An Observational Study of Acquired EGFR T790M-Dependent Resistance to EGFR-TKI Treatment in Lung Adenocarcinoma Patients in Taiwan

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In Taiwan, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (EGFR-TKIs), gefitinib, erlotinib, and afatinib are served as first-line therapy for non-small lung cell cancer (NSCLC) patients with EGFR sensitizing mutations. However, the majority of patients who initially respond to EGFR-TKIs, progress through acquiring EGFR T790M mutations (T790M), which is the most common resistant mechanism. Patients with T790M gain the opportunity of subsequent treatment with third-generation EGFR-TKI, osimertinib. This study aimed to evaluate the association between prior EGFR-TKI therapy and incidence of acquired T790M resistance in lung adenocarcinoma patients who have progressed on first/second-generation EGFR-TKI therapy. This retrospective study included lung adenocarcinoma patients who had a radiographically-confirmed progressive disease under EGFR-TKI treatment and had re-biopsy samples for T790M testing from seven medical centers in Taiwan from June 2013 to December 2018. Patients harboring de novo T790M or using more than one EGFR-TKI were excluded. Of the 407 patients enrolled, the overall T790M acquisition rate was 52.8%. The patients treated with gefitinib, erlotinib or afatinib had a statistically significant difference in the T790M rates (59.9, 45.5, and 52.7%, respectively; \( p = 0.037 \)) after disease progression. Patients with common baseline EGFR mutations (Del-19 and L858R) \( (p = 0.005) \) and longer treatment duration with EGFR-TKIs \( (p < 0.001) \) had higher chances of T790M acquisition. Multivariate logistic regression analysis further showed that patients with common baseline EGFR mutations, gefitinib (compared to erlotinib) administration, and longer treatment duration with EGFR-TKIs had higher T790M incidence. There was no significant difference in the incidence of acquired T790M between different re-biopsy...
Patients with NSCLC harboring EGFR activating mutation showed a good response to the first- (gefitinib, erlotinib) and second-generation (afatinib) of EGFR-tyrosine kinase inhibitors (TKIs), but they developed acquired resistance in about 9–13 months (6). Different mechanisms of acquired EGFR-TKIs resistance have been reported (7, 8). The most common mechanism involves the acquired EGFR T790M mutation, which accounts for about half of the acquired resistant cases (9, 10). To overcome T790M-mediated resistance, the third-generation EGFR-TKI, osimertinib, has shown improved median progression-free survival (PFS) in NSCLC patients with acquired T790M (11).

Tissue biopsy remains the most reliable specimen for re-biopsy analysis even considering the heterogeneous nature of tumors. Lung cytology (e.g., pleural fluid) and liquid biopsy are the less-invasive alternatives. Numerous detection platforms with high reliability and sensitivity have also been established, including direct sequencing, real-time polymerase chain reaction (qPCR), droplet digital PCR (ddPCR), next-generation sequencing (NGS), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), etc. (12). Liquid biopsy testing is now increasingly used in detecting targetable alterations in NSCLC (13).

Osimertinib has not been reimbursed in Taiwan until April 2020. To identify patients for subsequent osimertinib treatment, several studies have investigated the tendency of acquired T790M under first-line EGFR-TKI treatments (14–17). In Taiwan, two single-center studies reported different rates of T790M occurrence under the treatment of the three first-line EGFR-TKIs (15, 18). Since afatinib was approved much later than the first-generation EGFR-TKIs, previous studies included fewer patients treated with afatinib compared to those treated with gefitinib and erlotinib. Consequently, we aimed to conduct a nationwide study (ARISE study) with a sufficient number of lung adenocarcinoma patients who progressed with each of the three EGFR-TKI therapies and to investigate the association between prior EGFR-TKI therapies and the incidence of acquired T790M-associated resistance.

INTRODUCTION

Lung cancer is the most commonly diagnosed cancer and the leading cause of all cancer-related mortalities in Taiwan and worldwide (1, 2). Most of the lung cancers are diagnosed at advanced or metastatic stages with lower 5-year survival rates (1). In Taiwan, 54.1% of all the newly diagnosed lung cancer cases are at stage IV, with a median survival time of 9 months (2).

