ORIGINAL ARTICLE

WILEY

Resistance mechanisms of epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer patients: A meta-analysis

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Abstract

Background: Differences in the resistance mechanisms of epidermal growth factor receptor tyrosine kinase inhibitors in patients with non-small cell lung cancer (NSCLC) harboring epidermal growth factor receptor mutations are unknown. This meta-analysis aimed to clarify the differences in resistance mechanisms after treatment with various epidermal growth factor receptor tyrosine kinase inhibitors.

Methods: We systematically searched PubMed, Cochrane, and Web of Science on July 29, 2020, for relevant studies on acquired resistance mechanisms against epidermal growth factor receptor tyrosine kinase inhibitors. The primary outcome measure was differences in the resistance mechanism between individual or generations of epidermal growth factor receptor tyrosine kinase inhibitors.

Results: In total, 33 trials involving 2418 individuals were included and analyzed. T790M was significantly less frequent after afatinib treatment (40.2%, 95% confidence interval [CI]: 31.7%–48.7%) than after gefitinib and erlotinib treatments (52.5%, 95% CI: 48.7%–56.3%, p = 0.005). There were no significant differences between Asian and non-Asian patients in the incidence of T790M after gefitinib, erlotinib, and afatinib treatments. Regarding epidermal growth factor receptor pathway-independent resistant mechanisms, the incidences of small cell lung cancer transformation (osimertinib: 7.9%, 95% CI: 3.6%–12.2%, others: 2.3%, 95% CI: 0.8%–3.8%) and Kirsten rat sarcoma (KRAS) viral oncogene homolog mutation (osimertinib: 4.6%, 95% CI: 1.5%–7.7%, others: 0.2%, 95% CI: 0.0%–1.7%) were significantly higher following osimertinib treatment than with others.

Conclusions: Significant differences in the incidence of resistance mechanisms among epidermal growth factor receptor tyrosine kinase inhibitors exist, which should be taken into consideration when choosing the treatment strategy.

KEYWORDS
epidermal growth factor receptor tyrosine kinase inhibitors, NSCLC, resistance mechanisms, T790M

INTRODUCTION

Epidermal growth factor receptor (EGFR) mutation is the most prevalent driver oncogene mutation in lung carcinoma and is detected in almost half of all untreated non-small cell lung cancer (NSCLC) patients in Asia.[1] EGFR tyrosine kinase inhibitors (TKIs) have been established as standard first-line therapy for *EGFR*-mutant NSCLC patients owing to their superiority over conventional cytotoxic chemotherapy.[2–4] Osimertinib is a third-generation

EGFR-TKI that inhibits both major *EGFR*-activating and Thyr790Met (T790M) resistance mutations.[5] Based on the AURA3 phase III clinical trial results, osimertinib has been approved for the treatment of NSCLC patients with T790M resistance mutations upon disease progression after previous EGFR-TKI therapy. This clinical trial showed that osimertinib was superior to standard cytotoxic chemotherapy with respect to the objective response rate, progression-free survival (PFS), and tolerability in patients with T790M-mediated acquired resistance.[6] Furthermore, osimertinib has also been approved as a first-line treatment for NSCLC patients harboring EGFR exon 19 deletions or L858R mutations based on clinical evidence of a direct comparison with first-generation EGFR-TKIs.[7] When comparing first- and second-generation TKIs, the second-generation TKI, dacomitinib, yields better PFS and overall survival (OS) than the first-generation TKI, gefitinib.[8] However, no clinical trial to date has compared second-generation TKIs with osimertinib in the first-line setting.

Despite their survival benefit, most patients treated with EGFR-TKIs develop acquired resistance within two years. EGFR T790M-acquired mutations are the most frequent resistance mechanism after treatment with first- or second-generation EGFR-TKIs.[9,10] However, increasing evidence supports that there are several acquired resistance mechanisms after treatment with Osimertinib.[11] Unfortunately, there is limited information regarding the difference in resistance mechanisms with regard to EGFR-TKI treatment. Clarifying this difference may influence the treatment strategy for patients with activating *EGFR* mutations, possibly leading to the selection of better treatment options. Thus, this meta-analysis aimed to clarify the difference in resistance mechanisms among EGFR-TKIs and compare these differences between Asian and non-Asian populations.

METHODS

Ethics

The need for institutional review board approval and patient consent for this study was waived because it was a review. The systematic review and meta-analysis was performed according to the Meta-analysis of Observational Studies in Epidemiology guidelines (Table S1).[12] The study protocol was included on the University Hospital Medical Information Network Clinical Trials Registry (UMIN000040759).[13]

Study overview and search strategy

We systematically searched PubMed, the Cochrane Database, and Web of Science on July 29, 2020, for relevant studies on acquired resistance mechanisms against EGFR-TKIs. The search strategies are presented in Table S2. Gefitinib and erlotinib, afatinib and dacomitinib, and osimertinib were defined as first-, second-, and third-generation

EGFR-TKIs, respectively. Two investigators independently screened the titles and abstracts and scrutinized the full text. The reference list of all included articles was also manually checked to further identify other relevant studies. Papers that involved repeated participation of the same patient in multiple and/or duplicated studies were excluded. If conflicts arose between the review authors during the selection process, the inconsistencies were discussed, and a consensus was reached.

Study selection

Design

The inclusion criteria were as follows: (i) availability of data for the number of acquired resistance mechanisms against EGFR-TKIs: *EGFR* T790M mutation, *EGFR* C797S mutation, MET amplification, Kirsten rat sarcoma (*KRAS*) viral oncogene homolog mutation, phosphatidylinositol-4,-5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) mutation, and transformation to small cell lung cancer (SCLC); and (ii) written in English as a full article or a brief report regardless of its primary endpoint.

Patients

Patients with a pathologically or cytologically confirmed NSCLC diagnosis who relapsed after EGFR-TKI therapy and were examined for resistance mechanisms were included. There was no restriction based on age, sex, smoking history, clinical staging, performance status, and NSCLC pathological subtype. Patients treated with one of the following EGFR-TKIs were eligible: gefitinib, erlotinib, afatinib, dacomitinib, or osimertinib. Patients treated with combination chemotherapy of two or more EGFR-TKIs, EGFR-TKI, and any cytotoxic agent, EGFR-TKI and anti-vascular endothelial growth factor receptor agents, or EGFR-TKI and immune checkpoint inhibitors were excluded because such treatments may have influenced the resistance mechanism.

