EGFR mutation genotypes affect efficacy and resistance mechanisms of osimertinib in T790M-positive NSCLC patients

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Background: Osimertinib is a potent third-generation *EGFR* tyrosine kinase inhibitor (TKI) with robust activity in advanced *EGFR*-mutant non-small cell lung cancer (NSCLC), including those with T790M resistance mutation. However, a broad interpatient variability was observed. This study aimed to evaluate whether *EGFR*-mutant genotypes affect the clinical outcomes and resistance mechanisms in T790M-positive NSCLC patients receiving osimertinib therapy.

Methods: All NSCLC patients treated with osimertinib in our institute were screened. We included those with known *EGFR*-mutant genotypes and T790M positivity. Clinical outcomes including objective response rate (ORR), clinical benefit rate (CBR), progression-free survival (PFS), and overall survival (OS), were evaluated and compared between different *EGFR* genotypes. Patients with next-generation sequencing testing or tumor rebiopsy after osimertinib treatment were analyzed for resistance mechanisms.

Results: ORR, CBR, PFS, and OS were all non-significantly different among patients harboring *EGFR* exon 19 deletion (19Del, n=136), L858R (n=93), and uncommon mutations (n=6). However, a subset of tumors with deletion starting at E746 (ΔE746, n=98), but not non-ΔE746 tumors (n=38), had better clinical outcomes than L858R tumors (n=93). Frequencies of T790M loss and C797S acquisition after osimertinib treatment were similar between 19Del (n=56) and L858R tumors (n=33). However, compared with L858R tumors (n=33), those with 19Del ΔE746 subtype (n=40) had a higher whereas non-ΔE746 subtype (n=16) had a similar frequency of acquired C797S mutation. Combined analysis of our cohort and public cohort confirmed these findings.

Conclusions: Our findings indicate that the *EGFR* 19Del subtypes affect the clinical outcomes and resistance mechanisms to osimertinib in T790M-positive patients. Identifying patients with relatively worse treatment outcomes may be informative for establishing new therapies for these patients.

Keywords: Non-small cell lung cancer (NSCLC); epidermal growth factor receptor (*EGFR*); exon 19 deletion; osimertinib; C797S mutation

Introduction

Epidermal growth factor receptor (*EGFR*) mutations are found in almost half of Asian non-small cell lung cancer (NSCLC) patients (1,2). Treatment with *EGFR* tyrosine kinase inhibitors (TKIs) is a milestone achievement in the management of advanced *EGFR*-mutant NSCLC. However, it is also of note that increasing evidence indicates *EGFR*-mutant NSCLCs display broad molecular and clinical heterogeneity (3-11). Even the most two common EGFR TKI-activating mutations, exon 19 deletion (19Del) and L858R, have differences in sensitivities to first- and second-generation *EGFR* TKIs (e.g., gefitinib, erlotinib, and afatinib) (4-6). In addition, these two types of *EGFR* mutation also produced differences in resistance mechanisms to first- or second-generation TKIs, where the acquired T790M mutation occurred more frequently in the 19Del mutant tumors than in L858R mutant tumors (8,9). Recent studies still revealed that distinct *EGFR*-mutant tumors have differences in tumor mutation burden (TMB) and in outcomes with immune checkpoint blockade treatment, where 19Del mutant tumors have a significantly lower TMB and a reduced benefit of treatment with immune checkpoint inhibitors compared with L858R mutant tumors (10,11).

Moreover, in contrast to L858R mutation, 19Del mutations have a variety of variants and represent a more heterogeneous disease entity (12,13). Distinct 19Del variants also confer heterogeneous sensitivity to first- or second-generation *EGFR* TKIs (14-19), and even associate with different histology in NSCLCs (19). Additional studies still suggested that patients carrying the canonical variant p.E746_A750del have a higher proportion of acquired T790M mutation than those carrying other 19Del variants after progression on earlier-generation TKIs (20,21).

Osimertinib is a potent third-generation *EGFR* TKI with robust activity in advanced *EGFR*-mutant NSCLCs, including those with T790M resistance mutation (22). Although treatment responses and acquired resistance mechanisms to earlier-generation *EGFR* TKIs between *EGFR* mutation types or subtypes have been identified to be different in previous studies, few have comprehensively examined whether there is a discrepancy in sensitivity and resistance mechanisms to osimertinib between *EGFR*-mutant genotypes in the presence of T790M mutation. This study was thus performed with the purposes of assessing the differences in clinical outcomes and resistance mechanisms among patients with T790M-positive NSCLCs who were

specifically receiving osimertinib treatment and harboring different *EGFR* mutation types and subtypes.

Methods

Study population

Following the institutional review board approval at Sun Yat-sen University Cancer Center (SYSUCC), we identified all patients with advanced NSCLC treated with osimertinib up to February 2019 in our institutional lung cancer-specific database. Patients were included if they had a detection of T790M mutation before commencing osimertinib treatment, and a known *EGFR*-mutant genotype determined by next-generation sequencing (NGS). Patients were excluded if they received osimertinib as maintenance treatment or in combination with chemotherapy or other targeted therapy, as well as those with concurrent malignancy (*Figure S1*).

Data collection

Patient clinicopathologic features and treatment histories were retrospectively collected from electronic medical records. Clinical outcomes include overall response rate (ORR), clinical benefit rate (CBR), progression-free survival (PFS), and overall survival (OS). To determine the response, radiographic scans were reviewed by investigators (QZ and WF) based on Response Evaluation Criteria in Solid Tumors version 1.1 (23). ORR was defined as the proportion of patients with a best overall response of complete response (CR) or partial response (PR). CBR was defined as the proportion of patients whose best overall response is either CR, PR, or stable disease lasting for more than 24 weeks. PFS was defined as the date of commencing osimertinib treatment to the date of disease progression or death, whichever occurred first. Patients who were alive without disease progression were censored on the date of their last efficacy assessment. OS was defined as the date of commencing osimertinib treatment to death. Patients who were still alive were censored at the last contact date.

Identification of EGFR genotypes

EGFR genotypes were dominantly determined by using one of the following commercial NGS panels: the BGI Oseq-Drug panel, the Mygene Diagnostic panel, and the Personalized Diagnostics NGS panels of our hospital. During our study, the BGI Oseq-Drug panel expanded

from 206 genes to a 508-gene panel. The Mygene Diagnostic panel was used to sequence targeted hotspots for 22 lung cancer-related genes. The SYSUCC Personalized Diagnostics NGS panels include a 21-gene panel for circulating tumor DNA (ctDNA), a 150-gene ctDNA panel, and a 295-gene panel for solid tumor, and all panels have full coverage of *EGFR* gene. In addition, a subset of patients was sequenced using other targeted NGS panels at outside institutions (*Figure S2*).