Histologically, 85% of primary lung cancers are classified as non-small-cell lung cancer (NSCLC), with adenocarcinoma being the most common subtype. Somatic mutations in the EGFR gene are frequently found in adenocarcinomas (3, 4). The exon 19 deletion (Del-19) and exon 21 L858R (L858R) together account for 90% of the EGFR mutations. Other clinically relevant mutations include G719X, L861X, exon 20 insertions, etcetera (5).

Patients with NSCLC harboring EGFR activating mutation showed a good response to the first- (gefitinib, erlotinib) and second-generation (afatinib) of EGFR-tyrosine kinase inhibitors (TKIs), but they developed acquired resistance in about 9–13 months (6). Different mechanisms of acquired EGFR-TKIs resistance have been reported (7, 8). The most common mechanism involves the acquired EGFR T790M mutation, which accounts for about half of the acquired resistant cases (9, 10). To overcome T790M-mediated resistance, the third-generation EGFR-TKI, osimertinib, has shown improved median progression-free survival (PFS) in NSCLC patients with acquired T790M (11).

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MATERIALS AND METHODS

Patients

This retrospective study (ARISE study) included advanced lung adenocarcinoma patients with radiographically-confirmed progressive disease after EGFR-TKI treatment. The EGFR TKIs included two first-generation drugs gefitinib and erlotinib and one second-generation drug afatinib. The participating 7 hospitals included three hospitals in northern Taiwan (National Taiwan University Hospital, Taipei Veterans General Hospital, Chang Gung Memorial Hospital Linkou Branch), two hospitals in middle Taiwan (Taichung Veterans General Hospital, China Medical University Hospital), and two hospitals in southern Taiwan (Chang Gung Memorial Hospital Kaohsiung Branch, National Cheng Kung University Hospital). Patients were ≥ 20 years old at enrollment. The documentation of confirmed EGFR sensitizing mutations before initiation of EGFR-TKI treatment was required. Upon disease progression from the EGFR-TKI treatment, repeat biopsy (re-biopsy) samples were obtained for the assessment of T790M mutation from June 2013 to December 2018. Some patients were reported in prior studies (15, 18).

The three EGFR-TKIs, gefitinib, erlotinib, and afatinib, have been reimbursed by the National Health Insurance (NHI) of Taiwan for patients with locally advanced or metastatic (stage IIIb/IV) lung adenocarcinoma with EGFR mutations for first-line treatment since 2004, 2007, and 2014, respectively. Physicians had the opportunity to choose between the three EGFR-TKI based on patient’s conditions and preferences and clinical evidence.

The information on NSCLC treatments prior to re-biopsy, including surgery, chemotherapy, radiotherapy, and TKI therapy was also collected. Chemotherapy was referred to as conventional cytotoxic agents, not including EGFR TKIs.

This study was approved by the institutional/ethical review board (IRB) of each participating medical center. Written informed consent was obtained from each patient except those with a waiver granted from the IRB of each medical center.

Keywords: afatinib, epidermal growth factor receptor mutation, erlotinib, gefitinib, non-small cell lung cancer, tyrosine kinase inhibitor, osimertinib
Re-biopsy Specimen and T790M Detection Platform

For patients after the acquired resistance to the first/second-generation EGFR TKIs, T790M detection is a routine clinical practice. Re-biopsy specimens of primary or metastasis tumors were obtained either within the thorax (i.e., lung biopsies and pleural effusion) or out of thorax (i.e., non-lung biopsies, plasma, peritoneal fluid, and cerebrospinal fluid). The detection methods included the following: COBAS EGFR mutation test v2 (COBAS; Roche Molecular Systems Inc., New Jersey, USA), Therascreen EGFR RGQ PCR Kit (Therascreen; Scorpions & amplification refractory mutation system [ARMS], Qiagen Manchester Ltd, Manchester, UK), Beads, Emulsion, Amplification and Magnetics (BEAMing) digital PCR (dPCR) assay (BEAMing; OncoBEAM EGFR assay; Sysmex Inostics, Inc., Maryland, USA), MassARRAY genotyping (Mass; previously named SEQUENOM; Agena Bioscience, California, USA), competitive allele-specific TaqMan polymerase chain reaction (TaqMan; Life Technologies; Thermo Fisher Scientific, Inc., Massachusetts, USA), and laboratory-developed test (LDT). The laboratory-developed test for the detection of EGFR mutation included peptide nucleic acid locked nucleic acid sequencing (PNA-sequencing) and direct sequencing (19, 20). Each detection method was performed based on the manufacturer’s instructions. All the pathology laboratories of the hospitals were certified for clinical examination by the Taiwan Society of Pathology. For the detection of T790M, the BEAMing assay had a limit of detection (LoD) < 1%, whereas COBAS, Therascreen, Mass, and TaqMan tests had LoD > 1%.

