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ORIGINAL ARTICLE

# Mutational landscape and characteristics of ERBB2 in non-small cell lung cancer

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**Keywords**
Co-mutation; ERBB2 mutation; non-small cell lung cancer; oncogenic function; prognosis.

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## Abstract

**Background:** Tyrosine kinase domain (TKD) mutation and particularly exon 20 insertion mutations of *ERBB2* have been extensively reported in non-small cell lung cancer (NSCLC). Due to the increased accessibility of next-generation sequencing, more *ERBB2* mutations within the non-TKD can be detected in clinical practice. Nevertheless, the clinical significance of non-TKD mutations remains unknown. Hence, this study was designed to comprehensively outline the landscape and characteristics of *ERBB2* mutations in NSCLC.

**Methods:** A total of 1934 patients with NSCLC from cBioPortal were included in the study. An *ERBB2* mutation cohort was identified, while subsequent analyses revealed clinical and genomic characteristics.

**Results:** The frequency of *ERBB2* mutation was 4.5%, and it was determined to be more likely to occur in never-smokers. *ERBB2* mutations occurring in the non-TKD accounted for 57.5% of *ERBB2* mutations. In the non-TKD, missense mutation was the most recurrent mutation type, and S310F was the most recurrent mutation variant. *ERBB2* mutations within non-TKD also had a strong oncogenic ability where up to 37.5% of *ERBB2* oncogenic mutations were within non-TKD. The co-mutation of *EGFR* or *KRAS* was higher in the non-TKD mutation compared to the TKD mutation. Shorter overall survival was observed in *ERBB2*-mutant patients compared with *ERBB2* wild-type patients. There was no significant difference in overall survival between patients with non-TKD mutations and TKD mutations.

**Conclusions:** The present study showed that a considerable portion of non-TKD mutations were oncogenic. *ERBB2* mutation was a poor prognostic factor. The non-TKD mutation might also be used as a therapeutic target in ERBB2-directed target therapy.

**Key points**

• **Significant findings of the study**

*ERBB2* mutations were more abundant within a nontyrosine domain than those within the tyrosine domain. Up to 37.5% of *ERBB2* oncogenic mutations were within the nontyrosine domain. *ERBB2* mutation was a poor prognostic factor.

• **What this study adds**

The frequency of *EGFR* or *KRAS* co-mutations were significantly higher in *ERBB2* mutations within the nontyrosine kinase domain compared to *ERBB2* mutations within the tyrosine kinase domain. Nontyrosine domain mutations confer equal overall survival to tyrosine domain mutations.

 

## Introduction

The epidermal growth factor receptor (EGFR) target therapy has been the cornerstone for the precise treatment of NSCLC. Nowadays, the classification of NSCLC is not just built on the histology but is also based on tumor driver mutations. A driver mutation leads to abnormal activation of cellular signaling pathways, thus resulting in abnormal proliferation and survival of cancer cells.[1] Treatments that target driver gene mutations improve the prognosis in patients with NSCLC compared with conventional chemotherapy.[2] A previous study reported that in over 60% of patients with lung adenocarcinomas with detected driver mutations, 9%–14% were rare driver mutations, including erb-b2 receptor tyrosine kinase 2 (*ERBB2*).[3]

ERBB2 has been extensively studied in breast cancer. Its amplification or overexpression was a biomarker of anti-ERBB2 target therapy in breast cancer. Instead, the mutation is predominant in lung cancer, so conventional ERBB2-targeting drugs are not effective against *ERBB2* mutations in lung cancer. Therefore, a thorough analysis of the *ERBB2* mutation spectrum in NSCLC is necessary for the future study of targeted drugs. ERBB2 is composed of an extracellular domain that contains two receptor-L domain and furin-like cysteine-rich domain, a transmembrane domain (TMD), and an intracellular structure that contains a tyrosine kinase domain (TKD) and a carboxyl-terminal tail.[4] *ERBB2* TKD mutations and particularly exon 20 insertion mutations are classical driver mutations that have been extensively reported in NSCLC. However, *ERBB2* non-TKD mutation, such as V659E and G660D mutations within the TMD, can also act as driver mutations in NSCLC.[5] It has been reported that ERBB2 V659E has shown sensitivity to afatinib and lapatinib in in vitro models.[6,7] In addition, Pahuja *et al.* found multiple oncogenic mutations in the TMD and the juxtamembrane domain in human tumors.[8] They reported that small molecule inhibitors and ERBB2 inhibiting antibodies could efficiently inhibit non-TKD oncogenic mutations. Some recurring extracellular domain mutations of *ERBB2,* such as S310F, are also potently oncogenic but can be inhibited by treatment with small-molecule inhibitors of ERBB2.[9] All these preclinical studies indicated that the non-TKD mutations could be used as candidates for targeted anti-ERBB2 therapy.

Thanks to easier accessibility to next-generation sequencing, it is possible to detect more *ERBB2* mutations that occur within the non-TKD in clinical practice; yet, the clinical significance remains unknown in most of these mutations. Hence, this study was designed to comprehensively outline the landscape and characteristics of *ERBB2* mutations in NSCLC.

