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ORIGINAL RESEARCH

Exploratory cohort study and meta-analysis of *BIM* deletion polymorphism in patients with epidermal growth factor receptor-mutant non-small-cell lung cancer treated with epidermal growth factor receptor tyrosine kinase inhibitors

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Si Sun[1,2]
Hui Yu[1,2]
Huijie Wang[1,2]
Xinmin Zhao[1,2]
Xintai Zhao[3]
Xianghua Wu[1,2]
Jie Qiao[1,2]
Jianhua Chang[1,2]
Jialei Wang[1,2]

[1]Department of Medical Oncology, Fudan University Shanghai Cancer Center, [2]Department of Oncology, Shanghai Medical College, Fudan University, [3]Shanghai Shines Biomedical Co. Ltd., Shanghai, People's Republic of China

Correspondence: Jialei Wang
Department of Medical Oncology, Fudan University Shanghai Cancer Center, 270 Dong An Road, 200032 Shanghai, People's Republic of China
Tel +86 21 6417 5590 (ext 5006)
Fax +86 21 6443 0160
Email luwangjialei123@hotmail.com

Background: Non-small-cell lung cancer (NSCLC) patients with epidermal growth factor receptor (*EGFR*) mutations might develop primary and secondary resistance to tyrosine kinase inhibitors (TKIs). The proapoptotic protein Bcl-2-like 11 (BIM) is a key modulator of apoptosis triggered by EGFR-TKIs. The recent studies have indicated that some patients with positive *EGFR* mutations were refractory to EGFR-TKIs if they harbored a *BIM* deletion polymorphism. The purpose of this study was to investigate whether *BIM* polymorphism predicts treatment efficacy of EGFR-TKIs in Chinese NSCLC patients.

Patients and methods: A cohort of advanced NSCLC patients with *EGFR* mutations and treated with EGFR-TKIs (gefitinib or erlotinib) were recruited. We drew peripheral blood to determinate *BIM* deletion status and then compared patients' clinical outcomes according to the *BIM* deletion status. Additionally, we electronically searched eligible cohort studies and conducted a meta-analysis to pool event risk.

Results: The exploratory cohort study included 140 patients. Patients with and without the *BIM* deletion polymorphism had similar objective response rates (ORRs, 48.5 vs 63.0%, P=0.16), disease control rate (DCR, 93.9 vs 97.0%, P=0.60) and adverse reactions. Similar progression-free survival (PFS) and overall survival (OS) were noted in overall population (P=0.27 for PFS and P=0.61 for OS) and prespecified patient subgroups. The meta-analysis included 10 eligible cohort studies involving 1,317 NSCLC patients. It showed the positive BIM deletion was associated with shorter PFS (hazard ratio =1.45; P=0.02). Nonsignificant differences existed for ORR, DCR and OS.

Conclusion: The expanded meta-analysis results demonstrated the positive *BIM* deletion predicts shorter PFS in NSCLC patients after treatment with EGFR-TKIs while other clinical measures do not. A large multicenter well-designed cohort study involving other concurrent genetic alterations is warranted.

Keywords: *BIM*, *EGFR*, NSCLC, clinical outcome

Introduction

Non-small-cell lung cancer (NSCLC) is the leading cause of cancer-related death.[1] Like other cancers, NSCLC develops when cells initiate to uncontrollably drive mutations due to changes in their genes. Using targeted therapies could specifically attack

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these changes and block the growth of cancer cells without damaging the normal cells like cytotoxic chemotherapy.[2] The representative targeted therapy – tyrosine kinase inhibitor (TKI), which targets mutated epidermal growth factor receptors (EGFRs) – has turned into a better alternative for treating advanced NSCLC.[3] Consequently, it has surprisingly changed the treatment of advanced NSCLC.[4–6] NSCLC patients with *EGFR* mutations who receive first-line therapy with an EGFR-TKI, such as gefitinib or erlotinib, have longer progression-free survival (PFS) than those who are treated with platinum-based chemotherapy.[4,7–9] However, about 30% of these patients show primary resistance to EGFR-TKIs even if they have *EGFR* mutations; meanwhile, most patients who respond initially might acquire drug resistance after approximately 1 year of treatment.[4,5,9–11] Mechanisms of acquired resistance to EGFR-TKI include T790M secondary mutation, or subsequently C797S mutation responsible for resistance to T790M-targeting EGFR inhibitors, and MET amplification.[12–14]

BIM, also known as *Bcl-2-like 11* (*BCL2L11*), is a member of the Bcl-2 family of genes and encodes the protein BIM. By binding to all members of the prosurvival Bcl-2 subfamily with high affinity, BIM serves as a key element in promoting apoptosis. *BIM* deletion polymorphism is a 2,903-bp deletion located in exon 2 of the *BCL2L11* gene that leads to alternative splicing of the mRNA of *BIM*, which results in expression of *BIM* isoforms lacking the pro-apoptotic *BCL2*-homology domain 3 (*BH3*).[15] It is hypothesized that BIM might be involved in the apoptotic signaling following EGFR disruption by TKIs.[2] The intrinsic resistance and incomplete response may be due, in part, to downregulation of BIM expression.[11,12] A recent study has suggested that the BIM germline alteration would prevent apoptosis induced by EGFR-TKIs, which poses a potential mechanism conferring resistance.[16] Another study has showed that *BIM* deletion polymorphism is associated with primary drug resistance to EGFR-TKIs.[17] As shown by induction of apoptosis, the *EGFR*-mutant NSCLC cells with the *BIM* deletion polymorphism are much less sensitive to gefitinib than those with wild-type *BIM*.[15] Thus, therapies that upregulate BIM expression, such as histone deacetylase inhibitor, vorinostat, may resensitize some low *BIM*-expressing oncogene-addicted cancers to targeted therapies.[17]

Given that *EGFR*-mutated lung tumors occur more frequently in East Asians and the *BIM* polymorphism is also prevalent in East Asian population and seldom found in Caucasian counterparts,[16] we carried out this exploratory cohort study in the People's Republic of China to investigate the predictive role of *BIM* deletion polymorphism in advanced *EGFR*-mutant NSCLC patients treated with EGFR-TKIs. Besides, we sought to perform a meta-analysis incorporating all currently available evidences from cohort studies to compare the clinical outcomes according to the *BIM* polymorphism status in NSCLC patients with *EGFR* mutations after the treatment with EGFR-TKIs.

