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Prevalence and Clinicopathological Characteristics of *HER2* and *BRAF* Mutation in Chinese Patients with Lung Adenocarcinoma

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Abstract

Aims

To determine the prevalence and clinicopathological characteristics of *BRAF* V600E mutation and *HER2* exon 20 insertions in Chinese lung adenocarcinoma (ADC) patients.

Methods

Given the fact that the driver mutations are mutually exclusive in lung ADCs, 204 *EGFR*/*KRAS* wild-type cases were enrolled in this study. Direct Sanger sequencing was performed to examine *BRAF* V600E and *HER2* exon 20 mutations. The association of *BRAF* and *HER2* mutations with clinicopathological characteristics was statistically analyzed.

Results

Among the 204 lung ADCs tested, 11 cases (5.4%) carried *HER2* exon 20 insertions and 4 cases (2.0%) had *BRAF* V600E mutation. *HER2* mutation status was identified to be associated with a non-smoking history ($p<0.05$). *HER2* mutation occurs in 9.4% of never smokers (10/106), 8.7% of female (8/92) and 2.7% of male (3/112) in this selected cohort. All four *BRAF* mutated patients were women and three of them were never-smokers. No *HER2* mutant patients harbor *BRAF* mutation.

Conclusions

HER2 and *BRAF* mutations identify a distinct subset of lung ADCs. Given the high prevalence of lung cancer and the availability of targeted therapy, Chinese lung ADC patients without *EGFR* and *KRAS* mutations are recommended for *HER2* and *BRAF* mutations detection, especially for those never smokers.

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Introduction

Lung cancer is the leading cause of cancer-related death worldwide [1]. Adenocarcinoma (ADC), the most common type of lung cancer, is diagnosed in 1 million patients each year [2]. Targeted therapies have been succeeded in a subset of lung ADC patients with driver oncogenic mutations [3,4]. Currently a higher than 50% estimated frequency of actionable oncogenic drivers have been identified in lung ADCs. Sensitizing *EGFR* mutations occur in 30% -50% of Asian lung ADC patients, who are potential responders for EGFR tyrosine kinase inhibitors (TKIs) treatment. *ALK* and *ROS1* rearrangements, targeted by crizontinib, appear in approximately 6%-10% of lung ADC patients. Testing for somatic *EGFR* mutations and *ALK* rearrangements is now in clinical routine for advanced lung ADC patients. Another two actionable targets, *BRAF* and *HER2* mutations, have been identified in approximately 3% and 2% of lung ADC patients, respectively [5–7]. Vemurafenib, the selective BRAF kinase inhibitors, has been approved and succeed for the treatment of melanoma patients harboring *BRAF* V600E mutation. It provided a rationale for testing *BRAF* mutation in lung ADC patients. Very recently, dramatic response of Vemurafenib and Dabrafenib treatment has been observed in lung ADC patients with *BRAF* V600E mutation [8–10]. Meanwhile, *HER2* exon 20 insertions in lung ADC patients were identified to indicate efficacy of HER2-targeted drugs, i.e. trastuzumab and afatinib [11,12]. As a result, the importance of screening for *BRAF* and *HER2* mutations in lung ADC patients is recognized in clinical practice. However, as only a few patients would harbor the *BRAF* and *HER2* mutations, it is not plausible to examine these mutations in all lung ADC patients. Although efforts have been made to identify the clinicopathological factors of the lung ADC patients harboring the *BRAF* or *HER2* mutations, the studies were performed predominately on white and Japanese patients [7,11,13–16]. For Chinese lung ADC patients, *HER2* and *BRAF* mutations have been selectively examined in never-smokers [17–19]. Given the fact that the epidemiology and clinical behaviors of lung cancer is different between East Asians and Caucasians [20], we examined the *BRAF* V600E mutation and *HER2* exon 20 insertions in Chinese lung ADC patients in order to determine the frequency of these two mutations and identify their clinicopathological characteristics.

