

ORIGINAL ARTICLE

Predictive value of *K-ras* and *PIK3CA* in non-small cell lung cancer patients treated with EGFR-TKIs: a systemic review and meta-analysis

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ABSTRACT

Objective: A meta-analysis was performed to augment the insufficient data on the impact of mutative *EGFR* downstream phosphatidylinositol-3-kinase (*PI3K*) and mitogen-activated protein kinase (*MAPK*) pathways on the clinical efficiency of epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) treatment of non-small cell lung cancer (NSCLC) patients.

Methods: Network databases were explored in April, 2015. Papers that investigated the clinical outcomes of NSCLC patients treated with EGFR-TKIs according to the status of *K-ras* and/or *PIK3CA* gene mutation were included. A quantitative meta-analysis was conducted using standard statistical methods. Odds ratios (ORs) for objective response rate (ORR) and hazard ratios (HRs) for progression-free survival (PFS) and overall survival (OS) were calculated.

Results: Mutation in *K-ras* significantly predicted poor ORR [OR = 0.22; 95% confidence interval (CI), 0.13-0.35], shorter PFS (HR = 1.56; 95% CI, 1.27-1.92), and shorter OS (HR = 1.59; 95% CI, 1.33-1.91) in NSCLC patients treated with EGFR-TKIs. Mutant *PIK3CA* significantly predicted shorter OS (HR = 1.83; 95% CI, 1.05-3.20), showed poor ORR (OR = 0.70; 95% CI, 0.22-2.18), and shorter PFS (HR = 1.79; 95% CI, 0.91-3.53) in NSCLC patients treated with EGFR-TKIs.

Conclusion: *K-ras* mutation adversely affected the clinical response and survival of NSCLC patients treated with EGFR-TKIs. *PIK3CA* mutation showed similar trends. In addition to EGFR, adding *K-ras* and *PIK3CA* as routine gene biomarkers in clinical genetic analysis is valuable to optimize the effectiveness of EGFR-TKI regimens and identify optimal patients who will benefit from EGFR-TKI treatment.

KEYWORDS

Non-small cell lung cancer (NSCLC); tyrosine kinase inhibitor (TKI); targeted therapy; *K-ras*; *PIK3CA*; meta-analysis

Introduction

Lung cancer remains the leading cause of cancer death in both genders according to the most recent statistics of the American Cancer Society¹. Non-small cell lung cancer (NSCLC) accounts for more than 85% of lung cancers². Most patients present with

advanced NSCLC at the time of diagnosis, and chemotherapy becomes their palliative option. However, the poor improvement in the clinical response and survival outcomes of NSCLC patients who underwent chemotherapy over the last two decades highlights the need for more effective and less toxic treatments³.

Epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) is a small-molecule drug that targets the active adenosine triphosphate binding site of EGFR kinase. Recent studies on patients bearing sensitive EGFR mutation have shown that EGFR-TKIs effectively increase clinical response rate and improve patients' survival compared with standard

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Received March 30, 2015; accepted June 10, 2015.

Available at www.cancerbiomed.org

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chemotherapy, such as cisplatin plus gemcitabine or carboplatin plus paclitaxel, by inhibiting autophosphorylation and activation of downstream signaling pathways^{4,7}. NSCLC patients harboring *EGFR* mutations benefit more from EGFR-TKI treatment than those without *EGFR* mutations. However, several studies demonstrated that gene mutations on the *EGFR* downstream signal pathways are also significant for the response of NSCLC patients to EGFR-TKIs.

EGFR activation elicits its effects via the *K-ras/BRAF/mitogen-activated protein kinase (MAPK)* and phosphatidylinositol-3-kinase (*PI3K*)/*AKT/mTOR* pathways, which promote tumor proliferation, invasion, migration, and neovascularization⁸. Mutation in the downstream genes of *EGFR* signaling pathways may result in receptor-independent pathway activation that renders the tumors unresponsive to EGFR inhibition. *K-ras* and *PI3K* are the key regulators on the two aforementioned pathways, respectively. *K-ras* encodes RAS, a guanosine triphosphate (GTP)-binding protein, which phosphorylates and activates MAPK by interacting with downstream *BRAF*, leading to a cascade of kinase reactions⁹. *K-ras* mutation attenuates the intrinsic GTPase activity of RAS protein, resulting in prolonged RAS activation¹⁰. The *PIK3CA* gene encodes the p110 α catalytic subunit of PI3K protein, and its mutation leads to constitutive activation of protein kinase B signaling¹¹. Both pathways play an important role in various cell physiological and pathological processes, such as proliferation, differentiation, apoptosis, and cell migration¹²⁻¹⁴. Although the corresponding frequencies of *K-ras* and *PIK3CA* mutations are approximately 5%-15% and 3%-5%^{15,16}, many studies have reported that *K-ras* and *PIK3CA* mutations may have primarily induced resistance to EGFR-TKIs of NSCLC patients^{17,18}.

A previous meta-analysis¹⁹ indicated a significant correlation between *K-ras* mutation and clinical response of NSCLC patients treated with EGFR-TKIs. However, the study merely focused on the objective response rate (ORR), and valuable information on the impact of *K-ras* mutation on the survival of NSCLC patients treated with EGFR-TKIs was not provided because of insufficient data. Similar studies on *PIK3CA* mutation are rarely reported. Thus, limited information on the clinical significance of gene mutations in the *EGFR* downstream signal pathways, especially for *K-ras* and *PIK3CA*, in NSCLC patients treated with EGFR-TKIs is available.

Therefore, we performed a meta-analysis of published studies to assess the impact of *K-ras* and *PIK3CA* mutation on the ORR, progression-free survival (PFS), and overall survival (OS) of NSCLC patients treated with EGFR-TKIs to clarify whether these mutations attenuate the clinical benefits of EGFR-TKI treatment in NSCLC patients.

Materials and methods

Search strategy

We developed a search strategy. An internet search of PubMed, EBSCO, OvidSP, and Wiley Online database was performed in April, 2015. Gefitinib and erlotinib, which are the first-generation EGFR-TKIs, had similar efficacies in NSCLC patients^{20,21}. Thus, a combination of a disease domain ("lung cancer"), a treatment domain ("gefitinib", "erlotinib", or "EGFR TKI"), and a gene domain ("*kras*" or "*pik3ca*") was used in all fields. The language was limited to English. All retrieved results were sent to EndNote software (EndNote X6, THOMSON REUTERS, US) to automatically and manually check for duplicate studies. After removing the duplicates, the titles and/or abstracts of the remaining results were screened to exclude irrelevant articles. Full texts of relevant articles were obtained and screened further for eligible studies. Bibliographies of relevant articles were hand-searched to determine additional eligible studies.

