

Meta-analysis of the impact of de novo and acquired *EGFR* T790M mutations on the prognosis of patients with non-small cell lung cancer receiving EGFR-TKIs

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Purpose: The purpose of this meta-analysis was to explore the influences of pretreatment de novo and posttreatment-acquired epidermal growth factor receptor (*EGFR*) T790M mutations in patients with advanced non-small cell lung cancer (NSCLC) who had received tyrosine kinase inhibitors (TKIs).

Methods: We searched PubMed, Embase, and the China National Knowledge Infrastructure database for eligible literature. Data were extracted to assess the hazard ratios (HRs) for progression-free survival (PFS), overall survival (OS), and post-progression survival (PPS) and the relative ratios (RRs) for objective response rate (ORR).

Results: This meta-analysis included 22 studies comprising 1,462 patients with NSCLC who harbored activating *EGFR* mutations and were treated with EGFR-TKIs. Compared to pretreatment T790M mutation-negative NSCLC, pretreatment T790M mutation-positive NSCLC was associated with decreased PFS (HR 2.23, $P < 0.001$) and OS (HR 1.55, $P = 0.003$). A trend toward significance of worsening ORR (RR 0.86, $P = 0.051$) was evident. The acquired T790M mutation was correlated with improved PFS (HR 0.75, $P = 0.006$) and PPS (HR 0.57, $P < 0.001$), compared to patients without the T790M mutation who progressed after EGFR-TKI treatment. There were no significant differences in OS or ORR between patients with acquired T790M mutation-positive and T790M mutation-negative NSCLC. However, in the tumor tissue rebiopsy subgroup, patients with acquired T790M mutation had improved OS (HR 0.60, $P < 0.001$) compared to T790M mutation-negative patients. In the plasma ctDNA subgroup, acquired T790M mutation decreased the OS (HR 1.87, $P < 0.001$).

Conclusion: Pretreatment T790M mutation was associated with worse PFS and OS in patients with advanced NSCLC treated with EGFR-TKIs, while acquired T790M mutation was associated with longer PFS and PPS than T790M mutation-negative NSCLC. The effects on OS were different between acquired T790M mutation detected from rebiopsy of tumor tissue and that detected from plasma ctDNA.

Keywords: epidermal growth factor receptor, T790M, non-small cell lung cancer, pretreatment, mutation

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Background

Non-small cell lung cancer (NSCLC) accounts for more than 85% of lung cancers; half of the cases of NSCLC are classified as adenocarcinoma. Approximately 30%–50% of Asian and 10%–17% of Caucasian patients with lung adenocarcinoma harbor activating epidermal growth factor receptor (*EGFR*) mutations.^{1,2} EGFR-tyrosine

kinase inhibitors (TKIs) such as gefitinib and erlotinib are the preferred treatment for patients with activating *EGFR* mutations (a deletion in exon 19 [19del] and a point mutation in exon 21 leading to substitution of leucine for arginine at position 858 [L858R]). Treatment with EGFR-TKIs achieves a significantly improved objective response rate (ORR) of 60%–80% and a progression-free survival (PFS) of 9–13 months, which are significantly improved outcomes compared to those achieved with chemotherapy.^{3,4}

Nevertheless, most patients who initially respond to EGFR-TKIs will eventually acquire resistance. Approximately 50%–60% of the cases of resistance are mediated by a secondary T790M mutation (ie, a threonine-to-methionine substitution at amino acid position 790 in exon 20 [T790M]). The T790M mutation can induce steric hindrance to EGFR-TKIs and increase the affinity of the receptor to adenosine triphosphate, relative to its affinity to EGFR-TKIs, which abolishes the effect of EGFR-TKIs.⁵ AZD9291 (osimertinib [Tagrisso]) is the only third-generation EGFR-TKI approved by the US Food and Drug Administration for the treatment of acquired T790M mutation-positive advanced NSCLC after secondary resistance to first- or second-generation EGFR-TKIs. Significant improvements in PFS and ORR were observed in a phase I/II study of this drug.^{6–8} Some retrospective studies have observed that patients who experienced disease progression with or without acquired T790M mutation after EGFR-TKI therapy might have different prognoses.^{9–22} However, low rebiopsy rates and low sensitivities of detection methods after acquired resistance to EGFR-TKIs are challenging for clinical practice. Therefore, the development of noninvasive rebiopsy samples, such as plasma circulating tumor DNA (ctDNA), and high-sensitivity detection methods, such as digital polymerase chain reaction and next generation sequencing, is essential for monitoring dynamic changes in genes and selecting appropriate treatment strategies. Recently, the detection of *EGFR* mutations using plasma ctDNA and polymerase chain reaction-based or next generation sequencing methods has been confirmed as a feasible alternative strategy, if tumor tissue is not available. A moderate concordant rate of 65% in E20 T790M mutations between tumor and plasma ctDNA has been reported; this contrasts the high concordant rate of 90% in E19del and E21 L858R mutations.^{23,24}

Recently, researchers have also explored the relationship between prognosis and pretreatment T790M mutation.^{25–32} Increasing evidence has indicated that T790M may exist at a low frequency within the tumor cells before EGFR-TKI treatment and may become the dominant clone only after drug selection pressure of EGFR-TKI treatment.²⁵

Although reliable and widely accepted methods for detecting *EGFR* T790M mutation status have not yet been established, some researchers have attempted to detect T790M mutation before EGFR-TKI treatment using different assays with sensitivities ranging from 0.001% to 0.4%.^{25–32} This meta-analysis explored the influences of acquired T790M mutation following EGFR-TKI treatment and de novo T790M mutation prior to EGFR-TKI treatment on survival and prognosis in patients with advanced NSCLC who had activating *EGFR* mutations.

Methods

Literature search

PubMed, Embase, China National Knowledge Infrastructure database, and abstracts from major scientific meetings were searched for relevant articles published up to July 5, 2016. The following search terms were used: 1) lung cancer OR non-small cell lung cancer OR NSCLC; 2) T790M; and 3) progression-free survival (PFS) OR overall survival (OS) OR progression. The computer searches were supplemented with a manual search of the references listed in all retrieved review articles, primary studies, and meeting abstracts.

Study selection

Eligible studies for the pretreatment T790M group met several criteria. First, patients were confirmed to have advanced or recurrent NSCLC with activating *EGFR* mutations (19del or L858R mutation), and the status of the T790M mutation was detected before treatment with single-agent EGFR-TKI, that is, erlotinib or gefitinib (there was no limitation to the detection method). In the studies, EGFR-TKIs must have been used for the first time. Also, the study must have contained PFS or OS outcome data based on T790M mutation status; the corresponding hazards ratios (HRs) and 95% confidence intervals (CIs) could be directly obtained or calculated. Finally, PFS was defined as the time from the start of EGFR-TKI treatment to the first disease progression or death from any reason without progression; OS was defined as the time from the start of EGFR-TKI treatment or first diagnosis to the date of death by any cause or the date patients were last known to be alive. In all of the studies, the prevalence of T790M mutation was higher than 10%.