Materials and Methods

Patient selection

All included patients had received curative surgery and diagnosed as primary lung ADC. Mutation testing of *EGFR* and *KRAS* genes had been routinely performed for all the samples at the Cancer Hospital, Chinese Academy of Medical Sciences (CAMS), Beijing, China. Hematoxylin and eosin-stained (HE) sections of formalin-fixed paraffin-embedded (FFPE) tissue were reviewed for each sample to identify the section with the highest tumor density (at least 50% tumor content). Genomic DNA was extracted using the QIAamp DNA Mini Tissue kit (Qiagen, Germany) following the manufacturer's standard protocol. Clinical testing for *EGFR* was carried out using quantitative real-time PCR (qRT-PCR) (Beijing ACCB Biotech Ltd., China) for the detection of small indels in exons 19 and 20, the G719X mutation in exon18, the T790M mutation in exon 20 and the L858R and L861Q mutation in exon 21. *KRAS* testing was performed using qRT-PCR for the detection of the G12X and G13D mutations (Beijing ACCB Biotech Ltd., China). All DNA samples were kept in -80°C freezer after the mutation testing for long-term storage and the *EGFR* and *KRAS* mutation status were recorded electronically. According to the record, 215 cases were negative for *EGFR* (exons 18–21) and *KRAS* (G12 and G13) mutations between January 1, 2008 and December 31, 2012. Two hundred and four cases had enough stored DNA for *HER2* and *BRAF* mutation analysis. The clinicopathological

records of these patients were retrospectively collected from the Department of Pathology, CAMS, including sex, age, smoking history, tumor size, histological subtype, pT, pN and pTNM stages. Two of the most predominant histological subtypes for each tumor were used to further analysis. This study is retrospective and the data were analyzed anonymously. No images and private information of the patients were released. The Institute Review Board of the Cancer Hospital, CAMS, agreed to waive the need for consent for this study and approved the study protocol.

BRAF and *HER2* mutation analysis

BRAF V600E and *HER2* exon 20 mutation analysis was carried out using direct Sanger sequencing. Briefly, the *BRAF* V600E mutation was examined though amplifying the exon 15 using forward primer, 5'- TCATAATGCTTGCTCTGATAGGA-3' and reverse primer, 5'- GGCCAAAAATTTAATCAGTGGA-3'. The entire coding region of *HER2* exon 20 was amplified using forward primer, 5'- GCCATGGCTGTGGTTTGTGATGG-3' and reverse primer, 5'-ATCCTAGCCCCTTGTGGACATAGG-3'. The Refseq accession number for *HER2* gene analyzed in this study is NM_001289937.

Statistical analysis

The statistical analysis of the tumors' size and age was carried out using Student's t tests. The values are shown as mean ± SD. The relationship between *HER2* mutation and clinicopathological variables was analyzed with the chi-square test. Statistical significance was defined as $p < 0.05$.

Results

BRAF and *HER2* mutations

According to the known mutually exclusive nature of driver mutations, 204 lung ADC cases without activating *EGFR* (exons 18–21) and *KRAS* (G12 and G13) mutations were selected for *BRAF* and *HER2* mutation analysis. Among the 204 lung ADCs tested, 11 cases (5.4%) were with *HER2* exon 20 insertions and 4 cases (2.0%) were identified with *BRAF* V600E mutation. All *HER2* mutations in exon 20 were in-frame insertions ranged from 3 to 12 bp between codon 775 and 780 (Fig 1). The 12 bp insertion was the most common mutation (45.5%, 5/11). All these cases showed a duplication/insertion of 4 amino acids (YVMA) at codon 775. The 3 bp insertion at codon 776 was the second most common mutation (36.4%, 4/11). This insertion resulted in a replacement of codon 776 (G) by 2 amino acids (VC). Two cases (18.2%, 2/11) were identified with 9 bp insertion and resulted in a duplication/insertion of 3 amino acids (GSP) at codon 780.

Clinicopathological characteristics of patients with *HER2* or *BRAF* mutations in *EGFR/KRAS* wild-type lung ADCs

The clinicopathological characteristics of the *HER2* and *BRAF* mutations in *EGFR*/*KRAS* wild-type lung ADCs are summarized in Table 1. Compared to *HER2* wild-type group, although not significant, the patients with *HER2* mutations tend to be more in women (8/11) than men (3/11). All *BRAF* mutations occurred in women (4/4) in this cohort. In 92 female patients, 13% of them carried either *HER2* or *BRAF* mutations. Patients with *HER2* mutations were more likely to be never smoker (90.9%, 10/11) compared to ever smoker (9.1%, 1/11) ($p<0.05$). In 106 never smokers, 12.3% of patients carried either *HER2* or *BRAF* mutant tumors. There were no significant differences of *HER2* or *BRAF* mutations regarding tumor size, pT, pN factors or