Data extraction

Data regarding the study characteristics, type of EGFR-TKI used for the patient, incidences of detected resistant mechanisms, and risk of bias were independently extracted by two review investigators. Inconsistencies were discussed to reach a consensus.

Assessment of study quality

The Newcastle–Ottawa scale was used to evaluate the study quality. The scores ranged from 0–9, with 9 points
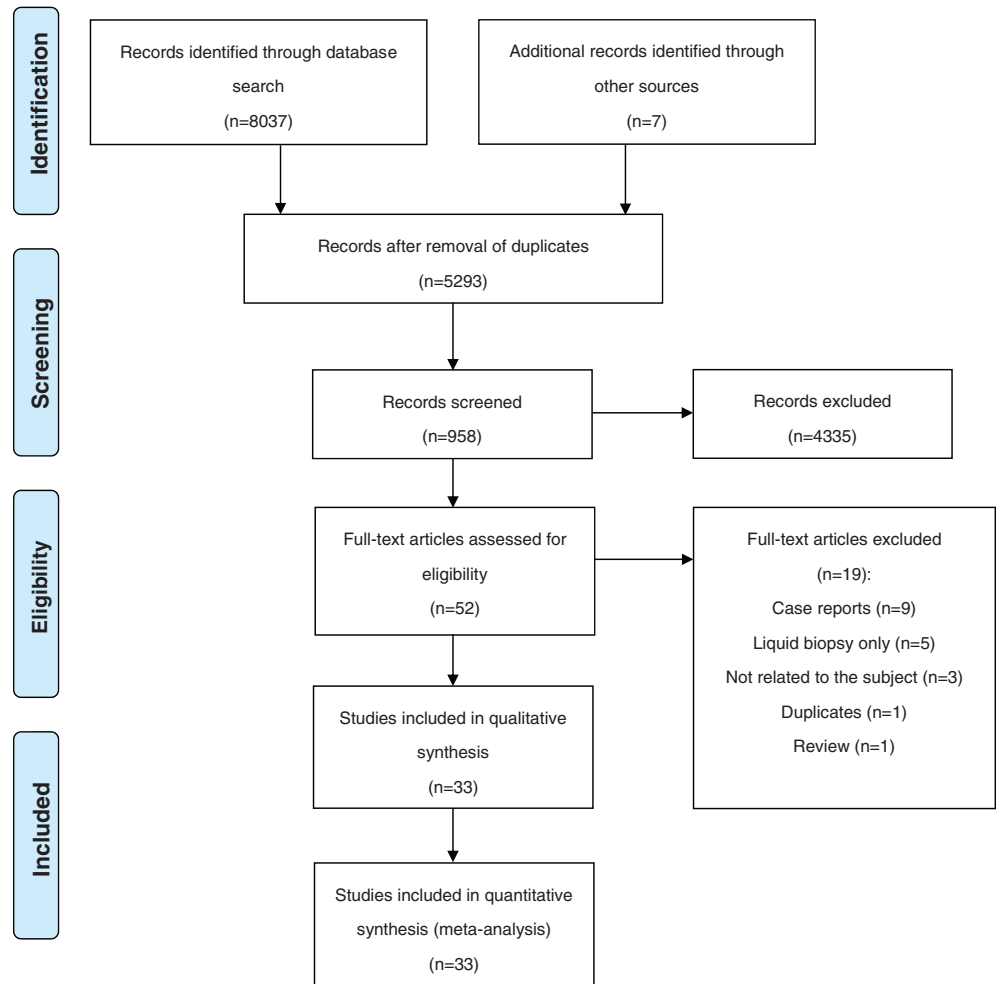
suggesting the best study quality. The included studies were assessed according to their methodological quality for patient selection, comparability, and outcome. Quality evaluation was independently conducted by two investigators. Disagreements among them were resolved through discussion to reach a consensus.

Outcome measures

The outcomes of this meta-analysis were the incidence of acquired resistance mechanisms, *EGFR* T790M mutations, *EGFR* C797S mutations, *MET* amplification, transformation to SCLC, PIK3CA mutations, and *KRAS* mutations. The primary outcome measure was the difference in resistance mechanisms among EGFR-TKIs or generations of EGFR-TKIs. The secondary outcome measure was the difference in the resistance mechanism between Asian and non-Asian patients.

Statistical analysis

We used the random-model generic inverse variance method.[14] Preceding the meta-analysis, the standard error was estimated using the Agrestia-Coull method, as we could not obtain standard error for outcomes with a prevalence of 0% through the commonly used method (standard error = standard deviation/square root of n).[15] Heterogeneity among studies was quantified using the I^2 statistic, with an I^2 value of 0% set to indicate no heterogeneity and higher values signifying increasing heterogeneity. In particular, heterogeneity was interpreted as follows: I^2 = 0% to 40%: may not be important; 30% to 60%: may represent moderate heterogeneity; 50% to 90%: may represent substantial heterogeneity; 75% to 100%: considerable heterogeneity.[16] Random-effect model meta-analysis was performed using Reviewing Manager software, ver. 5.4 (Cochrane Collaboration).[17]

RESULTS

Study characteristics

Among the 6746 articles initially reviewed, 33 studies that revealed resistance mechanisms after EGFR-TKI treatment in patients with advanced lung cancer were selected for full review (Figure 1, Table S3). Among the 33 studies, 17 were reported from Asian countries. The list of studies included in this meta-analysis is shown in Table S1. Most of the included studies were reported after 2016. In total, 13, 12,