Osimertinib resistance mechanism analysis

To evaluate the osimertinib resistance mechanisms among different *EGFR* genotypes, we further identified patients who conducted NGS testing at osimertinib resistance. Either tumor tissue or plasma cfDNA analysis was available. We focused on two common established mechanisms, the *EGFR* T790M loss and the C797S acquisition. Methodologies to detect *EGFR* resistance mutations included the BGI Oseq-Drug panel, the Mygene Diagnostic panel, and the SYSUCC Personalized Diagnostics NGS panels. In addition, a public cohort of 93 lung cancer patients with osimertinib-resistance mutation profiles from Yang *et al.* (24) was also used to evaluate osimertinib resistance mechanisms according to *EGFR* genotypes. The patients in public cohort were enrolled from multiple centers across China, and their mutation profiles were tested using a targeted NGS panel for 416 cancer-related genes. Moreover, we also identified patients who underwent tumor biopsy at osimertinib resistance. These patients were analyzed for the resistance mechanism of small-cell lung cancer (SCLC) transformation. All of their pathology slides were examined by expert lung cancer pathologists.

Statistical analysis

Differences in baseline characteristics between patients with *EGFR* mutation types or subtypes were compared using chi-square or Fisher's exact test for categorical data and the Wilcoxon rank-sum test for continuous data. PFS and OS were assessed by the Kaplan-Meier curve with log-rank test. Differences in tumor responses and *EGFR* resistance mechanisms were compared between patient groups by using chi-square tests. Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) were calculated by using univariate and multivariate Cox proportional hazards regression models to assess the effects of different variables on PFS and OS. Statistical analyses

were performed using STATA/MP 14.0 and GraphPad Prism software, version 7.0. Two-tailed P<0.05 was considered statistically significant.

Results

Characteristics of patients receiving osimertinib

We identified 540 advanced NSCLC patients treated with osimertinib between August 2015 and February 2019. From 337 patients with known mutant *EGFR* genotyping results determined by NGS, 90 had no detection of T790M mutation, 8 received a combination therapy, and 4 had concurrent malignant neoplasm. Finally, 235 patients were included in association analysis between *EGFR* genotypes and clinical outcomes of osimertinib treatment. Patient characteristics are summarized in *Table 1*. Among 235 patients [median age, 55 years (range, 27–81 years); 143 (60.9%) female; 164 (69.8%) non-smokers; 227 (96.6%) adenocarcinoma], 136 (57.9%) harbored EGFR 19Del, 93 (39.6%) harbored L858R, and only 6 (2.6%) harbored uncommon *EGFR* mutations (two G719A, one G719A+L861Q, one G719A+E709A, one V769M, and one exon 20 insertion [p.A763_ Y764insFQEA]). Patient characteristics grouped by *EGFR* mutation types are provided in *Table S1*. For T790M mutation status at baseline prior to osimertinib treatment, 101 (43.0%) patients were detected using plasma specimens, 98 (41.7%) using tumor tissue specimens, 8 (3.4%) using both ctDNA and tissue specimens, 15 (6.4%) using pleural/ peritoneal effusion specimens, 2 (0.9%) using cerebrospinal fluid specimens, and 11 (4.7%) were unspecified.

In addition, we identified 14 deletion variants from the 136 patients with *EGFR* 19Del (*Figure S3*). The most common deletion variants were p.E746_A750del (n=87, 64.0%), followed by p.L747_P753delinsS (n=15, 11.0%), p.L747_T751del (n=9, 7.1%), and p.L747_A750delinsP (n=6, 4.4%), consistent with previously reported frequencies for patients harboring 19Del (14-19). We classified these patients into two subgroups according to the deletion starting codon by Gazdar *et al.* (13): from E746 [ΔE746 subgroup, n=98 (72.1%)] or from other than E746 [non-ΔE746 subgroup, n=38 (27.9%)]. In non-ΔE746 subgroup, 35 patients harbored deletions from codon L747, two S752, and one T751. There were no clinicopathologic differences between two subgroups at baseline, except for a trend toward more non-adenocarcinoma in non-ΔE746 versus ΔE746 patients (7.9% *vs.* 1.0%; P=0.066; *Table 2*).

Table 1 Baseline characteristics of included patients in our cohort

Characteristics	Patients (n=235)
Age at osimertinib treatment	
Median [range], years	55 [27–81]
≤60 years	153 (65.1)
>60 years	82 (34.9)
Sex	
Female	143 (60.9)
Male	92 (39.1)
Smoking history	
Never	164 (69.8)
Current/ever	47 (20.0)
Unknown	24 (10.2)
Brain metastasis	
Yes	112 (47.7)
None	123 (52.3)
Histological type	
Adenocarcinoma	227 (96.6)
Others	8 (3.4)
Type of previous TKI	
Gefitinib	113 (48.1)
Erlotinib	65 (27.7)
Icotinib	45 (19.2)
Afatinib	1 (0.43)
None[†]	11 (4.7)
PFS of previous TKI[†]	
<6 months	25 (11.2)
≥6 months	199 (88.8)
Line of osimertinib treatment*	
1st line	11 (4.7)
2nd line	148 (63.0)
3rd or more line	76 (32.3)
EGFR mutation type	
Exon 19 deletion	136 (57.9)
L858R	93 (39.6)
Uncommon	6 (2.6)

[†], Eleven patients who harboring de novo T790M mutation received osimertinib as first-line treatment. *, Line of osimertinib treatment refers to the treatment line of osimertinib administration. For example, a patient who received osimertinib as third-line therapy will have previously received two treatment regimens in metastatic setting, which can include prior chemotherapy. TKI, tyrosine kinase inhibitor; PFS, progression-free survival; EGFR, epidermal growth factor receptor.