Eligible studies for the posttreatment-acquired T790M group met several criteria. First, patients were confirmed to have advanced or recurrent NSCLC before treatment with single-agent EGFR-TKI (erlotinib or gefitinib), and acquired resistance to EGFR-TKI was established according to the Jackman criteria³³ (ie, patients who were *EGFR* wild-type

or *EGFR* status unknown had an objective response [according to RECIST criteria] to *EGFR*-TKIs or had a period of durable stable disease [≥ 6 months] and eventually developed acquired resistance to *EGFR*-TKIs). Also, the status of the T790M mutation was detected after resistance to *EGFR*-TKIs following treatment with single-agent *EGFR*-TKI, that is, erlotinib or gefitinib (there was no limitation to the detection method). The study must have contained PFS, OS, or post-progression survival (PPS) outcome data; HRs and the corresponding 95% CIs for PFS, OS, and PPS based on T790M mutation status could be acquired or calculated. There was no upper limit for the number of lines of chemotherapy. Finally, PFS was defined as the time from the start of *EGFR*-TKI treatment to the first disease progression or death from any reason without progression; PPS was defined as time from the date of the first progression according to RECIST criteria version 1.1 to the second progression or death; and OS was defined as the time from the start of *EGFR*-TKI treatment to the date of death by any cause or the date patients were last known to be alive. In all of the studies, the prevalence of T790M mutation was higher than 10%.

Studies were excluded if they mentioned the use of third-generation *EGFR*-TKIs, repeated published studies, or included patients with small cell lung cancer. Studies were also excluded if patients simultaneously received other therapies or multiple targeted drug combinations. Finally, studies in which the data were insufficient and unable to meet the inclusion criteria were excluded.

Data extraction and quality assessment

The primary outcomes were PFS and OS, and the secondary outcomes were PPS and ORR. Two reviewers independently extracted author name, published date, total number of patients, method of *EGFR* detection, T790M mutation status, study outcomes (OS, PFS, PPS, and ORR) and the corresponding HR or relative ratio (RR), and patient characteristics. Discrepancies were discussed with a third investigator to reach an agreement. The Joanna Briggs Institute Prevalence Critical Appraisal Tool was used to assess the quality of the enrolled studies.³⁴

Statistical analysis

All data were analyzed using the STATA 12.0 statistical software. The statistical heterogeneity of the enrolled studies was assessed using the inconsistency index (I^2 statistic). If the I^2 was $>50\%$ indicating significant heterogeneity, a random-effects model was used;³⁵ otherwise, a fixed-effects model was used.³⁶ HRs were extracted from the original

studies or calculated from the reported number of events and the corresponding P -values of the log-rank statistics, as described by Tierney et al.³⁷ The PFS, OS, and PPS were pooled and the results were analyzed according to HR and the corresponding 95% CI; ORR was pooled and the results were analyzed according to RR and the corresponding 95% CI. A P -value <0.05 was considered statistically significant for all analyses. Publication bias was examined with Begg and Mazumdar's rank correlation test³⁸ and Egger's regression asymmetry test.³⁹

Results

Search results

In total, the meta-analysis included 22 studies comprising 1,462 patients according to the inclusion and exclusion criteria. Figure 1 illustrates the selection process.

Study characteristics

The pretreatment T790M mutation group included eight eligible studies involving 538 patients: 212 patients were T790M mutation positive and 326 patients were T790M mutation negative. The studies by Karachaliou et al²⁸ and Rosell et al³¹ might share the same patients, despite the difference in survival-related data. Overall, seven studies including 447 patients reported PFS_{total}-related data, six studies including 420 patients reported PFS_{first-line}-related data, four studies including 298 patients reported OS_{total}-related data,

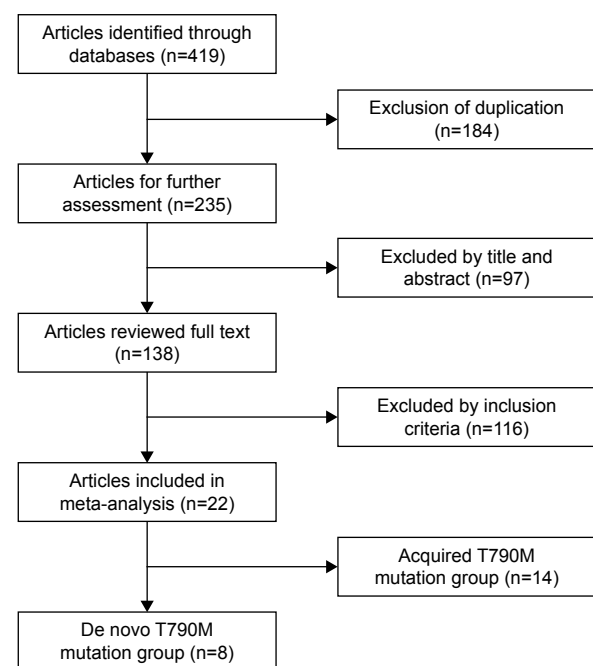


Figure 1 Flowchart of the literature search procedure.

and five studies including 385 patients reported ORR_{total} -related data. Table 1 lists the details of the studies included in the meta-analysis.

The posttreatment-acquired T790M mutation group included 14 eligible studies involving 924 patients: 445 patients were T790M mutation positive and 479 patients were T790M mutation negative. Three studies^{14,17,21} only provided initial data, so the *P*-values and corresponding HRs were calculated. Sorensen et al¹⁷ only reported patients who received second-line treatment with EGFR-TKIs. The study by Uramoto et al²¹ defined “TTP” as time-to-progression after gefitinib therapy, which was different from the definition in this meta-analysis, so TTP-related data were excluded. All the patients who had PPS-related data had rebiopsy tumor tissue specimens and almost received a rechallenge with TKIs (exclusive of third-generation TKIs) or standard chemotherapy regimens. Among the 14 studies included, nine studies including 405 patients reported PFS-related data, nine studies including 696 patients reported OS-related data, four studies including 282 patients reported PPS-related data, and five studies including 217 patients reported ORR-related data. Table 2 lists the details of the studies included in the analysis.

Quality assessment, heterogeneity analysis, and publication bias

The Joanna Briggs Institute Prevalence Critical Appraisal Tool was used to assess the quality of the enrolled studies (Tables 3 and 4). There was moderate heterogeneity in the pooled analysis of survival data (PFS_{total} , $PFS_{first-line}$, OS, and ORR), and a random-effects model was used for final analysis (Table 5). Publication bias was assessed according to Egger's and Begg's regression methods and no significant publication bias was observed ($P>0.05$; Table 5).