a

Study or Subgroup	Incidence	SE	Weight	Incidence IV, Random, 95% CI
GEF/ERL				
Bean J, 2007	46.5	7.3	3.3%	46.50 [32.19, 60.81]
Cardona AF, 2017	47.1	8.1	3.0%	47.10 [31.22, 62.98]
Huang YH, 2018	47.9	3.6	4.9%	47.90 [40.84, 54.96]
Iacono D, 2019	54.2	9.5	2.6%	54.20 [35.58, 72.82]
Ji W, 2013	42.3	9.1	2.7%	42.30 [24.46, 60.14]
Johnson ML, 2011	63.2	10.2	2.4%	63.20 [43.21, 83.19]
Ko R, 2016	37.5	6.3	3.7%	37.50 [25.15, 49.85]
Lee CK, 2017	63.2	10.2	2.4%	63.20 [43.21, 83.19]
Lee K, 2020	55.4	3.7	4.9%	55.40 [48.15, 62.65]
Li C, 2018	52.2	5.9	3.9%	52.20 [40.64, 63.76]
Lin YT, 2019	50	6.5	3.7%	50.00 [37.26, 62.74]
Nosaki K, 2016	50.4	3.3	5.0%	50.40 [43.93, 56.87]
Oxnard GR, 2011	62.4	4.9	4.3%	62.40 [52.80, 72.00]
Redig AJ, 2016	65.7	7.7	3.2%	65.70 [50.61, 80.79]
Sequist LV, 2011	48.6	7.8	3.2%	48.60 [33.31, 63.89]
Tseng JS, 2016	54.3	5	4.3%	54.30 [44.50, 64.10]
Wagener-Ryczek S, 2020	66.2	5.6	4.0%	66.20 [55.22, 77.18]
Yu HA, 2013	63.2	3.8	4.8%	63.20 [55.75, 70.65]
Subtotal (95% CI)			66.4%	53.98 [50.23, 57.73]

Heterogeneity: Tau² = 28.50; Chi² = 32.93, df = 17 (P = 0.01); I² = 48%
Test for overall effect: Z = 28.23 (P < 0.00001)

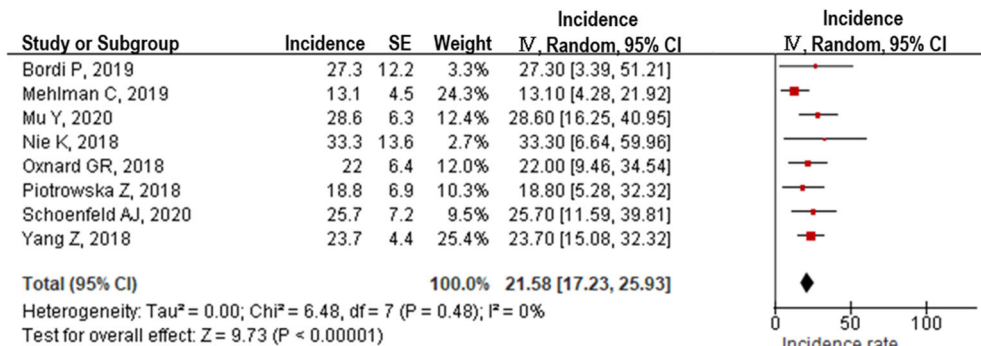| **AFA**				
Ho GF, 2019	41.9	8.4	2.9%	41.90 [25.44, 58.36]
Hochmair MJ, 2019	66.7	6.2	3.8%	66.70 [54.55, 78.85]
Huang YH, 2018	23.1	11.1	2.2%	23.10 [1.34, 44.86]
Iacono D, 2019	33.3	18.9	1.0%	33.30 [0.00, 70.34]
Ko R, 2016	0	13.9	1.6%	0.00 [0.00, 27.24]
Lee K, 2020	40.7	5.2	4.2%	40.70 [30.51, 50.89]
Lin YT, 2019	33.3	7.6	3.2%	33.30 [18.40, 48.20]
Nakamura T, 2018	40	11.4	2.1%	40.00 [17.66, 62.34]
Nosaki K, 2016	20	15.8	1.3%	20.00 [0.00, 50.97]
Tanaka K, 2017	43.2	7.8	3.2%	43.20 [27.91, 58.49]
Tseng JS, 2016	75	17.3	1.1%	75.00 [41.09, 100.00]
Wagener-Ryczek S, 2020	43.6	6.5	3.7%	43.60 [30.86, 56.34]
Wu SG, 2016	47.6	7.4	3.3%	47.60 [33.10, 62.10]
Subtotal (95% CI)			33.6%	40.60 [32.11, 49.09]

Heterogeneity: Tau² = 144.35; Chi² = 35.50, df = 12 (P = 0.0004); I² = 66%
Test for overall effect: Z = 9.37 (P < 0.00001)

| **Total (95% CI)** | | | 100.0% | 49.67 [45.64, 53.71] |

Heterogeneity: Tau² = 73.55; Chi² = 85.19, df = 30 (P < 0.00001); I² = 65%
Test for overall effect: Z = 24.12 (P < 0.00001)
Test for subgroup differences: Chi² = 7.98, df = 1 (P = 0.005), I² = 87.5%

Incidence rate (%)

b

Study or Subgroup	Incidence	SE	Weight	Incidence IV, Random, 95% CI
GEF/ERL				
Bean J, 2007	46.5	7.3	3.5%	46.50 [32.19, 60.81]
Cardona AF, 2017	47.1	8.1	3.2%	47.10 [31.22, 62.98]
Huang YH, 2018	47.9	3.6	5.3%	47.90 [40.84, 54.96]
Iacono D, 2019	54.2	9.5	2.7%	54.20 [35.58, 72.82]
Ji W, 2013	42.3	9.1	2.8%	42.30 [24.46, 60.14]
Johnson ML, 2011	63.2	10.2	2.5%	63.20 [43.21, 83.19]
Ko R, 2016	37.5	6.3	4.0%	37.50 [25.15, 49.85]
Lee CK, 2017	63.2	10.2	2.5%	63.20 [43.21, 83.19]
Lee K, 2020	55.4	3.7	5.2%	55.40 [48.15, 62.65]
Li C, 2018	52.2	5.9	4.1%	52.20 [40.64, 63.76]
Lin YT, 2019	50	6.5	3.9%	50.00 [37.26, 62.74]
Nosaki K, 2016	50.4	3.3	5.4%	50.40 [43.93, 56.87]
Oxnard GR, 2011	62.4	4.9	4.6%	62.40 [52.80, 72.00]
Redig AJ, 2016	65.7	7.7	3.3%	65.70 [50.61, 80.79]
Sequist LV, 2011	48.6	7.8	3.3%	48.60 [33.31, 63.89]
Tseng JS, 2016	54.3	5	4.6%	54.30 [44.50, 64.10]
Wagener-Ryczek S, 2020	66.2	5.6	4.3%	66.20 [55.22, 77.18]
Yu HA, 2013	63.2	3.8	5.2%	63.20 [55.75, 70.65]
Subtotal (95% CI)			70.3%	53.98 [50.23, 57.73]

