

# Blood as a Substitute for Tumor Tissue in Detecting *EGFR* Mutations for Guiding *EGFR* TKIs Treatment of Nonsmall Cell Lung Cancer

## *A Systematic Review and Meta-Analysis*

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**Abstract:** Tumor tissues are often absent or insufficient for testing epidermal growth factor receptor (*EGFR*) mutations to guide *EGFR* tyrosine kinase inhibitors (TKIs) treatment of patients with nonsmall cell lung cancer (NSCLC).

We conducted this systematic review and meta-analysis to assess whether blood can be used as a substitute for tumor tissue in detecting *EGFR* mutations.

MEDLINE, EMBASE, and the Cochrane Library were searched for studies that provided data to estimate the accuracy of blood testing against tissue testing in NSCLC patients and/or those directly compared the efficacy of *EGFR* TKIs in *EGFR* mutant and wild-type patients according to sources of specimens.

Sensitivity, specificity, and concordance rate were used as measures of the accuracy. Risk ratio (RR) for objective response and hazard ratio (HR) for progression-free survival (PFS) and overall survival (OS) were used as measures for treatment efficacy. We combined the effects by using the fixed-effects model unless there was evidence of heterogeneity, in which case a random-effects mode was used.

This systematic review included 25 studies with 2605 patients. The pooled overall sensitivity, specificity, and concordance rate were 0.61, 0.90, and 0.79, respectively. Serum showed lower sensitivity (0.56 vs 0.65) but higher specificity (0.95 vs 0.85) and higher concordance (0.86 vs 0.74) than plasma. *EGFR* mutations (exon 19 or 21) in blood were significantly associated with objective response (RR: 4.08; 95% confidence interval [CI] 2.48–6.70), PFS (HR: 0.72; 95% CI 0.64–0.80), and OS (HR: 0.71; 95% CI 0.50–0.99). Importantly, the association of the mutations with the 3 clinical outcomes for serum was similar to that for tumor tissue and higher than that for plasma.

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Blood, in particular serum, is a good substitute when tumor tissue is absent or insufficient for testing *EGFR* mutations to guide *EGFR* TKIs treatment in patients with NSCLC. *EGFR* mutation positivity in blood could be used to recommend *EGFR* TKIs treatment, but the absence of blood positivity should not necessarily be construed with confirmed negativity.

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**Abbreviations:** CI = confidence interval, *EGFR* = epidermal growth factor receptor, HR = hazard ratio, NSCLC = nonsmall cell lung cancer, OS = overall survival, PFS = progression-free survival, QUADAS-2 = Quality Assessment of Diagnostic Accuracy Studies-2, RR = risk ratio, TKIs = tyrosine kinase inhibitors.

## INTRODUCTION

Lung cancer is a leading cause of cancer-related deaths worldwide and some 85% of lung cancer patients were having nonsmall cell lung cancer (NSCLC).<sup>1,2</sup> Gefitinib and erlotinib, 2 tyrosine kinase inhibitors (TKIs) that are targeted at epidermal growth factor receptor (*EGFR*), are widely recommended for advanced NSCLC but only some 10% of patients respond to the treatment.<sup>3–5</sup> Clinical trials have shown that patients with mutations in the kinase domain of the *EGFR* gene are much more likely to respond to *EGFR* TKIs treatment than *EGFR* wild-type patients.<sup>6,7</sup> Testing *EGFR* mutations is now a common practice in selecting patients for *EGFR* TKIs treatment.

However, some two-third of NSCLC patients<sup>8</sup> are already at an advanced stage at the time of diagnosis for which surgical operation is normally not recommended. Biopsy is thus required to obtain tumor tissues for testing *EGFR* mutations.<sup>9</sup> Biopsies can fail in 10% to 50% of patients to obtain sufficient tumor tissues for *EGFR* mutation analysis.<sup>10</sup> Even in well-organized clinical trials, more than half of the patients did not have sufficient tumor tissues for the testing.<sup>11</sup> Surrogate biological samples for *EGFR* mutation testing have been investigated.

The level of circulating DNA in blood has been found to be higher in lung cancer patients than cancer-free patients.<sup>12,13</sup> Most of the excess circulating DNA is believed to be released from dying lung cancer cells at primary and/or metastatic sites.<sup>13</sup> Therefore, blood is a potential substitute for tumor tissues to provide a noninvasive, easily accessible, and repeatedly measurable source of genotypic information that may predict response and prognosis after treatment. *EGFR* mutations have been detected in plasma DNA<sup>14,15</sup> and serum DNA<sup>16,17</sup> and some consistency in *EGFR* mutation status is observed between blood and tumor tissues.<sup>14–17</sup> As a result, *EGFR*

mutations detected in blood may be a good predictor of response to EGFR TKIs treatment.<sup>14,17–20</sup>

We conducted this study to identify and summarize the current best research evidence to evaluate the accuracy of *EGFR* mutations status in blood against that in tumor tissues as the reference and to compare the power of *EGFR* mutations in blood and in tumor tissues in predicting clinical outcomes of EGFR TKIs treatment in patients with NSCLC.

## MATERIALS AND METHODS

### Data Sources and Search Strategy

We conducted a computerized literature search of the Cochrane Library, PubMed, and EMBASE from their inception to June 2013, with different combinations of the following keywords: “non-small cell lung cancer,” “epidermal growth factor receptor,” “mutation,” “plasma,” and “serum.” In addition, we searched the abstracts database of the American Society of Clinical Oncology using the previously mentioned terms. We subsequently manually searched the bibliographies of included studies and recent narrative reviews for additional studies. No language restrictions were applied. We considered both published and unpublished studies for inclusion, including those only published in abstracts.