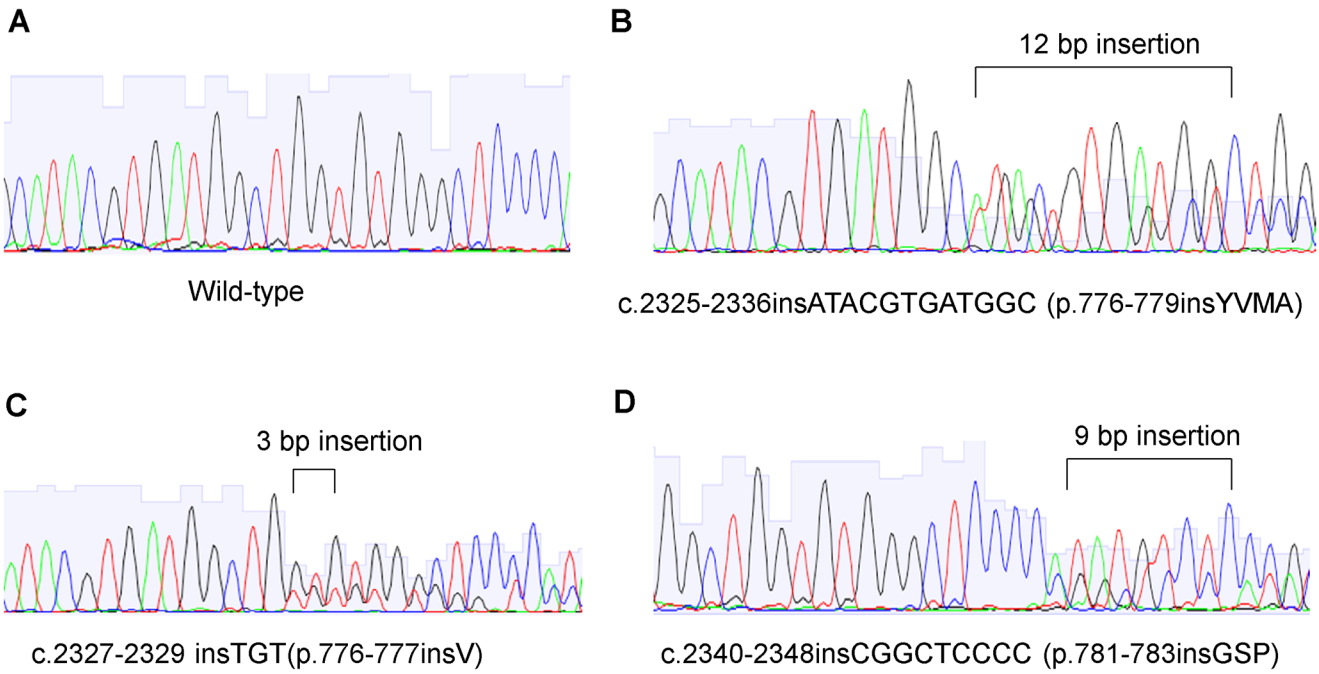

Fig 1. Sanger sequencing reads demonstrating mutational patterns of *HER2* exon 20. (A) Wild-type sequence. (B) A 12-bp duplication/insertion of the amino acid sequence YVMA between codon 776 and 779. (C) A 3-bp insertion of the amino acid sequence VC at codon 776. (D) A 9-bp insertion of the amino acid GSP between codon 781 and 783.

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pTNM stages. The predominant histological subtype of *HER2* mutated patients was acinar in 10/11 (90.9%) of cases, solid pattern in 6/11 (54.5%), papillary in 4/11 (36%), and micropapillary in 4/11 (36%). No *HER2* mutant patients harbor *BRAF* mutation.

Discussion

HER2 and *BRAF* genes represent relatively new biomarkers for NSCLC. *HER2* (also known as *EGFR2*, *ERBB2* or *NEU*) belongs to the ERBB family. Like other family members, HER2 is structurally constituted by three domains: an extracellular domain responsible for ligand binding and homo/heterodimers formation, a transmembrane domain that makes a single pass through the plasma membrane and a tyrosine kinase (TK) domain responsible for activation of two key signaling pathways, namely, the RAS/RAF/MAPK pathway, which stimulates proliferation, and the PI3K/Akt pathway, which promotes tumor cell survival. *HER2* mutations occur in the TK domain to cause a conformational change, which lead to an increased kinase activity compared to the wild-type form. Both *in-vitro* and *in-vivo* studies have confirmed the oncogenic potential of these mutations [21–23]. Given the fact that the driver mutations are mutually exclusive in lung ADCs [13,24], we selected 204 cases negative for the activating *EGFR* and *KRAS* mutations in this study. The frequency of *HER2* exon 20 mutations was 5.4% in this cohort. The incidence of *HER2* mutations has been reported previously to range from 1% to 6% in NSCLC, and the vast majority of *HER2* mutations were represented by a 12 bp duplication/insertion of the amino acid sequence YVMA in exon 20 at codon 776 [7,11,13,25,26]. The highest frequency described was 5% in *EGFR*/*KRAS* wild-type and 6% in *EGFR*/*KRAS*/*ALK* wild-type populations, respectively [13]. In Chinese lung ADC patients, *HER2* mutation was identified in 6% of never-smokers [18]. Although the incidence of *HER2* mutation in *EGFR*/*KRAS* wild-type NSCLC patients in this study was similar to others in white patients, there is a