Table 2 Characteristics of patients grouped by starting codon of deletion

Characteristics	19Del ΔE746 (n=98)	19Del non-ΔE746 (n=38)	P value
Age at osimertinib treatment			
Median [range], years	53 [27–81]	55 [33–76]	0.377
≤60 years	72 (73.5)	24 (63.2)	0.236
>60 years	26 (26.5)	14 (36.8)	
Sex			0.853
Female	61 (62.2)	23 (60.5)	
Male	37 (37.8)	15 (39.5)	
Smoking history			0.871
Never	72 (73.5)	28 (73.7)	
Current/ever	18 (18.4)	6 (15.8)	
Unknown	8 (8.2)	4 (10.5)	
Brain metastasis			0.612
Yes	46 (46.9)	16 (42.1)	
None	52 (53.1)	22 (57.9)	
Histological type			0.066
Adenocarcinoma	97 (99.0)	35 (92.1)	
Others	1 (1.0)	3 (7.9)	
Type of previous TKI			0.941
Gefitinib	53 (54.1)	21 (55.3)	
Erlotinib	26 (26.5)	10 (26.3)	
Icotinib	18 (18.4)	7 (18.4)	
None[†]	1 (1.0)	0 (0.0)	
PFS of previous TKI			0.316
<6 months	7 (7.1)	1 (2.6)	
≥6 months[‡]	91 (92.9)	37 (97.4)	
Line of osimertinib treatment*			0.991
1/2-line[†]	62 (63.3)	24 (63.2)	
3 or more line	36 (36.7)	14 (36.8)	

[†], One patient received osimertinib as first-line treatment since harboring de novo T790M mutation. [‡], One patient who received osimertinib as first-line treatment were included in this category. *, Line of osimertinib treatment refers to the treatment line of osimertinib administration. For example, a patient who received osimertinib as third-line therapy will have previously received two treatment regimens in metastatic setting, which can include prior chemotherapy. 19Del, exon 19 deletion; TKI, tyrosine kinase inhibitor; PFS, progression-free survival.

Distinct EGFR mutation types have similar outcomes with osimertinib treatment

At the time of analysis (data cutoff September 1, 2019), the median follow-up was 18.0 months. Overall, 233 patients were evaluable for response. The ORR and CBR to osimertinib in our cohort were 53.7% and 78.1%, respectively. Besides, 169 patients either had disease progression or had died at the time of data cutoff. The median PFS of overall population was 10.1 months (95% CI: 9.1–11.5 months; *Figure S4A*), and the median OS was 22.0 months (95% CI: 18.7–24.4 months; *Figure S4B*). We examined the effect of distinct *EGFR* mutation types on outcome with osimertinib treatment. We found that patients harboring 19Del, L858R, and uncommon mutations had non-significantly different ORRs (58.5%, 47.3%, and 40.0%, respectively, P=0.206, *Figure S5A*) and CBRs (81.5%, 73.1%, and 80%, respectively, P=0.322, *Figure S5B*). There were also no significant difference in PFSs (median: 11.3, 9.0, and 11.7 months, respectively, log-rank P=0.276, *Figure S5C*) and OSs (median: 22.6, 20.1 months, and not reached, respectively, log-rank P=0.173, *Figure S5D*) among patients with 19Del, L858R, and uncommon mutations.

Distinct EGFR 19Del subtypes have different outcomes with osimertinib treatment

Due to the absence of study reporting the association between *EGFR* 19Del subtypes and osimertinib efficacy, we subsequently investigated the impact of varying *EGFR* 19Del subtypes on clinical outcomes of osimertinib treatment in our cohort of 136 T790M-mutant cases. We compared these cases with patients with L858R tumors. We found ΔE746 tumors had a significantly higher ORR and CBR compared with L858R tumors (ORR: 64.9% *vs.* 47.3%, P=0.014; CBR: 86.6% *vs.* 73.1%, respectively, P=0.020), whereas non-ΔE746 tumors had similar ORR and CBR compared with L858R tumors (ORR: 42.1% *vs.* 47.3%, respectively, P=0.587; CBR: 68.4% *vs.* 73.1%, respectively, P=0.588) (*Figure 1A,B*). PFS and OS in ΔE746 patients were also significantly longer (median PFS: 12.2 *vs.* 9.0 months, log-rank P=0.027; median OS: 23.7 *vs.* 20.1 months, log-rank P=0.02) whereas non-ΔE746 patients had similar PFS and OS compared with the L858R patients (median PFS: 8.6 *vs.* 9.0 months, log-rank P=0.621; median OS: 15.7 *vs.* 20.1 months, log-rank P=0.955) (*Figure 1C,D*). Moreover, with regard to ΔE746 subgroup, no significant differences in PFS and OS were observed between the most

common 19Del variant p.E746_A750del and other ΔE746 variants (log-rank P=0.967, P=0.396, respectively; *Figure S6A,B*). Overall, these data suggested that patients with *EGFR* ΔE746 tumors have a significantly better benefit from osimertinib treatment.

Clinicopathologic features associated with osimertinib outcomes

We further evaluated the effect of clinical and pathologic features on PFS and OS to osimertinib in patients with T790M-mutant NSCLC. In univariate analysis, we found that tumor histological type, line of osimertinib therapy, and *EGFR* mutation subtype were significant prognostic factors for PFS (*Table S2*). In multivariate Cox regression analysis adjusting for potential confounders, only tumor histologic type and *EGFR* mutation subtype remained independent predictive value for PFS (*Table S2*). Specifically, *EGFR* non-ΔE746 (HR=1.70, 95% CI: 1.08–2.69, P=0.022) and L858R (HR=1.70, 95% CI: 1.19–2.43, P=0.004) had a significantly higher HR than ΔE746 subtype. Similarly, multivariate analysis also confirmed that L858R (HR=1.70, 95% CI: 1.06–2.73, P=0.026) was independently associated with worse OS than *EGFR* ΔE746 mutation subtype (*Table S3*). In addition, non-adenocarcinoma histology (HR=3.92, 95% CI: 1.49–10.27, P=0.006) and third- or more line of osimertinib therapy (HR=1.59, 95% CI: 1.03–2.46, P=0.035) were also independently associated with worse OS for osimertinib treatment.