Heterogeneity: Tau² = 28.50; Chi² = 32.93, df = 17 (P = 0.01); I² = 48%
Test for overall effect: Z = 28.23 (P < 0.00001)

OSM				
Bordi P, 2019	27.3	12.2	1.9%	27.30 [3.39, 51.21]
Le X, 2018	47.5	7.5	3.4%	47.50 [32.80, 62.20]
Mehlman C, 2019	31.5	5.3	4.4%	31.50 [21.11, 41.89]
Mu Y, 2020	49	6.9	3.7%	49.00 [35.48, 62.52]
Nie K, 2018	77.8	12.9	1.8%	77.80 [52.52, 100.00]
Oxnard GR, 2018	31.7	7	3.6%	31.70 [17.98, 45.42]
Piotrowska Z, 2018	38.7	8.3	3.1%	38.70 [22.43, 54.97]
Schoenfeld AJ, 2020	45.2	8.4	3.1%	45.20 [28.74, 61.66]
Yang Z, 2018	40.9	5	4.6%	40.90 [31.10, 50.70]
Subtotal (95% CI)			29.7%	41.61 [34.51, 48.72]

Heterogeneity: Tau² = 58.15; Chi² = 16.75, df = 8 (P = 0.03); I² = 52%
Test for overall effect: Z = 11.48 (P < 0.00001)

| **Total (95% CI)** | | | 100.0% | 50.29 [46.28, 54.29] |

Heterogeneity: Tau² = 65.95; Chi² = 74.89, df = 26 (P < 0.00001); I² = 65%
Test for overall effect: Z = 24.61 (P < 0.00001)
Test for subgroup differences: Chi² = 9.11, df = 1 (P = 0.003), I² = 89.0%

Incidence rate (%)

FIGURE 2 *EGFR* T790M mutation incidence among patients treated with EGFR-TKIs. (a) First-generation EGFR-TKIs (gefitinib and erlotinib) versus afatinib. (b) First-generation EGFR-TKIs (gefitinib and erlotinib) vs. osimertinib

nine, and eight studies investigated acquired resistance mechanisms after treatment with erlotinib, gefitinib, afatinib, and osimertinib, respectively. The median Newcastle–Ottawa scale score of the 33 studies was 8 points (range, 5–9), indicating good quality (Table S4).

Incidence of EGFR T790M mutations

T790M was significantly less frequent with afatinib treatment (40.2%, 95% confidence interval [CI]: 31.7%–48.7%, $I^2 = 66\%$) than with gefitinib and erlotinib treatments

Study or Subgroup	Incidence	SE	Weight	Incidence IV, Random, 95% CI
Bordi P, 2019	27.3	12.2	3.3%	27.30 [3.39, 51.21]
Mehlman C, 2019	13.1	4.5	24.3%	13.10 [4.28, 21.92]
Mu Y, 2020	28.6	6.3	12.4%	28.60 [16.25, 40.95]
Nie K, 2018	33.3	13.6	2.7%	33.30 [6.64, 59.96]
Oxnard GR, 2018	22	6.4	12.0%	22.00 [9.46, 34.54]
Piotrowska Z, 2018	18.8	6.9	10.3%	18.80 [5.28, 32.32]
Schoenfeld AJ, 2020	25.7	7.2	9.5%	25.70 [11.59, 39.81]
Yang Z, 2018	23.7	4.4	25.4%	23.70 [15.08, 32.32]
Total (95% CI)			**100.0%**	**21.58 [17.23, 25.93]**

Heterogeneity: Tau² = 0.00; Chi² = 6.48, df = 7 (P = 0.48); I² = 0%
Test for overall effect: Z = 9.73 (P < 0.00001)

(52.5%, 95% CI: 48.7%–56.3%, I^2 = 51%, *p* for heterogeneity = 0.005) (Figure 2(a)). However, T790M was significantly less frequent with osimertinib treatment (41.6%, 95% CI: 34.5%–48.7%, I^2 = 66%) than with gefitinib and erlotinib treatments (Figure 2(b)). There was no significant difference in resistance mechanisms between afatinib and osimertinib treatments (Figure S1A) or between gefitinib and erlotinib treatments (Figure S1B). All cases were confirmed to have T790M before osimertinib treatment. C757S, a major secondary *EGFR* mutation resistant to osimertinib, was seen in 21.5% (95% CI: 17.2%–25.9%, I^2 = 0%) of patients who developed progressive disease after osimertinib treatment (Figure 3). Heterogeneity analysis using the I^2 statistic indicated that the highest variation was observed with afatinib and osimertinib treatments.

Differences in the incidence of T790M acquired mutations between Asian and non-Asian populations

The included studies were divided into two groups based on patient information. The incidence of T790M mutations after gefitinib or erlotinib treatment was 50.7% (95% CI: 47.3%–54.1%, I^2 = 11%) among Asian and 52.8% (95% CI: 45.4%–60.2%, I^2 = 55%) among non-Asian patients (Figure 4(a)). However, the incidence of T790M mutations after afatinib was 35.4% (95% CI: 24.5%–46.2%, I^2 = 60%) among Asian and 39.3% (95% CI: 27.4%–51.2%, I^2 = 49%) among non-Asian patients (Figure 4(b)). There were no significant differences between Asian and non-Asian patients in the incidence of T790M mutation after gefitinib, erlotinib, and afatinib treatments.

Other resistance mechanisms against EGFR-TKIs through EGFR-independent signaling pathways

The incidence of other resistant mechanisms through EGFR-independent signaling pathways was compared between patients treated with osimertinib and those treated with first- or second-generation EGFR-TKIs (gefitinib, erlotinib, or afatinib) (Figure 5). SCLC transformation was significantly more frequent with osimertinib treatment (7.9%, 95% CI: 3.6%–12.2%) than with the other TKIs (2.3%, 95% CI: 0.8%–3.8%, *p* = 0.02, Figure 5(a)). *KRAS* mutations were also significantly more frequent with osimertinib treatment (4.6%, 95% CI: 1.5%–7.7%) than with the other TKIs (0.2%, 95% CI: 0.0%–1.7%, *p* = 0.01, Figure 5 (b)). However, there were no significant differences in the incidence of *MET* amplification (Figure 5(c)) and PIK3CA mutations (Figure 5(d)) among the EGFR-TKIs.