Study Endpoints

The primary endpoint was to compare the incidence of acquired T790M mutation in patients after acquired resistance to the three first-line EGFR-TKIs. This study also aimed to analyze the association of the T790M acquisition rate with baseline EGFR sensitizing mutations, treatment duration, and other clinical characteristics of patients.

FIGURE 1 | The flow chart of patient enrollment. *There were 12 patients who switched to another EGFR-TKI as a first-line treatment due to the side effects (see Supplementary Figure 1).
Statistical Analysis
The comparison of T790M acquisition from each first-line EGFR-TKI was analyzed through ANOVA or Kruskal-Wallis test for continuous data and Chi-square test for categorical data. If the sampling variability was \( \leq 5 \), Fisher's exact test was applied. The association between clinical factors and the acquisition of T790M resistant mutation was examined using univariate and multivariate regression analyses. Time to treatment discontinuation (TTD) curves was plotted using the Kaplan-Meier method and compared using the log-rank test. All analyses were performed using SPSS software (version 22.0 for Windows; SPSS Inc., Chicago, Illinois).

RESULTS
Patient Distribution and Baseline Clinical Characteristics
From June 2013 to December 2018, 547 patients who progressed from the first-line EGFR-TKI and had re-biopsy results were recruited (Figure 1). The final analysis contained 407 lung adenocarcinoma patients after the exclusion of 1 patient with osimertinib as first-line treatment, 4 patients with de novo T790M before the first-line EGFR-TKI treatment, 4 patients with adenosquamous cell carcinoma, and 131 patients with more than one EGFR-TKI treatments before re-biopsy. Twelve patients who had received short-term treatment with EGFR-TKIs (7–76 days) before switching to the second or third EGFR-TKIs for 241–565 days due to adverse events, were also included into the analysis (Supplementary Figure 1). The baseline mutations included 210 (51.6%) Del-19, 175 (43.0%) L858R, and 22 (5.4%) other mutation types (Supplementary Table 1). There was no significant difference in different EGFR mutation rates, including Del-19, L858R, or uncommon EGFR mutation, among the three areas in Taiwan (\( p = 0.384 \)) (Figure 2).

Among the 407 patients enrolled, 162 (39.8%), 154 (37.8%), and 91 (22.4%) used gefitinib, erlotinib, and afatinib, respectively, as first-line EGFR-TKI (Table 1). In addition, 58 (14.1%) patients were administered EGFR TKIs because of tumor recurrence after definitive surgery. There were 27 patients who received chemotherapy and 63 patients who underwent radiotherapy before EGFR TKIs administration.

In the gefitinib group, there were higher proportions of females (\( p < 0.001 \)) and patients with older age (\( p = 0.002 \)) than in the other two groups (Table 1). Patients under gefitinib treatment also had more solid tumor specimens for re-biopsy analysis (\( p = 0.004 \)). Patients who were treated with afatinib had fewer tumors harboring L858R (\( p < 0.001 \)) and used more BEAMing dPCR assay and LDTs for the T790M detection (\( p < 0.001 \)). Patients across the three EGFR-TKI groups showed no differences in the smoking status, disease stage, chemotherapy, or radiotherapy before re-biopsy.

Development of Acquired T790M
Of the 407 enrolled patients, 217 (52.8%) developed acquired T790M. The T790M acquisition rate was significantly different among patients under the three different treatments (gefitinib vs. erlotinib vs. afatinib: 59.9 vs. 45.5 vs. 52.7%, respectively; \( p = 0.037 \)) (Table 2 and Figure 3). A higher incidence was observed in patients treated with gefitinib than in those treated with erlotinib (\( p = 0.010 \)).

Incidence of Acquired T790M by Times to Treatment Discontinuation
The difference in median time to treatment discontinuation (TTD) among patients who received gefitinib (16.2 months), erlotinib (12.1 months), and afatinib (14.4 months) was significant (\( p = 0.001 \); Figure 4). The T790M acquisition rate was analyzed by predefined TTD, and 32.1% (52 of 162) of the patients who received gefitinib had TTD of more than 24 months (Supplementary Table 2). There was a significant difference in T790M incidence among patients who had TTD of \( < 6 \) months (30.6%), 6–12 months (41.1%), 12–18 (57.9%), 18–24 (59.0%) or \( > 24 \) months (62.2%) (\( p < 0.001 \)) (Table 2 and Figure 5).