Selection criteria

Two reviewers carefully and independently investigated all studies identified, and consensus was reached after discussion when disagreement in the inclusion or exclusion of studies was encountered. Inclusion criteria were as follows: (I) studies focused on NSCLC patients; (II) studies explored the relation between mutations of *K-ras* or *PIK3CA* and outcomes of NSCLC patients treated with EGFR-TKIs; and (III) studies assessed anti-tumor response using one or more of the following parameters: ORR, PFS, and OS. Distinguishing the predominant effect of the EGFR-TKI treatment was difficult when patients underwent combined therapy treatment. Therefore, exclusion criteria were as follows: (I) patients were not treated with single EGFR-TKIs; and (II) PFS and OS were not calculated from the initiation of EGFR-TKI treatment. When the same patient population was used in several publications, only the most recent, complete, or largest study was included in the meta-analysis.

Data extraction

Data from all eligible studies were extracted independently by two researchers with disagreement settled by discussion. The following data from eligible studies were collected: publication details (such as the first author's last name, publication year, and country in which the study was performed), trial information (such as inclusion criteria, number of patients assessed, therapy regimens, genes detected and detection methods, and type of

end points used), patient characteristics (such as age, gender, stage, and histology), and outcome measures [such as hazard ratios (HRs) for PFS and OS and their 95% confidence intervals (CIs), log-rank test *P* values, and ORRs]. PFS and OS were defined as starting from the initial EGFR-TKI treatment. For PFS and OS, the HRs and their 95% CIs were estimated by methods proposed by Tierney *et al.*²² in the absence of published HRs or their 95% CIs. For ORRs, the reported number of objective response (complete response + partial response) and no response (progressive disease + stable disease) in each arm was collected. Quality was assessed independently by two investigators using the Newcastle-Ottawa scale (NOS) for non-randomized studies (available at http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp) with consensus on all items through discussion.

Statistical analysis

The relationship between gene mutation and ORR was presented by odds ratio (OR) with 95% CI. The impact of gene mutation on PFS and OS was measured by HR with 95% CI. The pooled ORs were computed for dichotomous variables by the Mantel-Haenszel method, and the pooled HRs and their 95% CIs were estimated by a general variance-based method. Heterogeneity across studies was tested by the χ^2 -based Q-test and *I*² statistic. A *P* value greater than 0.10 for the Q-test and *I*² statistic with values no more than 50% indicate the lack of heterogeneity among studies. Thus, the fixed-effect model was used for meta-analysis; otherwise, the random-effect model was used. Sensitivity analysis was conducted for meta-analyses by removing one study at a time to test the robustness of the overall results. Potential publication bias was estimated using Begg's funnel plots and Egger's linear regression test. All statistical tests were performed with STATA 12.0 (STATA Corporation, College Station, TX). All reported *P* values were two-sided. Differences were considered statistically significant at *P*<0.05.

Results

Literature search and study characteristics

The initial search on PubMed, EBSCO, OvidSP, and Wiley Online database in April, 2015 retrieved 2,795 studies. A total of 2,294 articles remained after 501 duplicates were removed. After preliminary screening of titles and/or abstracts, 2,087 non-original or irrelevant studies, 90 book sections, and 74 abstracts or posters of conferences were excluded. Hand search on bibliographies of relevant articles retrieved five additional articles. Thus, full texts

of 48 relevant studies were obtained for further investigation. Thirteen articles were further excluded because they were out of scope (12) and they lack relevant data (1). Finally, 19 articles²³⁻⁴¹ published before 2010, 10 articles^{17,18,42-49} after 2010 and another 6 articles⁵⁰⁻⁵⁵ were included. The selection flow diagram is summarized in **Figure 1**.

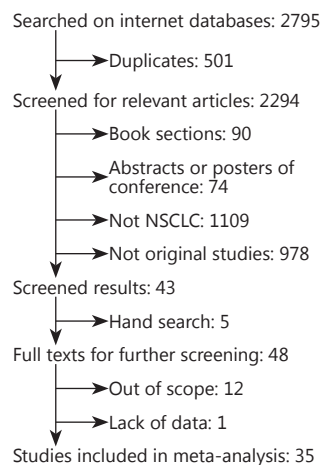


Figure 1 Flow diagram of selection process.

The 35 studies were published from 2006 to 2014. These studies were conducted worldwide: nine from Italy^{18,26,34-36,40,44,49,53}, five from multi-centers (more than two countries or regions)^{33,39,42,51,52}, five from the United States^{28,30,38,50,54}, two from Netherlands^{29,48}, three from Japan^{23,27,37}, two from Korea^{17,25}, two from Germany^{31,55}, and the rest were from Switzerland²⁴, Greece⁴⁵, France⁴³, Czech Republic⁴⁷, China⁴¹, Mexico⁴⁶, and Taiwan³². The median age reported in 28 studies ranged from 58 to 75. A total of 3,958 patients were included with a mean sample size of 113 (ranged from 15 to 393). Most studies included patients with NSCLC, with only five studies focused on lung adenocarcinoma^{17,35,36,49,50}, and one focused on lung squamous carcinoma⁴⁷. Except in two studies^{17,32}, all patients had inoperable stage IIIB or IV or recurrence. Previous treatments included chemotherapy, radiotherapy, surgery, or none. Current treatments of all included studies were monotherapy with EGFR-TKIs. In studies with treatment details, patients were treated with erlotinib or gefitinib according to international standard with one patient who received PF00299804, an irreversible TKI of EGFR, HER2, and HER4, in a study¹⁷. Clinical response was evaluated using RECIST criteria⁵⁶ in 31 studies and WHO criteria⁵⁷ in three studies, with one study not reported. Patients with complete or partial responses were classified as responders in all studies. ORR was the end point of 30 studies, PFS in seven studies, and OS in 11 studies. HR and corresponding 95% CI for PFS and OS were calculated from the

primary data reported in the text of one study¹⁷, and estimated from the reported summary statistics with method recommended by Tierney in two studies^{44,47}. The quality of all included studies was assessed with NOS. The quality scores of all studies were above 7, with mean score of 8.3.

Biomarker analysis

A total of 33 studies provided the technological details for detecting gene mutations, and 16 studies performed mutation screening using direct sequencing (DS). The rest of the articles included pyrosequencing (1), denaturing capillary electrophoresis (DCE) (3), performance of amplification refractory mutation system (2), polymerase chain reaction (PCR)-restriction fragment length polymorphism (2), mutant-enrich sequencing (ME) (2), and denaturing high-performance liquid chromatography (2). A combination of the aforementioned methods was used in five studies. Mutation in *K-ras* exons 1, 2, and/or 3 was assessed in 34 studies, and *PIK3CA* exons 9 and/or 20 in 5 studies. Mutation of *EGFR* exons 18-21 was detected in all studies.