Meta-analysis of pretreatment de novo T790M mutation

The rate of pretreatment T790M mutation-positive status ranged from 22.22% to 80% in the included studies. In the pretreatment T790M group, there was no significant heterogeneity between trials in the analysis of OS_{total} ($I^2=23.3\%$, $P=0.271$) or ORR_{total} ($I^2=0.00\%$, $P=0.499$), so a fixed-effects model was used for analysis. A random-effects model was used when moderate heterogeneity existed between trials in the analysis of PFS_{total} ($I^2=60.6\%$, $P=0.019$) and $PFS_{first-line}$ ($I^2=56.5\%$, $P=0.042$). The heterogeneity did not decrease with the sensitivity analysis of the enrolled studies. Compared to T790M-negative patients, T790M-positive patients had

Table 1 Studies of pretreatment T790M mutation

Study	Published year	Number of patients	T790M detection method	Detection limit (%)	Specimen	TKI treatment	EGFR mutation: Del19+/L858R+	TKI treatment line: first/second	Histology: Ade/non-Ade	Prevalence of T790M (%)	Extracted data
Costa et al ²⁶	2014	50	PNA-clamping PCR	0.02	Tumor tissue	Erlotinib	33/17	50/0	47/3	68.00	ORR, PFS
Fujita et al ²⁷	2012	35	CH	0.01	Tumor tissue	Gefitinib	22/13	35/0	35/0	80.00	PFS
Karachaliou et al ²⁸	2013	91	TaqMan assay	0.02	Tumor tissue	Erlotinib	57/34	49/42	70/21	38.46	OS
Lee et al ²⁹	2014	124	MALDI-TOF MS	2.50	Tumor tissue	Gefitinib (n=108), erlotinib (n=16)	78/46	50/74	120/4	25.00	ORR, OS, PFS
Maheswaran et al ³⁰	2008	26	SARMs assay	0.10	Tumor tissue	Gefitinib, erlotinib	15/7/others	26/0	26/0	38.46	ORR, PFS
Rosell et al ³¹	2011	129	TaqMan assay	0.02	Tumor tissue	Erlotinib	81/48	65/64	129/0	34.88	ORR, PFS
Su et al ²⁵	2012	56	MALDI-TOF MS	0.4–2.2	Tumor tissue	Erlotinib (n=17), gefitinib (n=55)	24/32	56/0	53/3	41.07	ORR, OS, PFS
Zhao et al ³²	2016	27	ACB-ARMS PCR	0.01	Tumor tissue	Gefitinib	13/14	9/18	26/1	22.22	PFS, OS

Abbreviations: ACB-ARMS PCR, allele-specific competitive blocker-amplification refractory mutation system polymerase chain reaction; Ade, adenocarcinoma; CH, colony hybridization; EGFR, epidermal growth factor receptor; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PNA-clamping PCR, peptide nucleic acid-clamping polymerase chain reaction; SARMs, scorpion amplification refractory mutation system; TKI, tyrosine kinase inhibitor.

Table 2 Studies of posttreatment-acquired T790M mutation

Study	Number of patients	T790M detection method	Detection limit (%)	Specimens	TKI treatment	EGFR mutation: Del19+/L858R+/other	TKI treatment line: first/second	Histology: Ade/non-Ade	Prevalence of T790M (%)	Extracted data
Hata et al ⁹	78	PNA-LNA clamp PCR assay	Unclear	Tumor tissue	Gefitinib (n=65), erlotinib (=13)	42/33/3	31/47	71/6	33.33	ORR, PPS
He et al ^{10*}	33	Mutant-enriched PCR	0.10	ctDNA	Gefitinib	Del19+/L858R+: 16, others: 17	Unclear	29/4	36.36	ORR, PFS
Isobe et al ¹¹	12	Direct sequence method or PNA-LNA PCR	Unclear	Tumor tissue	Gefitinib	8/4/0	3/9	12/0	33.33	ORR, OS, PFS
Ji et al ¹²	26	Asan-Panel assay	1–5	Tumor tissue	Gefitinib	16/10/0	Unclear	25/1	42.30	OS, PFS
Li et al ¹³	54	PCR-based testing	Unclear	Tumor tissue	Gefitinib (n=15), erlotinib (n=36), icotinib (n=3)	29/24/1	11/43	48/6	53.70	OS, PFS, PPS
Nakamura et al ^{14*}	19	MBP-QP assay	0.30	ctDNA	EGFR-TKIs	9/5/5	Unclear	19/0	52.63	TTP
Oxnard et al ¹⁵	93	LNA-PCR assay	0.1	Tumor tissue	Gefitinib (n=23), erlotinib (n=64)	70/23/0	72/21	93/0	62.36	OS, PPS, TTP
Sakai et al ^{16*}	75	SABER technique	0.30	ctDNA	Gefitinib or erlotinib	Del19+/L858R+: 60 others: 15	Unclear	71/4	28	ORR, PFS
Sorensen et al ¹⁷	23	Allele-specific PCR assay	Unclear	ctDNA	Erlotinib	17/5/1	0/23	23/0	39.13	TTP
Soria et al ¹⁸	247	BEAMing	Unclear	ctDNA	Gefitinib	Unclear	247/0	Unclear	57.49	OS
Sueoka-Aragane et al ¹⁹	58	MBP-QP assay	0.30	ctDNA	EGFR-TKIs	31/27/0	47/11	58/0	39.66	OS
Sun et al ^{20*}	70	DS-PCR and PNA-clamping PCR	0.01	Tumor tissue	Gefitinib (n=38), erlotinib (n=32)	31/18/21	13/57	67/3	51.43	OS, PFS, PPS
Uramoto et al ²¹	19	PCR-based analyses	Unclear	Tumor tissue	Gefitinib	7/12/0	9/10	18/1	42.10	ORR, OS
Zheng et al ²²	117	ddPCR	0.04	ctDNA	EGFR-TKIs	Activating EGFR mutation	29/88	108/9	47	OS

Notes: *Contained EGFR wild-type or EGFR status unknown patients. Asan-Panel, mass spectrometric genotyping technology.

Abbreviations: Ade, adenocarcinoma; ctDNA, circulating tumor DNA; ddPCR, droplet digital polymerase chain reaction; DS-PCR, directional sequencing of polymerase chain reaction; EGFR, epidermal growth factor receptor; LNA, locked nucleic acid; MBP-QP assay, mutation-biased polymerase chain reaction and quenching probe assay; ORR, objective response rate; OS, overall survival; PCR, polymerase chain reaction; PFS, progression-free survival; PNA-LNA clamp PCR, peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp method; PPS, post-progression survival; SABER technique, single allele base extension reaction technique; TKI, tyrosine kinase inhibitor; TTP, time-to-progression.