We included all studies that provided enough raw data to create the  $2 \times 2$  diagnostic tables for *EGFR* mutation status in tumor tissue specimens and blood samples in NSCLC patients and/or those that directly compared the clinical outcomes of EGFR TKIs in *EGFR* mutant and wild-type patients according to sources of specimens. Two reviewers independently reviewed the titles, abstracts, and full texts of all citations that were likely to meet the predefined selection criteria. The discrepancies were resolved by discussion or by consulting with a third reviewer. For duplicate publications, we only included the most recent and complete version of studies.

### Quality Assessment and Data Extraction

Two reviewers independently extracted data using a pre-designed data abstraction form and critiqued the quality of the studies, with a third reviewer contacted in case of disagreement. We constructed  $2 \times 2$  tables that contained the number of true positives (*EGFR* mutations detected in both tumor tissue and blood samples), true negatives (wild-type *EGFR* detected in both tumor tissue and blood samples), false positives (*EGFR* mutations detected in blood samples but not in tumor tissue samples), and false negatives (wild-type *EGFR* detected in blood samples but not in tumor tissue samples). In addition, the following data were extracted from each study: study characteristics (such as first author’s name, year of publication, study design, and number of patients enrolled), patients’ characteristics (such as mean or median of age, percentage of male, smoking status, and histology), *EGFR* mutation testing methods, clinical outcomes of EGFR TKIs treatment stratified by *EGFR* mutation status (such as objective response, progression-free survival [PFS], and overall survival [OS]) and items necessary to assess study quality. We assessed the methodological quality of studies with the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool.<sup>21</sup>

### Statistical Analysis

We used the *EGFR* mutation status in tumor tissue samples as reference standard, and calculated sensitivity, specificity, and concordance rate for each study according to the  $2 \times 2$  tables

mentioned in the previous subsection. The association between the status of *EGFR* mutations and clinical outcomes of EGFR TKIs treatment was measured by risk ratio (RR) for objective response and hazard ratio (HR) for PFS and OS. We combined the *EGFR* mutation rate, sensitivity, specificity, concordance rate, RR, and HR by using the fixed-effects model<sup>22</sup> unless there was evidence of heterogeneity, in which case a random-effects model<sup>23</sup> was used. In the meta-analysis of *EGFR* mutation rate of all exons, we only included the data of direct sequencing, the most commonly used mutation test method, when  $\geq 2$  mutation test methods were used in the same original trials. Heterogeneity was explored by the *Q* test with degree of freedom equal to the number of analyzed studies minus one.<sup>24</sup> A *P* value of  $\leq 0.10$  in the *Q* test is deemed statistically significant and suggests presence of heterogeneity across studies. We performed subgroup analyses to detect the source of heterogeneity according to ethnicity, sex, age, histology, tumor stages, smoking history, type of blood samples, and *EGFR* mutation testing methods, wherever deemed appropriate. We performed funnel plots to assess the possible presence of publication bias and Egger test to assess the symmetry of the funnel plot.<sup>25</sup> We employed a 2-tailed significance level of 0.05 for all the statistical tests except for the assessment of heterogeneity. Data analyses were carried out with STATA Version 13 (STATA Corporation, College Station, TX), Review Manager (RevMan 5.2.9) and MetaAnalyst Version Beta 3.13 (Tufts MedicalCenter, Boston, MA). The study was reported according to the guidelines provided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses report.<sup>26</sup>

## RESULTS

### Study Characteristics

A total of 6261 citations were retrieved from the Cochrane Library, MEDLINE, EMBASE, and other resources. After excluding duplicates and screening of titles and abstracts, 353 citations were left for full-text screening, among which 328 were excluded, leaving 25 studies<sup>14–20,27–44</sup> including 2605 patients who were eligible for inclusion. The details for study selection are indicated in Figure 1.

Of all included studies, 19 studies<sup>14–17,19,27–33,35,37,39,41–44</sup> were carried out mainly in Asians and 6<sup>18,20,34,36,38,40</sup> in Caucasians. The participants in 12 included studies<sup>14–18,20,31–33,35,41,42</sup> were in stages III–IV and 6 studies<sup>19,27,37,40,43,44</sup> in I–IV. Thirteen studies<sup>14,15,18–20,28,31,35–38,42,44</sup> tested *EGFR* mutations in plasma and 12 studies<sup>16,17,27,29,30,32–34,39–41,43</sup> in serum. Direct sequencing and scorpion amplification refractory mutation system (SARMS) were the main methods used for mutation test, which were adopted in 8<sup>16–19,28,30,39,40</sup> and 7<sup>18,29,34–36,38,41</sup> studies, respectively. The detailed baseline characteristics are presented in Table 1.