A total of 573 out of 3,377 evaluable patients were *K-ras*-mutation positive (17.0%), and 18 out of 473 patients were *PIK3CA*-mutation positive (3.8%). A total of 16 studies reported that *K-ras* mutation was mutually exclusive with *EGFR* mutation, and five other studies reported that 10 out of 178 patients positive for *K-ras* mutation were concomitant with *EGFR* mutation. Three studies reported that 6 out of 11 patients positive for *PIK3CA* mutation were concomitant with *EGFR* mutation. **Table 1** shows the main characteristics of studies included in the meta-analysis.

Predictive value of *K-ras* mutation

The impact of *K-ras* mutation on the ORR of NSCLC patients treated with EGFR-TKI therapy was evaluated based on 29 studies (**Table 2**). *K-ras* mutation was associated with reduced objective response in NSCLC patients with a pooled OR of 0.22 (95% CI, 0.13-0.35) (**Figure 2A**). Fixed-effect model was used because heterogeneity across the trials was not significant ($I^2=0\%$; $P=0.999$). The sensitivity analysis indicated that no individual study changed the pooled OR significantly (**Figure 2B**), suggesting that the result was reliable. Publication bias was significant in Begg's test ($P=0.049$), but not in Egger's test ($P=0.090$) (**Figure 2C**). Patients included in two studies^{39,54} apparently originated from the same center. Given that the independence of the two studies could not be confirmed, another analysis excluding the prior one of the aforementioned

studies was conducted considering the possibility of duplicate patient population. The pooled OR was 0.22 (95% CI, 0.13-0.35) in a fixed effect model ($I^2=0\%$; $P=0.998$), with publication bias reduced significantly (P values in Egger's and Begg's tests were 0.101 and 0.072, respectively).

Data for assessing the impact on PFS according to *K-ras* mutation status was available in six studies. *K-ras* mutant patients had shorter PFS compared with wild-type patients with pooled HR of 1.56 (95% CI, 1.27-1.92) (**Figure 3A**). Fixed-effect model was used when calculating pooled HR for PFS because heterogeneity across trials was not significant ($I^2=0\%$; $P=0.748$). Sensitivity analysis indicated that this result was robust (**Figure 3B**). Egger's test revealed slight publication bias ($P=0.046$), contrary to Begg's test ($P=0.260$) (**Figure 3C**). Thus, a non-parametric "trim-and-fill" method was utilized to adjust the publication bias (**Figure 3D**). After the trim-and-fill adjustment, two missing studies were added, and the estimated pooled HR was 1.46, with 95% CI ranging from 1.21 to 1.74.

Ten studies were available for analyzing the impact on OS according to *K-ras* mutation. Results showed that NSCLC patients with *K-ras* mutation had shorter OS than wild-type patients with pooled HR of 1.59 (95% CI, 1.33-1.91) (**Figure 4A**). A fixed-effect model was used in calculating pooled HR for OS because heterogeneity across the trials was not significant ($I^2=22.8\%$, $P=0.233$). Sensitivity analysis indicated that the result was stable (**Figure 4B**). Publication bias was not significant in both Egger's ($P=0.098$) and Begg's tests ($P=0.210$) (**Figure 4C**).

To determine the slight heterogeneity across trials in the analysis of the impact of *K-ras* mutation on the OS of NSCLC patients treated with EGFR-TKIs, we conducted subgroup analysis based on whether *K-ras* mutation is concomitant with *EGFR* mutation, previous treatment, and mutation detection method (**Table 3**). Heterogeneity across trials decreased in most subgroups (**Table 3**). In addition, a negative effect of *K-ras* mutation on the OS of NSCLC patients with EGFR-TKI treatment was observed in all subgroups, which further confirmed the robustness of the general result.

Predictive value of *PIK3CA* mutation

Five studies investigated the predictive role of *PIK3CA* mutation in NSCLC patients (**Table 2**). Among these, ORR data were available in four studies, PFS data in two studies, and OS data in three studies. *PIK3CA* mutant NSCLC patients exhibited similar response to EGFR-TKIs compared with wild-type patients with corresponding pooled OR of 0.70 (95% CI, 0.22-2.18) (**Figure 5A**). Fixed-effect model was used because heterogeneity across studies was not significant ($I^2=34.9\%$; $P=0.203$). The pooled HR of 1.79