Association of Baseline Mutations With Acquired T790M
Patients with baseline EGFR mutations of Del-19 (58.1%) or L858R (50.3%) had a significantly higher incidence of acquired T790M than those with other uncommon EGFR mutations (22.7%; \( p = 0.005 \)) (Table 2 and Figure 6). There was no significant difference in the incidence of acquired T790M between patients with Del-19 and those with L858R (\( p = 0.125 \)).

Potential Predictive Clinical Factors for Acquired EGFR T790M
The association between patient baseline clinical characteristics and the incidence of acquired EGFR T790M was assessed using multivariate logistic regression analyses (Table 2). Compared with the patients who received gefitinib, those who received
TABLE 1 | Clinical characteristics of EGFR-TKI-treated patients who received re-biopsy for detection of acquired T790M.

|                          | All patients | Gefitinib | Erlotinib | Afatinib | P       |
|--------------------------|--------------|-----------|-----------|----------|---------|
| Total                    | 407          | 162 (39.8%) | 154 (37.8%) | 91 (22.4%) |         |
| Age, median, years       | 65           | 67        | 64        | 63       | 0.002< |
| (range)                  | (37–96)      | (41–96)   | (37–93)   | (37–83)  |         |
| Gender                   |              |           |           |          | <0.001  |
| Female                   | 260          | 121 (74.7%) | 92 (59.7%) | 47 (61.6%)|         |
| Male                     | 147          | 41 (25.3%)  | 62 (40.3%) | 44 (38.4%)|         |
| Smoking status           |              |           |           |          | 0.091   |
| Never-smokers            | 320          | 138 (45.2%) | 117 (41.0%) | 65 (38.8%)|         |
| Current-smokers          | 14           | 3 (21.4%)  | 7 (42.9%)  | 4 (25.9%) |         |
| Former-smokers           | 73           | 21 (28.8%)  | 30 (40.8%) | 22 (29.8%)|         |
| EGFR mutation            |              |           |           |          | <0.001  |
| Del-19*                  | 210          | 80 (49.4%)  | 71 (46.1%) | 59 (60.5%)|         |
| L858R                    | 175          | 77 (44.5%)  | 77 (44.5%) | 66 (60.3%)|         |
| Others                   | 22           | 5 (22.7%)  | 6 (27.3%)  | 11 (40.7%)|         |
| Disease stage            |              |           |           |          | 0.179   |
| Tumor recurrence         | 58           | 28 (47.5%)  | 22 (45.8%) | 8 (3.3%)  |         |
| Advanced stage           | 349          | 134 (38.7%) | 132 (38.2%) | 83 (24.0%)|         |
| Chemotherapy before biopsy|            |           |           |          | 0.683   |
| No chemotherapy          | 287          | 110 (46.7%) | 113 (44.9%) | 64 (70.3%)|         |
| Before EGFR-TKI          | 27           | 10 (47.6%)  | 9 (47.4%)  | 8 (47.4%) |         |
| After EGFR-TKI           | 93           | 42 (45.4%)  | 32 (45.4%) | 19 (45.4%)|         |
| Radiotherapy before biopsy|            |           |           |          | 0.267   |
| No radiotherapy          | 301          | 127 (42.2%) | 106 (69.2%) | 68 (74.7%)|         |
| Before EGFR-TKI          | 63           | 18 (28.6%)  | 20 (32.8%) | 14 (22.2%)|         |
| After EGFR-TKI           | 43           | 17 (40.9%)  | 17 (40.9%) | 9 (20.9%) |         |
| Re-biopsy samples        |              |           |           |          | 0.004   |
| Tumor specimens          | 257          | 115 (45.0%) | 94 (61.0%) | 48 (62.7%)|         |
| Body fluid cells<        | 82           | 28 (34.5%)  | 37 (45.3%) | 16 (19.6%)|         |
| Plasma                   | 68           | 19 (28.6%)  | 23 (34.3%) | 14 (20.6%)|         |
| T790M detection method   |              |           |           |          | <0.001  |
| LoD >1%<                 | 349          | 139 (39.5%) | 144 (42.0%) | 66 (72.5%)|         |
| LoD <1%<                 | 11           | 3 (27.3%)   | 2 (18.2%)  | 6 (54.5%) |         |
| Other<                   | 47           | 20 (42.6%)  | 8 (17.0%)  | 19 (40.3%)|         |