Overall, the methodological quality of included studies is moderate. In terms of patient selection, 11 studies<sup>14,17,30–34,38,39,41,44</sup> enrolled a consecutive or random sample of patients, 14 studies<sup>14,16,18,29–31,33,35,36,38,41–44</sup> avoided a case–control design, and inappropriate exclusion was avoided in 3 studies.<sup>34,38,44</sup> As for the index test, most of the included studies did not clearly report whether the interpretation of index test results was blinded and if a threshold was prespecified. As for a reference standard, only 2 studies<sup>34,44</sup> clearly stated that the reference standard results were interpreted without knowledge of the results of the index test. In terms of flow and timing, the patients of all studies received the same

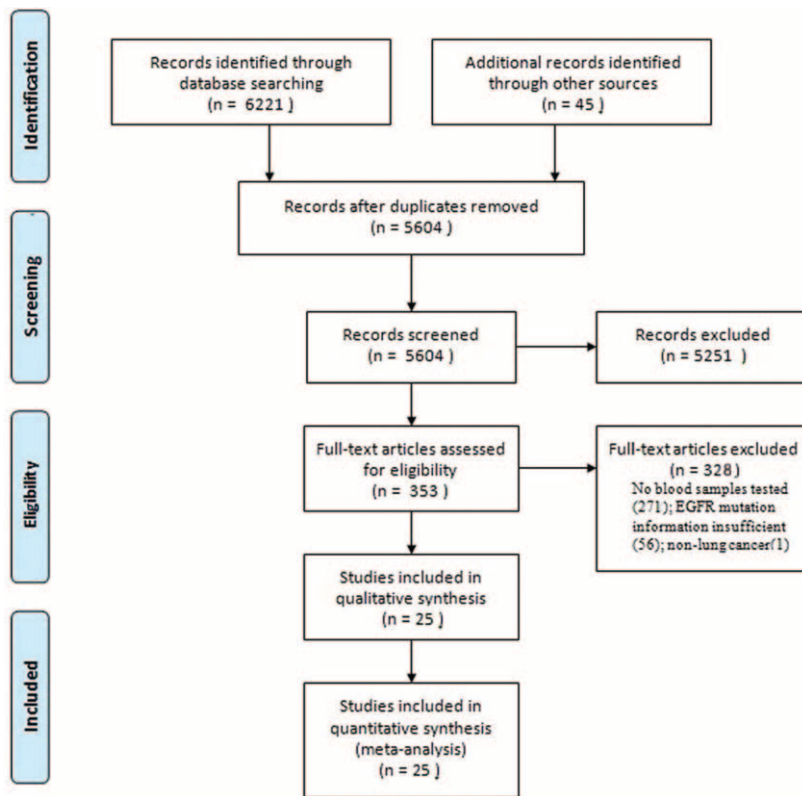


FIGURE 1. Flowchart of study selection.

reference standard, but 15 studies<sup>14–16,18,19,29,34–36,38,40–44</sup> did not include all the study population in the analysis. The quality assessment results are indicated in Supplemental Digital Content—Table 1, <http://links.lww.com/MD/A260>.

### Characteristics of EGFR Mutations in Blood Samples

The number of studies contributed to the meta-analysis of the EGFR mutation rate in blood samples ranged from 3 to 25 (Table 2). The pooled rate of blood EGFR mutations of all exons was 35.0% (95% CI 26.0%–44.0%), with 27.20% (95% CI 16.0%–38.3%) of the mutations in exon 19, 18.8% (95% CI 10.4%–27.2%) in exon 21, 3.9% (95% CI 0.0%–9.2%) in exon 18, and 2.9% (95% CI 0.0%–6.4%) in exon 20.

The EGFR mutation rates of all exons were pooled separately according to ethnicity, sex, smoking status, and age. The mutation rate was 35.8% (95% CI 25.3%–46.2%) in the Asian ethnicity and 6.6% (95% CI 0.0%–13.2%) in the Caucasians ethnicity. The mutation rate in females (43.0%; 95% CI 20.8%–65.2%) was higher than that in males (31.4%; 95% CI 9.1%–53.7%) but the difference was not statistically significant (RR: 0.73; 95% CI 0.37%–1.45%). Smoking status was significantly associated with EGFR mutations in blood, with a higher mutation rate in never smokers than in ever smokers (37.4% vs 11.50%; RR: 2.77; 95% CI 1.52–5.08). No difference was shown in the mutation rate between those aged ≥60 and those aged <60 (44.5% vs 41.0%; RR: 1.00; 95% CI 0.72–1.41). Subgroup analysis by tumor stage (stages I–II vs stages III–IV) was not done due to insufficient data for patients

with stages I–II NSCLC. The pooled mutation rate in patients with stages III–IV NSCLC was 35.9%.

### Consistency of EGFR Mutations in Tumor Tissue and Blood

Table 3 presents the meta-analysis of diagnostic tests for EGFR mutation in blood using EGFR mutation in tumor tissues as the gold standard. Overall, the sensitivity, specificity, and concordance rate were 0.61 (95% CI 0.50–0.71), 0.90 (95% CI 0.85–0.94), and 0.79 (95% CI 0.73–0.84), respectively. There was no major difference in the subgroup analysis for mutations in exons 19 and 21 when compared with the mutations of all exons. As for the subgroup analysis according to the type of sample, the serum was generally associated with lower sensitivity but higher specificity and concordance when compared with plasma. As for mutation test methods, the direct sequencing showed the highest sensitivity (0.70; 95% CI 0.46–0.87) and SARMS showed the highest specificity (0.92; 95% CI 0.81–0.97). Subgroup analysis by tumor stage was not done due to insufficient data for patients with stages I–II NSCLC. The pooled sensitivity, specificity, and concordance rate in patients with stages III–IV NSCLC were 0.80 (95% CI 0.73–0.85), 0.91 (95% CI 0.88–0.94), and 0.88 (95% CI 0.83–0.94), respectively. Of note, the meta-analyses of diagnostic test were generally associated with high heterogeneity.

