2* WT group and patients with *KRAS* mutations. While we noted differences in stage at presentation between the *HER2* and both the *BRAF* and *ALK* subsets, this is difficult to explain biologically and of unclear clinical significance. In this cohort of patients with *HER2*-mutated lung cancers, survival was numerically similar to other molecularly defined cohorts.

Previous studies have shown that *HER2* mutations are confined to the adenocarcinoma subtype of NSCLCs but specific histologic subtype associations have only been assessed by a single group (17). In this study, Buttita *et al* reported that *HER2* mutations are significantly more frequent in ADC with bronchioloalveolar carcinoma features (43). In our study, we do not find this association. Instead we observe significant heterogeneity and a predominance of high grade morphologic features. Most tumors (82%) were of mixed phenotype with papillary, acinar, solid and micropapillary patterns representing the most common components, in decreasing order. The vast majority of tumors (92%) were moderately or poorly differentiated, as also reported in an Asian cohort (28).

In our analysis of *HER2* copy number status, we did not find an association of *HER2* mutations and gene amplification. While copy number gains were present in most of the cases studied, either as high or low polysomy, this finding was also present among the WT group without significant differences. Although the number of samples studied is small, this suggests that the presence of a *HER2* mutation does not necessarily drive copy number gains of the mutated allele. By comparison, a recent study by Li and colleagues reports a significant association with 7 of 8 mutant tumors showing *HER2* gains (4 amplification and 3 high polysomy). While copy number variations in their WT group was not reported, previous studies in unselected patients report the presence of *HER2* gene amplification by FISH in up to 23% of unselected NSCLC cases (14, 34, 35), suggesting that both amplification and high copy number gains can be present in a significant proportion of cases in the absence of mutations. Although discrepancies could be attributed to the limited number of cases, the combined findings would seem to suggest that, similar to what has been found in *EGFR*-mutated lung cancer, amplification cannot serve as a surrogate marker for activating *HER2* mutations.

In summary, mutations in the tyrosine kinase domain of *HER2* identify a distinct subset of lung adenocarcinomas with a higher prevalence among never-smokers. *HER2* mutations are mutually exclusive with other activating mutations and independent of *HER2* gene amplification. Given the high incidence of lung adenocarcinomas, there may be several thousand patients with this uncommon, yet important, mutation diagnosed every year in the United States, and their identification will allow for assignment to one of many investigational agents targeting this pathway. Testing for activating *HER2* kinase domain aberrations, both point mutations and exon 20 insertions, should therefore be incorporated into standard multiplex molecular screening in lung ADC.

Disclosure of Potential Conflicts of Interest

M.G. Kris has a commercial research grant from Pfizer, Inc and Boehringer Ingelheim and is a consultant/advisory board member of Pfizer Inc, Boehringer Ingelheim, and Genentech. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M.E. Arcila, J.E. Chaft, M.G. Kris, M. Ladanyi
Development of methodology: M.E. Arcila, K. Nafa, C. Lau, M.G. Kris, M. Ladanyi
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.E. Chaft, J.E. Chaft, C. Lau, P.K. Paik, M.G. Kris
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.E. Arcila, J.E. Chaft, K. Nafa, S. Roy-Chowdhuri, C. Lau, M. Zaidinski, M.F. Zakowski, M.G. Kris, M. Ladanyi
Writing, review, and/or revision of the manuscript: M.E. Arcila, J.E. Chaft, K. Nafa, S. Roy-Chowdhuri, C. Lau, P.K. Paik, M.F. Zakowski, M.G. Kris, M. Ladanyi
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K. Nafa, M. Zaidinski, M.G. Kris, M. Ladanyi
Study supervision: M. Ladanyi

Acknowledgments

The authors thank Dr. Laetitia Borsu and Angela Marchetti for assistance with the Sequenom assays; Talia Mitchell, Justyna Sadowska, and Jacklyn Casanova for technical assistance with *EGFR* and *KRAS* assays; and Edyta B. Brzostowski for help with the MSKCC Lung Cancer Mutation Analysis Project database.

Grant Support

This work was supported by NIH: P01 CA129243 (to M.G. Kris and M. Ladanyi).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 19, 2012; revised May 15, 2012; accepted June 17, 2012; published OnlineFirst July 3, 2012.