Distinct EGFR genotypes have different resistance mechanisms to osimertinib treatment

Given the reported association between mechanisms of osimertinib resistance and duration of osimertinib treatment (25-27), we investigated the osimertinib resistance mechanisms across variable *EGFR* mutation subtypes in our cohort. Of 169 patients developed resistance to osimertinib, we identified 90 patients who underwent NGS testing after resistance to osimertinib, including 34 (37.8%) using tissue biopsy specimens, 46 (51.1%) using plasma specimens, 7 (7.8%) using pleural effusion specimens, and 3 (3.3%) using both tissue and plasma specimens. Across all *EGFR* mutation subtypes, T790M loss was seen in 52 patients (57.8%); while acquired C797S mutation was developed in 20 patients (22.2%), all with maintained T790M. Of these C797S mutations, 15 (75.0%) were in cis to T790M, 2 (10.0%) were in trans, but 3 (15.0%) were not specified.
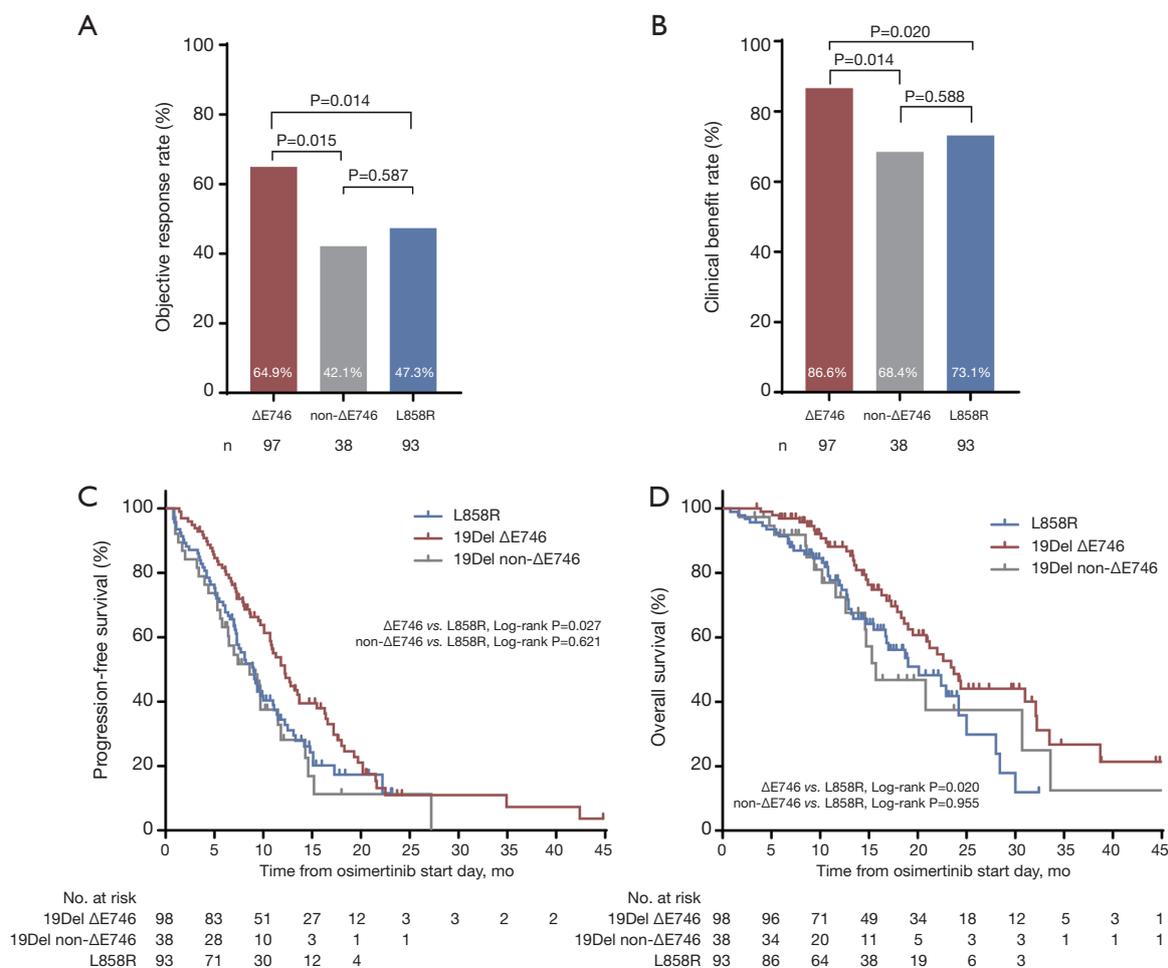
Figure 1 Clinical outcomes of patients with different exon 19 deletion subtypes compared with L858R. (A) Overall response rate and (B) Clinical benefit rate in tumors with *EGFR* exon 19 deletions from E746 (ΔE746, n=97) and non-ΔE746 (n=38) and L858R mutations (n=93). (C) Progression-free survival in tumors with *EGFR* ΔE746 (n=98) (log-rank P=0.027) or non-ΔE746 (n=38) (log-rank P=0.621) compared with tumors with L858R mutations (n=93). (D) Overall survival in tumors with *EGFR* ΔE746 (n=98) (log-rank P=0.02) or non-ΔE746 (n=38) (log-rank P=0.955) compared with tumors with L858R mutations (n=93).

As expected, patients with T790M loss had a significantly lower ORR (37.3% *vs.* 63.2%, P=0.016; *Figure 2A*), lower CBR (60.8% *vs.* 89.5%, P=0.003; *Figure 2B*), and shorter duration of osimertinib treatment (6.4 *vs.* 11.3 months, Log-rank P=0.008; *Figure 2C*) compared with patients with T790M maintained. Patients with acquired C797S mutation had a significantly higher ORR (75.0% *vs.* 40.6%, P=0.007; *Figure 2D*), higher CBR (100% *vs.* 65.2%, P=0.002; *Figure 2E*), and longer duration of osimertinib treatment (14.2 *vs.* 7.3 months, Log-rank P=0.002; *Figure 2F*) compared with patients without acquired C797S mutation.