### Association of EGFR Mutations and Response to EGFR TKIs Treatment

Five studies<sup>16–18,30,41</sup> including 185 patients contributed to the meta-analysis of EGFR mutations (exons 19 and 21) and

TABLE 1. Characteristics of Included Studies

Study	Country	N	Age, Median (Range)	Sex, Male/Female	Race, Asians/Caucasians	Histology, Adenocarcinoma/Nonadenocarcinoma	Never Smokers/ Ever Smoker	Tumor Stage	Mutation Detection Method for Tumor Tissues	Mutation Detection Method for Blood Samples	Type of Blood Samples
Kimura et al <sup>16</sup>	Japan	12	N/A	9/3	12/0	8/4	N/A	IIIb-IV	Direct sequencing	Direct sequencing	Serum
Huang et al <sup>30</sup>	China	20	63 (41-71)	8/12	20/0	13/5	N/A	II-III	Direct Sequencing	Direct sequencing	Serum
Kimura et al <sup>17</sup>	China	42	58 (40-81)	28/14	42/0	31/1	14/28	IIIb-IV	Direct sequencing	Direct sequencing	Serum
Maheswaran et al <sup>36</sup>	United Kingdom	23	N/A	N/A	N/A	N/A	N/A	N/A	SARMS	SARMS	Plasma
Bai et al <sup>14</sup>	China	230	N/A	123/107	230/0	N/A	127/103	IIIb-IV	DHPLC	DHPLC	Plasma
Yung et al <sup>15</sup>	Hong Kong	35	59 (33-91)	20/15	35/0	N/A	N/A	III-IV	Digital PCR, CSGE/sequencing	Digital PCR	Plasma
Mack et al <sup>18</sup>	United States	14	N/A	N/A	N/A	N/A	N/A	IIIb-IV	Direct sequencing, SARMS	Direct sequencing, SARMS	Plasma
He et al <sup>19</sup>	China	134	N/A	85/49	134/0	101/33	N/A	I-IV	ME-PCR, direct sequencing	ME-PCR, direct sequencing	Plasma
Kuang et al <sup>34</sup>	United Kingdom	54	N/A	10/44	N/A	N/A	N/A	N/A	SARMS, direct sequencing	SARMS, direct sequencing	Plasma
Song et al <sup>39</sup>	China	50	N/A	27/23	50/0	24/24	N/A	I-IIIa	Direct sequencing	Direct sequencing	Serum
Brevet et al <sup>20</sup>	United States	31	62 (38-86)	15/16	N/A	30/1	17/14	III-IV	ME-PCR, Sequenom	ME-PCR, Sequenom	Plasma
Jiang et al <sup>32</sup>	China	58	56 (43-80)	40/18	58/0	42/16	22/36	IIIb-IV	ME sequencing	ME sequencing	Serum
Sriram et al <sup>40</sup>	Australia	522	65 (33-85)	345/177	6/498	294/228	35/489	I-IV	ME-PCR, direct sequencing, HRM	ME-PCR, direct sequencing, HRM	Serum
Yasuda et al <sup>43</sup>	Japan	23	68 (48-83)	9/14	23/0	20/1	N/A	I-IV	PNA-LNA	PNA-LNA	Serum
Goto et al <sup>29</sup>	Japan	86	N/A	N/A	86/0	N/A	N/A	N/A	SARMS	SARMS	Serum
Nakamura et al <sup>37</sup>	Japan	39	N/A	19/20	39/0	N/A	21/18	I-IV	WIP-QP, MBP-QP	WIP-QP, MBP-QP	Plasma
Xu et al <sup>41</sup>	China	51	N/A	31/20	51/0	43/8	32/19	IIIb-IV	SARMS	SARMS, DHPLC, ME-Liquidchip	Serum
Zhao et al <sup>44</sup>	China	111	59 (27-87)	76/35	111/0	73/38	54/57	I-IV	ME-PCR	ME-PCR	Plasma
Yam et al <sup>42</sup>	Hong Kong	37	68 (38-94)	17/20	37/0	37/0	N/A	III-IV	PNA-LNA	PNA-LNA	Plasma
Punnoose et al <sup>38</sup>	Australia	37	N/A	21/16	4/33	24/9	6/31	N/A	SARMS	SARMS	Plasma
Huang et al <sup>31</sup>	China	822	N/A	438/384	822/0	641/181	482/340	IIIb-IV	DHPLC	DHPLC	Plasma
Chen et al <sup>28</sup>	Taiwan	54	N/A	21/33	54/0	N/A	39/15	N/A	Direct sequencing	Direct sequencing	Plasma
Kim et al <sup>33</sup>	Korea	57	64 (28-84)	35/22	57/0	40/17	25/32	IIIb-IV	Direct sequencing	PNA-LNA	Serum
Ly et al <sup>35</sup>	China	11	55 (42-61)	5/6	11/0	N/A	6/5	IIb-IIIa	DHPLC	DHPLC	Plasma
Akea et al <sup>27</sup>	Japan	52	N/A	39/13	52/0	36/16	15/37	I-IV	Pyrosequencing, dideoxy sequencing	Pyrosequencing, dideoxy sequencing	Serum

CI = confidence interval, CSGE = conformation-sensitive gel electrophoresis, DHPLC = denaturing high-performance liquid chromatography, HRM = high-resolution melt, MBP-QP = mutation-biased polymerase chain reaction quenching probe system, ME-PCR = mutant-enriched polymerase chain reaction, N/A = not available, PNA-LNA = peptide nucleic acid-locked nucleic acid, SARMS = scorpion amplification refractory mutation system, WIP-QP = wild inhibiting polymerase chain reaction and quenched probe system.