## Methods

### Patient cohorts

A total of 5222 patients with NSCLC pooled from The Cancer Genome Atlas cohort and other available studies[10–15] via a public database cBioPortal for Cancer Genomics (https://www.cbioportal.org/), were initially screened.[16,17] Briefly, 2725 duplicated patients and 563 patients without ERBB2 sequencing were excluded. Finally, 1934 patients were included in the analysis.

### Mutation analyses

The next-generation sequencing was applied in the present study.[10–15] The mutation domain was defined as the region where *ERBB2* mutation occurs. Mutation domain was referred to the Pfam database (http://pfam.xfam.org/), including receptor-L domain (amino acid position: 52–173 and 366–486), furin-like cysteine-rich domain (183–343), growth factor receptor domain IV (510–643), transmembrane domain (654–675), and tyrosine kinase domain (TKD) (720–976). Nontyrosine kinase domain (non-TKD) was defined as *ERBB2* domains mentioned above, except for the TKD. The oncogenic function of mutation was first referred to the OncoKB (https://oncokb.org/), a

**Table 1** Clinical characteristics of patients included in the study

| Variables | *ERBB2* mutation *n* = 84 | *ERBB2* wild-type *n* = 1850 | *P*-value |
|---|---|---|---|
| Age, mean (SD) | 65.5 (9.1) | 66.9 (8.6) | 0.159 |
| Sex (%) | | | 0.14 |
| Female | 47 (60.3) | 847 (51.7) | |
| Male | 31 (39.7) | 791 (48.3) | |
| Unknown | 6 | 212 | |
| Stage (%) | | | 0.937 |
| I | 22 (30.6) | 417 (28.3) | |
| II | 8 (11.1) | 188 (12.7) | |
| III | 12 (16.7) | 226 (15.3) | |
| IV | 30 (41.7) | 644 (43.7) | |
| Unknown | 12 | 375 | |
| Pathology (%) | | | 0.062 |
| LUAD | 65 (79.3) | 1554 (86.2) | |
| LUSC | 17 (20.7) | 242 (13.4) | |
| LUNE | 0 (0.0) | 6 (0.3) | |
| NSCLC[a] | 2 | 48 | |
| Smoker (%) | | | 0.002 |
| Yes | 43 (62.3) | 1119 (78.5) | |
| No | 26 (37.7) | 307 (21.5) | |
| Unknown | 15 | 424 | |

[a] Specific pathological type was unknown. LAUD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; LUNE, lung neuroendocrine carcinoma; NSCLC, non-small cell lung cancer.

  

precision oncology knowledge base containing information on the biological effects and treatment implications of specific cancer gene alterations.[18] Mutations with unknown oncogenic function in the OncoKB, including missense mutation and splice site mutation, were analyzed using the Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and Human Splicing Finder (http://www.umd.be/HSF/), respectively, to predict whether a given mutation had an impact on the ERBB2 protein. The oncogenic function was defined as the ability to induce tumor of specific *ERBB2* mutations, catalogued as oncogenic, benign, and unknown function. *ERBB2* synonymous mutations were generally excluded from the *ERBB2* mutation cohort, but synonymous mutations in splice sites were included due to their potential impact on alternative splicing. Splice site was defined as a region near the intron/exon junction or two base pairs into an intron adjacent to the intron/exon junction, referring to Sequence Ontology (http://www.sequenceontology.org/).

### Clinical characteristics

Age at diagnosis, sex, smoking history, tumor pathology, and stage was summarized after identifying patients with *ERBB2* mutation. Overall survival (OS) was defined as the time from initial diagnosis until death. Survival analysis was performed between *ERBB2*-mutant patients and *ERBB2* wild-type patients.

### Statistical analysis

Measurement data were tested using Student's *t*-test. Categorical data were analyzed using the chi-square test or Fisher's exact test, and the odds ratio or risk ratio was assessed for the association. The Kaplan-Meier method was used to estimate the event-time distribution, and the log-rank test was used to compare OS between *ERBB2*-mutant patients and *ERBB2* wild-type patients. Multivariate survival analysis was performed using the Cox regression model. To balance confounding factors, *ERBB2*-mutant patients with survival data were matched to *ERBB2* wild-type patients at a ratio of 1:3 using the propensity score matching method. Matched factors included age at diagnosis, sex, smoking history, pathology, stage, and common oncogenes (*EGFR* and *KRAS*). Propensity score matching was performed using R software (version 3.6.1) with matchit package. All statistical tests were two-sided, and the *P*-value < 0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS, version 23.0 (Armonk, NY).