Table 3 Quality assessment of studies included in the pretreatment T790M mutation group

Study	Costa et al ²⁶	Fujita et al ²⁷	Karachaliou et al ²⁸	Lee et al ²⁹	Maheswaran et al ³⁰	Rosell et al ³¹	Su et al ²⁵	Zhao et al ³²
Was the sample representative of the target population?	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Were the study participants recruited in an appropriate way?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was the sample size adequate?	Not applicable (not designed to explore the prevalence of T790M)							
Were the study subjects and the setting described in detail?	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Was the data analysis conducted with sufficient coverage of the identified sample?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Were objective, standard criteria used for the measurement of the condition?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was the condition measured reliably?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was there appropriate statistical analysis?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Are all important confounding factors/subgroups/differences identified and accounted for?	Yes	No	Yes	Yes	No	Yes	Yes	Yes
Were subpopulations identified using objective criteria?	Yes	No	Yes	Yes	No	Yes	Yes	Yes

significantly shorter PFS_{total} (HR 2.23, 95% CI 1.44–3.45, $P<0.001$; Figure 2A) and OS_{total} (HR 1.55, 95% CI 1.16–2.07, $P=0.003$; Figure 2B) in all treatment lines, as well as shorter PFS_{first-line} (HR 1.91, 95% CI 1.23–2.97, $P=0.004$; Figure 3A) in first-line treatments. Additionally, pretreatment T790M-positive patients had a decreased ORR_{total} compared to T790M-negative patients; there was a trend toward significance (RR 0.86, 95% CI 0.74–1.00, $P=0.051$; Figure 3B). Data showed that pretreatment T790M mutation may be predictive of worse survival in patients with NSCLC.

Meta-analysis of posttreatment-acquired T790M mutation

The rate of acquired T790M mutation-positive status after resistance to EGFR-TKI therapy ranged from 28% to 62.36% in the included studies. In the posttreatment-acquired T790M group, there was no significant heterogeneity between studies in the analysis of PFS ($I^2=30.2\%$, $P=0.1.77$) or PPS ($I^2=41.90\%$, $P=0.16$), so a fixed-effects model was used for analysis. The meta-analysis showed that T790M-positive patients had significantly longer PFS (HR 0.75, 95% CI 0.61–0.92, $P=0.006$; Figure 4A) and PPS (HR 0.57, 95% CI 0.44–0.73, $P<0.001$; Figure 4B) than T790M-negative patients. Heterogeneity was apparent in OS ($I^2=74.4\%$, $P<0.001$) and ORR ($I^2=66.9\%$, $P=0.017$) among the studies included, so a random-effects model was used. The heterogeneity of ORR did not change with the sensitivity analysis. The results showed that acquired T790M mutation-positive patients had similar OS (HR 0.86, 95% CI 0.55–1.36, $P=0.526$; Figure 5A) and ORR (RR 1.21, 95% CI 0.89–1.70, $P=0.256$; Figure 5B) compared to T790M-negative patients. PFS, OS, and ORR were further analyzed in two subgroups on the basis of type of rebiopsy specimen used for detection of *EGFR* mutation: tumor tissue or plasma

ctDNA detection. In the tumor tissue rebiopsy subgroup, acquired T790M mutation significantly improved PFS_{tissue} (HR 0.76, 95% CI 0.59–0.98, $P=0.037$) and OS_{tissue} (HR 0.60, 95% CI 0.48–0.77, $P<0.001$) compared to T790M-mutation negative patients, but it did not increase ORR_{tissue} (HR 0.93, 95% CI 0.85–1.49, $P=0.759$). In the plasma ctDNA subgroup, acquired T790M mutation significantly decreased OS_{ctDNA} (HR 1.87, 95% CI 1.49–2.36, $P<0.001$); no differences in PFS_{ctDNA} (HR 0.73, 95% CI 0.51–1.03, $P=0.072$) or ORR_{ctDNA} (HR 2.02, 95% CI 0.59–6.87, $P=0.262$) were observed between the two subgroups.

Discussion

The results of our meta-analysis indicate that pretreatment T790M mutation had a negative impact on PFS and OS in patients with NSCLC who harbored activating *EGFR* mutations and received EGFR-TKI treatment. In contrast, patients with acquired T790M mutation after resistance to EGFR-TKIs had significantly prolonged PFS and PPS, compared to patients without acquired T790M mutation. The subgroup analysis showed that OS benefit differed on the basis of the type of rebiopsy samples used for acquired T790M detection. In the tissue rebiopsy subgroup, OS was significantly improved, but, in the plasma ctDNA subgroup, OS was significantly inferior in patients with T790M mutation compared to those without T790M mutation.

Recently, highly sensitive genetic detection methods have been developed. Researchers are now largely able to identify pretreatment de novo T790M mutation existing at baseline before EGFR-TKI treatment. This achievement has attracted great interest related to drug sensitivity and survival prognosis. Pretreatment T790M mutation was reported to have no significant associations with the majority of the clinicopathologic characteristics such as age, stage, tumor

Table 4 Quality assessment of studies included in the posttreatment-acquired T790M mutation group

Study	Hata et al ⁹	He et al ¹⁰	Isobe et al ¹¹	Ji et al ¹²	Li et al ¹³	Nakamura et al ¹⁴	Oxnard et al ¹⁵	Sakai et al ¹⁶	Sorensen et al ¹⁷	Soria et al ¹⁸	Sueoka-Aragane et al ¹⁹	Sun et al ²⁰	Uramoto et al ²¹	Zheng et al ²²
Was the sample representative of the target population?	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Were the study participants recruited in an appropriate way?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was the sample size adequate?	Yes	Not applicable (not designed to explore the prevalence of T790M)	Yes	Yes*	Yes	Yes*	Yes	Yes*	Yes	Yes	Yes	Yes	Yes	Yes
Were the study subjects and the setting described in detail?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was the data analysis conducted with sufficient coverage of the identified sample?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Were objective, standard criteria used for the measurement of the condition?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was the condition measured reliably?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was there appropriate statistical analysis?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Are all important confounding factors/subgroups/differences identified and accounted for?	Yes	Yes	No	No	No	No	Yes	Yes	No	Yes	Yes	Yes	No	No
Were subpopulations identified using objective criteria?	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes	Yes	Yes	No	Yes

Note: *No available information about lines of tyrosine kinase inhibitor therapy.