Patients and methods

Patients

In this exploratory cohort study, a total of 140 NSCLC patients harboring *EGFR* mutation who were treated with EGFR-TKIs were recruited from June 2009 through May 2013. This study was approved by the Ethics Committees of Shanghai Cancer Center, Fudan University, and was carried out in accordance with the World Medical Association's Declaration of Helsinki (1964) and its later amendments. Informed consent was obtained from each participating patient before any study-related procedure was performed.

Patients received either oral gefitinib (250 mg per day) or oral erlotinib (150 mg per day). Every 2 months, patients were assessed for response using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.[18] According to this criteria, overall response rate (ORR) was defined as the proportion of patients who had complete response and partial response, while disease control rate (DCR) was defined as the proportion of patients who had a best response rating of complete response, partial response or stable disease. PFS was calculated from the date EGFR-TKIs therapy was initiated to the date of either tumor progression or death from any cause. Overall survival (OS) was defined as the time from the initiation of EGFR-TKIs therapy to death from any cause. Adverse events related to EGFR-TKIs treatment were evaluated using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 4.0 (2009).

EGFR mutations and *BIM* deletion polymorphism

We used direct sequencing to determinate *EGFR* (exons 18–21) mutations in polymerase chain reaction (PCR) fragments amplified with genomic DNA from formalin-fixed paraffin-embedded tissue.[19,20] *BIM* deletion polymorphism analysis (the presence of wild-type or deletion alleles) was performed on genomic DNA extracted (QIAamp DNA blood mini kit; Qiagen NV, Venlo, the Netherlands) from peripheral blood samples using PCR amplification and agarose gel electrophoresis. The primer sequences were as

follows: wild-type *BIM* forward primer, 5′-ACTGTAAAACGACGGCCAGTCCTCATGATGAAGGCTAACTCAA-3′; and reverse primer, 5′-ACCAGGAAACAGCTATGACCAACCTCTGACAAGTGACCACCA-3′. For the *BIM* deletion polymorphism, the forward primer sequence was the same as that used for wild-type *BIM*, and the reverse sequence was 5′-ACCAGGAAACAGCTATGACCGGCACAGCCTCTATGGAGAACA-3′. The PCR conditions were 95°C for 3 minutes, and then 40 cycles of 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 30 seconds. The final elongation step was performed at 72°C for 5 minutes. The PCR products were subjected to electrophoresis in 2% agarose gel stained with ethidium bromide and visualized using an ultraviolet illuminator.

Statistical analysis

R version 3.1.2 and SAS®9.2 software were used for all statistical analyses, including those in the meta-analysis. Two-sided *P*-values of less than 0.05 were considered statistically significant.

In the exploratory study, demographic and clinicopathological characteristics and adverse reactions were summarized by *BIM* deletion polymorphism status using descriptive statistics. ORR and DCR between patients with and without *BIM* deletion polymorphism were compared using Pearson's Chi-square test. Survival curves were drawn by the Kaplan–Meier method, and statistical test was performed using log-rank test. To calculate hazard ratios (HRs) and 95% confidence intervals (CIs), Cox regression analysis was applied among both overall population and those prespecified subgroups according to the following prognostic factors: age, gender, smoking status, type of *EGFR* mutation, chemotherapy history and EGFR-TKIs treatment line.

In the meta-analysis, risk ratios (RRs) for binary data (ORR and DCR) as well as HRs for survival time (PFS and OS) were pooled along with 95% CIs using fixed-effect model and additionally displayed using forest plots. Statistical heterogeneity was considered significant when *P*-value was less than 0.10 for the *Q*-test. Publication bias was evaluated using funnel plot and Begg's and Egger's tests.[21,22]

Results

Demographic and clinicopathological characteristics

The relevant characteristics of the study patients at the initiation of EGFR-TKI treatment are summarized in Table 1. The median age of all the included patients was 58.5 years, 94 (67.1%) patients were female and 117 (83.6%) did not report family history of lung cancer. Approximately three-fourths of patients were nonsmokers and could not undergo radical surgery as well. These patients had previously received a median number of four treatment cycles. The vast majority of patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 1 (86.4%) and the pathological diagnosis of adenocarcinoma (91.4%). In addition, there were 54 (38.6%) patients receiving EGFR-TKIs as first-line treatment. The most common *EGFR* mutation was seen in exon 19, accounting for 52.1% of mutations, followed

Table 1 Demographic and clinicopathological characteristics of the patients included in cohort study