## Results

### Prevalence and clinical characteristics

As shown in Table 1, a total of 87 *ERBB2* mutations (4.5%, 87/1934) were found, and three patients carried double *ERBB2* mutations. Exon 20 insertion mutations accounted

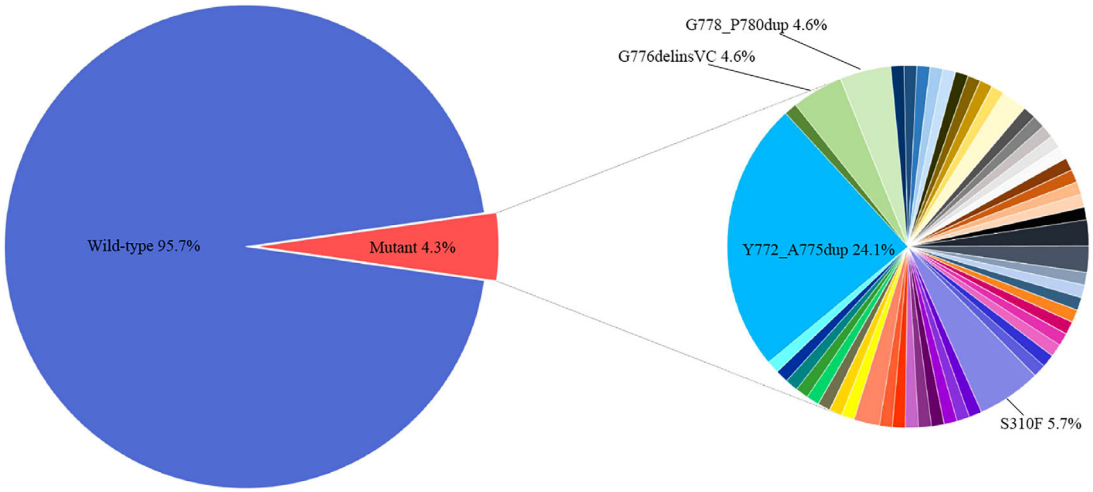


**Figure 1** Mutational landscape of *ERBB2* in 1934 NSCLC patients. Wild-type, R47H, V94I, P122L, G152V, K200N, G222C, D277Y, G292C, A293P, S310F, N302K, V308M, Q329L, S335C, R340P, Q396K, S418T, L651V, V659E, I661V, Q680H, V697L, Q711H, G727A, L755A, L755P, V777M, Y772_A775dup, G776delinsAVGC, G776delinsVC, G778_P780dup, R840W, W906*, Q943*, G1015E, E1021Q, G1057V, G1188W, P1233S, A1232Gfs*45, ERBB2-CTTN, ERBB2-PPP1R1B, ERBB2-TCAP, SHC1-ERBB2, CASC3-ERBB2, ST14-ERBB2, L215=, P300=, X192_splice, X254_splice, X408_splice, X1054_splice

 

for 34.4% (30/87) of ERBB2 mutations; all mutation variants are summarized in Fig 1. A total of 53 *ERBB2* mutation variants were defined in 84 patients. The most recurrent mutation variant was Y772_A775dup (25.0%, 21/84), followed by S310F (6.0%, 5/84), G776delinsVC (4.8%, 4/84), and G778_P780dup (4.8%, 4/84). All other mutations occurred in two or fewer patients, with frequency ranging from 1.2% to 2.4%. *ERBB2* mutation was associated with smoking history, but not with age, sex, stage, and pathology (never-smokers vs. smokers, odds ratio = 2.2, 95% confidence interval [CI], 1.3–3.6; $P = 0.002$).

## Mutational characteristics

A total of 42.5% (37/87) and 57.5% (50/87) of *ERBB2* mutations occurred within the TKD and the non-TKD, respectively. Within the non-TKD, mutation rate was ranked by furin-like cysteine-rich region (17.2%, 15/87), splice site (10.3%, 9/87), receptor-L domain (5.7%, 5/87), and TMD (4.6%, 4/87) (shown in Fig 2a). Missense mutation (43.7%, 38/87) was the most frequent mutation type, followed by in-frame insertion, splice site mutation, rearrangement, nonsense mutation, and frameshift insertion (34.5%, 10.3%, 8.0%, 2.3%, and 1.1%, respectively). All splice site mutations were predicted to have an impact on alternative splicing except for X192_splice mutation. *ERBB2* rearrangements were discovered in seven patients, and five were concurrent with *ERBB2* copy number amplification.

Human Splicing Finder and Polyphen-2 were used to predict the biological effects of *ERBB2* variants. Moreover, 47.2% (25/53) of mutation variants were oncogenic (Table 2). Oncogenic function was significantly stronger in TKD mutation compared with non-TKD mutation (risk ratio = 1.9, 95% CI, 1.3–2.6; $P = 0.03$), although up to 37.5% of *ERBB2* oncogenic mutations were within the non-TKD.