size, or number of metastatic lymph nodes.⁴⁰ However, patients with pretreatment T790M mutation tended to present with higher proportions of never-smoker status and brain metastasis.⁴¹ A previous meta-analysis by Ding et al⁴² indicated that pretreatment T790M mutation predicted inferior PFS in patients with NSCLC who harbored activating *EGFR* mutations and received EGFR-TKI treatment. Nevertheless, the relationship between pretreatment T790M mutation and OS has not been evaluated. This analysis revealed that pretreatment T790M mutation had a negative impact on OS. In a randomized phase III trial, patients with pretreatment T790M mutation-positive NSCLC had decreased PFS compared to patients with T790M mutation-negative NSCLC (9.7 vs 15.8 months, $P=0.0185$) when given erlotinib treatment.²⁶ Among patients receiving chemotherapy, PFS was 6 months for T790M mutation-positive patients and 5.1 months for T790M mutation-negative patients ($P<0.0001$).¹¹ Despite the prediction of poor prognosis for pretreatment T790M mutation, patients harboring activating *EGFR* mutations with or without the T790M mutation had longer PFS and better ORR compared to wild-type *EGFR* mutations when given EGFR-TKI therapy.²⁵

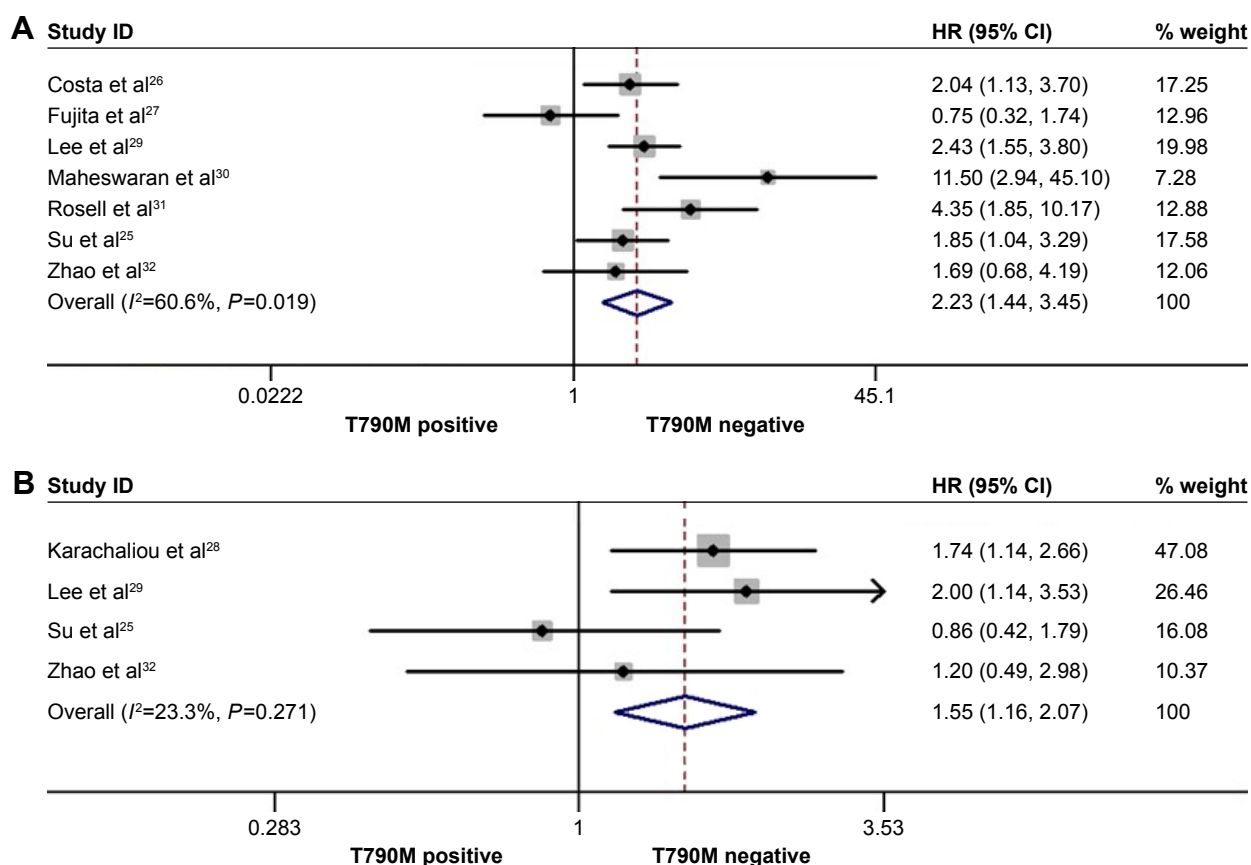
It is not very clear why pretreatment T790M mutation predicts a negative effect on PFS and OS. A preclinical study showed that lung cancer cell lines with double-mutant T790M/L858R exhibited increased phosphorylated EGFR protein expression compared to cells with L858R mutation alone.⁴³ When the T790M mutation was present in cells at a low percentage, the sensitivity to EGFR-TKI was similar to the sensitivity in cells harboring an activating *EGFR* mutation. When the T790M mutation in cells reached a certain percentage, the sensitivity to EGFR-TKIs obviously decreased.^{26,44} Clinical studies have shown that, with an increased abundance of pretreatment T790M mutation, patients had worse clinical outcomes in response to EGFR-TKI therapy.^{26,28,45} However, the European BELIEF study showed that patients with pretreatment T790M mutation benefited more from erlotinib combined with bevacizumab than patients with T790M mutation-negative NSCLC (PFS 16.0 vs 10.5 months).⁴⁶ Similarly, it is unclear whether patients harboring pretreatment T790M mutation will benefit more from third-generation EGFR-TKIs than patients without pretreatment T790M mutation. The ongoing FLAURA study might help to explore this important question.