**TABLE 2.** Pooled Rates of EGFR Mutation in Blood Samples

	Number of Studies (Patients)	EGFR Mutation Rate		Risk Ratio Between Subgroups	
		Mutation Rate (95% CI)	Heterogeneity, $I^2$ (P)	Risk Ratio (95% CI)	Heterogeneity, $I^2$ (P)
All exons	25 (1910)	35.0% (26.0% to 44.0%)	95.4% (<0.001)	N/A	N/A
Exon 19	13 (480)	27.2% (16.0% to 38.3%)	87.7% (<0.001)	N/A	N/A
Exon 21	11 (464)	18.8% (10.4% to 27.2%)	94.5% (<0.001)	N/A	N/A
Exon 18	1 (51)	3.9% (0.0% to 9.2%)	N/A	N/A	N/A
Exon 20	3 (297)	2.9% (0.0% to 6.4%)	50.6% (<0.001)	N/A	N/A
Race (all exons)					
Asians*	18 (1728)	35.8% (25.3% to 46.2%)	94.9% (<0.001)	N/A	N/A
Caucasians	3 (102)	6.6% (0.0% to 13.2%)	33.6% (0.222)		
Sex (all exons)					
Male	7 (153)	31.4% (9.1% to 53.7%)	91.2% (<0.001)	0.73 (0.37 to 1.45)	74.5% (0.001)
Female	7 (108)	43.0% (20.8% to 65.2%)	85.9% (<0.001)		
Smoking status (all exons)					
Never smoker	3 (77)	37.4% (12.7% to 62.1%)	70.7% (0.033)	2.77 (1.52 to 5.08)	27.8% (0.25)
Ever smoker	3 (103)	11.5% (5.3% to 17.6%)	0.0% (0.884)		
Age (all exons)					
≥60	3 (87)	44.5% (-15.5% to 104.4%)	98.5% (<0.001)	1.00 (0.72 to 1.41)	0.0% (0.49)
<60	3 (80)	41.0% (-2.4% to 84.4%)	95.0% (<0.001)		
Tumor stage (all exons)					
III-IV	10 (529)	35.9% (20.5% to 51.4%)	93.5% (<0.001)	N/A	N/A
I-IV	6 (336)	18.5% (7.6% to 39.4%)	89.4% (<0.001)		

CI = confidence interval, EGFR = epidermal growth factor receptor, N/A = not applicable.

\*The risk ratio between Asians and Caucasians was not available because no study provided data for direct within-study comparison.

**TABLE 3.** Accuracy and Agreement of EGFR Mutations in Blood Against Mutation Status in Tumor Tissue as the Reference

Subgroups	Number of Studies (Matched Specimen)	Pooled Sensitivity		Pooled Specificity		Pooled Concordance Rate	
		Estimate, 95% CI	$I^2$ , %	Estimate, 95% CI	$I^2$ , %	Estimate, 95% CI	$I^2$ , %
All exons							
Total	24 (1894)	0.61 (0.50 to 0.71)	75.4	0.90 (0.85 to 0.94)	59.1	0.79 (0.73 to 0.84)	44.1
Type of sample							
Plasma	12 (1391)	0.65 (0.51 to 0.77)	80.2	0.85 (0.76 to 0.91)	64.9	0.74 (0.65 to 0.81)	44.3
Serum	10 (412)	0.56 (0.35 to 0.75)	69.0	0.95 (0.89 to 0.98)	52.7	0.86 (0.75 to 0.93)	44.5
Mutation test methods							
Direct sequencing	6 (222)	0.70 (0.46 to 0.87)	69.5	0.90 (0.49 to 0.99)	83.9	0.80 (0.62 to 0.92)	45.1
SARMS	6 (200)	0.55 (0.31 to 0.76)	80.5	0.92 (0.81 to 0.97)	15.2	0.70 (0.54 to 0.82)	39.8
DHPLC	3 (1062)	0.68 (0.44 to 0.85)	91.6	0.86 (0.82 to 0.90)	63.4	0.81 (0.70 to 0.88)	45.0
Tumor stage							
III-IV	10 (529)	0.80 (0.73 to 0.85)	71.3	0.91 (0.88 to 0.94)	65.8	0.88 (0.83 to 0.94)	75.5
I-IV	6 (336)	0.54 (0.44 to 0.63)	87.1	0.98 (0.95 to 0.99)	43.0	0.83 (0.74 to 0.93)	87.5
Exon 19							
Total	15 (548)	0.57 (0.41 to 0.71)	61.7	0.94 (0.85 to 0.98)	79.5	0.81 (0.72 to 0.88)	42.3
Plasma	7 (207)	0.61 (0.40 to 0.79)	58.0	0.89 (0.65 to 0.97)	85.2	0.76 (0.57 to 0.89)	43.8
Serum	7 (255)	0.60 (0.32 to 0.83)	71.7	0.96 (0.92 to 0.98)	79.8	0.86 (0.76 to 0.92)	37.4
Exon 21							
Total	14 (543)	0.52 (0.33 to 0.70)	67.1	0.95 (0.86 to 0.98)	76.9	0.85 (0.76 to 0.91)	42.9
Plasma	6 (211)	0.66 (0.28 to 0.91)	81.3	0.91 (0.68 to 0.98)	83.9	0.85 (0.68 to 0.94)	44.0
Serum	6 (235)	0.42 (0.19 to 0.70)	65.7	0.96 (0.87 to 0.99)	60.8	0.86 (0.72 to 0.93)	42.3

CI = confidence interval, DHPLC = denaturing high performance liquid chromatography, SARMS = scorpion amplification refractory mutation system.