References

1. Daly JM, Jannot CB, Beerli RR, Graus-Porta D, Maurer FG, Hynes NE. Neu differentiation factor induces ErbB2 down-regulation and apoptosis of ErbB2-overexpressing breast tumor cells. Cancer Res 1997;57:3804–11.
2. Graus-Porta D, Beerli RR, Hynes NE. Single-chain antibody-mediated intracellular retention of ErbB-2 impairs Neu differentiation factor and epidermal growth factor signaling. Mol Cell Biol 1995;15:1182–91.
3. Riese DJ II, Stern DF. Specificity within the EGF family/ErbB receptor family signaling network. Bioessays 1998;20:41–8.
4. Pinkas-Kramarski R, Soussan L, Waterman H, Levkowitz G, Alroy I, Klapper L, et al. Diversification of Neu differentiation factor and epidermal growth factor signaling by combinatorial receptor interactions. EMBO J 1996;15:2452–67.
5. Tzahar E, Waterman H, Chen X, Levkowitz G, Karunagaran D, Lavi S, et al. A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. Mol Cell Biol 1996;16:5276–87.
6. Klapper LN, Glathe S, Vaisman N, Hynes NE, Andrews GC, Sela M, et al. The ErbB-2/HER2 oncoprotein of human carcinomas may function solely as a shared coreceptor for multiple stroma-derived growth factors. Proc Natl Acad Sci U S A 1999;96:4995–5000.
7. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235:177–82.
8. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989;244:707–12.
9. Gravalos C, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. Ann Oncol 2008;19:1523–9.
10. Slomovitz BM, Broaddus RR, Burke TW, Sneige N, Soliman PT, Wu W, et al. Her-2/neu overexpression and amplification in uterine papillary serous carcinoma. J Clin Oncol 2004;22:3126–32.
11. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344:783–92.
12. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. 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 2010;376:687–97.
13. Langer CJ, Stephenson P, Thor A, Vangel M, Johnson DH. Trastuzumab in the treatment of advanced non-small-cell lung cancer: is there a role? Focus on Eastern Cooperative Oncology Group study 2598. J Clin Oncol 2004;22:1180–7.
14. Pellegrini C, Falleni M, Marchetti A, Cassani B, Miozzo M, Buttitta F, et al. HER-2/Neu alterations in non-small cell lung cancer: a comprehensive evaluation by real time reverse transcription-PCR, fluorescence in situ hybridization, and immunohistochemistry. Clin Cancer Res 2003;9:3645–52.
15. Hirsch FR, Varella-Garcia M, Franklin WA, Veve R, Chen L, Helfrich B, et al. Evaluation of HER-2/neu gene amplification and protein expression in non-small cell lung carcinomas. Br J Cancer 2002;86:1449–56.
16. Gatzemeier U, Groth G, Butts C, Van Zandwijk N, Shepherd F, Ardizzoni A, et al. Randomized phase II trial of gemcitabine-cisplatin with or without trastuzumab in HER2-positive non-small-cell lung cancer. Ann Oncol 2004;15:19–27.
17. Buttitta F, Barassi F, Fresu G, Felicioni L, Chella A, Paolizzi D, et al. Mutational analysis of the HER2 gene in lung tumors from Caucasian patients: mutations are mainly present in adenocarcinomas with bronchioloalveolar features. Int J Cancer 2006;119:2586–91.
18. Davies H, Hunter C, Smith R, Stephens P, Greenman C, Bignell G, et al. Somatic mutations of the protein kinase gene family in human lung cancer. Cancer Res 2005;65:7591–5.
19. Sasaki H, Shimizu S, Endo K, Takada M, Kawahara M, Tanaka H, et al. EGFR and erbB2 mutation status in Japanese lung cancer patients. Int J Cancer 2006;118:180–4.
20. Shigematsu H, Takahashi T, Nomura M, Majmudar K, Suzuki M, Lee H, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. Cancer Res 2005;65:1642–6.
21. Sonobe M, Manabe T, Wada H, Tanaka F. Lung adenocarcinoma harboring mutations in the ERBB2 kinase domain. J Mol Diagn 2006;8:351–6.
22. Stephens P, Hunter C, Bignell G, Edkins S, Davies H, Teague J, et al. Lung cancer: intragenic ERBB2 kinase mutations in tumours. Nature 2004;431:525–6.
23. Wang SE, Narasanna A, Perez-Torres M, Xiang B, Wu FY, Yang S, et al. HER2 kinase domain mutation results in constitutive phosphorylation and activation of HER2 and EGFR and resistance to EGFR tyrosine kinase inhibitors. Cancer Cell 2006;10:25–38.
24. Perera SA, Li D, Shimamura T, Raso MG, Ji H, Chen L, 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 U S A 2009;106:474–9.
25. Shimamura T, Ji H, Minami Y, Thomas RK, Lowell AM, Shah K, 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;66:6487–91.
26. Suzuki T, Fujii A, Ohya J, Amano Y, Kitano Y, Abe D, et al. Pharmacological characterization of MP-412 (AV-412), a dual epidermal growth factor receptor and ErbB2 tyrosine kinase inhibitor. Cancer Sci 2007;98:1977–84.
27. Suzuki T, Fujii A, Ohya J, Nakamura H, Fujita F, Koike M, et al. Antitumor activity of a dual epidermal growth factor receptor and ErbB2 kinase inhibitor MP-412 (AV-412) in mouse xenograft models. Cancer Sci 2009;100:1526–31.
28. Li C, Sun Y, Fang R, Han X, Luo X, Wang R, et al. Lung adenocarcinomas with HER2-Activating mutations are associated with distinct clinical features and HER2/EGFR copy number gains. J Thorac Oncol 2012;7:85–9.