Table 1. Clinicopathologic comparisons between *HER2* and *BRAF* mutation positive and negative Lung adenocarcinomas.

	Overall n = 204	HER2 mutation n = 11 (5.4%)	HER2 wild-type n = 193 (94.6%)	*P* value	BRAF mutation n = 4 (2.0%)	BRAF wild-type n = 200 (98.0%)	*P* value
Age	Mean±SD	55.4±5.9	58.4±10.2	0.861	66.8±6.3	58.0±10.1	0.735
	Median	56	58		67	58	
	Range	44–62	25–81		59–74	25–81	
Sex	Male	3	109	0.114	0	112	0.085
	Female	8	84		4	88	
	Nonavailable						
Smoking	Never smoker	10	96	0.035	3	103	0.785
	Ever smoker	1	83		1	83	
	Nonavailable	0	14		0	14	
Tumour size (mm)	< = 2	2	48	1.000	0	50	0.424
	2–5	8	112		3	117	
	>5	1	32		1	32	
	Nonavailable	0	1		0	1	
pT status	pT1	1	20	1.000	0	21	1.000
	pT2	6	105		4	107	
	pT3-T4	2	25		0	27	
	Nonavailable	2	43		0	45	
pN status	pN0	4	78	0.294	2	80	1.000
	pN1	1	43		0	44	
	pN2-3	6	58		2	62	
	Nonavailable	0	14		0	14	
Clinical stage	I	2	69	0.074	2	69	1.000
	II	1	47		0	48	
	III-IV	8	74		2	80	
	Nonavailable		3		0	3	

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difference regarding the frequencies of different mutation subtypes. The most common mutation subtype of *HER2* was still the 12 bp duplication/insertion of the amino acid sequence YVMA in exon 20 at codon 776 in this study, however, the frequency of this mutation subtype (45.5%) was lower compared to other studies in white patients (~80%). The result in this study could not compare to those performed on Asian lung ADC patients, in which the detailed information of insertion site in exon 20 of *HER2* gene was absent [7,17,18,27]. *In vitro* studies have shown that tumor cells harboring the most prevalent $HER2^{YVMA}$ are able to activate EGFR in a ligand-independent fashion and irrespective of the presence of an activating *EGFR* mutation [22,28]. In addition, the tumor cells harboring $HER2^{YVMA}$ mutations have been demonstrated to be resistant to reversible EGFR-TKIs such as gefitinib and erlotinib, while they remain sensitive to HER2 and dual EGFR/HER2 inhibitors [28]. In the largest published series, Mazieres *et al.* reported an impressive response rate of nearly 60% for $HER2^{YVMA}$ mutation positive subjects receiving trastuzumab and chemotherapy [11]. However, it is still not clear if other two mutations, $HER2^{V}$ and $HER2^{GSP}$, would benefit from trastuzumab. Thus, further clinical trials are required.

Based on published studies, the presence of *HER2* mutations seems associated with female gender and never smokers in lung ADC patients [11,29,30]. In this study, *HER2* mutations were confirmed to be associated with never smokers (90.9%, 10/11) ($p<0.05$) and more in women than men (72.7% vs. 27.3%). In this *EGFR*/*KRAS* wild-type lung ADC patient cohort,

HER2 mutation occurs in 9.4% of never smokers (10/106), 8.7% of female (8/92) and 2.7% of male (3/112).

BRAF mutations have been reported in 2% to 4.9% of white patients and less than 1% of Asian patients with NSCLC [6,14,19,25,31,32]. In this selected patient cohort, the frequency of *BRAF* mutation only reached to 2% (4/204). Therefore, it seems that *BRAF* mutation is more common in the white NSCLC patients than in the Asian. Due to the low frequency, there is no agreement so far regarding *BRAF* mutations associated clinicopathological characteristics including sex and smoking history. In a study with the largest series of patients with *BRAF* mutant lung cancers, most patients were identified to be heavy smokers [33]. However, in this study, all four *BRAF* mutated patients are female and three of them are never-smokers.