*By Kruskal-Wallis test.
†Sampled from pleural effusion, CSF or ascites.
‡COBAS EGFR Mutation Test, Scorpions & ARMS (Amplification Refractory Mutation System), competitive allele-specific TaqMan polymerase chain reaction, MassARRAY genotyping (SEQUENOM) and Therascreen test.
§Including BEAMing.
φIncluding laboratory developed test (LDT) and others.
EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor; LoD, limit of detection.

erlotinib had a significantly lower T790M acquisition rate (odds ratio [OR]: 0.58; 95% confidence interval [CI]: 0.36–0.96; p = 0.032), while patients under afatinib treatment had similar rates of acquired T790M (OR: 0.72; 95% CI: 0.40–1.33; p = 0.296). Patients with other uncommon baseline EGFR mutations were less likely to develop acquired T790M compared to those with Del-19 (OR: 0.18; 95% CI: 0.06–0.55; p = 0.003). In addition, compared to patients with TTD < 6 months of EGFR-TKI treatments, higher T790M acquisition were seen in those with 12–18 months of TTD (OR: 3.29; 95% CI: 1.39–7.79; p = 0.007), 18–24 months of TTD (OR: 5.48; 95% CI: 2.09–14.40; p = 0.001), and > 24 months of TTD (OR: 3.59; 95% CI: 1.46–8.84; p = 0.005).

Acquired T790M in Different Re-biopsy Tissue Specimens and Complications

Table 3 showed the different re-biopsy samples. The acquired T790M incidence rates were 54.4% in primary tumors, 50.0% in metastatic tumors, and 55.9% in plasma samples (p = 0.286). After the exclusion of plasma samples, no significant difference was noted between the re-biopsy tissues located in intra- and extra-thoracic lesions (54.4
### TABLE 2 | Multivariate analysis of factors for acquired T790M in patients treated with EGFR-TKIs.

| Factors                          | Number of patients | T790M (%) | p          | Odds ratio 95% CI | p       |
|----------------------------------|--------------------|-----------|------------|-------------------|---------|
| **Gender**                      |                    |           |            |                   |         |
| Female                           | 260                | 141 (54.2)| 0.450      |                   |         |
| Male                             | 147                | 74 (50.3) | 0.75 (0.44–1.29) | 0.296  |
| **Age**                          |                    |           | 0.109      |                   |         |
| ≤ 65 y/o                         | 210                | 119 (56.7) | 1         |                   |         |
| > 65 y/o                         | 197                | 96 (48.7)  | 0.74 (0.48–1.14) | 0.174  |
| **Smoking status**               |                    |           | 0.265      |                   |         |
| Never-smokers                    | 320                | 170 (53.1)| 1         |                   |         |
| Current-smokers                  | 14                 | 10 (71.4)  | 3.19 (0.86–11.79) | 0.083  |
| Former-smokers                   | 73                 | 35 (47.9)  | 1.00 (0.52–1.95) | 0.990  |
| **EGFR mutation**                |                    |           | 0.005      |                   |         |
| Del-19*                          | 210                | 122 (58.1)| 1         |                   |         |
| L858R                            | 175                | 88 (50.3)  | 0.79 (0.51–1.23) | 0.292  |
| Others                           | 22                 | 5 (22.7)   | 0.18 (0.06–0.55) | 0.003  |
| **EGFR-TKI**                     |                    |           | 0.037      |                   |         |
| Gefitinib                        | 162                | 97 (59.9)  | 1         |                   |         |
| Erlotinib                        | 154                | 70 (45.5)  | 0.58 (0.36–0.96) | 0.032  |
| Afatinib                         | 91                 | 48 (52.7)  | 0.72 (0.40–1.33) | 0.296  |
| **EGFR-TKI TTD**                 |                    |           | <0.001     |                   |         |
| < 6 mo                           | 36                 | 11 (30.6)  | 1         |                   |         |
| 6–12 mo                          | 124                | 51 (41.1)  | 1.58 (0.68–3.65) | 0.289  |
| 12–18 mo                         | 107                | 62 (57.9)  | 3.29 (1.39–7.79) | 0.007  |
| 18–24 mo                         | 58                 | 40 (69.0)  | 5.48 (2.09–14.40) | 0.001  |
| > 24 mo                          | 82                 | 51 (62.2)  | 3.59 (1.46–8.84) | 0.005  |
| **Chemotherapy before re-biopsy**|                    |           | 0.356      |                   |         |
| No chemotherapy                  | 287                | 158 (55.1)| 1         |                   |         |
| Before EGFR-TKI                  | 27                 | 12 (44.4)  | 0.70 (0.29–1.70) | 0.432  |
| After EGFR-TKI                   | 93                 | 45 (48.4)  | 0.85 (0.51–1.40) | 0.516  |
| **Re-biopsy samples**            |                    |           | 0.840      |                   |         |
| Tumor specimens                  | 257                | 135 (52.5)| 1         |                   |         |
| Body fluid cells†                | 82                 | 42 (51.2)  | 1.08 (0.62–1.88) | 0.790  |
| Plasma                           | 68                 | 36 (55.9)  | 1.13 (0.58–2.22) | 0.715  |
| **T790M detection method**       |                    |           | 0.034      |                   |         |
| LoD > 1‰                         | 349                | 182 (52.1)| 1         |                   |         |
| LoD < 1‰                         | 11                 | 10 (90.9)  | 8.31 (0.95–73.03) | 0.056  |
| Otherφ                           | 47                 | 23 (48.9)  | 0.73 (0.34–1.57) | 0.419  |