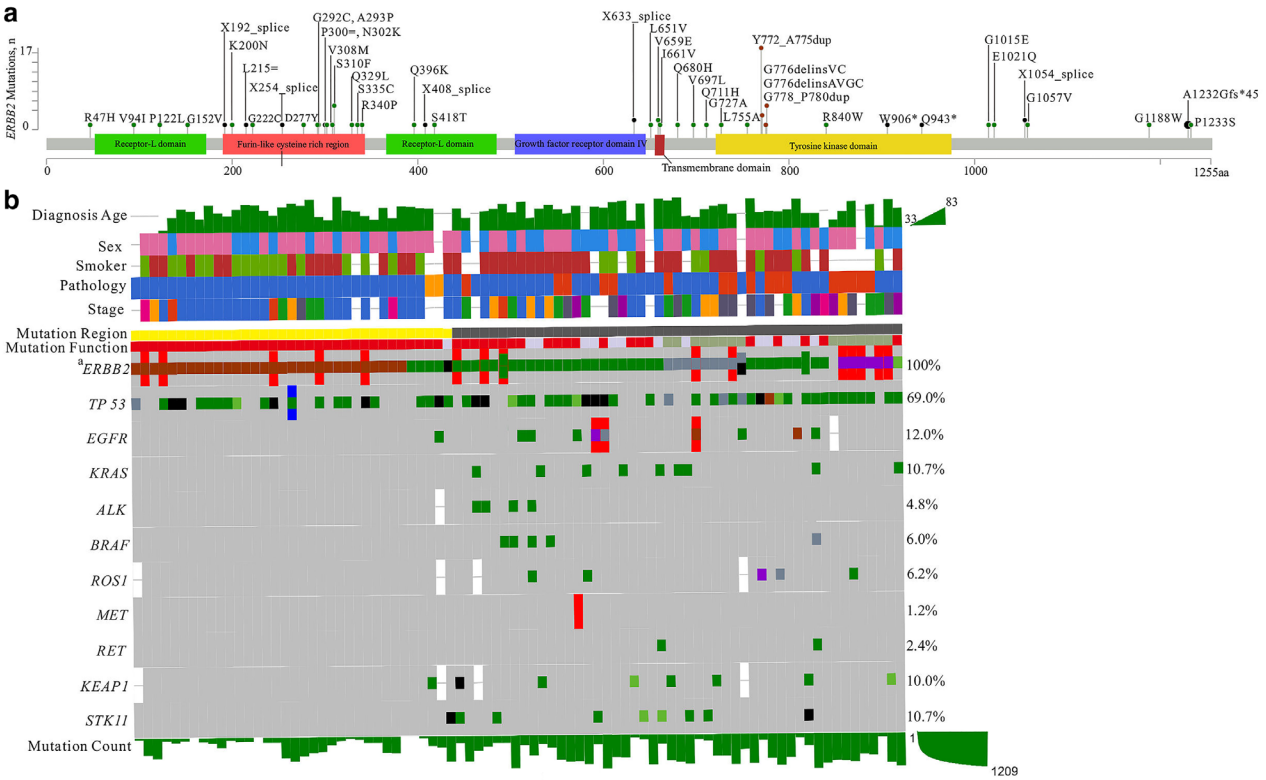


**Figure 2** Clinical and molecular characteristics in *ERBB2* mutations. (**a**) An overview of the *ERBB2* mutation region; mutation region is referred to as the Pfam database. (**b**) Concurrent mutations of oncogenes and tumor suppressor genes in patients with *ERBB2* mutations. [a]Three patients carried double *ERBB2* mutations illustrated by longer bars based on the mutation types: S310F and D277Y; G727A and Q711H; X254_splice and W906*. No data, Female, Male No data, Yes, No No Data, LUAD, LUSC, NSCLC, IA, IB, IIA, IIB, IIIA, IIIB, IV No data, TKD, non-TKD, Oncogenic, Benign, Unknow. Mutation type: Inframe mutation, Missense mutation, Frame shift mutation, No sense mutation, Splice site, Rearrangement, Amplification, Deep deletion, No alterations, Not profiled. Abbreviations: LAUD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; NSCLC, non-small cell lung cancer. TKD, tyrosine kinase domain; non-TKD, nontyrosine kinase domain.

  