In summary, *HER2* and *BRAF* mutations identify a distinct subset of lung ADCs. In Chinese *EGFR*/*KRAS* wild-type lung ADCs, 7.4% of the patients and 12.3% of never smokers carry *HER2* or *BRAF* mutations. Given the high prevalence of lung cancer and the availability of targeted therapy, Chinese lung ADC patients without *EGFR* and *KRAS* mutations are recommended for *HER2* and *BRAF* mutations detection, especially for those never smokers.

Author Contributions

Conceived and designed the experiments: LS JY. Performed the experiments: LS TQ YL LG BZ BW WL LL. Analyzed the data: LS JY. Contributed reagents/materials/analysis tools: LS BZ BW. Wrote the paper: LS JY.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011; 61: 69–90. doi: 10.3322/caac.20107 PMID: 21296855
2. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014; 64: 9–29. doi: 10.3322/caac.21208 PMID: 24399786
3. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med. 2004; 350: 2129–2139. PMID: 15118073
4. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science. 2004; 304: 1497–1500. PMID: 15118125
5. Kris MG, Johnson BE, Berry LD, Kwiatkowski DJ, Iafrate AJ, Wistuba II, et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. JAMA. 2014; 311: 1998–2006. doi: 10.1001/jama.2014.3741 PMID: 24846037
6. Chen D, Zhang LQ, Huang JF, Liu K, Chuai ZR, Yang Z, et al. BRAF mutations in patients with non-small cell lung cancer: a systematic review and meta-analysis. PLoS One. 2014; 9: e101354. doi: 10.1371/journal.pone.0101354 PMID: 24979348
7. Serizawa M, Koh Y, Kenmotsu H, Isaka M, Murakami H, Akamatsu H, et al. Assessment of mutational profile of Japanese lung adenocarcinoma patients by multitarget assays: a prospective, single-institute study. Cancer. 2014; 120: 1471–1481. doi: 10.1002/cncr.28604 PMID: 24700479
8. Peters S, Michielin O, Zimmermann S. Dramatic response induced by vemurafenib in a BRAF V600E-mutated lung adenocarcinoma. J Clin Oncol. 2013; 31: e341–344. doi: 10.1200/JCO.2012.47.6143 PMID: 23733758
9. Robinson SD, O'Shaughnessy JA, Cowey CL, Konduri K. BRAF V600E-mutated lung adenocarcinoma with metastases to the brain responding to treatment with vemurafenib. Lung Cancer. 2014; 85: 326–330. doi: 10.1016/j.lungcan.2014.05.009 PMID: 24888229
10. Planchard D, Mazieres J, Riely GJ, Rudin CM, Barlesi F, Quoix EA, et al. Interim results of phase II study BRF113928 of dabrafenib in BRAF V600E mutation–positive non-small cell lung cancer (NSCLC) patients. J Clin Oncol 2013; 31 (suppl; abstr 8009).
11. Mazieres J, Peters S, Lepage B, Cortot AB, Barlesi F, Beau-Faller M, et al. Lung cancer that harbors an HER2 mutation: epidemiologic characteristics and therapeutic perspectives. J Clin Oncol. 2013; 31: 1997–2003. doi: 10.1200/JCO.2012.45.6095 PMID: 23610105