Until now, research about the predictive role of acquired T790M mutation after EGFR-TKI therapy has been inconsistent. Rebiopsy of tumors after acquired resistance is vital to identify the mechanisms of resistance and choose subsequent

Table 5 Summary of heterogeneity and publication bias in all studies included in the meta-analysis

	Study number	Pooled HR with 95% CI	HRs (P-value)	Heterogeneity (I^2 , %)	Heterogeneity (P-value)	Analysis model	Begg's test (P-value)	Egger's test (P-value)
Pretreatment T790M group								
PFS _{total}	7	2.23 (1.44–3.45)	<0.001	60.60	0.019	Random	0.548	0.606
PFS _{first-line}	6	1.91 (1.23–2.97)	0.004	56.50	0.042	Random	0.452	0.396
OS _{total}	4	1.55 (1.16–2.07)	0.003	23.30	0.271	Fixed	0.734	0.319
ORR _{total}	5	0.86 (0.74–1.00)	0.051	0.00	0.499	Fixed	0.806	0.494
Posttreatment-acquired T790M group								
PFS	9	0.75 (0.61–0.92)	0.006	30.20	0.177	Fixed	0.175	0.113
PFS _{tissue}	5	0.76 (0.59–0.98)	0.037	38.50	0.164	Fixed		
PFS _{ctDNA}	4	0.73 (0.51–1.03)	0.072	38.90	0.178	Fixed		
OS	9	0.86 (0.55–1.36)	0.526	84.30	<0.001	Random	1.000	0.719
OS _{tissue}	6	0.60 (0.48–0.77)	<0.001	2.50	0.401	Random		
OS _{ctDNA}	3	1.87 (1.49–2.36)	<0.001	0.00	0.874	Random		
PPS	4	0.57 (0.44–0.73)	<0.001	44.70	0.143	Fixed	0.308	0.192
ORR	5	1.21 (0.87–1.70)	0.256	66.90	0.017	Random	0.806	0.712
ORR _{tissue}	3	0.93 (0.58–1.49)	0.759	53.00	0.119	Random		
ORR _{ctDNA}	2	2.02 (0.59–6.87)	0.262	88.80	0.003	Random		

Abbreviations: CI, confidence interval; HR, hazard ratio; ORR, objective response rate; ORR_{ctDNA}, objective response rate according to the mutation status assessed by rebiopsy with liquid ctDNA; ORR_{tissue}, objective response rate according to the mutation status assessed by rebiopsy with tissue sample; ORR_{total}, objective response rate for all lines of epidermal growth factor-tyrosine kinase inhibitor treatment; OS, overall survival; OS_{ctDNA}, overall survival according to the mutation status assessed by rebiopsy with ctDNA; OS_{tissue}, overall survival according to the mutation status assessed by rebiopsy with tissue sample; OS_{total}, overall survival for all lines of epidermal growth factor-tyrosine kinase inhibitor treatment; PFS, progression-free survival; PFS_{first-line}, progression-free survival for first-line epidermal growth factor-tyrosine kinase inhibitor treatment; PFS_{total}, progression-free survival for all lines of epidermal growth factor-tyrosine kinase inhibitor treatment; PPS, post-progression survival; PFS_{tissue}, progression-free survival according to the mutation status assessed by rebiopsy with tissue sample; PFS_{ctDNA}, progression-free survival according to the mutation status assessed by rebiopsy with ctDNA.

**Figure 2** Forest plots of pooled HRs and 95% CIs for PFS_{total} (A) and OS_{total} (B) according to pretreatment de novo T790M mutation status.

Note: Weights are from random effects analysis.

Abbreviations: CI, confidence interval; HR, hazard ratio; OS_{total}, overall survival for all lines of epidermal growth factor-tyrosine kinase inhibitor treatment; PFS_{total}, progression-free survival for all lines of epidermal growth factor-tyrosine kinase inhibitor treatment.

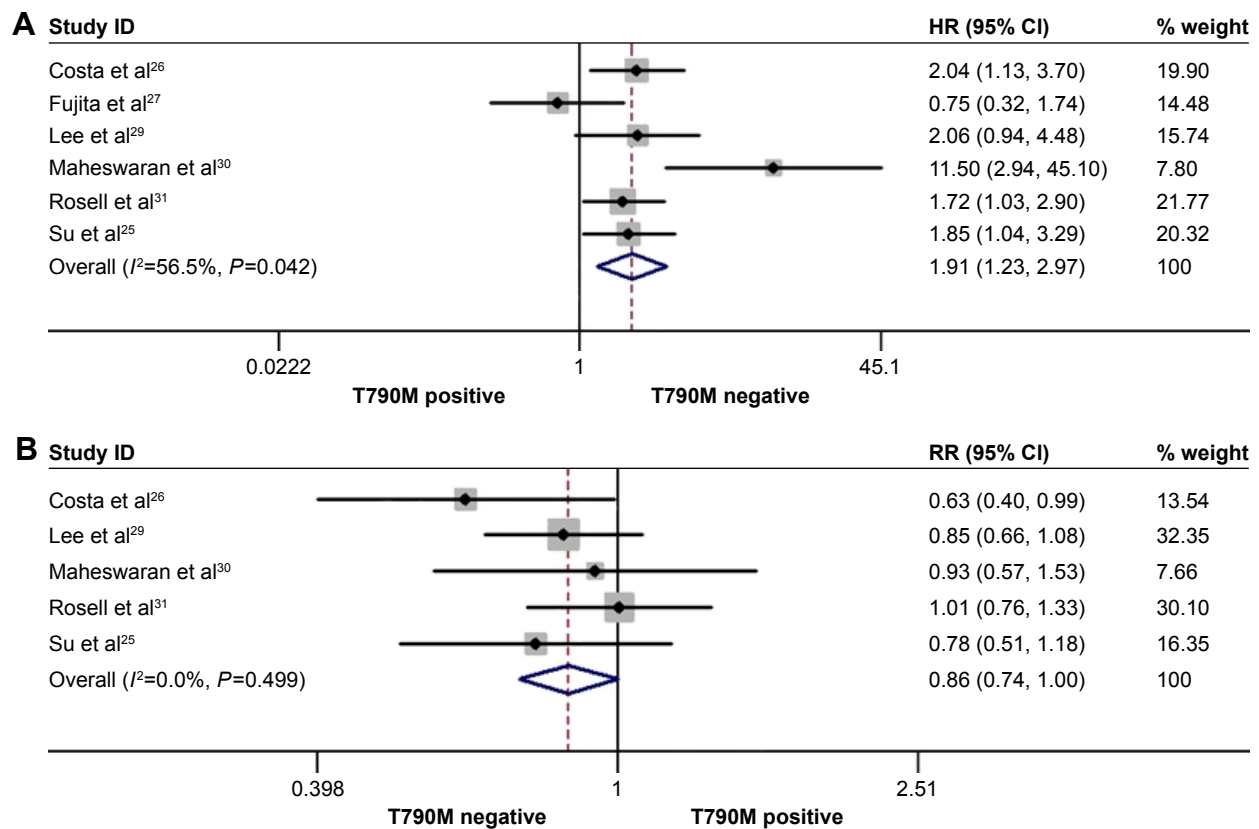


Figure 3 Forest plots of pooled HRs for all PFS_{first-line} (A) and RRs for ORR_{total} (B) according to pretreatment de novo T790M mutation status.

Note: Weights are from random effects analysis.