**Table 2** Oncogenic function of *ERBB2* mutation variants identified in the present study

| Variants | Mutation region | Exon | Oncogenic function | Source |
|---|---|---|---|---|
| R47H | - | 2 | Benign | Polyphen-2 |
| V94I | Receptor-L domain | 3 | Benign | Polyphen-2 |
| P122L | Receptor-L domain | 3 | Benign | Polyphen-2 |
| G152V | Receptor-L domain | 4 | Oncogenic | Polyphen-2 |
| K200N | Furin-like cysteine rich region | 5 | Benign | Polyphen-2 |
| G222C | Furin-like cysteine rich region | 6 | Oncogenic | OncoKB |
| D277Y | Furin-like cysteine rich region | 7 | Oncogenic | OncoKB |
| G292C | Furin-like cysteine rich region | 7 | Oncogenic | OncoKB |
| A293P | Furin-like cysteine rich region | 7 | Oncogenic | OncoKB |
| N302K | Furin-like cysteine rich region | 8 | Oncogenic | Polyphen-2 |
| V308M | Furin-like cysteine rich region | 8 | Oncogenic | Polyphen-2 |
| S310F | Furin-like cysteine rich region | 8 | Oncogenic | OncoKB |
| Q329L | Furin-like cysteine rich region | 8 | Oncogenic | Polyphen-2 |
| S335C | Furin-like cysteine rich region | 8 | Oncogenic | OncoKB |
| R340P | Furin-like cysteine rich region | 8 | Benign | Polyphen-2 |
| Q396K | Receptor-L domain | 10 | Benign | Polyphen-2 |
| S418T | Receptor-L domain | 11 | Benign | Polyphen-2 |
| L651V | Transmembrane domain | 17 | Oncogenic | OncoKB |
| V659E | Transmembrane domain | 17 | Oncogenic | OncoKB |
| I661V | Transmembrane domain | 17 | Benign | Polyphen-2 |
| Q680H | - | 17 | Benign | Polyphen-2 |
| V697L | - | 18 | Oncogenic | OncoKB |
| Q711H | - | 18 | Benign | Polyphen-2 |
| G727A | Tyrosine kinase domain | 18 | Oncogenic | Polyphen-2 |
| L755A | Tyrosine kinase domain | 19 | Oncogenic | OncoKB |
| L755P | Tyrosine kinase domain | 19 | Oncogenic | OncoKB |
| Y772_A775dup | Tyrosine kinase domain | 20 | Oncogenic | OncoKB |
| G776delinsAVGC | Tyrosine kinase domain | 20 | Oncogenic | OncoKB |
| G776delinsVC | Tyrosine kinase domain | 20 | Oncogenic | OncoKB |
| G778_P780dup | Tyrosine kinase domain | 20 | Oncogenic | OncoKB |
| V777M | Tyrosine kinase domain | 20 | Oncogenic | OncoKB |
| R840W | Tyrosine kinase domain | 21 | Oncogenic | Polyphen-2 |
| W906* | Tyrosine kinase domain | 22 | Unknown | - |
| Q943* | Tyrosine kinase domain | 23 | Unknown | - |
| E1021Q | - | 25 | Oncogenic | Polyphen-2 |
| G1015E | - | 25 | Benign | Polyphen-2 |
| G1057V | - | 26 | Benign | Polyphen-2 |
| G1188W | - | 27 | Oncogenic | Polyphen-2 |
| A1232Gfs*45 | - | 27 | Oncogenic | OncoKB |
| P1233S | - | 27 | Benign | Polyphen-2 |
| ERBB2-CTTN | - | - | Unknown | - |
| ERBB2-PPP1R1B | - | - | Unknown | - |
| ERBB2-TCAP | - | - | Unknown | - |
| CASC3-ERBB2 | - | - | Unknown | - |
| SHC1-ERBB2 | - | - | Unknown | - |
| ST14-ERBB2 | - | - | Unknown | - |
| L215= | Splice site | 6 | Unknown, affecting splicing | Human Splicing Finder |
| P300= | Splice site | 7 | Unknown, affecting splicing | Human Splicing Finder |
| X192_splice | Splice site | 5 | Benign | Human Splicing Finder |
| X254_splice | Splice site | 7 | Unknown, Affecting splicing | Human Splicing Finder |
| X408_splice | Splice site | 11 | Unknown, affecting splicing | Human Splicing Finder |
| X633_splice | Splice site | 16 | Unknown, affecting splicing | Human Splicing Finder |
| X1054_splice | Splice site | 26 | Unknown, affecting splicing | Human Splicing Finder |

 

## Concurrent mutations of cancer gene and tumor suppressor gene

Among *ERBB2*-mutant patients, 17 cancer genes or tumor suppressor genes were observed with a co-mutation rate not less than 10%, including *TP53*, *EGFR*, *KRAS*, *STK11*, and *KEAP1*. Concurrent mutations of the aforementioned five genes and other well-known oncogenes in NSCLC (*ALK*, *BRAF*, *MET*, *ROS1*, and *RET*) were analyzed in *ERBB2*-mutant patients.

*TP53* was the most frequently co-mutated gene in *ERBB2* mutations (69.0%) (Fig 2b). No concurrent oncogene mutations were found in the TKD mutation cohort except for one *EGFR* co-mutation. Further, a comparison of concurrent oncogene mutations between TKD mutation and non-TKD mutation was performed. The frequency of *EGFR* and *KRAS* mutations was higher in non-TKD mutation (*EGFR*: 19.1% vs. 2.9%, $P$ = 0.038; *KRAS*: 19.1% vs. 0.0%, $P$ = 0.017), but no difference was observed for *ALK*, *BRAF*, *MET*, *ROS1*, and *RET*. Tumor suppressor genes *KEAP1* and *STK11* concurrently mutated with 10.0% and 10.7% of *ERBB2* mutations, respectively, similar to *ERBB2* wild-type patients (*ERBB2* wild-type: *KEAP1*: 16.3%, $P$ = 0.134; *STK11*: 15.1%, $P$ = 0.272). Likewise, a comparison of *KEAP1* or *STK11* comutation between TKD mutation and non-TKD mutation was performed. Although the frequency of *STK11* and *KEAP1* mutations was higher in non-TKD mutation cohort, no statistical difference was observed for both of them (non-TKD mutation vs. TKD mutation: *STK11*: 14.9% vs. 2.9%, $P$ = 0.094; *KEAP1*: 12.8% vs. 2.9%, $P$ = 0.164).