Table 1 Main characteristics of studies included in the meta-analysis

| Reference | Location | No. of patients | Histology | Stage | Prior treatment | Current treatment | Gene | Mutation positive/total | End points | Response criteria | NOS score |
|-----------------------------------|----------------|-----------------|-----------|-------------------------|---|----------------------------|---|-------------------------|--------------|-------------------|-----------|
| Rotella 2014 ⁴⁹ | Italy | 88 | ADC | IIIB or VI | Surgery or chemotherapy or radiotherapy | E: 150 mg/d | <i>K-ras</i> (exon 2) | 13/88 | ORR | RECIST | 8 |
| Kim 2014 ¹⁷ | Korea | 55 | ADC | I-IV | Chemotherapy | E, G, PANHER | <i>PIK3CA</i> (exon 9, 20) | 3/55 | ORR, PFS, OS | RECIST | 7 |
| Kerner 2013 ⁴⁸ | Netherlands | 45 | NSCLC | NR | NR | EGFR-TKI | <i>K-ras</i> (exon 2) | 108/368 | OS | RECIST | 8 |
| Fiala 2013 ⁴⁷ | Czech Republic | 179 | SLC | IIIB or IV | Chemotherapy | G: 250 mg/d or E: 150 mg/d | <i>K-ras</i> (codon 12,13) <i>PIK3CA</i> (exon 9) | 14/174 6/170 | PFS, OS | NR | 9 |
| Campos-Parra 2013 ⁴⁶ | Mexico | 353 | NSCLC | IIIB or IV | Chemotherapy | E or G | <i>K-ras</i> (codon 12, 13) | NR | PFS | RECIST | 8 |
| Murray 2012 ⁴⁵ | Greece | 30 | NSCLC | IIIB or IV or recurrent | Chemotherapy | G: 250 mg/d or E: 150 mg/d | <i>K-ras</i> (codon 12, 13) | 3/30 | OS | RECIST | 8 |
| Metro 2014 ²⁴ | Italy | 67 | NSCLC | IIIB or IV | Chemotherapy | E or G | <i>K-ras</i> (exon 2, 3) | 18/67 | ORR, PFS, OS | RECIST | 9 |
| Ludovini 2012 ¹⁸ | Italy | 166 | NSCLC | III or IV | Chemotherapy | G: 250 mg/d or E: 150 mg/d | <i>K-ras</i> (exon 2, 3) <i>PIK3CA</i> (exon 9, 20) | 11/162 6/145 | ORR, OS | RECIST | 9 |
| Cadranel 2012 ⁴³ | France | 307 | NSCLC | Advanced | Chemotherapy | E: 150 mg/d | <i>K-ras</i> (exon 2) | 42/307 | PFS, OS | WHO | 9 |
| Hirsch 2011 ⁴² | Multicenter | 94 | NSCLC | IIIB or IV | NR | E: 150 mg/d | <i>K-ras</i> (exon 2) | 20/94 | ORR | RECIST | 8 |
| Zhu 2010 ⁴¹ | China | 17 | NSCLC | IIIB or IV | NR | E: 150 mg/d | <i>K-ras</i> | 3/17 | ORR | RECIST | 7 |
| Tiseo 2010 ⁴⁰ | Italy | 63 | NSCLC | III or IV | Chemotherapy | G: 250 mg/d | <i>K-ras</i> (exon 2) | 7/63 | ORR | RECIST | 8 |
| Douillard 2010 ³⁹ | Multicenter | 275 | NSCLC | IIIB or IV | Chemotherapy | G: 250 mg/d | <i>K-ras</i> (codon 12, 13) | 49/275 | ORR | RECIST | 9 |
| Amann 2010 ⁸ | USA | 41 | NSCLC | IIIB or IV or recurrent | Chemotherapy | E: 150 mg/d | <i>K-ras</i> (exon 2) | 3/41 | ORR, OS | RECIST | 9 |
| Varella-Garcia 2009 ³⁷ | Japan | 30 | NSCLC | Recurrent | Surgery | G: 250 mg/d | <i>K-ras</i> (codon 12, 13) | 4/30 | ORR | RECIST | 9 |
| Marchetti 2009 ³⁶ | Italy | 83 | ADC | IIIB or IV | Chemotherapy | G: 250 mg/d or E: 150 mg/d | <i>K-ras</i> (exon 2) | 30/83 | ORR, PFS, OS | WHO | 8 |
| Boldrini 2009 ³⁵ | Italy | 411 | ADC | IV | Surgery | G: 250 mg/d or E: 150 mg/d | <i>K-ras</i> (codon 12, 13) | 2/19 | ORR | RECIST | 7 |
| Zucali 2008 ³⁴ | Italy | 49 | NSCLC | III or IV | Chemotherapy or no | G: 250 mg/d | <i>K-ras</i> (exon 1, 2) | 15/49 | ORR | RECIST | 8 |
| Zhu 2008 ³³ | Multicenter | 206 | NSCLC | IIIB or IV | Chemotherapy | E: 150 mg/d | <i>K-ras</i> (exon 2) | 30/206 | ORR | RECIST | 9 |
| Wu 2008 ³² | Taiwan | 53 | NSCLC | IB-IIIb | Surgery | E or G | <i>K-ras</i> (exon 1,2) | 1/53 | ORR | RECIST | 8 |
| Schneider 2008 ³¹ | Germany | 393 | NSCLC | IIIB or IV | Chemotherapy or radiotherapy | E: 150 mg/d | <i>K-ras</i> (exon 2,3) | 17/114 | ORR, PFS, OS | RECIST | 9 |
| Miller 2008 ³⁰ | USA | 82 | NSCLC | IIIB or IV | Chemotherapy or no | E: 150 mg/d | <i>K-ras</i> (exon 2) | 18/80 | ORR | RECIST | 9 |
| Felip 2008 ³⁵ | Germany | 39 | NSCLC | Advanced | Chemotherapy or no | E: 150 mg/d | <i>K-ras</i> (exon 2,3) | 7/39 | ORR | RECIST | 8 |

Table 1 (continued)

Table 1 (continued)

| Reference | Location | No. of patients | Histology Stage | Prior treatment | Current treatment | Gene | Mutation positive/total | End points | Response criteria | NOS score |
|-------------------------------|-------------|-----------------|-----------------------------|-------------------------|----------------------------|---|-------------------------|------------|-------------------|-----------|
| Zandwijk 2007 ²⁹ | Netherlands | 15 | NSCLC NR | Chemotherapy | G | K-ras (codon 2) | 3/15 | ORR | RECIST | 8 |
| Massarelli 2007 ⁵⁴ | USA | 70 | NSCLC IIIB or VI | Chemotherapy or no | G: 250 mg/d or E: 150 mg/d | K-ras (exon 1) | 16/70 | ORR | RECIST | 9 |
| Loprevite 2007 ⁵³ | Italy | 21 | NSCLC Advanced | Chemotherapy | G: 250 mg/d | K-ras (exon 2) | 1/21 | ORR | RECIST | 8 |
| Jackman 2007 ²⁸ | USA | 41 | NSCLC IIIB or IV | Surgery or Radiotherapy | E: 150 mg/d | K-ras (exon 2,3) | 6/41 | ORR | RECIST | 9 |
| Ichihara 2007 ²⁷ | Japan | 99 | NSCLC Advanced or recurrent | Surgery or chemotherapy | G: 250 mg/d | K-ras (exon 2) | 8/87 | ORR | WHO | 8 |
| Hirsch 2007 ³² | Multicenter | 138 | NSCLC III or IV | Chemotherapy | G: 250 mg/d or E: 150 mg/d | K-ras (exon 2) | 36/138 | ORR | RECIST | 9 |
| Cappuzzo 2007 ²⁶ | Italy | 37 | NSCLC IIIB or IV | Chemotherapy or no | G: 250 mg/d | K-ras (exon 2) | 1/37 | ORR | RECIST | 7 |
| Hirsch 2006 ⁵¹ | Multicenter | 152 | NSCLC III or IV | Chemotherapy | G: 250 mg/d | K-ras (exon 2) | 12/152 | ORR | RECIST | 9 |
| Han 2006 ²⁵ | Korea | 69 | NSCLC IIIB or IV | NR | G:250 mg/d | K-ras (exon 2) | 9/69 | ORR | WHO | 9 |
| Giaccone 2006 ²⁴ | Switzerland | 53 | NSCLC IIIB or IV | Surgery or Radiotherapy | E: 150 mg/d | K-ras (exon 1, 2) PIK3CA (exon 9, 20) | 10/25; 1/25 | ORR | RECIST | 9 |
| Pao 2005 ⁵⁰ | USA | 59 | ADC NR | NR | G: 250 mg/d or E: 150 mg/d | K-ras (exon 2) | 9/47 | ORR | RECIST | 7 |
| Endoh 2006 ²³ | Japan | 78 | NSCLC Recurrent | Surgery or chemotherapy | G: 250 mg/d | K-ras (codon 12, 13, 61); PIK3CA (open reading frame) | 7/78; 2/78 | ORR, OS | RECIST | 8 |