Characteristic	*BIM* deletion status		All
	Heterozygous (N=37)	Wild type (N=103)	(N=140)
Age (years)			
Mean (SD)	56.1 (11.05)	58.5 (9.72)	57.9 (10.11)
Median	56.3	58.9	58.5
<65	28 (75.7)	75 (72.8)	103 (73.6)
≥65	9 (24.3)	28 (27.2)	37 (26.4)
Gender			
Male	8 (21.6)	38 (36.9)	46 (32.9)
Female	29 (78.4)	65 (63.1)	94 (67.1)
Family history of lung cancer			
No	33 (89.2)	84 (81.6)	117 (83.6)
Yes	4 (10.8)	19 (18.4)	23 (16.4)
Smoking			
No	30 (81.1)	76 (73.8)	106 (75.7)
Yes	7 (18.9)	27 (26.2)	34 (24.3)
Radical surgery			
No	22 (59.5)	74 (71.8)	96 (68.6)
Yes	15 (40.5)	29 (28.2)	44 (31.4)
ECOG performance status			
0	4 (10.8)	6 (5.8)	10 (7.1)
1	29 (78.4)	92 (89.3)	121 (86.4)
2	4 (10.8)	5 (4.9)	9 (6.4)
Histology			
Adenocarcinoma	33 (89.2)	95 (92.2)	128 (91.4)
Other	4 (10.8)	8 (7.8)	12 (8.6)
Number of metastatic organs*			
≤2	26 (70.3)	68 (66.7)	94 (67.6)
>2	11 (29.7)	34 (33.3)	45 (32.4)
EGFR mutation			
18 mutation	1 (2.7)	3 (2.9)	4 (2.9)
19 mutation	22 (59.5)	51 (49.5)	73 (52.1)
20 mutation	2 (5.4)	2 (1.9)	4 (2.9)
21 mutation	12 (32.4)	47 (45.6)	59 (42.1)
Clinical stage			
III	4 (10.8)	5 (4.9)	9 (5.4)
IV	33 (89.2)	98 (95.1)	131 (93.6)
EGFR-TKIs treatment			
First line	12 (32.4)	42 (40.8)	54 (38.6)
Second or more line	25 (67.6)	61 (59.2)	86 (61.4)

Notes: Data presented as n (%) unless stated otherwise. *Data missing for one patient.
Abbreviations: ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; TKIs, tyrosine kinase inhibitors; SD, standard deviation.

by exon 21 mutation (42.1%). In this cohort, 37 (26.4%) patients were identified with heterozygous (eg, positive) *BIM* deletion polymorphism.

Clinical responses and survival

We analyzed the association between the *BIM* deletion polymorphism status and clinical outcomes. In total, 133 patients were eligible for response assessment. The ORR and DCR in patients with heterozygous *BIM* deletion and treated with an EGFR-TKI were 48.5% (95% CI: 30.8%–66.5%) and 93.9% (95% CI: 79.8%–99.3%), respectively, which were not significantly different from those (63.0% [95% CI: 52.8%–72.4%] and 97.0% [95% CI: 91.5%–99.4%], respectively) observed in patients without the *BIM* deletion (P=0.16 and P=0.60, respectively) (Table 2).

The median follow-up duration was 29 months (range 2–61) for the entire patient cohort. At the time of the data analysis, 125 patients developed disease progression, including 32 (86.5%) in the heterozygous *BIM* deletion group and 93 (90.3%) in the wild-type group. The median PFS was 21 months (95% CI: 12–22) for patients with heterozygous *BIM* deletion polymorphism and 17 months (95% CI: 12–19) for the wild-type population. The Kaplan–Meier curve for PFS showed no significant difference between the heterozygous and wild type population after EGFR-TKIs therapy (P=0.27; Figure 1A). The possible predictive factors of EGFR-TKIs treatment efficacy in terms of PFS were further investigated using prespecified subgroups (Table 3). Each subgroup analysis showed patients with or without the deletion polymorphism did not differ on PFS. Seventy-eight patients (55.7%) died, including 24 (64.9%) in the heterozygous *BIM* deletion group and 54 (52.4%) in the wild-type group. The median OS was 34 months for patients with the *BIM* deletion and 33 months for those without the *BIM* deletion (P=0.61; Figure 1B). The median OS was also not significantly different between the heterozygous *BIM* and wild-type groups (P=0.61; Figure 1B). Furthermore, no significant differences in OS were found between patients with or without the deletion polymorphism with respect to selected patient subgroups (Table 4).

Table 2 Clinical response and adverse reactions after EGFR-TKIs therapy in cohort study

	BIM deletion status		*P*-value
	Heterozygous (N=37)	Wild type (N=103)	
Clinical response, n (%)			
ORR	16 (48.5)	63 (63.0)	0.16
95% CI	30.8–66.5	52.8–72.4	
DCR	31 (93.9)	97 (97.0)	0.60
95% CI	79.8–99.3	91.5–99.4	
Any adverse events, n (%)	18 (48.6)	55 (53.4)	
Rash	16 (43.2)	50 (48.5)	
Diarrhea	7 (18.9)	10 (9.7)	
Liver function impaired	4 (10.8)	13 (12.6)	
Paronychia	2 (5.4)	2 (1.9)	
Epistaxis	0	3 (2.9)	

Abbreviations: CI, confidence interval; DCR, disease control rate; EGFR, epidermal growth factor receptor; ORR, objective response rate; TKIs, tyrosine kinase inhibitors.

Adverse reactions

The study patients after EGFR-TKI treatment had similar adverse reactions of all types regardless of their *BIM* deletion polymorphism status (48.6% [heterozygous] vs 53.4% [wild type]). Rash and diarrhea were the most reported adverse reactions (Table 2).

Meta-analysis of *BIM* deletion status and clinical outcomes

One hundred sixty-nine records were identified in PubMed (from 1965 to November 2015), Embase (from 1965 to November 2015) and Cochrane Library databases according to the search strategy that used key words associated with "Lung cancer", "BIM or (BCL2L11 deletion) or (Bcl-2-like protein 11 deletion)" and "EGFR-mutant or (epidermal growth factor receptor mutation) or EGFR" without language limit. Finally, nine eligible previous cohort studies,[15,23–30] together with our present cohort study, were included for the meta-analysis, which involved a total of 1,317 NSCLC patients with *EGFR* mutations that referred to the efficacy of EGFR-TKIs (gefitinib, erlotinib or afatinib) stratified by *BIM* polymorphism status. The flow chart of study selection is summarized in Figure S1, and the characteristics of all the studies included in the meta-analysis are presented in Table S1. All of the ten studies presented HR of PFS data for pooling; nonetheless, data of ORR, DCR and OS were not available in several distinct studies, and so they were excluded from their respective pooling. Study quality was assessed by using the Newcastle–Ottawa Scale.[31] In general, the overall quality of included cohort studies could be rated as good (data not shown). Funnel plots (Figure S2), Egger's tests and Begg's test with regard to PFS indicated potential publication bias (Egger's P=0.02; Begg's P=0.02). Given the absence of heterogeneity (Q [df=9] =8.78; P=0.46) in the ten included studies, the results of fixed-effects models were used to draw study conclusions.