## Prognostic values of *ERBB2* mutation in patients with NSCLC

Overall survival data were available for 31 *ERBB2*-mutant patients and 478 *ERBB2* wild-type patients (stage I–IV). The median OS was 28.4 months (95% CI, 24.1–32.7) and 50.3 months (95% CI, 41.1–59.5) for *ERBB2*-mutant patients and *ERBB2* wild-type patients, respectively

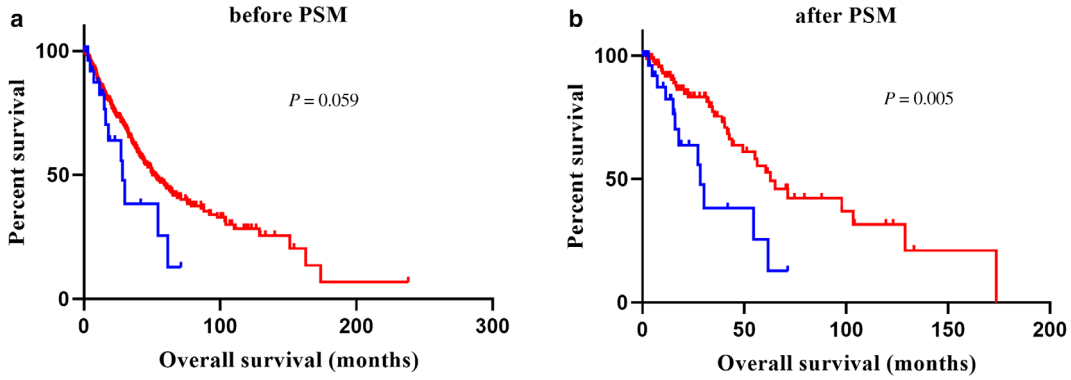


**Figure 3** Overall survival in patients from *ERBB2* mutation and wild-type cohorts. (**a**) Survival curve before PSM. ERBB2 mutation, median = 28.4 months, ERBB2 wild-type, median = 50.3 months. P = 0.059. (**b**) Survival curve after PSM. ERBB2 mutation, median = 28.4 months, ERBB2 wild-type, median = 62.8 months. $P$ = 0.005. Abbreviations: PSM, propensity score matching.

**Table 3** Multivariate Cox regression analysis of overall survival in patients with NSCLC

| | Before PSM | | | | After PSM | | | |
|---|---|---|---|---|---|---|---|---|
| | Univariate | | Multivariate | | Univariate | | Multivariate | |
| Variables | HR (95% CI) | *P*-value | HR (95% CI) | *P*-value | HR (95% CI) | *P*-value | HR (95% CI) | *P*-value |
| Age (<65 years vs. ≥65 years) | 0.80 (0.59–1.07) | 0.135 | | | 0.85 (0.47–1.54) | 0.587 | | |
| Gender (male vs. female) | 1.14 (0.86–1.51) | 0.363 | | | 0.84 (0.45–1.55) | 0.569 | | |
| Pathology (LUSC vs. LUAD) | 0.93 (0.81–1.07) | 0.288 | | | 1.00 (0.51–1.96) | 0.996 | | |
| Smoker (no vs. yes) | 0.87 (0.27–2.74) | 0.806 | | | 0.76 (0.17–3.35) | 0.719 | | |
| Stage (IIIB-IV vs. IA–IIIA) | 1.88 (1.21–2.93) | 0.005 | 1.88 (1.21–2.93) | 0.005 | 3.43 (1.04–11.30) | 0.043 | 3.54 (1.07–11.71) | 0.038 |
| *ERBB2* (mutation vs. wild-type) | 1.75 (0.97–3.14) | 0.063 | - | 0.098 | 2.69 (1.35–5.35) | 0.005 | 2.54 (1.25–5.18) | 0.010 |

NSCLC, non-small cell lung cancer; LAUD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PSM, propensity score matching; HR, hazard ratio.