ADC: adenocarcinoma; SLC: squamous lung cancer; NSCLC: non-small cell lung cancer; NR: not reported; E: erlotinib; G: gefitinib; PANHER: PF00299804, an irreversible TKI of EGFR, HER2, and HER4; ORR: objective response rate; PFS: progression-free survival; OS: overall survival; No.: number of patients assessed; NOS: Newcastle-Ottawa scale

Table 2 Pooled results of meta-analysis of the predictive value of K-ras and PIK3CA mutation in patients with NSCLC

| End points | No. of studies | Heterogeneity | | Fixed model | | Random model | | Begg's test | | Egger's test | |
|---------------|----------------|--------------------|---------|------------------|---------|------------------|---------|-------------|---------|--------------|---------|
| | | I ² (%) | P value | OR/HR (95% CI) | P value | OR/HR (95% CI) | P value | Z | P value | t (bias) | P value |
| <i>K-ras</i> | | | | | | | | | | | |
| ORR | 29 | 0.0 | 0.999 | 0.22 (0.13-0.35) | 0.000 | 0.26 (0.16-0.43) | 0.000 | 1.97 | 0.049 | -1.76 | 0.090 |
| PFS | 6 | 0.0 | 0.748 | 1.56 (1.27-1.92) | 0.000 | 1.56 (1.27-1.92) | 0.000 | 1.13 | 0.260 | 2.86 | 0.046 |
| OS | 10 | 22.8 | 0.233 | 1.59 (1.33-1.91) | 0.000 | 1.61 (1.31-2.02) | 0.000 | 1.25 | 0.210 | 1.88 | 0.098 |
| <i>PIK3CA</i> | | | | | | | | | | | |
| ORR | 4 | 34.9 | 0.203 | 0.70 (0.22-2.18) | 0.534 | 0.67 (0.12-3.62) | 0.642 | - | - | - | - |
| PFS | 2 | 0.0 | 0.893 | 1.79 (0.91-3.53) | 0.094 | 1.79 (0.91-3.53) | 0.094 | - | - | - | - |
| OS | 4 | 26.9 | 0.255 | 1.83 (1.05-3.20) | 0.034 | 1.82 (0.94-3.53) | 0.075 | - | - | - | - |

ORR, objective response rate; PFS, progression-free survival; OS, overall survival; OR, odds ratio; HR, hazard ratio.

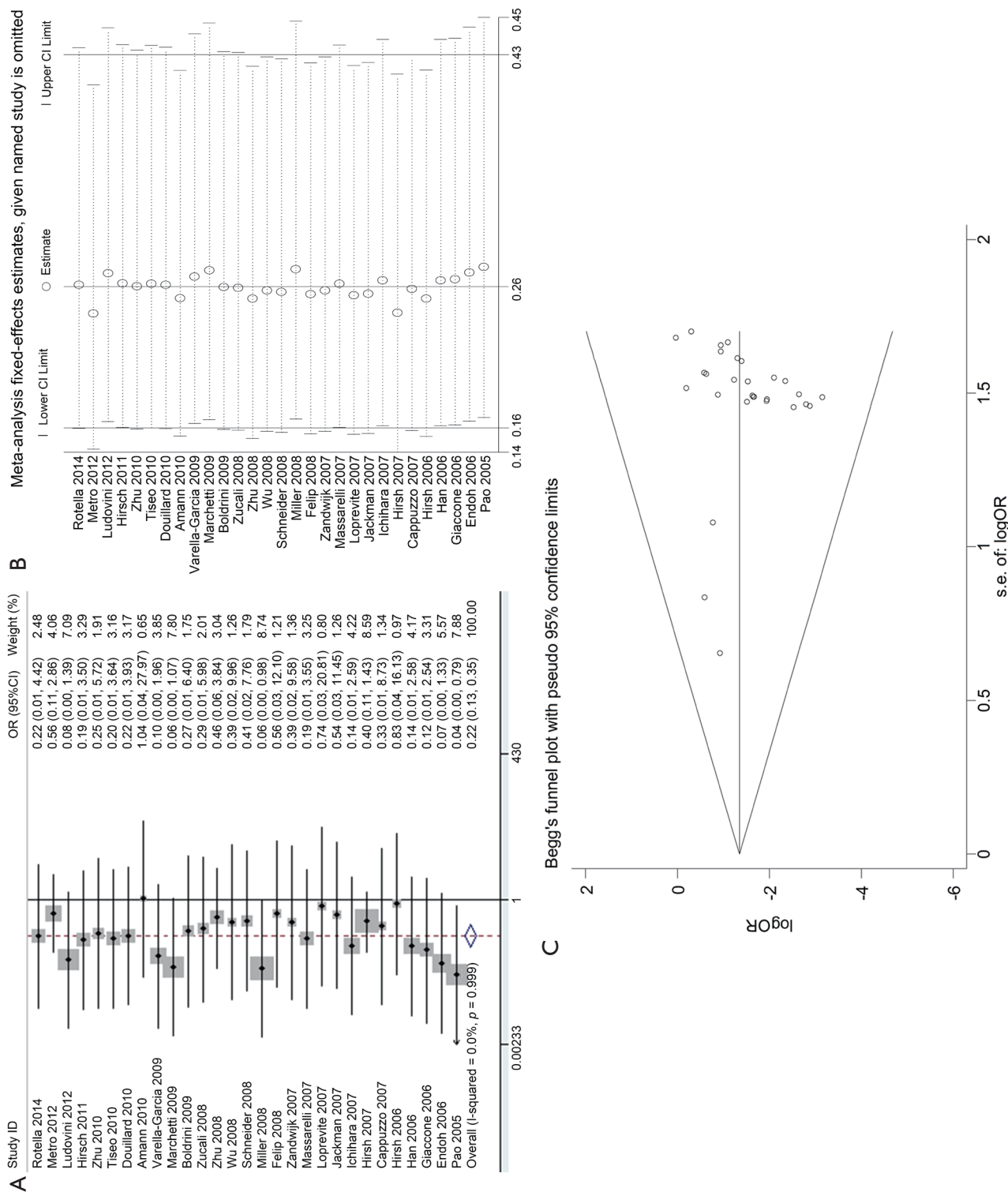


Figure 2 Meta-analysis of the predictive value of *K-ras* mutation for ORR. (A) Forest plots of OR and 95% CI; (B) Results of sensitivity analysis; and (C) Begg's funnel plot analysis of publication bias. OR, odds ratio; ORR, objective response rate; s.e., standard error.