In the ten included studies, the positive *BIM* polymorphism did not show a completely consistent effect on PFS

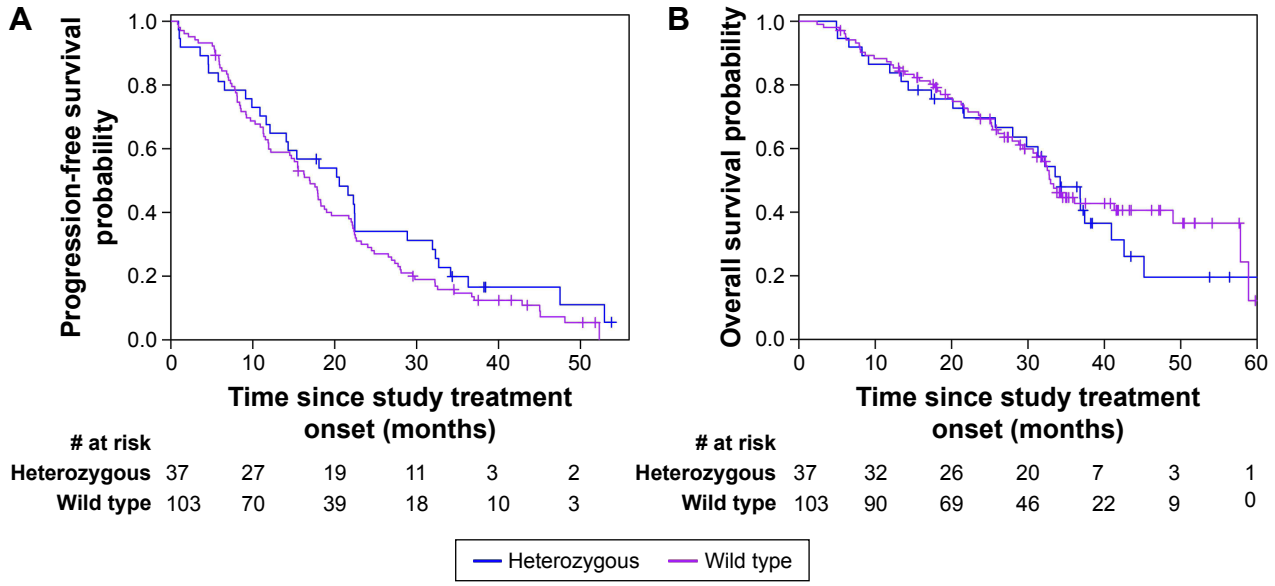


Figure 1 Kaplan–Meier curves for (**A**) progression-free survival and (**B**) overall survival according to *BIM* deletion status.

(Figure 2A). With a large sample size after pooling, however, a significant difference was then found between patients with or without the deletion polymorphism (HR =1.45, 95% CI: 1.06–1.99; *P*=0.02; Figure 2A). However, such difference was not observed in terms of OS (HR =1.23, 95% CI: 0.74–2.05; *P*=0.43; Figure 2B), ORR (RR =0.90, 95% CI: 0.55–1.48; *P*=0.69; Figure 2C) and DCR (RR =0.99, 95% CI: 0.93–1.05; *P*=0.64; Figure 2D).

Discussion

To the best of our knowledge, the predictive role of *BIM* deletion polymorphism in efficacy of EGFR-TKIs among NSCLC patients with *EGFR* mutations remains elusive. *BIM* deletion polymorphism is only found in East Asian descent.[15] A recent study randomly selected a wide range of 6,858 participants and used real-time PCR assay with high-resolution melting to detect *BIM* and *EGFR* mutation. The results showed that

Table 3 Progression-free survival analysis in patient subgroups according to *BIM* deletion status