DISCUSSION

Evidence on the difference in resistance mechanisms with regard to EGFR-TKI treatment is scarce. In this study, T790M mutations were significantly less frequent in patients who had disease progression after treatment with afatinib than in those treated with first-generation EGFR-TKIs. After osimertinib treatment, T790M disappeared in 58.4% of patients (Figure 2(b)), whereas C757S was detected in 20.8% of patients. There was no significant difference between Asian and non-Asian patients in the incidence of T790M after treatment with first- or second-generation TKI. SCLC transformations and *KRAS* mutations were more frequent after treatment with osimertinib than after treatment with other TKIs. To the best of our knowledge, this is the first meta-analysis on the resistance mechanisms involved in various generations of EGFR-TKI treatments among patients with *EGFR* mutations.

No phase 3 trial has directly compared second-generation EGFR-TKIs with osimertinib treatment. Therefore, we investigated the optimal EGFR-TKI for first-line treatment that would result in longer OS. Osimertinib as first-line treatment was reportedly superior to the first-generation EGFR-TKIs with respect to PFS (osimertinib: 18 months, first-generation: 10 months) and OS (osimertinib: 38.6 months, first-generation: 31.8 months).[7] However, the second-generation EGFR-TKI, dacomitinib, as first-line treatment yielded a longer PFS of 14.7 months[8] than osimertinib as second-line treatment (10.7 months).[18] Therefore, treatment with second-generation EGFR-TKIs as the first-line treatment, followed by osimertinib as the second-line treatment after a successful detection of T790M, could confer better PFS than first-line osimertinib
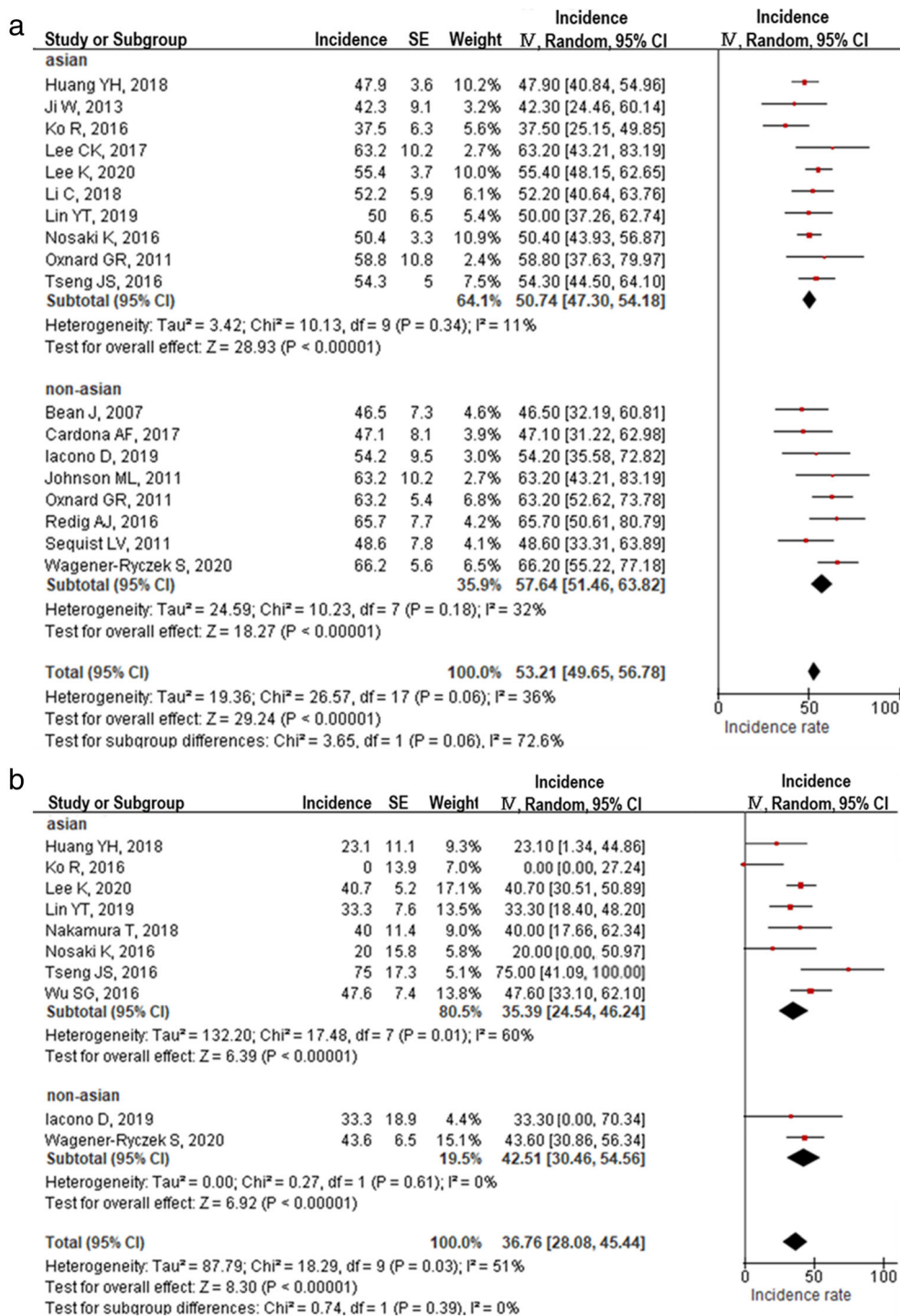
FIGURE 4 EGFR T790M mutation incidence in Asian and non-Asian patients who developed acquired resistance to EGFR-TKIs. (a) T790M incidence after first-generation EGFR-TKI treatment (gefitinib or erlotinib). (b) T790M incidence after afatinib treatment

treatment. To prove this concept, a retrospective observational study clarified the utility of afatinib as a first-line treatment, followed by osimertinib.[19] The combined PFS was 28.7 months, as expected. However, this was only observed in patients proven to have a T790M mutation in the EGFR gene, using specimens obtained during progression after treatment with second-generation TKIs. In contrast, our data revealed that T790M was significantly less frequent after afatinib treatment than after treatment using first-generation TKIs (Figure 2(a)). This implies that fewer patients may obtain this ideal PFS associated with the sequential therapy of afatinib followed by osimertinib.