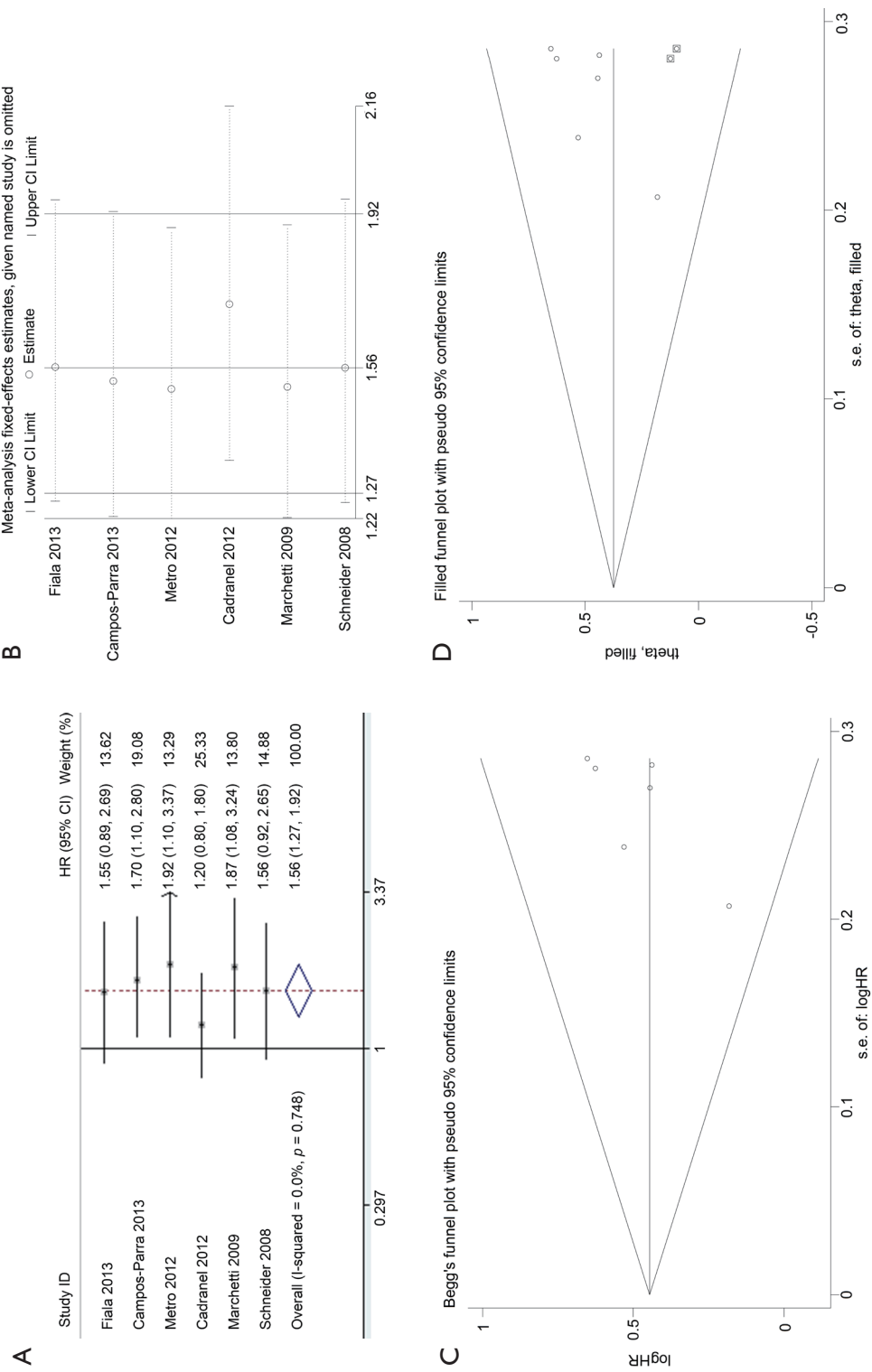


Figure 3 Meta-analysis of the predictive value of K-ras mutation for PFS. (A) Forest plots of HR and 95% CI; (B) Results of sensitivity analysis; (C) Begg's funnel plot analysis of publication bias; and (D) Filled funnel plot using trim-and-fill method. ○, indicates observed studies; ◐, indicates missed studies. HR, hazard ratio; s.e., standard error.