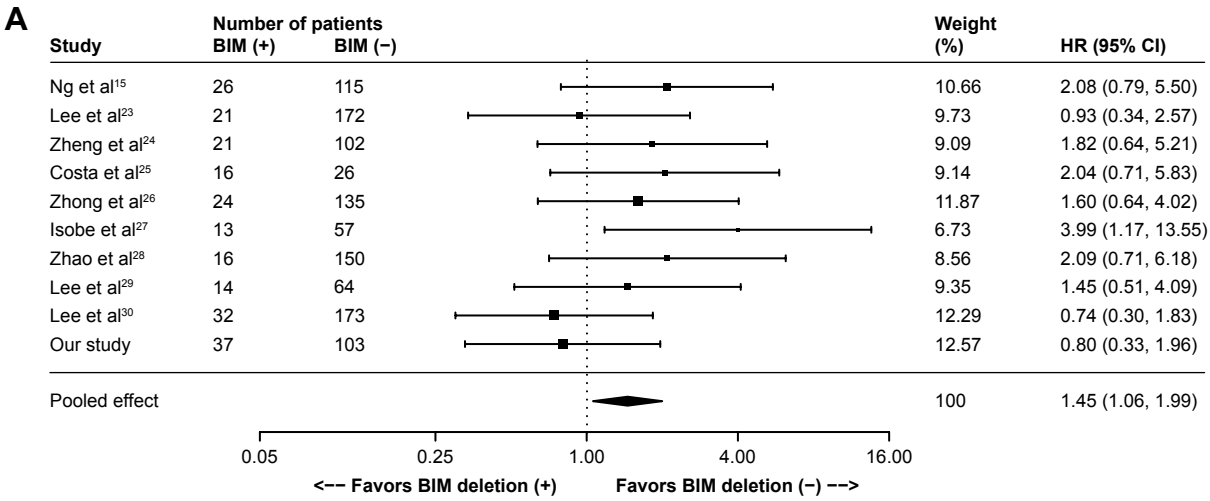
Subgroup	Number of patients	Number of events (%)		Hazard ratio (95% CI)
		Heterozygous	Wild type	
Overall	140	32 (86.5)	93 (90.3)	0.80 (0.53–1.20)
Age (years)				
≤65	103	24 (85.7)	68 (90.7)	0.80 (0.50–1.29)
>65	37	8 (88.9)	25 (89.3)	0.74 (0.33–1.66)
Gender				
Male	46	7 (87.5)	36 (94.7)	0.41 (0.17–1.01)
Female	94	25 (86.2)	57 (87.7)	0.98 (0.61–1.59)
Smoking				
No	106	26 (86.7)	69 (90.8)	0.90 (0.57–1.43)
Yes	34	6 (85.7)	24 (88.9)	0.53 (0.21–1.33)
EGFR mutation				
Exon 19	73	18 (81.8)	46 (90.2)	0.73 (0.42–1.29)
Exon 21	59	12 (100)	42 (89.4)	1.16 (0.61–2.21)
Others	8	2 (66.7)	5 (100)	0.49 (0.09–2.67)
Prior chemotherapy				
No	39	7 (100)	30 (93.8)	1.00 (0.42–2.43)
Yes	101	25 (83.3)	63 (88.7)	0.74 (0.46–1.17)
EGFR-TKIs treatment				
First line	54	11 (91.7)	39 (92.9)	0.96 (0.48–1.92)
Second or more line	86	21 (84.0)	54 (88.5)	0.73 (0.44–1.22)

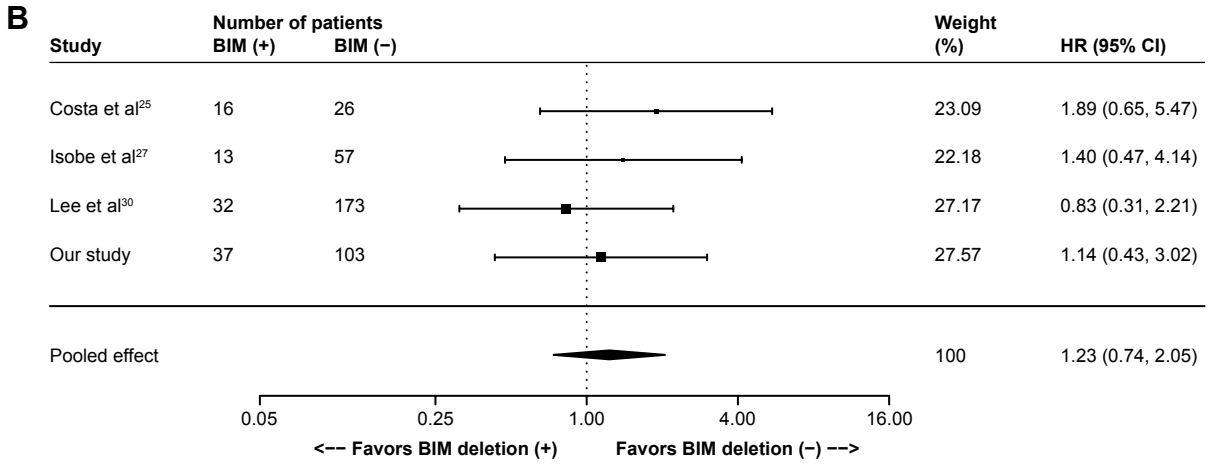
Abbreviations: CI, confidence interval; EGFR, epidermal growth factor receptor; TKIs, tyrosine kinase inhibitors.

Table 4 Overall survival analysis in patient subgroups according to *BIM* deletion status

Subgroup	Number of patients	Number of events (%)		Hazard ratio (95% CI)
		Heterozygous	Wild type	
Overall	140	24 (64.9)	54 (52.4)	1.14 (0.70–1.84)
Age (years)				
≤65	103	17 (60.7)	38 (50.7)	1.18 (0.66–2.09)
>65	37	7 (77.8)	16 (57.1)	0.94 (0.38–2.32)
Gender				
Male	46	5 (62.5)	25 (65.8)	0.59 (0.22–1.57)
Female	94	19 (65.5)	29 (44.6)	1.64 (0.91–2.95)
Smoking				
No	106	20 (66.7)	36 (47.4)	1.52 (0.87–2.63)
Yes	34	4 (57.1)	18 (66.7)	0.50 (0.16–1.50)
EGFR mutation				
Exon 19	73	14 (63.6)	21 (41.2)	1.49 (0.76–2.93)
Exon 21	59	8 (66.7)	30 (63.8)	0.87 (0.40–1.91)
Others	8	2 (66.7)	3 (60.0)	1.21 (0.16–9.34)
Prior chemotherapy				
No	39	6 (85.7)	18 (56.3)	1.44 (0.57–3.67)
Yes	101	18 (60.0)	36 (50.7)	1.06 (0.60–1.86)
EGFR-TKIs treatment				
First line	54	10 (83.3)	24 (57.1)	1.56 (0.74–3.28)
Second or more line	86	14 (56.0)	30 (49.2)	0.98 (0.52–1.86)

Abbreviations: CI, confidence interval; EGFR, epidermal growth factor receptor; TKIs, tyrosine kinase inhibitors.