Comparison of T790M loss between patients with 19Del

versus L858R did not reveal any significant differences (32 of 56 patients, 57.1% *vs.* 19 of 33 patients, 57.6%, P=0.968, *Figure S7A*). Both *EGFR* ΔE746 and non-ΔE746 tumors had equivalent rate of T790M loss compared with L858R tumors (20 of 40 patients, 50.0% *vs.* 19 of 33 patients, 57.6%, P=0.518; 12 of 16 patients, 75.0% *vs.* 19 of 33 patients, 57.6%, P=0.235; respectively, *Figure 3A*). However, it is noteworthy that borderline statistical significance was observed when comparing difference in acquired C797S mutation between 19Del and L858R mutation types (16 of 56 patients, 28.6% *vs.* 4 of 33 patients, 12.1%, P=0.073, *Figure S7B*). Moreover, ΔE746 tumors had a significantly
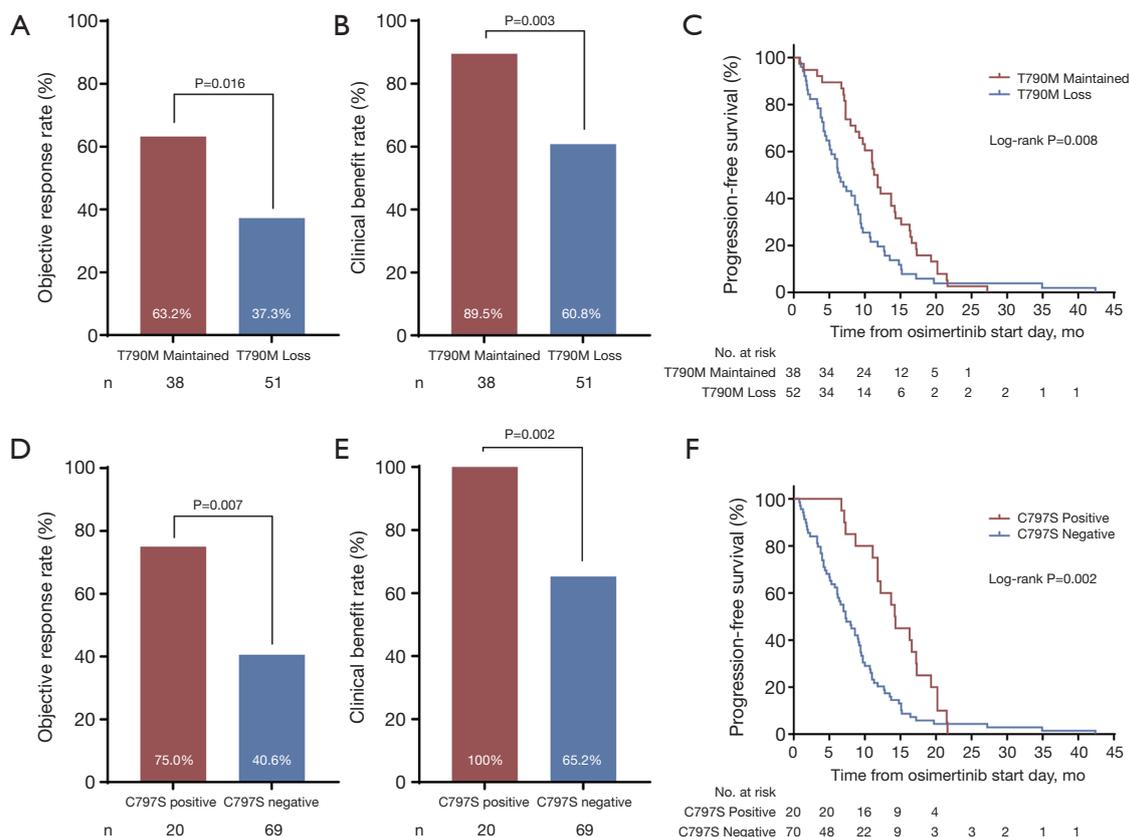
Figure 2 Clinical outcomes between patients with different resistance mechanisms. (A) Overall response rate (ORR) and (B) Clinical benefit rate (CBR) and (C) Duration of osimertinib treatment in tumors with T790M maintained (n=38) and T790M loss (n=51). (D) ORR and (E) CBR and (F) Duration of osimertinib treatment in tumors with positive C797S (n=20) and negative C797S (n=69).

higher (14 of 40 patients, 35.0% *vs.* 4 of 33 patients, 12.1%, P=0.024; *Figure 3B*) whereas non-ΔE746 tumors had a similar (2 of 16 patients, 12.5% *vs.* 4 of 33 patients, 12.1%, P=0.970; *Figure 3B*) prevalence of developing acquired C797S mutation compared with L858R tumors. According to the T790M and C797S mutation status, we classified the resistance mechanisms into three types including T790M loss, T790M maintained without C797S acquisition, and T790M maintained with C797S acquisition. Comparison of these resistance mechanisms between patients with 19Del versus L858R revealed a borderline statistical difference (P=0.077, *Figure S7C*). Further comparisons between ΔE746 and non-ΔE746 versus L858R tumors suggested that resistance pattern of ΔE746 was different from L858R (P=0.051, *Figure 3C*), while non-ΔE746 was similar (P=0.384, *Figure 3C*). A combined analyses of our institutional cohort and the public multicenter cohort (24) confirmed no differences in T790M loss between 19Del versus L858R tumors (59 of 107 patients, 55.1% *vs.* 43

of 66 patients, 65.2%, P=0.193, *Figure S7D*), or between ΔE746 and non-ΔE746 versus L858R tumors (40 of 79 patients, 50.6% *vs.* 43 of 66 patients, 65.2%, P=0.078; 19 of 28 patients, 67.9% *vs.* 43 of 66 patients, 65.2%, P=0.80; respectively, *Figure 3D*). In addition, a strong tendency towards statistical difference was found in acquired C797S mutation between 19Del and L858R mutation types (30 of 107 patients, 28.0% *vs.* 10 of 66 patients, 15.2%, P=0.051, *Figure S7E*). Moreover, combined analyses also confirmed ΔE746 tumors had a significantly higher frequency of acquired C797S mutation than non-ΔE746 (27 of 79 patients, 34.2% *vs.* 3 of 28 patients, 10.7%, P=0.018, *Figure 3E*) and L858R tumors (27 of 79 patients, 34.2% *vs.* 10 of 66 patients, 15.2%, P=0.009, *Figure 3E*). Resistance pattern was not different between 19Del and L858R tumors (P=0.149, *Figure S7F*), as well as between non-ΔE746 and L858R (P=0.848, *Figure 3F*), but was significantly different between ΔE746 and L858R (P=0.033, *Figure 3F*).