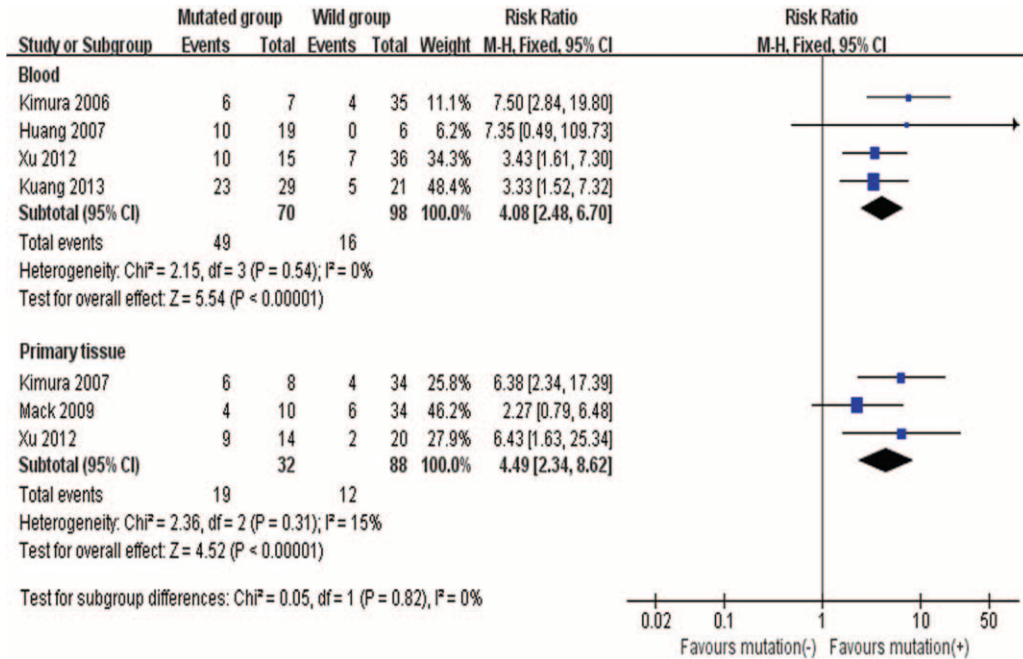


FIGURE 2. Association between objective response to TKIs treatment and EGFR mutations in blood or tumor tissue samples.

response to EGFR TKIs treatment. Meta-analysis demonstrated that the association between objective response to TKIs treatment and *EGFR* mutations were significant in both blood and tumor tissue, but the association was slightly stronger in tumor tissue (RR: 4.49; 95% CI 2.34–8.62) than in blood (RR: 4.08; 95% CI 2.48–6.70), though the subgroup difference was not significant ( $P = 0.82$ ) (Figure 2). The Egger test ( $P = 0.628$ ) and funnel plot suggested that there was no publication bias (Supplemental Digital Content—Figure 1, <http://links.lww.com/MD/A260>).

**Association of *EGFR* Mutations and Survival**

Eight studies<sup>14,16–19,28,30,38,41</sup> with a total of 564 patients contributed to the meta-analysis of *EGFR* mutations (exons 19 and 21) and survival. The pooled HR for PFS was larger in blood (HR: 0.72; 95% CI 0.64–0.80) than in tumor tissue (HR: 0.57; 95% CI 0.39–0.85) (Figure 3). Although no significant subgroup difference was identified ( $P = 0.20$ ), the *EGFR* mutation in serum (HR: 0.58; 95% CI 0.4–0.83) tended to have better predictive value than that in plasma (HR: 0.73; 95% CI 0.65–0.83).

Likewise, the pooled HR for OS was 0.71 (95% CI 0.50–0.99) for *EGFR* mutations in blood and 0.54 (95% CI 0.38–0.76) in tumor tissue (Figure 4). The subgroup analysis indicated that the HR was significant in serum (HR: 0.59; 95% CI 0.39–0.91), but not in plasma (HR: 0.95; 95% CI 0.54–1.67). No significant subgroup difference was seen ( $P = 0.19$ ).