*Deletion in exon 19.
†Sampled from pleural effusion, CSF or ascites.
¶COBAS EGFR Mutation Test, Scorpions & ARMS (Amplification Refractory Mutation system), competitive allele-specific TaqMan polymerase chain reaction, MassARRAY genotyping (SEQUENOM) and Therascreen test.
φIncluding BEAMing.
*Including laboratory-developed test (LDT) and others.
#By multivariate logistic regression analyses.

CI, confidence interval; Del-19: deletion in exon 19; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor; LoD, limit of Detection; Mo, months; TTD, Time to treatment discontinuation.

vs. 43.1%; p = 0.101). For metastatic tumor samples, there was also no significant difference in acquired T790M incidence rates between different metastatic lesions of bone, liver, lymph nodes, or others (p = 0.112). It is worth noting that acquired T790M mutation was detected only in one of seven (14.3%) cerebral spinal fluids samples. Twenty-four (5.9%) patients suffered from re-biopsy complications after percutaneous needle biopsy. There were 20 pneumothoraxes, 3 haemothorax/hemopneumothorax, and one hydro pneumothorax. There was no significant difference in the re-biopsy complication rate between patients with and without acquired T790M (p = 0.259).
FIGURE 3 | The comparison of acquired T790M incidence between the three different EGFR-TKIs (Gefitinib vs. Erlotinib vs. Afatinib: 59.9 vs. 45.5 vs. 52.7%; respectively, p = 0.037).

FIGURE 4 | Kaplan-Meier survival curves of time to treatment discontinuation (TTD) in the three EGFR-TKI-treated adenocarcinoma patient cohorts. There was a significant difference between gefitinib (n = 162), erlotinib (n = 154), and afatinib (n = 91) (gefitinib vs. erlotinib vs. afatinib: 16.2 months vs. 12.1 months vs. 14.4 months, respectively; p = 0.001).

**DISCUSSION**

This was the first nationwide study for the comparison of the T790M acquisition rate between the three first-line EGFR-TKIs, gefitinib, erlotinib, and afatinib. The study utilized a wide range of specimen types and detection platforms and revealed a T790M acquisition rate of 52.8%. Patients who progressed from the first-line of gefitinib, erlotinib, and afatinib treatments had an incidence of T790M acquisition rate at 59.9, 45.5, and 52.7%, respectively. Patients with first-line of gefitinib treatment (compared to erlotinib), common EGFR mutations at baseline, and longer treatment duration had significantly higher rates of T790M. In addition, there was no significant difference in acquired T790M incidence rates neither between the different re-biopsy tissue samples nor with regard to re-biopsy-associated complications.