Conventional chemotherapy is currently commonly adopted after first-line osimertinib treatment, which is expected to have a limited effect on extending survival.[20] Our data revealed that T790M disappeared in almost half of the patients treated with osimertinib (Figure 2(b)). Moreover, C757S, an acquired mutation to osimertinib that was observed in one of five patients, could be treated with first- or second-generation TKIs. These data suggest that first-line
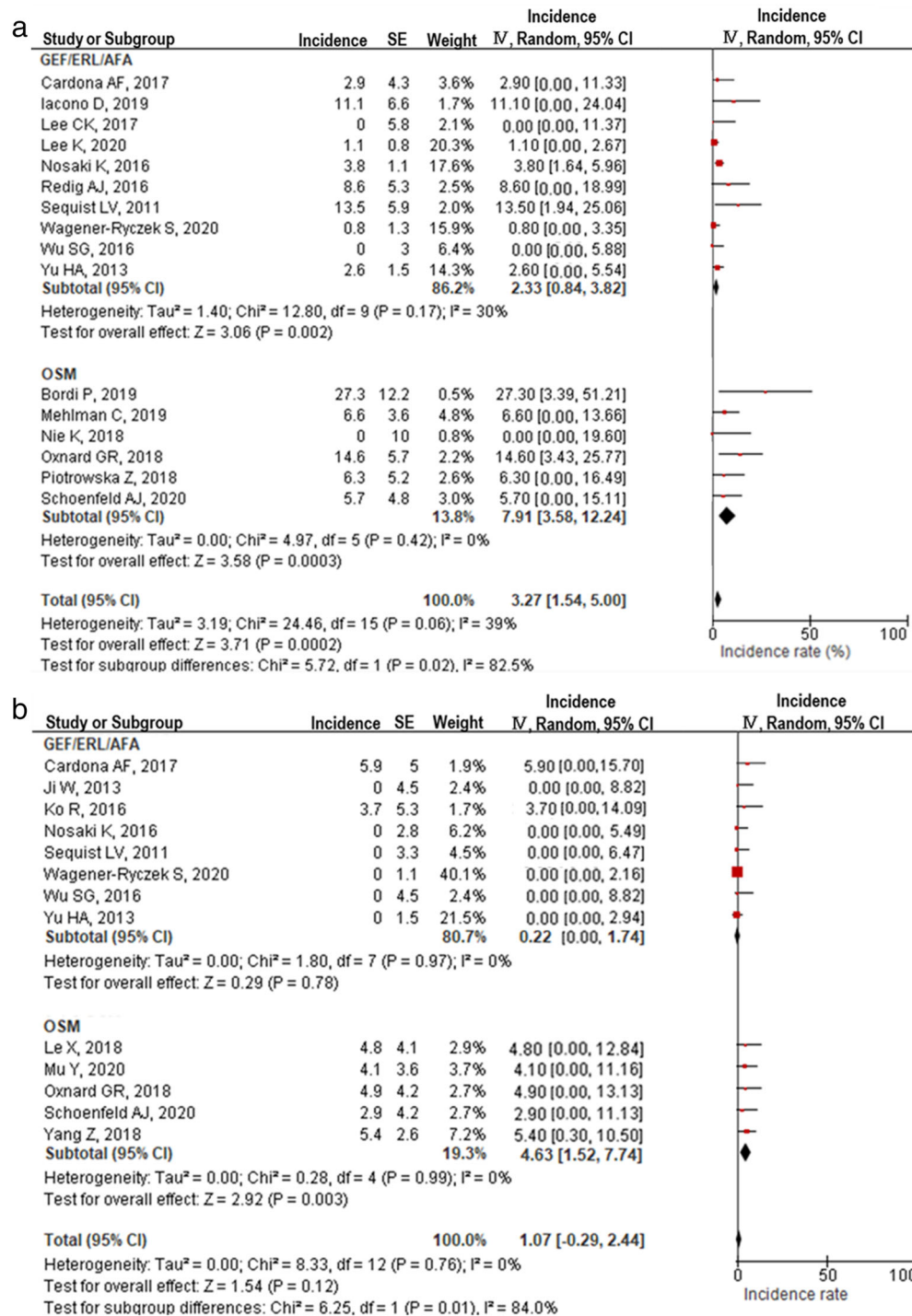
FIGURE 5 EGFR pathway-independent resistance mechanisms in patients who developed acquired resistance to EGFR-TKIs. (a) Incidence of small cell lung cancer transformation between gefitinib/erlotinib/afatinib and osimertinib. (b) *KRAS* mutation incidence between gefitinib/erlotinib/afatinib and osimertinib. (c) *MET* amplification incidence between gefitinib/erlotinib/afatinib and osimertinib. (d) PIK3A mutation incidence between gefitinib/erlotinib/afatinib and osimertinib

osimertinib treatment followed by previous-generation EGFR-TKIs could also be effective for specific patients. Several clinical trials exploring the efficacy of first- or second-generation EGFR-TKIs after osimertinib treatment have already been conducted.[21,22] Clinical practice may have already changed based on the results of these trials.

In the FLAURA trial, a randomized clinical trial that compared osimertinib and first-generation EGFR-TKIs as first-line treatment, the OS was similar in both treatment arms of the Asian population.[7] Our meta-analysis results indicate that there was no significant difference in the incidence of T790M between the Asian and non-Asian

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Study or Subgroup	Incidence	SE	Weight	Incidence IV, Random, 95% CI	Incidence IV, Random, 95% CI
GEF/ERL/AFA					
Bean J, 2007	20.9	6.2	3.3%	20.90 [8.75, 33.05]	
Cardona AF, 2017	8.8	5.5	4.0%	8.80 [0.00, 19.58]	
Iacono D, 2019	10.5	7.9	2.1%	10.50 [0.00, 25.98]	
Ji W, 2013	11.5	6.8	2.8%	11.50 [0.00, 24.83]	
Johnson ML, 2011	0	10	1.4%	0.00 [0.00, 19.60]	
Ko R, 2016	7.4	6	3.5%	7.40 [0.00, 19.16]	
Lee CK, 2017	0	5.8	3.7%	0.00 [0.00, 11.37]	
Li C, 2018	14.3	8	2.1%	14.30 [0.00, 29.98]	
Nakamura T, 2018	0	6.3	3.2%	0.00 [0.00, 12.35]	
Oxnard GR, 2011	10.5	5.4	4.2%	10.50 [0.00, 21.08]	
Redig AJ, 2016	2.9	4.2	6.2%	2.90 [0.00, 11.13]	
Sequist LV, 2011	6.9	5.7	3.8%	6.90 [0.00, 18.07]	
Wagener-Ryczek S, 2020	13.8	3.2	8.9%	13.80 [7.53, 20.07]	
Yu HA, 2013	5.3	3	9.7%	5.30 [0.00, 11.18]	
Subtotal (95% CI)			58.8%	8.11 [5.09, 11.13]	