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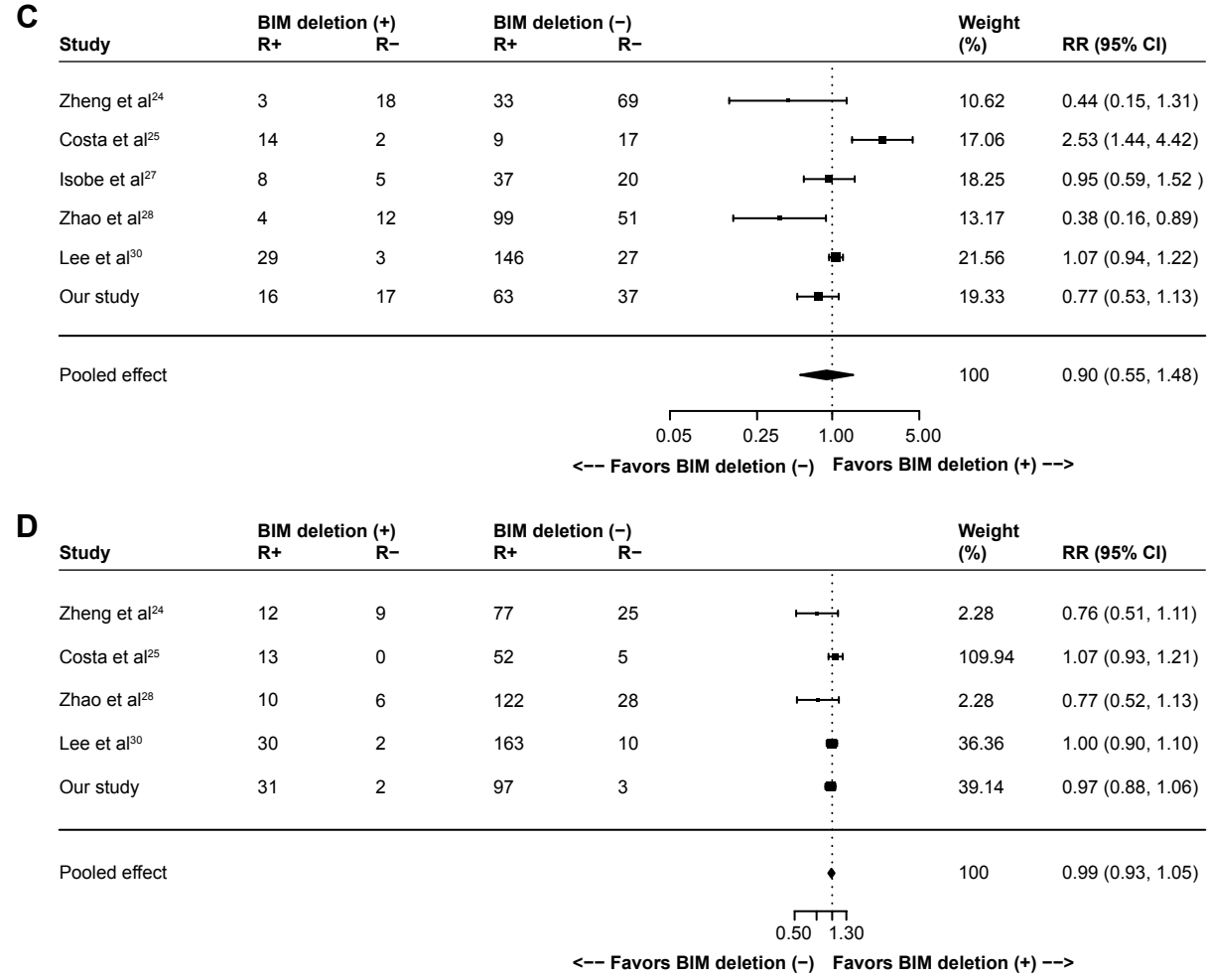

Figure 2 Meta-analyses of (**A**) PFS, (**B**) OS, (**C**) ORR and (**D**) DCR according to *BIM* deletion status in *EGFR*-mutant non-small-cell lung cancer patients receiving EGFR-TKIs. (**C** and **D**) R+ represents responders and R− represents nonresponders.

Abbreviations: CI, confidence interval; DCR, disease control rate; EGFR, epidermal growth factor receptor; HR, hazard ratio; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; RR, relative risk; TKIs, tyrosine kinase inhibitors.

there were four outcomes of *BIM*: non-detection of 2,903 bp *BIM* (NA), non-deletion of 2,903 bp *BIM* (homozygous non-deletion-type DNA, II), 2,903 bp deletion *BIM* (homozygous deletion-type DNA, DD) and heterozygote (ID).[32] We conducted our present study in the People's Republic of China to investigate whether the *BIM* polymorphism status would affect clinical efficacy of EFGR-TKIs and prognosis of NSCLC patients with *EGFR* mutations treated with EFGR-TKIs. Furthermore, we included all of the eligible cohort studies or appropriate subgroups of cohort studies in our meta-analysis to achieve an adequate sample size to draw a reliable conclusion. The present exploratory cohort study did not show positive *BIM* deletion was associated with poorer clinical outcomes in advanced and metastatic NSCLC patients after EGFR-TKIs treatment. With a substantially expanded sample size (n=1,317) in the current meta-analysis, however, the positive *BIM* deletion displayed significant predictive effects on shorter PFS (P=0.02), while it failed to demonstrate significant difference regarding the other three common clinical outcome measures OS (P=0.43), ORR (P=0.69) and DCR (P=0.64).