Abbreviations: CI, confidence interval; HRs, hazard ratios; PFS_{first-line}, progression-free survival for first-line of epidermal growth factor-tyrosine kinase inhibitor treatment; ORR_{total}, objective response rate for all lines of epidermal growth factor-tyrosine kinase inhibitor treatment; RR, relative ratio.

therapy strategies. However, this is often not easily accomplished. In this meta-analysis, acquired T790M mutation after resistance to EGFR-TKI treatment predicted longer PFS, which was contrary to outcomes associated with the

pretreatment T790M mutation. This could be explained by the fact that cells with acquired T790M mutation are characterized by indolent biologic behaviors;^{15,44} other complicated mechanisms of resistance to EGFR-TKIs might lead to

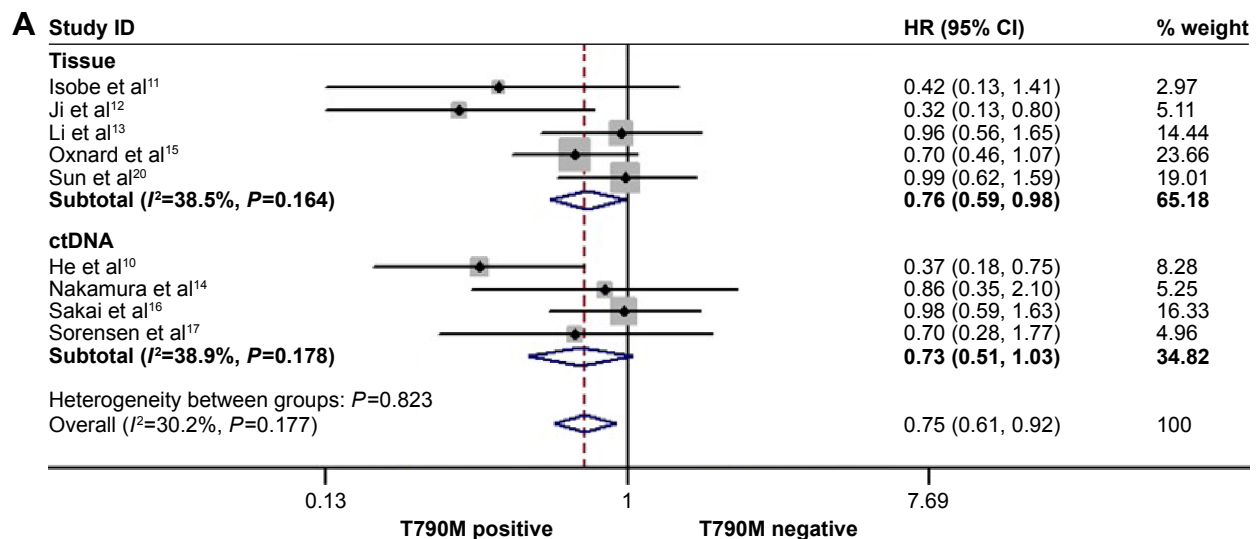


Figure 4 (Continued)

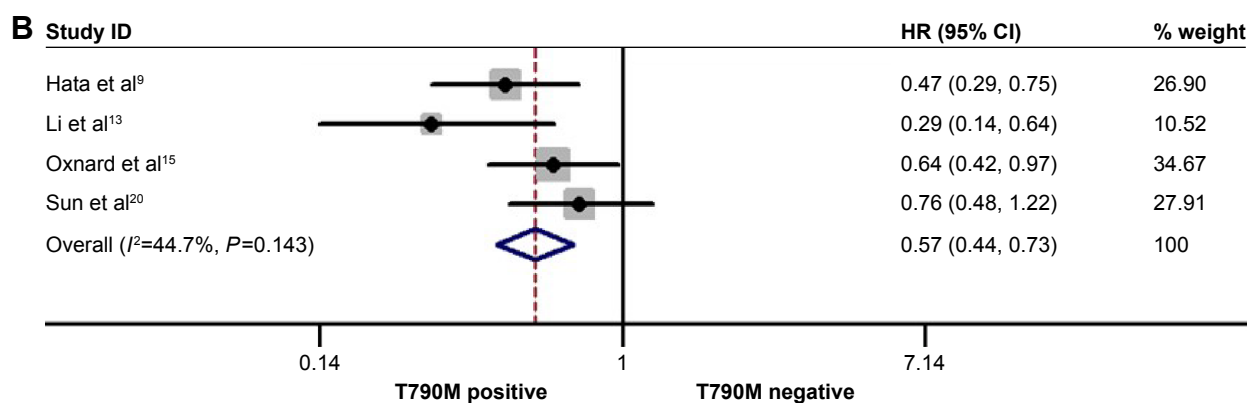


Figure 4 Forest plots of HRs and 95% CIs for PFS (A) and PPS (B) according to acquired T790M mutation status.

Abbreviations: CI, confidence interval; ctDNA, circulating tumor DNA; HRs, hazard ratios; PFS, progression-free survival; PPS, post-progression survival.

patients being more refractory to subsequent treatment. In fact, the low abundance of pretreatment T790M mutation in tumor cells might gradually increase after EGFR-TKI therapy under selective pressure from drugs.^{25,27,47} One

study involving 83 patients with activating *EGFR* mutations showed that patients with an increasing trend for T790M quantity from pretreatment to posttreatment of EGFR-TKIs had superior PFS and OS, compared to patients with a