12. De Greve 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: 123–127. doi: 10.1016/j.lungcan.2012.01.008 PMID: 22325357
13. Arcila ME, Chaft JE, Nafa K, Roy-Chowdhuri S, Lau C, Zaidinski M, et al. Prevalence, clinicopathologic associations, and molecular spectrum of ERBB2 (HER2) tyrosine kinase mutations in lung adenocarcinomas. Clin Cancer Res. 2012; 18: 4910–4918. doi: 10.1158/1078-0432.CCR-12-0912 PMID: 22761469
14. Cardarella S, Ogino A, Nishino M, Butaney M, Shen J, Lydon C, et al. Clinical, pathologic, and biologic features associated with BRAF mutations in non-small cell lung cancer. Clin Cancer Res. 2013; 19: 4532–4540. doi: 10.1158/1078-0432.CCR-13-0657 PMID: 23833300
15. Villaruz LC, Socinski MA, Abberbock S, Berry LD, Johnson BE, Kwiatkowski DJ, et al. Clinicopathologic features and outcomes of patients with lung adenocarcinomas harboring BRAF mutations in the Lung Cancer Mutation Consortium. Cancer. 2014.
16. Sasaki H, Shitara M, Yokota K, Okuda K, Hikosaka Y, Moriyama S, et al. Braf and erbB2 mutations correlate with smoking status in lung cancer patients. Exp Ther Med. 2012; 3: 771–775. PMID: 22969966
17. Sun Y, Ren Y, Fang Z, Li C, Fang R, Gao B, et al. Lung adenocarcinoma from East Asian never-smokers is a disease largely defined by targetable oncogenic mutant kinases. J Clin Oncol. 2010; 28: 4616–4620. doi: 10.1200/JCO.2010.29.6038 PMID: 20855837
18. Li C, Fang R, Sun Y, Han X, Li F, Gao B, et al. Spectrum of oncogenic driver mutations in lung adenocarcinomas from East Asian never smokers. PLoS One. 2011; 6: e28204. doi: 10.1371/journal.pone.0028204 PMID: 22140546
19. An SJ, Chen ZH, Su J, Zhang XC, Zhong WZ, Yang JJ, et al. Identification of enriched driver gene alterations in subgroups of non-small cell lung cancer patients based on histology and smoking status. PLoS One. 2012; 7: e40109. doi: 10.1371/journal.pone.0040109 PMID: 22768234
20. Zhou W, Christiani DC. East meets West: ethnic differences in epidemiology and clinical behaviors of lung cancer between East Asians and Caucasians. Chin J Cancer. 2011; 30: 287–292. PMID: 21527061
21. Perera SA, Li D, Shimamura T, Raso MG, Ji H, Chen L, et al. HER2YVMA 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: 474–479. doi: 10.1073/pnas.0808930106 PMID: 19122144
22. 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. PMID: 16843263
23. 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: 6487–6491. PMID: 16818618
24. Landi L, Cappuzzo F. HER2 and lung cancer. Expert Rev Anticancer Ther. 2013; 13: 1219–1228. doi: 10.1586/14737140.2013.846830 PMID: 24134423
25. Li C, Hao L, Li Y, Wang S, Chen H, Zhang L, et al. Prognostic value analysis of mutational and clinicopathological factors in non-small cell lung cancer. PLoS One. 2014; 9: e107276. doi: 10.1371/journal.pone.0107276 PMID: 25198510
26. Feng S, Ling H, Guo H, Tong L, Hu G, Liao L, et al. Infrequent ERBB2 mutations in Chinese patients with non-small cell lung cancer. J Thorac Dis. 2014; 6: 503–506. doi: 10.3978/j.issn.2072-1439.2014.03.20 PMID: 24822110
27. Hu H, Pan Y, Li Y, Wang L, Wang R, Zhang Y, et al. Oncogenic mutations are associated with histological subtypes but do not have an independent prognostic value in lung adenocarcinoma. Onco Targets Ther. 2014; 7: 1423–1437. doi: 10.2147/OTT.S58900 PMID: 25152623
28. Agus DB, Akita RW, Fox WD, Lewis GD, Higgins B, Pisacane PI, et al. Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. Cancer Cell. 2002; 2: 127–137. PMID: 12204533
29. 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: 1642–1646. PMID: 15753357
30. 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. 2011; 74: 139–144. doi: 10.1016/j.lungcan.2011.01.014 PMID: 21353324
31. Brustugun OT, Khattak AM, Tromborg AK, Beigi M, Beiske K, Lund-Iversen M, et al. BRAF-mutations in non-small cell lung cancer. Lung Cancer. 2014; 84: 36–38. doi: 10.1016/j.lungcan.2014.01.023 PMID: 24552757

32. Marchetti A, Felicioni L, Malatesta S, Grazia Sciarrotta M, Guetti L, Chella A, et al. Clinical features and outcome of patients with non-small-cell lung cancer harboring BRAF mutations. J Clin Oncol. 2011; 29: 3574–3579. doi: 10.1200/JCO.2011.35.9638 PMID: 21825258

33. Litvak AM, Paik PK, Woo KM, Sima CS, Hellmann MD, Arcila ME, et al. Clinical characteristics and course of 63 patients with BRAF mutant lung cancers. J Thorac Oncol. 2014; 9: 1669–1674. doi: 10.1097/JTO.0000000000000344 PMID: 25436800