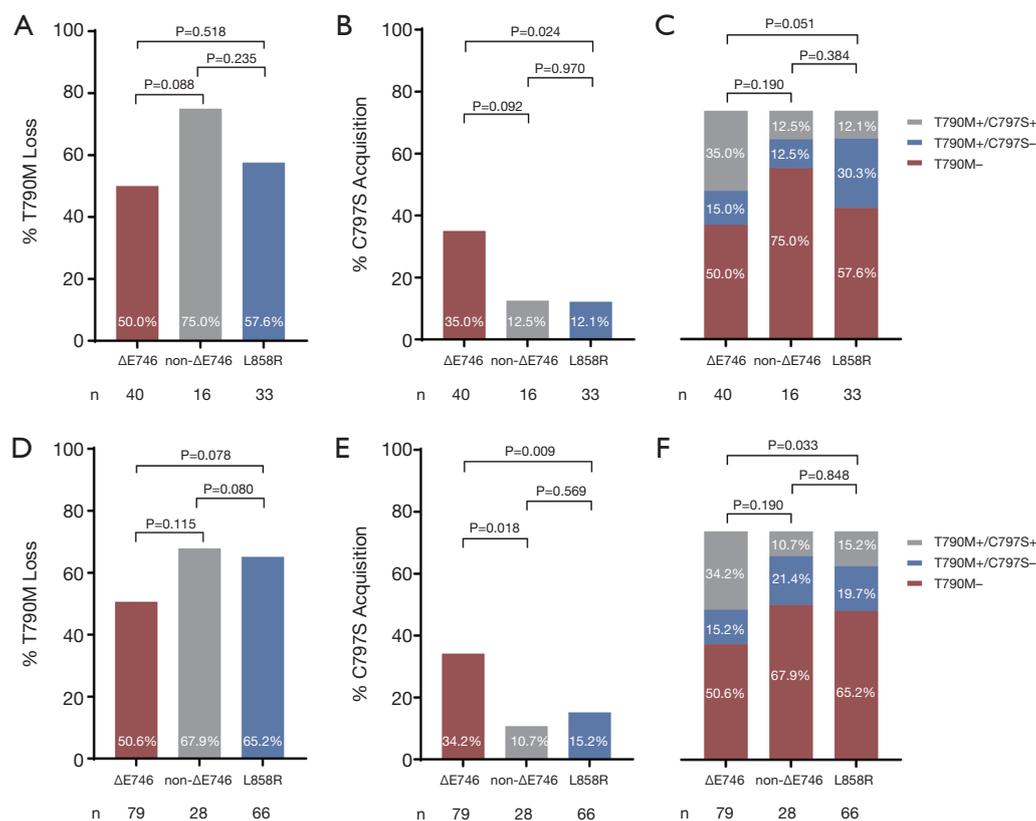Of the 169 osimertinib-resistant patients, 71 underwent

Figure 3 Frequency of T790M loss and C797S acquisition among different EGFR genotypes. (A) T790M loss in tumors with *EGFR* exon 19 from E746 (ΔE746, n=40) or exon 19 deletions from other than E746 (non-ΔE746, n=16) compared with L858R mutation (n=33) in our cohort. (B) C797S acquisition in tumors with *EGFR* ΔE746 (n=40) or *EGFR* non-ΔE746 (n=16) compared with L858R (n=33) in our cohort. (C) Distribution of three resistance mechanisms in patients with *EGFR* ΔE746 (n=40) and *EGFR* non-ΔE746 (n=16) and L858R (n=33) in our cohort. (D) T790M loss in tumors with *EGFR* ΔE746 (n=79) or *EGFR* non-ΔE746 (n=28) compared with L858R mutation (n=66) in combined analysis of our cohort and public cohort. (E) C797S acquisition in tumors with *EGFR* ΔE746 (n=79) or *EGFR* non-ΔE746 (n=28) compared with L858R (n=66) in combined analysis of our cohort and public cohort. (F) Distribution of three resistance mechanisms in patients with *EGFR* ΔE746 (n=79) and *EGFR* non-ΔE746 (n=28) and L858R (n=66) in combined analysis of our cohort and public cohort.

a post-progression tumor biopsy, and 68 (95.8%) of them succeeded to obtain tumor tissue. Four (5.9%) of 68 patients developed transformation to small-cell lung cancer (SCLC). All of them had 19Del mutation types (three ΔE746 and one non-ΔE746). However, statistical significance was not reached when compared with L858R tumors (4 of 45, 8.9% *vs.* 0 of 22, 0%, respectively, P=0.294). In addition, ORR, CBR, and duration of osimertinib treatment were all not significantly different between patients who developed SCLC transformation and those not developed SCLC transformation (*Figure 4*).

Discussion

In this study, we did not identify T790M-positive NSCLC patients carrying *EGFR* 19Del mutations had a significantly better therapeutic response or survival benefit to osimertinib compared with those carrying L858R or uncommon mutations. However, we did observe a subset of 19Del mutant patients (ΔE746) had superior clinical outcomes with osimertinib treatment than L858R mutant patients. Our study also found ΔE746 tumors had a higher whereas non-ΔE746 tumors had a similar proportion of acquired C797S mutation at resistance to osimertinib compared
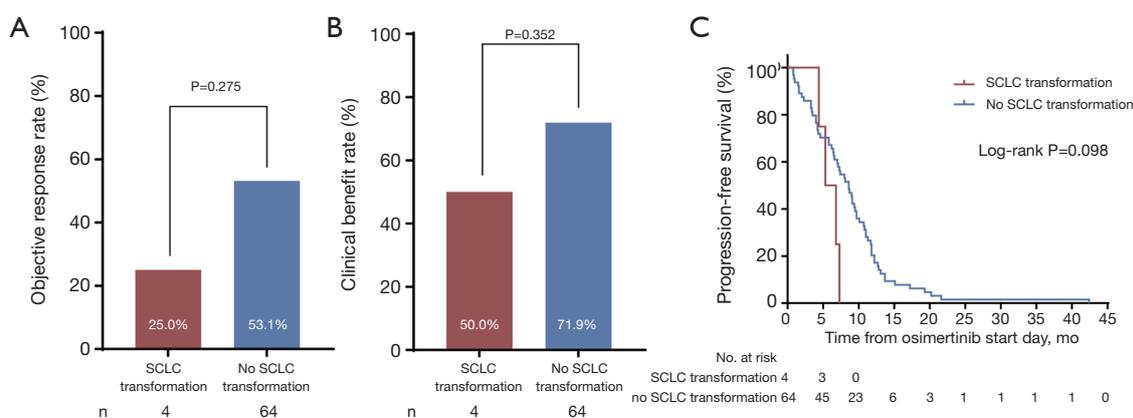
Figure 4 Clinical outcomes between patients with or without small-cell lung cancer (SCLC) transformation. (A) ORR and (B) CBR and (C) Duration of osimertinib treatment in tumors with SCLC transformation (n=4) and no SCLC transformation (n=64).

with L858R tumors. To the best of our knowledge, this is the first study to date to investigate the clinical effect of 19Del variants on outcomes of osimertinib treatment in T790M-positive NSCLCs, and the first analysis to compare osimertinib resistance mechanisms according to 19Del variants.