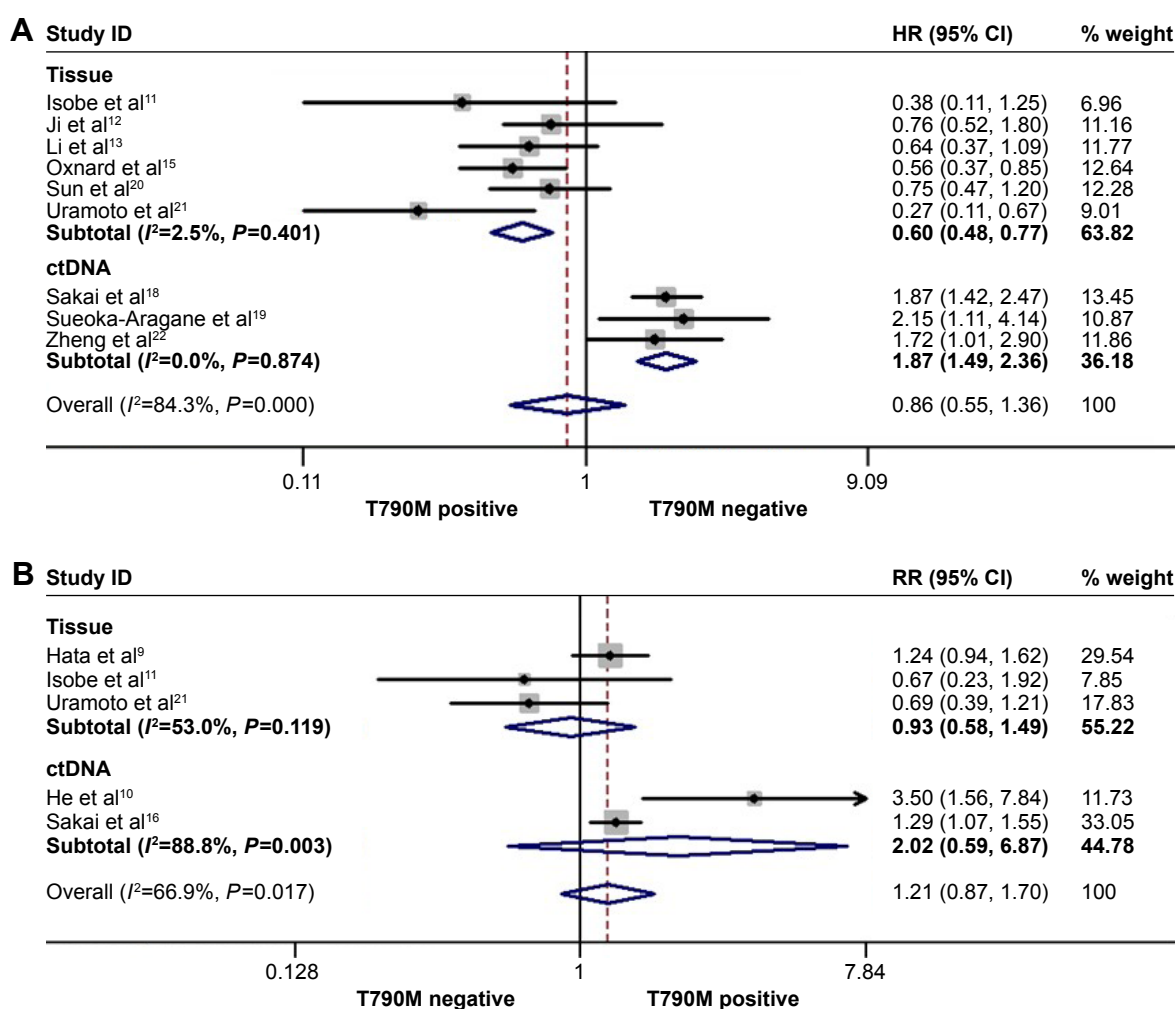


Figure 5 Forest plots of HRs for OS (A) and RR for ORR (B) according to acquired T790M mutation status.

Note: Weights are from random effects analysis.

Abbreviations: CI, confidence interval; ctDNA, circulating tumor DNA; HRs, hazard ratios; ORR, objective response rate; OS, overall survival; RR, relative ratio.

decreasing trend of T790M quantity.^{44,48} Thanks to recent advances in the era of third-generation EGFR-TKIs such as osimertinib, it is better understood that patients with acquired T790M mutation will benefit more from osimertinib treatment than patients without T790M mutation. Similar to resistance to first-generation EGFR-TKIs, acquired resistance to osimertinib is almost inevitable after a progression-free period of approximately 10 months. *EGFR* C797S, L718Q mutation, and amplification of *HER-2*, *MET*, and *ERBB2* were found to be responsible for this resistance. Importantly, another drug, EAI045, has been developed to partially overcome the acquired resistance to AZD9291.⁴⁹ In this analysis, all the included studies neither described the third generation of EGFR-TKIs nor mentioned that patients had ever received third-generation EGFR-TKI treatment. Therefore, our pooled results were not influenced by third-generation EGFR-TKI treatment. We believe acquired T790M mutation, relative to other resistance mechanisms after secondary resistance to EGFR-TKIs, suggests a better prognosis.

Several studies support these results. One study by Kuiper et al⁴⁸ reported that acquired T790M mutation had a positive effect on PFS compared to T790M mutation-negative status after EGFR-TKI resistance (14.2 vs 11.1 months, $P=0.034$); no difference in OS was observed between the two arms (45.9 vs 29.8 months, $P=0.213$). Another study by Yu et al⁵⁰ indicated that patients with acquired T790M mutation had improved PPS compared to T790M mutation-negative patients (1.9 vs 1.6 years, $P=0.015$). However, another study by Otsuka et al⁵¹ showed that acquired T790M mutation had a negative effect on PFS compared to T790M mutation-negative status after EGFR-TKI resistance (3.3 vs 4.1 months, $P=0.048$); no difference in OS was observed between the groups (15.1 vs 13.5 months, $P=0.996$). Interestingly, a randomized, controlled phase III trial (the IMPRESS study) indicated that patients who developed resistance to first-line gefitinib treatment failed to achieve benefits in PFS and OS from continuous gefitinib combined with chemotherapy compared to patients who received chemotherapy alone, regardless of T790M status.¹⁸ Other studies showed patients who progressed without the T790M mutation did benefit from the combination of continuous EGFR-TKIs and chemotherapy.^{9,13,15}

This meta-analysis showed that acquired T790M mutation was not predictive of improved OS. Nevertheless, the subgroup analysis showed that OS was significantly superior in the tissue rebiopsy subgroup, but significantly inferior in the plasma ctDNA subgroup in patients with the T790M mutation compared to those without the T790M mutation.

These findings suggest that high plasma levels of T790M mutation might be associated with an increased tumor burden, as well as tendencies for tumor progression and metastases.^{19,22} Thus, in order to individualize treatment, assessment of T790M status with both qualitative and quantitative analyses may be required. Combined detection of T790M in both tumor tissue and plasma ctDNA is a promising method for screening patients who might be appropriate candidates for osimertinib treatment.^{52,53}

Limitations

There are some limitations to this meta-analysis. Significant heterogeneities were observed among the included studies. First, the enrolled studies used different *EGFR* detection methods with different sensitivities and specificities; these methods likely yielded false-negative and false-positive results.^{54,55} Second, the mutation tended to be heterogeneously distributed within the tumor tissue or plasma and some mutations (especially the T790M mutation) are only present in low proportions.⁵⁶ In addition, the asymmetrical distribution of patient characteristics also influenced the results.

Conclusion

The clinical data included in this meta-analysis indicated that pretreatment T790M mutation was associated with worse PFS and OS in patients with advanced NSCLC who harbored activating *EGFR* mutations and received EGFR-TKI treatment, compared to patients without pretreatment T790M mutation. In contrast, acquired T790M mutation after resistance to EGFR-TKIs was associated with longer PFS and PPS, compared to T790M mutation-negative patients. Despite the fact that no significant difference was observed in OS in the total group, acquired T790M mutation detected from rebiopsy of tumor tissue had a positive effect on OS and mutation detected from plasma ctDNA had a negative effect on OS.

Disclosure

The authors report no conflicts of interest in this work.

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