Identification of acquired T790M is vital since patients with T790M gain the benefit of the second-line osimertinib treatment (11). In addition, cancer cells harboring T790M mutations grow slower and have a more indolent phenotype (21). Furthermore, patients with T790M-mediated resistance tend to have longer PFS and post-progression survival compared to those without T790M (22, 23). The present study revealed a comparable T790M acquisition rate to historical data (9, 10). Some previous studies showed that the T790M acquisition rate was higher in the first-generation of EGFR-TKIs, gefitinib (50–55%), and erlotinib (38–57%), compared to that in the second-generation of afatinib (20–41%) (14–17). Our study revealed that the T790M acquisition rates were similar between gefitinib and afatinib, while it was significantly higher in patients who progressed from gefitinib than in those treated with erlotinib.

These differences in acquired T790M incidence may result from some major differences in the experimental approach when compared with previous studies (14–17). First, various assays were used for EGFR T790M mutation detection. The more sensitive test could detect low allele frequencies of T790M. Second, the present study enrolled a larger number of patients studied when compared to the previous single-center studies. In particular, there were more erlotinib-treated (N = 154) and afatinib-treated patients (N = 91) in the present study. Lin et al. enrolled 16 erlotinib-treated and 36 afatinib-treated patients (18). Huang et al. enrolled 13 afatinib-treated patients (15). Nosaki et al. reported 5 afatinib-treated patients (16). Third, the present study enrolled patients from multicenter in different areas of Taiwan to avoid the selection biases from in a single hospital. This study cohort was a true real-world practice in Taiwan. In addition, this study would be more generalizable to the real...
clinical practice of the NSCLC patient population in Taiwan. In addition, it is known that T790M mutation is located in a region where a single nucleotide polymorphism is positioned nearby the GC-rich sequences, rendering it challenging to detect and amplify.

The mechanism of acquisition T790M is still unclear. El Kadi et al. reported that deamination of the 5-methylcytosine to thymidine at position c.2369 generates the T790M change that alters TKI-binding affinity and causes resistance (24). In addition, the BELIEF trial showed that T790M at disease progression can be derived from the selection of preexisting EGFR T790M-positive clones or emerge de novo in initially negative cells (25). Advanced studies are required to explore the definite mechanism that the incidences of acquired T790M are different after variable EGFR TKIs treatment.

Our study also identified that patients with common baseline EGFR mutations of Del-19 and L858R had a higher T790M acquisition rate compared to those with uncommon baseline EGFR mutations. Lin et al. also reported that uncommon EGFR mutation had less secondary T790M acquisition (adjusted OR 0.14, 95% CI, 0.02–0.97; p = 0.047) compared with

![FIGURE 5](image)

**FIGURE 5** | The comparison of acquired T790M incidence between different times to treatment discontinuation (TTD) of EGFR-TKIs (p < 0.001 by Chi-square test).

| All patients | T790M(+) | T790M(-) | p     |
|--------------|---------|---------|------|
| Total        | 407     | 215     | 192  | 0.286 |
| Re-biopsy tissue specimens |        |         |      |       |
| Primary lung tumors | 149     | 81      | 68   |       |
| Pleural effusions/ascites | 75      | 41      | 34   |       |
| Cerebrospinal fluids | 7       | 1       | 6    |       |
| Plasma        | 68      | 38      | 30   |       |
| Metastatic tumors | 108     | 54      | 54   | 0.112 |
| Bone          | 65      | 33      | 32   |       |
| Liver         | 13      | 10      | 3    |       |
| Lymph node    | 20      | 7       | 13   |       |
| Others        | 10      | 4       | 6    |       |
| Re-biopsy tumor location* |        |         |      | 0.101 |
| Intra-thoracic | 274     | 149     | 125  |       |
| Extra-thoracic | 65      | 26      | 37   |       |
| Re-biopsy complication |        |         |      | 0.259 |
| Yes           | 24      | 10      | 14   |       |
| No            | 383     | 206     | 178  |       |

*Exclude plasma samples.