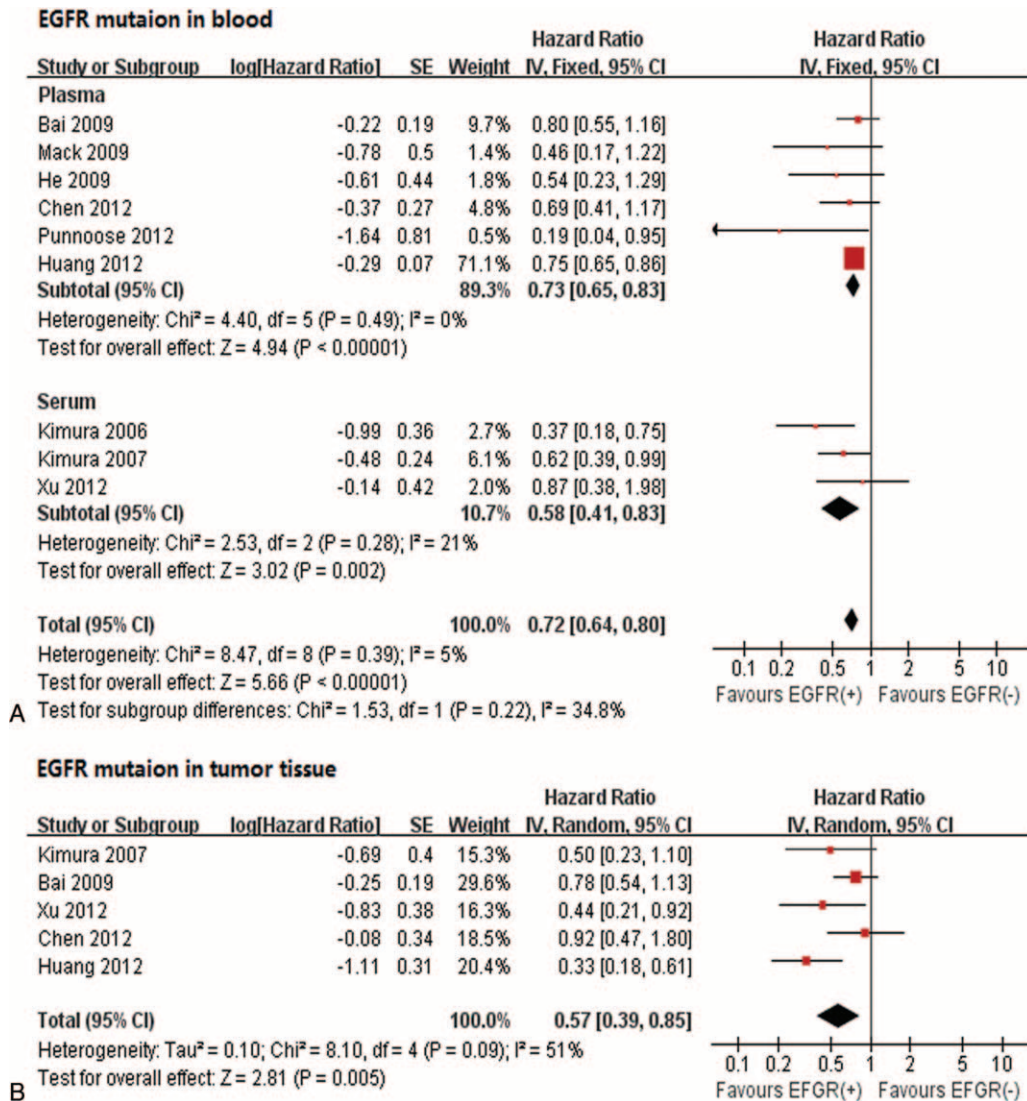
The risk of publication bias was not assessed in the meta-analyses in tumor tissues as the number of included studies was  $\leq 5$ . In terms of *EGFR* mutations in blood, the funnel plot for PFS showed some asymmetry (Egger test:  $P = 0.04$ ) (Supplemental Digital Content—Figure 2, <http://links.lww.com/MD/A260>) but the funnel plots for OS did not (Egger test:  $P = 0.69$ ) (Supplemental Digital Content—Figure 3, <http://links.lww.com/MD/A260>).

**DISCUSSION**

Testing *EGFR* mutations in tumor tissue plays an important role in selecting patients for EGFR TKIs treatment,<sup>45,46</sup> but in many patients, tumor tissues are either lacking or insufficient for mutations analysis. Blood may be a potential substitute for tumor tissues for this purpose. The present study reviewed 25 studies including 2605 patients and provided the most comprehensive analysis about the relation of *EGFR* mutations in blood to those in tumor tissues, and the value of *EGFR* mutations in blood in predicting the clinical outcomes of EGFR TKIs treatment.

We found a high overall concordance rate in *EGFR* mutation status between blood and tumor tissues, although the sensitivity can be further improved. Importantly, the high specificity of the blood testing against tissue analysis will guarantee high positive predictive values and avoid unnecessary treatment in those who have a false-positive result and will not respond to the treatment. This will be particularly true in populations that have a relatively high *EGFR* mutation rate. For example, based on the overall sensitivity of 0.64 and specificity of 0.89 and assuming a mutation rate of 10%, 30%, and 60% representing various populations, the positive predictive value will be 40%, 72%, and 90%, respectively. Interestingly, testing *EGFR* mutations in serum seems to have a higher specificity than in plasma, although the differences are not statistically significant.

Given the high positive predictive values, it is anticipated that those who are blood test positive would respond similarly or be slightly less well to EGFR TKIs treatment than those who are tissue test positive. Indeed, the difference in response to the treatment between *EGFR* mutation-positive and negative patients is very similar when either blood or tumor tissues are used for the testing. As PFS and OS are concerned, this difference is also evident when blood testing is used but slightly lower than that with tumor tissue testing.



**FIGURE 3.** Associations between progression-free survival and EGFR mutations in blood (A) and tumor tissue samples (B). EGFR = epidermal growth factor receptor.