Indeed, several key studies have suggested that, in the presence of acquired T790M mutation, patients harboring *EGFR* 19Del mutations have a non-significantly higher response and longer PFS than those harboring L858R when receiving osimertinib treatment (22,28,29). Our analyses also observed this association in patients with *EGFR* T790M mutation treated with osimertinib. In contrast to these findings, however, there were some studies showed significant differences in PFS and OS between these two *EGFR* mutation types (30,31). These conflicting results may be due partly to differences in clinicopathologic characteristics and previous treatment histories among these studies. In our study, we observed that none but one patient received afatinib before osimertinib treatment. This was very different from other studies (22,28-31). We think that it may be associated with the facts that afatinib provides only a modest improvement in PFS but has higher rate of serious drug-related adverse events than gefitinib (32,33), and has not been covered by the basic medical insurance system in China before October, 2018.

In addition, it is known that *EGFR* 19Del mutants constitute a heterogeneous group of genetic alterations characterized by in-frame deletions, substitutions and insertions. In our study, we did observe 19Del ΔE746 tumors had higher sensitivity to osimertinib and more

likelihood of developing acquired C797S mutation at resistance than L858R tumors, but non-ΔE746 tumors did not, which has never been previously reported. The underlying causes responsible for these discrepancies are still unclear, though it may be related to the variable sample sizes across different subgroups. The outcomes of osimertinib treatment were similar to those of first- and second-generation *EGFR* TKIs, where *EGFR* ΔE746 tumors are found to be associated with longer treatment duration of earlier-generation TKIs compared with non-ΔE746 tumors in several studies (17,18), highlighting potential differences between *EGFR* 19Del subtypes. The inconsistent findings of the effect of 19Del and L858R mutations on osimertinib efficacy in different studies may also due in part to different proportion of patients with various 19Del subtypes between studies (22,28-31). In fact, *in vitro* cellular experiments have observed that distinct 19Del variants confer different sensitivities to *EGFR* TKIs, including osimertinib (15,34,35). In addition, one recent study using molecular dynamics simulation analysis has also identified that structural consequences of p.L747_A750delinsP mutation, one common non-ΔE746 variant, reduces the sensitivity of osimertinib by impairing drug binding (15). L747 was a pivotal hydrophobic core stabilizing the inactive form of *EGFR*, changing in this site may disrupt the hydrophobic core and favored the active conformation of *EGFR* (36). Although these studies were not performed in the context of the T790M mutation, structural change in the deletion mutants may lead to different osimertinib sensitivity between ΔE746 and non-ΔE746 tumors. However, further prospective studies

with larger sample sizes to identify the impact of *EGFR* genotypes on outcomes with osimertinib treatment are warranted, as well as more in-depth fundamental researches to explore the intrinsic mechanisms.

The emergence of *EGFR* T790M in 19Del tumor is higher than that in L858R tumors after earlier-generation *EGFR* TKIs targeted therapy (8,9). This may be the result of higher sensitivity and thus longer exposure to *EGFR* TKIs in 19Del tumors than in L858R tumors. Similarly, T790M-positive *EGFR* mutant tumors which gain more durable benefit from osimertinib targeted therapy could be more dependent on *EGFR* pathway. This may be necessitated for the development of on-target acquired resistance (C797S) to osimertinib. Our study results also supported this notion, where ΔE746 tumors have higher sensitivity to osimertinib and consequently have more likelihood of developing on-target acquired resistance to osimertinib. Moreover, patients with acquired C797S mutation did have better response and survival, with a CBR of 100%, and a higher ORR and longer treatment duration of osimertinib than patients without developing acquired C797S mutation.

Recent reports have also identified *EGFR* G724S as an emerging mutation mediating acquired resistance to osimertinib (37-41). Moreover, G724S as an allele-specific osimertinib-resistance mutation exists exclusively in the context of specific 19Del mutants but not the most common 19Del variant p.E746_A750del or L858R (41), supporting that potentially different mechanisms confer resistance to different mutant tumors. Our study results also identified two patients with G724S resistant mutations, both with original *EGFR* genotype of p.E746_S752delinsV. This incidence was similar to most previous reports (37-39), except for the study from Fassunke *et al.* (40). However, because of the relatively low occurrence frequency, no separate comparison was performed between patient groups in our study.

Some limitations of present study should be concerned. First, this retrospective, single-center study was still limited in the number of patients with rare mutations, as well as the total number of rare 19Del subtypes, which may not be representative of all NSCLC patients with *EGFR* rare mutations. Further large, prospective, multicentre studies are warranted to confirm and expand on our findings. Second, the NGS testing in this study was not performed using a single central panel, and not all resistant patients had performed post-osimertinib NGS testing and rebiopsy. However, we deemed that the various panels used in our study would not be limited in their ability of detecting specific *EGFR* 19Del variants or resistance mutations

such as T790M and C797S. Additionally, other specific resistance mechanisms of osimertinib treatment, such as *c-MET* amplification, *HER2* amplification, and *BRAF* mutations, etc. (42), were not assessed in our analysis. Moreover, osimertinib has been recently approved as first-line treatment before acquiring T790M mutation (43). Whether different sensitivity to osimertinib continuing to be observed in first-line setting remains unanswered.

Conclusions

our study provided evidence supporting that not all *EGFR* 19Del tumors have equally favorable outcomes to osimertinib treatment in the context of the T790M mutation, where ΔE746 tumors suggest preferable sensitivity to osimertinib. Our analyses also revealed ΔE746 tumors have a higher frequency to develop the acquired C797S mutation after osimertinib treatment, highlighting potential different resistance mechanisms between *EGFR* 19Del variants. Genotyping of 19Del mutations should be precisely identified at diagnosis in order to determine the prognostic and predictive value and select the better treatment. Further researches are needed to determine the intrinsic mechanisms of our findings.