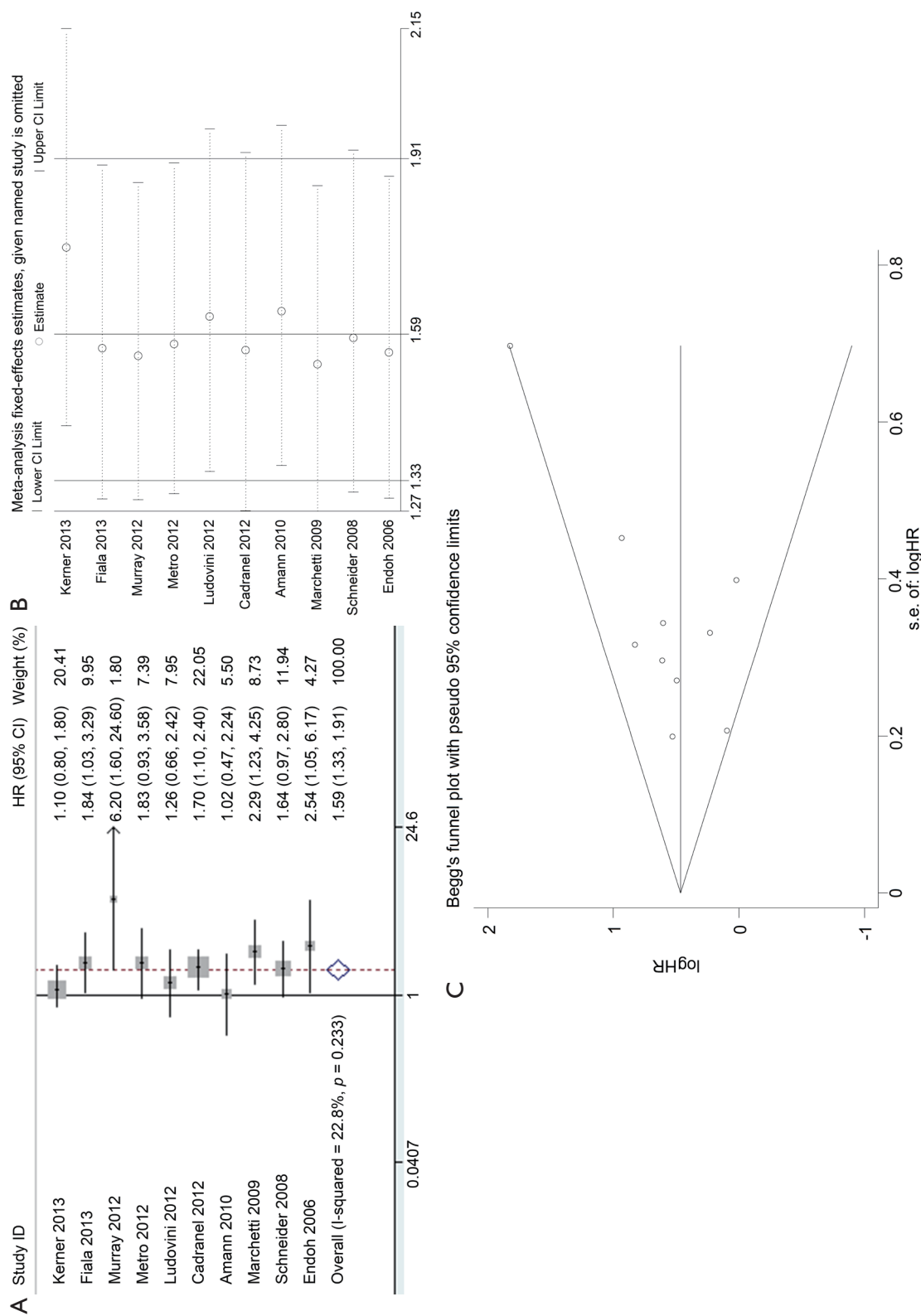


Figure 4 Meta-analysis of the predictive value of *K-ras* mutation for OS. (A) Forest plots of HR and 95% CI; (B) Results of sensitivity analysis; and (C) Begg's funnel plot analysis of publication bias. HR, hazard ratio; s.e., standard error.

Table 3 Results of subgroup analysis of pooled HRs for OS of patients harboring *K-ras* mutation with EGFR-TKI treatment

| Subgroups | No. of study | Heterogeneity | | Fixed model | | Random model | |
|--------------------------------|--------------|--------------------|-------|-------------------|-------|-------------------|-------|
| | | I ² (%) | P | HR (95% CI) | P | HR (95% CI) | P |
| Concomitant with EGFR mutation | | | | | | | |
| No | 5 | 19.5 | 0.290 | 1.79 (1.35-2.36) | 0.000 | 1.83 (1.31-2.02) | 0.000 |
| Yes | 2 | 73.4 | 0.052 | 1.37 (0.98-1.92) | 0.069 | 1.53 (0.75-3.12) | 0.246 |
| NR | 3 | 0.0 | 0.477 | 1.55 (1.09-2.21) | 0.013 | 1.55 (1.09-2.21) | 0.013 |
| Previous treatment | | | | | | | |
| CT | 7 | 12.9 | 0.331 | 1.73 (1.37-2.18) | 0.000 | 1.73 (1.34-2.23) | 0.000 |
| Combination | 2 | 0.0 | 0.406 | 1.84 (1.17-2.90) | 0.009 | 1.84 (1.17-2.90) | 0.009 |
| NR | 1 | – | – | 1.10 (0.80-1.80) | 0.645 | 1.10 (0.80-1.80) | 0.645 |
| Mutation detection | | | | | | | |
| DS | 3 | 14.2 | 0.312 | 1.58 (1.07-2.35) | 0.022 | 1.59 (1.03-2.45) | 0.037 |
| DCE | 2 | 0.0 | 0.643 | 1.63 (1.13-2.35) | 0.008 | 1.63 (1.13-2.35) | 0.008 |
| RFLP | 1 | – | – | 6.20 (1.58-24.31) | 0.009 | 6.20 (1.58-24.31) | 0.009 |
| ME | 1 | – | – | 2.29 (1.23-4.26) | 0.009 | 2.29 (1.23-4.26) | 0.009 |
| Combination | 2 | 56.5 | 0.129 | 1.38 (1.04-1.83) | 0.025 | 1.37 (0.90-2.10) | 0.146 |

CT, chemotherapy; DS, direct sequencing; DCE, denaturing capillary electrophoresis; ME, mutant-enrich sequencing; NR, not reported; RFLP, polymerase chain reaction-restriction fragment length polymorphism; HR, hazard ratio.

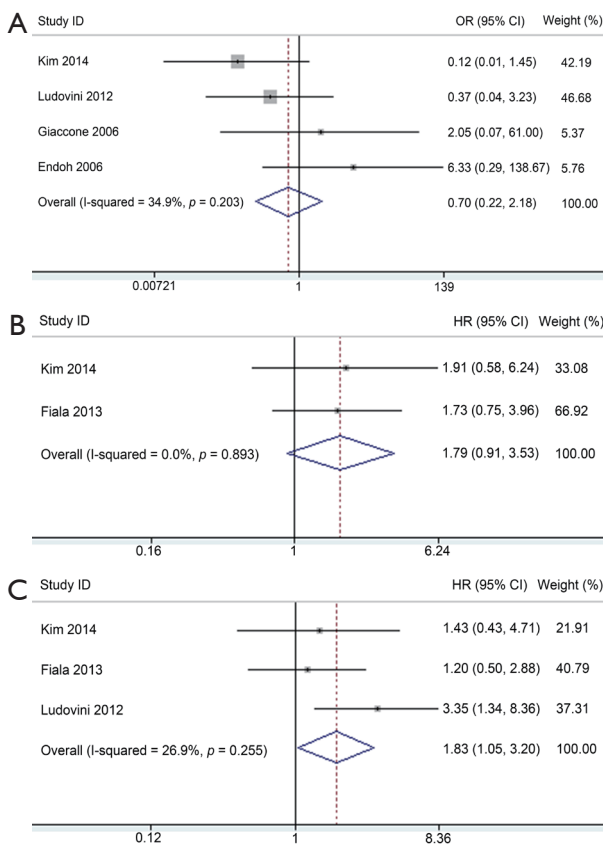


Figure 5 Meta-analysis of the predictive value of *PIK3CA* mutation. (A) Forest plots of OR and 95% CI for ORR; (B) Forest plots of HR and 95% CI for PFS; and (C) Forest plots of HR and 95% CI for OS. OR, odds ratio; HR, hazard ratio.