# | The comparison between the different metastatic tumors. The italic value demonstrated the subgroups of different metastatic tumors.

| All patients | T790M(+) | T790M(-) | p     |
|--------------|---------|---------|------|
| Total        | 407     | 215     | 192  | 0.286 |
| Re-biopsy tissue specimens |        |         |      |       |
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| Re-biopsy complication |        |         |      | 0.259 |
| Yes           | 24      | 10      | 14   |       |
| No            | 383     | 206     | 178  |       |
del-19 of EGFR mutation (18). In addition, the previous studies showed T790M was more frequent in patients with Del-19 compared to those with L858R (15, 17, 26–28), which was also observed as a trend but without statistical significance in our study. Prior studies also revealed that the incidence of T790M was higher in patients under more extended EGFR-TKI treatment before re-biopsy (14, 15, 26–29). We further stratified the data by a series of timeframe and showed that the incidence of T790M acquisition increased in accordance with the prolongation of treatment duration. We started to see the difference after 12–18 months of treatment, which is comparable to the findings of previous studies.

For body fluid or liquid biopsy, various methodologies, with high sensitivity and detection of genetic number and type alteration, are being used for the detection of EGFR T790M (7). Cobas EGFR Mutation Test v2 shows 61.4% of sensitivity and 78.6% of specificity (30). In addition, the sensitivity and specificity of T790M detection are 93 and 94% in the next-generation sequencing (NGS) (31, 32), 70 and 69% for beads, emulsion, amplification and magnets (BEAMing) (33), and 77 and 63% for digital droplet polymerase chain reaction (ddPCR) (34).

There was no significant difference in the incidence of acquired T790M among different re-biopsy tissue samples, re-biopsy lesion locations (intra- vs. extra-thoracic), or different metastatic organs (bone, liver, lymph nodes, or others). However, CSF seems to have lower acquired T790M incidence. The prior reports showed that there was discordance in EGFR mutation status between primary tumor and CSF (35). It may result from low cellularity or ctDNA in the small amount of CSF or the low concentrations of the first and second generation of EGFR TKIs in CSF inadequate to drive the occurrence of T790M (36).

The main limitation of this study was the nature of the retrospective study design and potential bias. The imbalanced baseline characteristics of age, gender, EGFR mutation, re-biopsy sample type, and the usage of the T790M detection methods seen in this study might result from the physician’s preference and the differences in technology accessibility at each medical center. Future studies utilizing randomized controlled design and unified companion diagnostic devices will help to strengthen the data. Nonetheless, the enrollment of a large population across the country and the utilization of various high-sensitive detection platforms give the strength to provide valuable information for future clinical decision-making. In addition, this study was based on a single nationality.

Osimertinib has recently been approved as the first-line treatment in patients with Del-19/L858R based on the groundbreaking results of the FLAURA trial (37). First-line osimertinib treatment reduced the risk of death by 20% compared to first-generation EGFR-TKIs and achieved a 3-year median OS of 38.6 months, which was 6.8 months longer than that achieved in the first-generation of EGFR-TKIs (37, 38). Although sequential second-line therapy of osimertinib in patients who progressed with acquired T790M attained recognized benefits, emerging evidence suggested that osimertinib as a first-line was preferable (39). In addition to the interest of reducing multiple systemic EGFR-TKI exposures that increase harmful side effects, the use of the first-line osimertinib could minimize the potential of patient loss (known as first-line treatment attrition) as a result of treatment intolerance and adverse events. It was estimated that more than 2/3 of patients might never receive clinical benefits of osimertinib due to the fact of the first-line treatment attrition and difficulties of re-biopsy and T790M detection. Despite standing as a preferable choice, the first-line osimertinib is hampered by law or price barriers in some countries. Alternatively, utilization of a first-line EGFR-TKI that is associated with a higher T790M acquisition rate remains favorable for the subsequent clinical management.

In conclusion, this study established that patients under the first-line of gefitinib treatment, bearing baseline common EGFR mutations, and with more than 1 year of treatment had a higher incidence rate of acquired T790M at progression, which could be managed with subsequent second-line of osimertinib treatment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional/ethical review board (IRB) of National Taiwan University Hospital, Taipei Veterans General Hospital, Chang Gung Memorial Hospital Taoyuan branch and Kaohsing Medical Center, National Cheng Kung University Hospital, China Medical University Hospital and Taichung Veterans General Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

S-GW and J-YS: study design, literature search, and drafting of the manuscript. C-LC, C-YL, C-CW, P-LS, T-CH, J-YS, and G-CC: patient specimen collection and data collection. J-YS and G-CC: study supervision. All authors approved the final draft of the submitted manuscript.

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SUPPLEMENTARY MATERIAL

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The handling editor declared a shared affiliation, though no other collaboration, with one of the authors P-LS.

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