Heterogeneity: Tau² = 4.87; Chi² = 15.31, df = 13 (P = 0.29); I² = 15%
Test for overall effect: Z = 5.27 (P < 0.00001)

Study or Subgroup	Incidence	SE	Weight	Incidence IV, Random, 95% CI	
OSM					
Bordi P, 2019	9.1	10.3	1.3%	9.10 [0.00, 29.29]	
Le X, 2018	14.3	5.6	3.9%	14.30 [3.32, 25.28]	
Mehlman C, 2019	13.1	4.5	5.6%	13.10 [4.28, 21.92]	
Mu Y, 2020	6.1	4	6.6%	6.10 [0.00, 13.94]	
Nie K, 2018	0	10	1.4%	0.00 [0.00, 19.60]	
Oxnard GR, 2018	9.8	5.1	4.6%	9.80 [0.00, 19.80]	
Piotrowska Z, 2018	21.9	7.2	2.5%	21.90 [7.79, 36.01]	
Schoenfeld AJ, 2020	8.6	5.3	4.3%	8.60 [0.00, 18.99]	
Wang Y, 2018	38.5	12	1.0%	38.50 [14.98, 62.02]	
Yang Z, 2018	7.5	2.9	10.0%	7.50 [1.82, 13.18]	
Subtotal (95% CI)			41.2%	10.65 [6.68, 14.62]	

Heterogeneity: Tau² = 10.11; Chi² = 12.21, df = 9 (P = 0.20); I² = 26%
Test for overall effect: Z = 5.25 (P < 0.00001)

Total (95% CI) 100.0% 9.08 [6.70, 11.45]
Heterogeneity: Tau² = 6.22; Chi² = 28.33, df = 23 (P = 0.20); I² = 19%
Test for overall effect: Z = 7.49 (P < 0.00001)
Test for subgroup differences: Chi² = 0.99, df = 1 (P = 0.32), I² = 0%

Incidence rate (%) 0 50 100

d

Study or Subgroup	Incidence	SE	Weight	Incidence IV, Random, 95% CI	Incidence IV, Random, 95% CI
GEF/ERL/AFA					
Cardona AF, 2017	14.7	6.3	3.3%	14.70 [2.35, 27.05]	
Ji W, 2013	3.8	5.4	4.2%	3.80 [0.00, 14.38]	
Ko R, 2016	3.7	5.3	4.3%	3.70 [0.00, 14.09]	
Li C, 2018	4.8	6.5	3.1%	4.80 [0.00, 17.54]	
Sequist LV, 2011	5.4	4.6	5.2%	5.40 [0.00, 14.42]	
Wagener-Ryczek S, 2020	0	1.1	14.3%	0.00 [0.00, 2.16]	
Wu SG, 2016	0	4.5	5.4%	0.00 [0.00, 8.82]	
Yu HA, 2013	0	1.5	13.1%	0.00 [0.00, 2.94]	
Subtotal (95% CI)			53.0%	0.93 [0.00, 2.85]	

Heterogeneity: Tau² = 0.76; Chi² = 7.68, df = 7 (P = 0.36); I² = 9%
Test for overall effect: Z = 0.95 (P = 0.34)

Study or Subgroup	Incidence	SE	Weight	Incidence IV, Random, 95% CI	
OSM					
Le X, 2018	14.3	5.6	4.0%	14.30 [3.32, 25.28]	
Mehlman C, 2019	3.3	3	8.5%	3.30 [0.00, 9.18]	
Mu Y, 2020	2	3.1	8.3%	2.00 [0.00, 8.08]	
Nakamura T, 2018	33.3	13.6	0.8%	33.30 [6.64, 59.96]	
Oxnard GR, 2018	4.9	4.2	5.9%	4.90 [0.00, 13.13]	
Piotrowska Z, 2018	12.5	6.2	3.4%	12.50 [0.35, 24.65]	
Schoenfeld AJ, 2020	0	3.8	6.7%	0.00 [0.00, 7.45]	
Wang Y, 2018	7.7	9.2	1.7%	7.70 [0.00, 25.73]	
Yang Z, 2018	10.8	3.3	7.8%	10.80 [4.33, 17.27]	
Subtotal (95% CI)			47.0%	6.53 [2.53, 10.54]	

Heterogeneity: Tau² = 15.16; Chi² = 14.47, df = 8 (P = 0.07); I² = 45%
Test for overall effect: Z = 3.20 (P = 0.001)