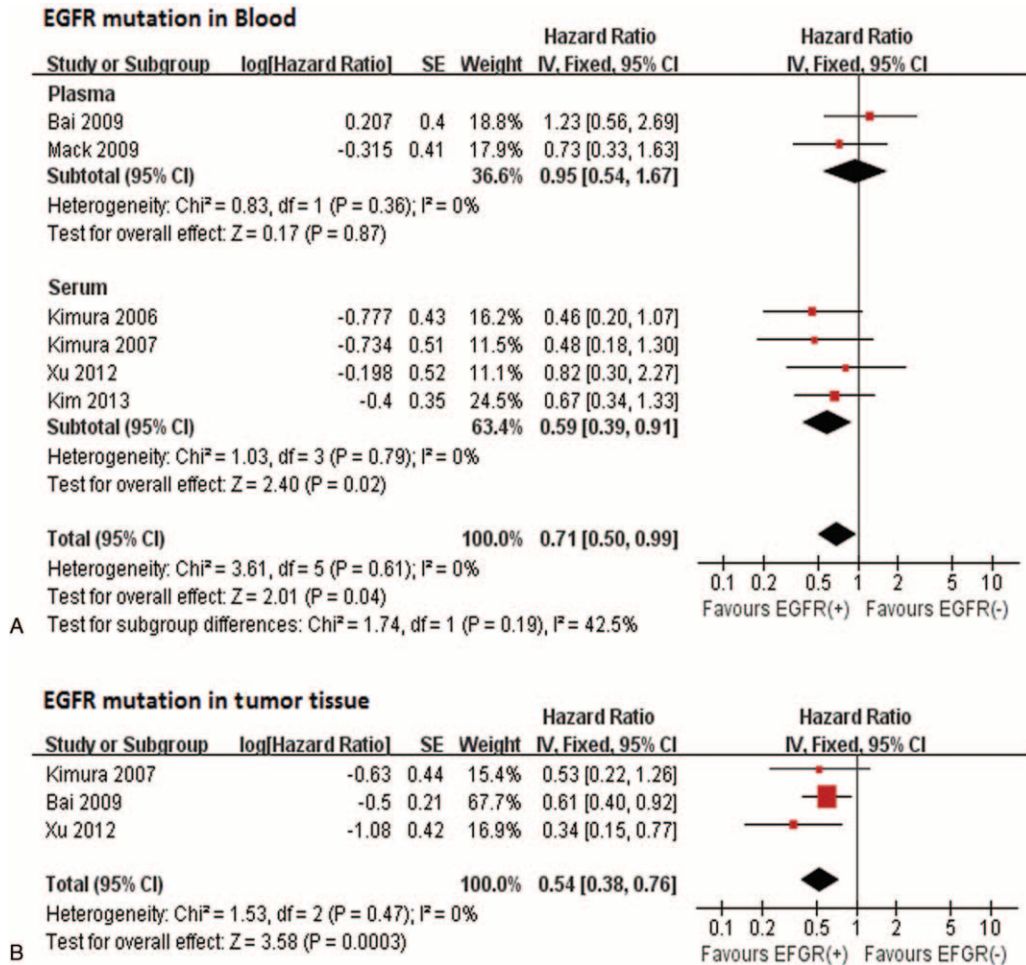
For similar reasoning, we would also expect plasma test-positive patients benefit less than serum test-positive patients, which is confirmed with empirical data in our study. Importantly, the response to EGFR TKIs treatment in serum test-positive patients relative to that in serum-negative patients is almost as large as that observed when mutation status is determined based on tissue testing. This seems to give strong support for serum as a good surrogate for tumor tissue for EGFR mutation testing. Having said this, it appears that more future research is required to confirm the superiority of serum over plasma in this context.

In using EGFR mutation status to guide EGFR TKIs treatment, it is important to achieve a high positive predictive value so that this expensive treatment that also has severe side effects will not be used in those are false positive and will not benefit from the treatment. It is known that the positive predictive value is also determined by the prevalence of the condition (which is EGFR mutation in our case) in addition to the sensitivity and specificity of the testing method. This

means that the higher the prevalence of the mutation, the higher the positive predictive value, the more useful blood testing would be.

It has been shown that the prevalence of EGFR mutations is higher in females, nonsmokers, adenocarcinoma, and Asians.<sup>47,48</sup> These associations were also reflected in the EGFR mutation rate in blood in our study although the data here are limited to a few small studies relevant to this project and biased by the false positives. This is important information for determining the applications of the blood mutation testing. We thus suggest blood testing, in particular serum testing, to be used in females, nonsmokers, adenocarcinoma, and Asians in whom the positive predictive value could reach >90% as they usually have a mutation rate >50%.

Our study also suggested that the EGFR mutation test methods may have different sensitivity and specificity. The sensitivity by direct sequencing, the current gold standard for testing EGFR mutations,<sup>49</sup> was the highest in our study. Different from the work by Ellison et al,<sup>50</sup> we found that SARMS had a



**FIGURE 4.** Associations between overall survival and EGFR mutations in blood (A) or tumor tissue samples (B). EGFR = epidermal growth factor receptor.

relatively lower sensitivity than other methods. Further studies are needed as the sample size is generally small in the studies included. The difference in the accuracy of the methods might be partially a result of the hidden cutoff used in different methods.

Limitations of this meta-analysis primarily arise from the quality of original studies reviewed. Owing to insufficient reporting of the original studies, the assessment of many QUADAS items could only be judged as unclear, which suggests that there may be a risk of bias in patient selection and interpretation of reference and index test. Regarding the estimate of the efficacy of treatment, the bias would affect both the blood test and tissue test groups, and these may cancel each other out. Second, the sample sizes in most included studies were small and some important subgroup differences may truly exist but cannot be confirmed. Third, like many other meta-analyses of single rates, our study showed large statistical heterogeneity that could not be explained. We employed the random-effects model in combining the result. Last, the level of circulating DNA could be related to tumor volume that largely depends on tumor stage, so tumor stage could be a potential factor that may influence the results. Owing to insufficient data for patients with stages I–II NSCLC, we were unable to perform subgroup analysis according to tumor stage. However, we pooled the mutation rate, sensitivity, specificity, and

concordance rate in studies including stages III–IV and I–IV NSCLC, separately. As expected, the pooled sensitivity and concordance rate in studies including stages III–IV NSCLC were relatively higher than those in studies including all stages NSCLC. This suggests that blood *EGFR* mutation testing is particularly suitable for patients in advanced NSCLC, though further investigation is still needed.

In conclusion, blood, in particular serum, is a good substitute when tumor tissue is absent or insufficient for testing *EGFR* mutations to guide EGFR TKIs treatment in patients with NSCLC. *EGFR* mutation positivity in blood could be used to recommend EGFR TKIs treatment, but the absence of blood positivity should not necessarily be construed with confirmed negativity. Further research is necessary to further improve the sensitivity and identify the best testing methods for testing *EGFR* mutations in blood.

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