  

**Table 4** Clinical characteristics of patients included in the survival analysis

| Variable | *ERBB2* mutation *n* = 31 | *ERBB2* wild-type Before PSM *n* = 478 | *P*-value | *ERBB2* wild-type After PSM *n* = 93 | *P*-value |
|---|---|---|---|---|---|
| Age (%) | | | 0.234 | | 0.678 |
| <65 years | 15 (48.4) | 180 (37.7) | | 49 (52.7) | |
| ≥65 years | 16 (51.6) | 298 (62.3) | | 44 (47.3) | |
| Gender (%) | | | 0.086 | | 0.852 |
| Female | 18 (60.0) | 210 (43.9) | | 54 (58.1) | |
| Male | 12 (40.0) | 268 (56.1) | | 39 (41.9) | |
| Unknown | 1 | 0 | | 0 | |
| Stage[a](%) | | | 0.138 | | 0.663 |
| IA | 10 (33.3) | 105 (22.0) | | 35 (37.6) | |
| IB | 10 (33.3) | 150 (31.4) | | 31 (33.3) | |
| IIA | 1 (3.3) | 40 (8.4) | | 2 (2.2) | |
| IIB | 5 (16.7) | 73 (15.3) | | 12 (12.9) | |
| IIIA | 3 (10.0) | 64 (13.4) | | 11 (11.8) | |
| IIIB | 0 (0.0) | 21 (4.4) | | 0 (0.0) | |
| IV | 1 (3.3) | 15 (3.1) | | 2 (2.2) | |
| Unknown | 1 | 0 | | 0 | |
| Pathology (%) | | | 0.534 | | 0.533 |
| LUSC | 15 (48.4) | 204 (42.7) | | 51 (54.8) | |
| LUAD | 16 (51.6) | 274 (57.3) | | 42 (45.2) | |
| Smoker (%) | | | 0.013 | | 0.489 |
| Yes | 17 (81.0) | 280 (95.9) | | 54 (87.1) | |
| No | 4 (19.0) | 12 (4.1) | | 8 (12.9) | |
| Unknown | 10 | 31 | | 31 | |
| *KRAS* wild-type (%) | 26 (83.9) | 390 (79.6) | 0.75 | 74 (79.6) | 0.793 |
| *EGFR* wild-type (%) | 31 (100.0) | 424 (88.7) | 0.093 | 93 (100.0) | - |

[a] Difference between stage was tested by Mann-Whitney Wilcoxon test. LAUD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma. PSM, propensity score matching.

(Fig 3, $P = 0.059$). The multivariate survival analysis showed that OS was significantly associated with stage ($P = 0.005$), but not with *ERBB2* mutation ($P = 0.098$) (Table 3). Considering that confounding factors and sample size varied between two cohorts, a propensity score matching method was performed to match *ERBB2*-mutant patients and *ERBB2* wild-type patients. Age, sex, smoking, pathology, stage, and matched oncogenes were balanced between two cohorts (Table 4). The multivariate survival analysis after propensity score matching showed that both *ERBB2* mutation and advanced stage were poor prognostic factors (*ERBB2* mutation vs. wild-type: hazard ratio = 2.54, 95% CI, 1.25–5.18, $P = 0.010$; IIIB–IV vs. IA–IIIA: hazard ratio = 3.54, 95% CI, 1.07–11.71, $P = 0.038$) (Table 3).

Next, we compared the OS between patients with TKD and non-TKD mutations. Subgroup analysis showed that OS was longer in patients with non-TKD mutations; the observed difference was not statistically significant (OS: non-TKD vs. TKD, 30.1 months vs. 15.0 months; $P = 0.475$). After excluding patients with benign *ERBB2* mutation, the OS was longer in patients with non-TKD mutations (OS: non-TKD vs. TKD, 28.4 months vs. 15.0 months; $P = 0.177$).

## Discussion

In this study, the prevalence of *ERBB2* mutation was 4.5%, which was relatively high in comparison to previous studies.[19–22] This discrepancy is reasonable if we take into account that these studies were limited to the detection of TKD mutations, particularly exon 20 mutation in *ERBB2*. Our results showed that a considerable number of non-TKD mutations may have significant oncogenic capacity.

In the present study, Y772_A775dup, G776delinsVC, G778_P780dup, and S310F were the most recurrent *ERBB2* mutations in NSCLC, which is consistent with a previous report.[22] In colorectal cancer, the most recurrent *ERBB2* mutations are I655V, V842I, and R678Q.[23] The difference indicated different preferred mutant variants among different types of cancer with the same oncogene. In the present study, smoking status was the only factor associated with *ERBB2* mutation, which was not in line with previous studies reporting that *ERBB2* mutation was associated with

 

lung adenocarcinoma, female sex, and never-smokers.[24,25] Previous studies have mostly focused on TKD mutations, particularly exon 20-insertion mutation, barely detecting *ERBB2* non-TKD mutations. Excluding two mutations that occurred in NSCLC (specific histology not defined), all TKD mutations identified in the present study occurred in lung adenocarcinoma. Hence, the results of the present study do not contradict previous studies but provide more comprehensive information regarding mutations throughout *ERBB2*. Smoking status is usually related to sex. Two previous studies showed that sex was not associated with *EGFR* mutations after balancing the smoking status of participants, considering that sex-related smoking was a confounding factor.[26,27]