Total (95% CI) 100.0% 4.21 [1.70, 6.73]
Heterogeneity: Tau² = 10.28; Chi² = 31.71, df = 16 (P = 0.01); I² = 50%
Test for overall effect: Z = 3.28 (P = 0.001)
Test for subgroup differences: Chi² = 6.12, df = 1 (P = 0.01), I² = 83.7%
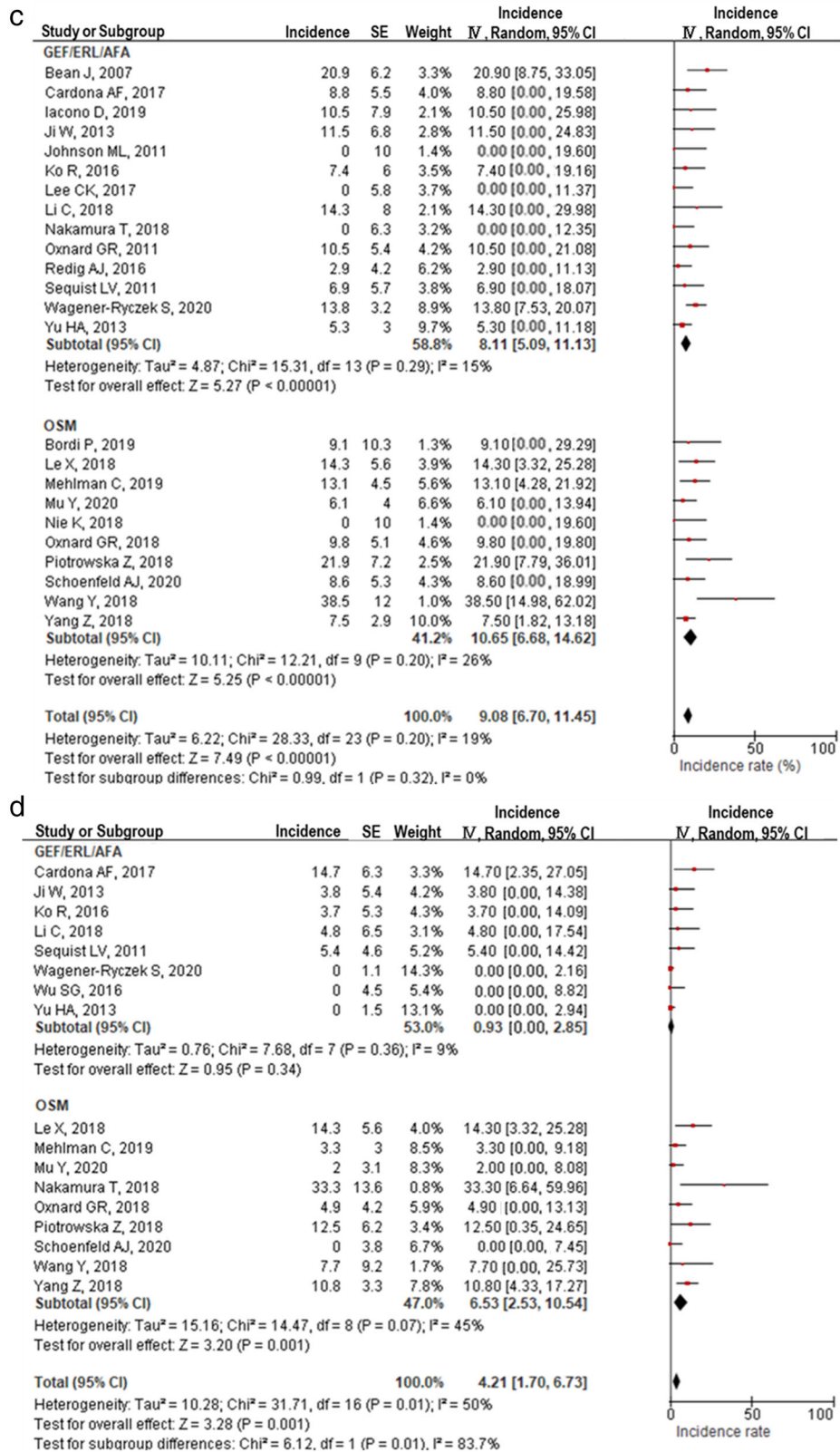
Incidence rate 0 50 100

FIGURE 5 (Continued)

populations. C757S was another possible mechanism that could explain the lesser efficacy of osimertinib in Asian patients. Unfortunately, we could not examine the difference in the incidence of C757S among various racial groups because of the limited number of studies on this topic. We speculate that the C757S mutation could be more frequent

in Asian patients than in non-Asian patients and could, therefore, limit the efficacy of osimertinib.

Our data also provide novel insights into the mechanism of osimertinib treatment. The incidence of T790M after osimertinib treatment was only 41.6%. Oxnard et al. revealed that alterations in the EGFR-independent signaling pathway, such as an acquired abnormality in other *EGFR* genes including *KRAS*, *MET* amplification, and PIK3CA mutation, or the transformation to SCLC, were more frequent in patients whose T790M mutations disappeared.[11] In our comparison of the incidence of the EGFR-independent mechanisms between osimertinib and other TKIs, *KRAS* mutations and SCLC transformations were significantly more frequent with osimertinib treatment than with treatment using first- and second-generation EGFR-TKIs. Moreover, the incidence of *KRAS* mutations was almost 10-fold higher with osimertinib treatment than with other TKIs. Although there were no significant differences in the incidence of *MET* amplification and PIK3CA mutations among TKIs, both mutations were more frequent after osimertinib treatment than after treatment with other TKIs. Therefore, identifying acquired resistance mechanisms is more beneficial for patients treated with osimertinib. This will allow for a more specific and effective treatment, such as KRAS inhibitors (e.g., AMG510, and MRTX489) for patients with *KRAS* mutations,[23] combination therapy with osimertinib plus savolitinib[24] or crizotinib[25] for patients with *MET* amplification, and pictilisib or PX-866 for patients with PIK3CA mutations.[26,27] Our data support that the fact that resistance mechanisms in patients who acquire resistance to osimertinib should be examined to improve prognosis through specific treatment.

The major reasons for the differences in resistance mechanisms among TKIs remain unknown. Regarding the incidence of T790M after first-generation TKI or afatinib, Byung et al. revealed that afatinib could inhibit the growth of gefitinib-resistant cancer cells with low T790M allele frequencies.[28] This effect of afatinib is the possible reason for the lower incidence of T790M after resistance than in first-generation TKIs. Afatinib is unique in its multi-inhibitory activity targeting the pan-HER family, including EGFR, HER2, ErbB3, and ErbB4 compared to other TKIs.[29] This difference might affect other differences in resistance mechanisms.

Our study has some limitations. First, the detection methods for analyzing the various resistance mechanisms varied. The difference may have influenced the results of the incidence of resistance mechanisms. Second, while analyzing the differences between the Asian and non-Asian populations, some reports could not be included because of the lack of information on race. Finally, the resistance mechanism against osimertinib in the first-line setting is unknown because there are no reports in this setting to date.

In conclusion, there are significant differences in the incidence of resistance mechanisms among EGFR-TKIs. These findings provide new insights into the difference in resistance mechanisms among EGFR-TKIs, as well as the influence of the therapeutic sequence to be chosen. Our data suggest that resistance mechanisms should be identified to pursue a more specific treatment for patients with acquired resistance to osimertinib.

CONFLICT OF INTERST
The authors have no conflicts of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.