In our cohort, the positive *BIM* deletion polymorphism occurred in 37 (26.4%) patients, which is relatively higher than the rate (9.6%–20%) reported in other cohort studies included for meta-analysis,[15,23,24,26–30] except for one study which has also quantitatively reported low/intermediate *BIM* mRNA expression.[25] The characteristics of the patients at baseline indicated that our cohort patients with heterozygous *BIM* deletion polymorphism were likely associated with marginally better prognosis factors in terms of younger age, less smoking, better performance status, less metastasis and earlier disease stage as of TKIs treatment onset. These slight inequalities of distribution may partially contribute to the estimated HR of <1 observed for PFS (HR =0.80; with vs without *BIM* deletion polymorphism). Even so, similar observation of variants was reported in two published

studies conducted in Korea (HR =0.74, 95% CI: 0.30–1.83; HR =0.93, 95% CI: 0.34–2.57, respectively).[23,30] The authors of these studies and Chinese counterparts[9] discussed these findings using the following potential arguments: (1) uncertainties may be by chance due to small size of included study patients; (2) they did not consider other proapoptotic Bcl-2 family members such as BAX, BAK, and other BH3-only proteins including BAD and PUMA which might be key players in the apoptotic response in oncogene-addicted cancer; (3) unconsidered concomitant genetic alterations beyond *EGFR* mutations could conceivably accelerate or delay cancer progression; and (4) *BIM* RNA levels in treatment-naïve tissue were not measured; these measurements could be helpful for better understanding of the meaning of *BIM* deletion in patients with *EGFR*-mutant NSCLC. All of these highlighted points were echoed in our current study indeed. Subject to limited number of included studies and data availability, our study did not further analyze the role of *EGFR* subtypes with *BIM* polymorphism in predicting efficacy of EGFR-TKIs, either. Nevertheless, we analyzed toxic effects and obtained similar findings, with rash and diarrhea being the most common adverse reactions as in the EGFR-TKI group of the randomized control trials.[4,5,7,8]

Several prior studies[15,24–29] reported that patients with *BIM* deletion polymorphism had significantly shorter PFS after EGFR-TKI treatment than did patients without *BIM* deletion polymorphism. As a result, our meta-analysis of these studies also reflected this finding. Although several previous meta-analyses[9,33–36] mentioned similar findings regarding PFS, the current pooled analysis containing more eligible studies further provided a possibility to analyze other clinical outcome measures. As a comprehensive meta-analysis of PFS, OS, ORR and DCR with the largest sample size to date, our study provided a more reliable answer regarding the impact of *BIM* polymorphism status on treatment efficacy of EGFR-TKIs in advanced and metastatic NSCLC patients with *EGFR* mutations.

Despite the comprehensive findings, there exist several limitations in our cohort study and meta-analysis. First, unlike randomized controlled trials, this present observational study, especially of small sample size, predisposes to imbalanced distribution of baseline characteristics. In this case, we did a serial of subgroup analyses to ascertain possibly consistent effect. In addition, the data of the cohort study are from a single hospital in the People's Republic of China, which would potentially limit any extrapolations of the study conclusions. For the part of meta-analysis, reported aggregate data from several cohort studies were used rather than individual patient data, which may not provide robust estimation for the comparative efficacy. Publication bias might exist, although we did citations search without any language limit. Moreover, the quality of meta-analysis was subject to the quality of individual studies included. Second, although the prevalence of *BIM* deletion polymorphism was examined carefully in this study, we did not consider other coexisting genetic alterations beyond that of *BIM* deletion polymorphism. The underlying biology of *EGFR*-mutant NSCLC and tumor prognosis should be complex enough.[3,9,37,38] Therefore, more efforts should be made to investigate the potential mechanisms of the primary and secondary resistance to EGFR-TKIs induced by *BIM* polymorphism and other ones in order to find oriented solutions and develop new therapies.

In summary, our meta-analysis of studies demonstrated that *BIM* deletion polymorphism is associated with shorter PFS after EGFR-TKIs treatment in advanced NSCLC EGFR-mutant patients than those without *BIM* polymorphism. Even so, additional large multicenter well-designed cohort studies comprising essential *BIM* gene alteration and other concurrent genetic alterations are warranted to uncover more underlying biology of *EGFR*-mutant NSCLC used for predicting clinical prognosis in the future. This further clarification will provide benefits for new drug development in the relevant therapeutic area.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

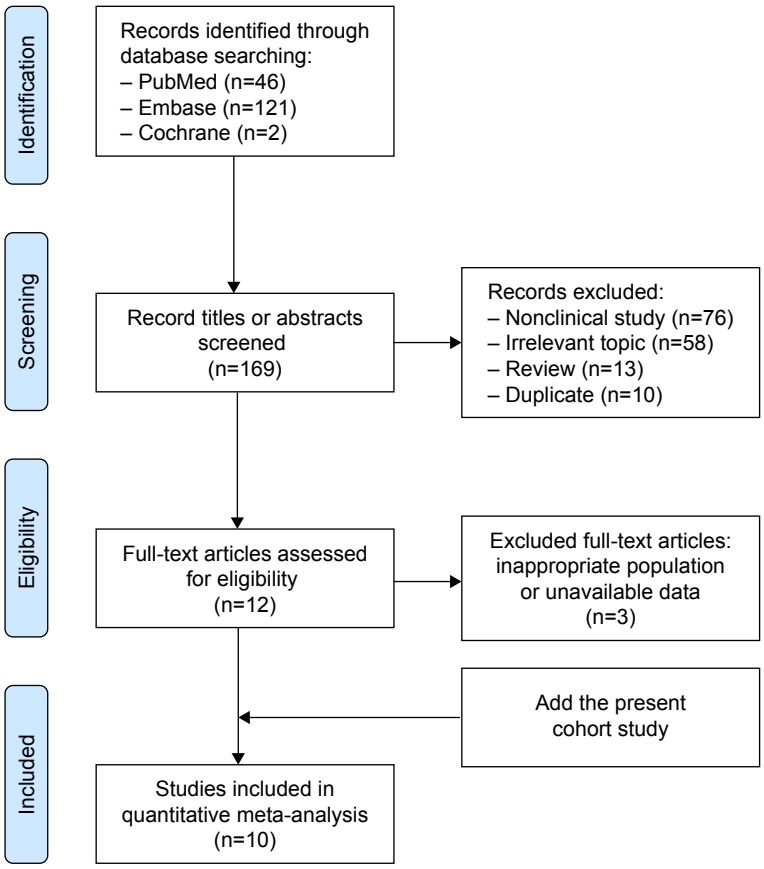

Figure S1 PRISMA flow diagram of the studies search and selection process.
Abbreviation: PRISMA, preferred reporting items for systematic reviews and meta-analyses.