(95% CI, 0.91-3.53) for PFS in a fixed-effect model ($I^2=0\%$; $P=0.893$) suggested that *PIK3CA* mutant NSCLC patients had similar PFS compared with wild-type patients when treated with EGFR-TKIs (Figure 5B). However, *PIK3CA* mutation showed a trend toward a significant adverse effect on OS with a pooled HR of 1.83 (95% CI, 1.05-3.20) in NSCLC patients treated with EGFR-TKIs (Figure 5C). Between-study heterogeneity was not significant; thus, the analysis was performed in the fixed-effect model ($I^2=26.9\%$; $P=0.255$).

Sensitivity analysis and publication bias of all above analyses was not performed because of the relatively limited eligible studies. Subgroup analysis was not conducted because of the relatively small size of included articles.

Discussion

EGFR inhibitor elicits multiple downstream effects, primarily moderated by *RAS/RAF/MAPK* and *PI3K/AKT/mTOR* signaling pathways. Rational use of target therapy requires the optimal selection of patients whose tumors are dependent on the activation of these two pathways. The predictive value of gene mutations on these two pathways downstream of *EGFR* for EGFR-TKI treatment is gradually recognized. This meta-analysis reveals an independent predictive value of *K-ras* and *PIK3CA* genetic status on EGFR-TKI therapy.

Coincident with previous report, our results again demonstrated that NSCLC patients harboring *K-ras* mutation had poor response to EGFR-TKIs. Exclusion of possible duplicate study reduced publication bias significantly and did not alter the pooled result, thus proving the stability of our result. More importantly, we quantitatively demonstrated that such patients had shorter PFS and OS compared with wild-type patients. Given that heterogeneity was zero across the studies in the analysis of the impact of *K-ras* mutation on PFS of NSCLC patients treated with EGFR-TKIs, a trim-and-fill method was applied to adjust publication bias. The adjusted pooled HR did not alter significantly the primary result, suggesting the dependability of our results. Slight heterogeneity was observed in the meta-analysis of the impact of *K-ras* mutation on the OS of NSCLC patients treated with EGFR-TKIs. Subgroup analysis showed that if *K-ras* mutation is concomitant with EGFR mutation, previous treatment and mutation detection method (Table 3) might affect the result. However, a negative effect of *K-ras* mutation on the OS of NSCLC patients with the EGFR-TKI treatment was observed in all subgroups. All these results indicated the adverse impact of mutant *K-ras* on the response and survival outcomes of NSCLC patients treated with EGFR-TKIs. This adverse effect has been proved in other cancers⁵⁸.

Mutant *PIK3CA* proteins increase catalytic activity resulting in enhanced downstream signaling and oncogenic transformation *in vitro*⁵⁹. Preclinical data showed that introducing activated *PIK3CA* mutations into *EGFR*-mutated lung cancer cell lines confers resistance to EGFR-TKIs⁶⁰. Consistent with this result, our analysis revealed significantly shorter OS, poor ORR, and shorter PFS in *PIK3CA* mutant NSCLC patients treated with EGFR-TKIs.

The most common mutation of *PIK3CA* was found in exons 9 and 20⁶¹, corresponding to the helical and kinase domains, respectively. The predictive value of *PIK3CA* as a negative biomarker for anti-EGFR response in colorectal cancer differed in exons 9 and 20⁶². However, similar study in NSCLC was rarely reported in published articles, and no clear evidence was obtained to show that the impact of mutations in exons 9 and 20 of *PIK3CA* on anti-EGFR response differ in NSCLC. Thus, further analysis of the predictive value of these two exons separately with enlarged samples size is needed to achieve definite conclusion.

Slight heterogeneity was observed in the analysis of the impact of *PIK3CA* mutation. Although subgroup analysis could not be conducted because of insufficient data, some diversity on whether *PIK3CA* mutation was concomitant with *EGFR* mutation, mutation detecting method, and data extraction method was observed. Coexistence of *PIK3CA* mutations with *EGFR* is frequent in lung cancer^{15,63,64}. However, the predictive value of *PIK3CA* to anti-EGFR treatment in *EGFR* mutant or wild-type NSCLC is ambiguous at present. The accuracy and specificity of different mutation detection methods also varied, which led to different false positive and false negative rates³⁶. Although extracting time-to-event data according to Tierney was preferable, it failed to circumvent the potential biases associated with relying on published data for meta-analysis as mentioned by the authors. Therefore, despite the slight heterogeneity of the included studies in the analysis of the impact of mutant *PIK3CA* on the response and survival outcomes of NSCLC patients, our result would be consolidated by increasing sample size.

Despite our efforts to provide an accurate and comprehensive analysis, limitations of our meta-analysis should be addressed. First, most of the included studies were retrospective. Second, not all published studies presented adjusted estimates or had been adjusted by similar potential confounders. Third, limited studies presented *PIK3CA* mutation data, in which only four studies provided ORR information, two studies provided PFS information, and three studies provided OS information. Thus, increasing sample size of studies will further increase the creditability of adverse effect of *PIK3CA* mutation on clinical prognosis of NSCLC patients receiving EGFR-TKI treatment.

In conclusion, this meta-analysis indicated that *K-ras* mutation is probably a valuable predictive biomarker for assessing the clinical response and survival outcomes of NSCLC patients treated with EGFR-TKIs. More importantly, similar trends for *PIK3CA* mutation were shown in this meta-analysis, although the trends in ORR and PFS were not significant. Increasing sample size of studies will further increase the creditability of adverse effect of *PIK3CA* mutation on the clinical prognosis of NSCLC patients receiving EGFR-TKI treatment. Mutations of *K-ras* and *EGFR* are usually mutually exclusive, and coexistence of mutation in *PIK3CA* and *EGFR* is common. Thus, determining the status of *K-ras* and *PIK3CA* is valuable to distinguish the optimal patients who will benefit from EGFR-TKI treatment.

Acknowledgements

This work was supported by Key Projects in the National Science & Technology Pillar Program (Grant No. 2013ZX09303001, 2015BAI12B12, and 2015BAI12B15), National Natural Science Foundation of China (Grant No. 81472473 and 81272360), and Tianjin Municipal Commission of Science & Technology Key Research Program (Grant No.13ZCZCSY20300). We thank Dr. Wei-Jia Zhang from the Department of Medicine, Icahn School of Medicine at Mount Sinai for providing constructive suggestions for manuscript preparation.

Conflict of interest statement

No potential conflicts of interest are disclosed.

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- Cite this article as:** Chen JY, Cheng YN, Han L, Wei F, Yu WW, Zhang XW, Cao S, Yu JP. Predictive value of *K-ras* and *PIK3CA* in non-small cell lung cancer patients treated with EGFR-TKIs: a systemic review and meta-analysis. *Cancer Biol Med* 2015;12:126-139. doi: 10.7497/j.issn.2095-3941.2015.0021