29. Pan Q, Pao W, Ladanyi M. Rapid polymerase chain reaction-based detection of epidermal growth factor receptor gene mutations in lung adenocarcinomas. J Mol Diagn 2005;7:396–403.
30. Su Z, Dias-Santagata D, Duke M, Hutchinson K, Lin YL, Borger DR, et al. A platform for rapid detection of multiple oncogenic mutations with relevance to targeted therapy in non-small-cell lung cancer. J Mol Diagn 2011;13:74–84.
31. Arcila M, Lau C, Nafa K, Ladanyi M. Detection of KRAS and BRAF mutations in colorectal carcinoma roles for high-sensitivity locked nucleic acid-PCR sequencing and broad-spectrum mass spectrometry genotyping. J Mol Diagn 2011;13:64–73.
32. Chaft JE, Arcila ME, Paik PK, Lau C, Riely GJ, Pietanza MC, et al. Coexistence of PIK3CA and other oncogene mutations in lung adenocarcinoma-rationale for comprehensive mutation profiling. Mol Cancer Ther 2012;11:485–91.
33. Rekhtman N, Paik PK, Arcila ME, Tafe LJ, Oxnard GR, Moreira AL, et al. Clarifying the spectrum of driver oncogene mutations in biomarker-verified squamous carcinoma of lung: lack of EGFR/KRAS and presence of PIK3CA/AKT1 mutations. Clin Cancer Res 2012;18:1167–76.
34. Cappuzzo F, Varella-Garcia M, Shigematsu H, Domenichini I, Bartolini S, Ceresoli GL, et al. Increased HER2 gene copy number is associated with response to gefitinib therapy in epidermal growth factor receptor-positive non-small-cell lung cancer patients. J Clin Oncol 2005;23:5007–18.
35. Hirsch FR, Varella-Garcia M, McCoy J, West H, Xavier AC, Gumerlock P, et al. Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study. J Clin Oncol 2005;23:6838–45.
36. Engelman JA, Zejnullahu K, Gale CM, Lifshits E, Gonzales AJ, Shimamura T, et al. PF00299804, an irreversible pan-ERBB inhibitor, is effective in lung cancer models with EGFR and ERBB2 mutations that are resistant to gefitinib. Cancer Res 2007;67:11924–32.
37. Cappuzzo F, Bemis L, Varella-Garcia M. HER2 mutation and response to trastuzumab therapy in non-small-cell lung cancer. N Engl J Med 2006;354:2619–21.
38. DeGreve J, Teugels E, Geers C, Decoster L, Galdermans D, De Mey J, 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;76:123–7.
39. Ross HJ, Blumenschein GR Jr, Aisner J, Damjanov N, Dowlati A, Garst J, et al. Randomized phase II multicenter trial of two schedules of lapatinib as first- or second-line monotherapy in patients with advanced or metastatic non-small cell lung cancer. Clin Cancer Res 2010;16:1938–49.
40. Yap TA, Vidal L, Adam J, Stephens P, Spicer J, Shaw H, 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;28:3965–72.
41. Yasuda H, Kobayashi S, Costa DB. EGFR exon 20 insertion mutations in non-small-cell lung cancer: preclinical data and clinical implications. Lancet Oncol 2012;13:e23–31.
42. Cappuzzo F, Ligorio C, Janne PA, Toschi L, Rossi E, Trisolini R, et al. Prospective study of gefitinib in epidermal growth factor receptor fluorescence in situ hybridization-positive/phospho-Akt-positive or never smoker patients with advanced non-small-cell lung cancer: the ONCOBELL trial. J Clin Oncol 2007;25:2248–55.
43. Tomizawa K, Suda K, Onozato R, Kosaka T, Endoh H, Sekido Y, et al. Prognostic and predictive implications of HER2/ERBB2/neu gene mutations in lung cancers. Lung Cancer 2007;74:139–44.