Table S1 Characteristics of cohort studies included in meta-analyses

Study, country	EGFR-TKIs; n (%) as first line	Population; clinical stage	Pathological type (n)	Specimen; method	*BIM* deletion rate, n (%)	ORR, n (%)	Median PFS (months, with vs without BIM deletion)	Median OS (months, with vs without BIM deletion)	Adjusted covariates for hazard ratio
Ng et al,[1] Singapore, Japan	Gefitinib or erlotinib; 93 (66.0)	Patients with EGFR-NSCLC; III/IV/recurrent	AC (128); BAC (4); others (9); total (141)	Peripheral blood or biopsy slides and blocks; DNA polymorphism	26 (18.4)	NR	6.6 vs 11.9	NR	Age, gender, histology, smoking history, type of EGFR mutation by exon and specific mutation, stage, first- or second-line TKI therapy, race, country, TKI type and ECOG status
Lee et al,[2] Korea	Gefitinib or erlotinib; 67 (34.0)	Patients with NSCLC harboring EGFR-activating mutations; IIIB/IV/postoperative relapse	AC (191); ASC (1); NSCLC, NOS (5); total (197)	Tumor tissue; DNA polymorphism	21 (10.9)	154 (77.7)	11.9 vs 11.3	NR	NR
Zheng et al,[3] People's Republic of China	Gefitinib or erlotinib; 0	Patients with advanced NSCLC; IIIB/IV	AC (97); others (26); total (123)	Peripheral blood; DNA polymorphism	21 (17.1)	36 (29.3)	3.5 vs 6.0	NR	Age, gender, histology, smoking history, stage, line of TKI therapy, TKI type and performance status
Costa et al,[4] European	Erlotinib; 50 (100)	Patients with advanced EGFR-mutation-positive NSCLC; IIIB (malignant effusion)/IV/unknown (n=1)	AC (47); others (3); total (50)	Tumor tissue; mRNA expression	Low (<1.83) or intermediate (1.83–2.96) in 53 (64.0) and high (≥2.96) in 30 (36.1)	28 (56.0)	7.2 vs 12.9	20.8 vs 24.5	Potential risk factors as covariates
Zhong et al,[5] People's Republic of China	Gefitinib or erlotinib; overall 35.5%	Patients with advanced EGFR-mutation-positive NSCLC; overall – IIIa (4.5); IIIb (7.6); IV (78.7)	AC (159)	Patient blood samples; DNA polymorphism	Overall, 15.5%	Overall, 24.5%	7.3 vs 9.5	21.9 vs 21.9 (overall)	NR
Isobe et al,[6] Japan	Gefitinib or erlotinib; 70 (100)	Patients with EGFR-mutation-positive NSCLC; IV/recurrent	AC (65); SCC (7); total (72)	Peripheral blood; DNA polymorphism	18.6	64.30	7.5 vs 17.6	38.9 vs 45.1	Sex, bone metastasis and smoking history
Zhao et al,[7] People's Republic of China	Gefitinib or erlotinib; 69 (41.6)	Patients with activating EGFR mutations – NSCLC; IIIB/IV	AC (140); SCC (8); ASC (9); others (9); total (166)	Tumor tissue; DNA polymorphism	9.6	62.0	4.7 vs 11.0	NR	Age, gender and exon 19 deletion vs L858R
Lee et al,[8] People's Republic of China	Gefitinib, erlotinib and afatinib; overall 153 (75)	Patients with activating EGFR mutations – NSCLC; IIIB/IV	Overall: AC (189); non-AC (12); unspecified (3)	Peripheral blood; DNA polymorphism	20.0	51.0	7.4 vs 9.4	18.3 vs 24.9	Age, gender, EGFR mutation and non-AC
Lee et al,[9] Korea	Gefitinib or erlotinib; 68 (33)	Patients with EGFR-mutant NSCLC who received EGFR-TKIs; IIIB/IV/postoperative relapse	AC (203); SCC (2); total (205)	Peripheral blood; DNA polymorphism	15.6	85.0	11.9 vs 10.9	31.2 vs 30.3	Age, gender, smoking history, performance status, pathology, stage, number of metastases, type of EGFR mutation, EGFR-TKIs type, and line of EGFR-TKIs

(*Continued*)

Table S1 (*Continued*)

Study, country	EGFR-TKIs; n (%) as first line	Population; clinical stage	Pathological type (n)	Specimen; method	*BIM* deletion rate, n (%)	ORR, n (%)	Median PFS (months, with vs without **BIM** deletion)	Median OS (months, with vs without **BIM** deletion)	Adjusted covariates for hazard ratio
Present study, People's Republic of China	Gefitinib or erlotinib; 54 (38.6)	Patients with EGFR-mutant NSCLC who received EGFR-TKIs; IIIB/IV	AC (128); others (12); total (140)	Peripheral blood; DNA polymorphism	26.4	56.4	20.6 vs 17.0	34.2 vs 33.0	None; prespecified subgroup analyses done

Abbreviations: AC, adenocarcinoma; ASC, adenosquamous carcinoma; BAC, bronchioalveolar carcinoma; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; NOS, not otherwise specified; NR, not reported; NSCLC, non-small-cell lung cancer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; SCC, squamous cell carcinoma; TKIs, tyrosine kinase inhibitors.

Figure S2 Funnel plot of hazard ratio for PFS and standard error of hazard ratio.
Abbreviation: PFS, progression-free survival.

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