As shown in the present study, the oncogenic function varied among mutations in different *ERBB2* domains. Most of the oncogenic mutations occurred in TKD. The extracellular domain of *ERBB2* included four parts: two receptor-L domains, a furin-like cysteine-rich domain, and a growth factor receptor domain IV. The receptor-l domain is related to leucine-rich segments that participate in ligand binding in EGFR, but ligand binding to ERBB2 has not been discovered.[4] This might explain why 80% (4/5) of mutations in the receptor-L domain found in the present study were benign. Furin-like cysteine-rich domain and growth factor receptor domain IV contain numerous cysteine residues that participate in disulfide bond formation, and in homodimer and heterodimer formation with other ErbB family members.[4] A high frequency of oncogenic mutations (86.7%) was observed in the furin-like cysteine-rich domain. A total of 15n mutations were found in furin-like cysteine-rich domain, including a recurrent mutation S310F ($n$ = 5). Two oncogenic mechanisms were found in the extracellular domain mutation. The oncogenic mechanism of S310F implied an elevation of C-terminal phosphorylation,[9] and then again, cysteine substitution in the furin-like cysteine-rich domain was identified as another oncogenic mechanism mediated by the formation of disulfide-linked dimers.[9] The TMD not only serves as a membrane anchor but also has a significant role in receptor dimerization.[28] In the present study, we identified three mutation variants in TMD (V659E, L651V, and I661V). V659E and G660D are oncogenic mutations that respond to afatinib treatment.[7] All these results implied that the oncogenic function of a specific mutation depended on the biological function of the mutation domain.

Our findings indicated that concurrent driver mutation was excluded by *ERBB2* TKD mutation. However, a significantly higher frequency of co-mutation with *EGFR* and *KRAS* was observed in non-TKD mutation. This could be explained by the weaker oncogenic function of non-TKD mutation compared with TKD mutation. Similarly, the mutation count was much higher in non-TKD mutation compared with TKD mutation ($P < 0.001$), but it should be carefully interpreted for different gene test methods applied in the present study. *KEAP1* and *STK11* mutations were frequently observed in our patient population (16.0% for *KEAP1* and 14.9% for *STK11*). *KEAP1* mutation is a poor prognostic factor, while *STK11* mutation is a negative predictor of immune checkpoint inhibitors.[29,30] *STK11* regulates cellular metabolism/energy homeostasis, growth, and polarity.[31] Inactivation of *STK11* mediated by mutation is associated with a "cold" tumor immune microenvironment and a decreased density of infiltrating cytotoxic CD8+ T lymphocytes in both genetically engineered murine models and human tumors.[32] Both *KEAP1* and *STK11* mutations rarely coexisted with *ERBB2* TKD mutations. Interestingly, a greater portion of these poor-prognosis and immune-negative genes concurrently mutated in the non-TKD mutation cohort with a higher mutation count, although without statistical significance.

Previous studies have also shown a tendency toward shorter OS in both *ERBB2*-mutant NSCLC and *ERBB2*-mutant colorectal cancer.[23,25] In the present study, the survival of *ERBB2*-mutant patients was significantly shorter compared to *ERBB2* wild-type patients after propensity score matching. This might indicate that *ERBB2* mutation was a poor prognostic factor. Although the previous case report showed that *ERBB2* mutated NSCLC patient could benefit from afatinib treatment,[33] a retrospective study in China suggested that compared with chemotherapy, afatinib was not more beneficial to *ERBB2* mutated NSCLC patients.[34] Also, most of the clinical trials, including afatinib, dacomitinib, and neratinib, focused on *ERBB2* exon 20 insertion mutations and failed with a low objective response rate of 11%–19%.[35–37] Two-phase II studies showed a promising response of poziotinib and pyrotinib in advanced NSCLC with *ERBB2* exon 20 mutation. The objective response rate and median PFS were 50% and 5.1 months for poziotinib, and 55% and 6.2 months for pyrotinib, respectively.[38,39] None of these clinical trials included *ERBB2* mutations that occurred in non-TKD. However, basket trials verified the efficacy of tyrosine kinase inhibitor as well as ado-trastuzumab emtansine on non-TKD mutations in patients with NSCLC.[40,41] This indicated that non-TKD mutation was targetable and could be considered as a target during the management of *ERBB2*-mutant patients. It is necessary to identify mutations that can benefit from such treatment, which should be initiated by defining a subset of oncogenic mutations.

The present study has certain limitations. First, the patients included in the present analysis were from different cohorts. Also, different gene test methods were used, leading to a mutation detection bias. The aim of the present study was mainly to characterize mutations throughout the entire *ERBB2* and emphasize that they might also

  

be important in carcinogenesis and have the potential to be used as therapeutic targets. Second, survival data were only available for a small sample of *ERBB2*-mutant patients. Hence, a larger population of *ERBB2*-mutant patients is needed to validate the prognostic value of *ERBB2* mutation. Finally, the oncogenic function of specific *ERBB2* mutation predicted in this study still needs further validation.

Above all, the present study demonstrated that the non-TKD mutation accounted for over half of *ERBB2* mutations. A considerable portion of non-TKD mutations were oncogenic, while *ERBB2* mutation resulted in a poor prognostic factor. The non-TKD mutation might also be used as a therapeutic target in ERBB2-directed target therapy.

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## Disclosure

The authors declare that they have no competing interests.

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