Acknowledgments

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tlcr.2020.03.35). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the Institutional Review Board of Sun Yat-sen University Cancer Center (B2019-127-01), and was in accordance with the guidelines of the Helsinki Declaration (as revised in 2013) and individual consent for this retrospective analysis was waived.

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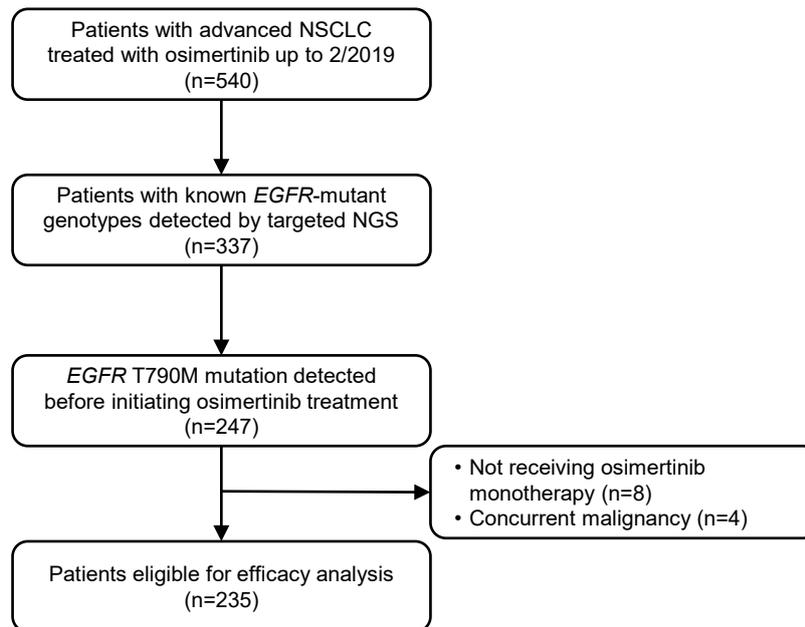
Figure S1 The patient collection flow chart. NSCLC, non-small cell lung cancer; NGS, next-generation sequencing; *EGFR*, epidermal growth factor receptor.
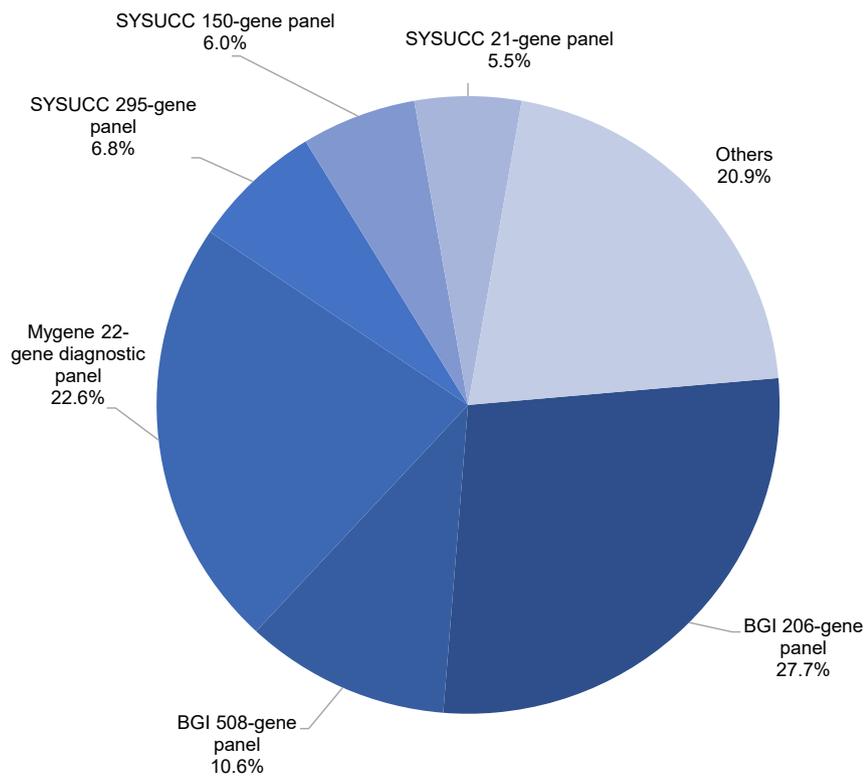

Figure S2 Identification of *EGFR* mutation genotypes. BGI, Beijing Genomic Institute; SYSUCC, Sun Yat-sen University Cancer Center.
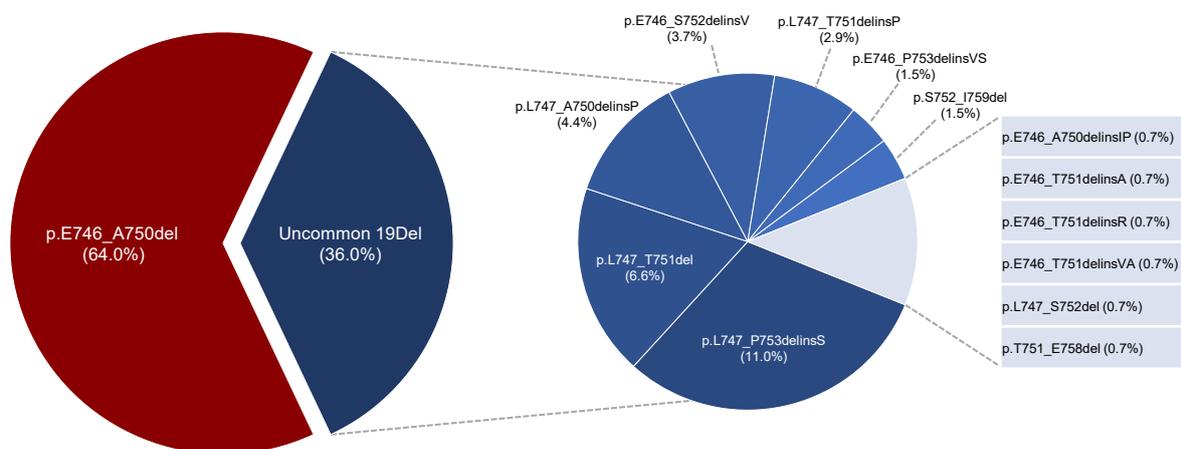
Figure S3 Frequency of *EGFR* exon 19 deletion (19Del) mutation subtypes in the patient group for investigating therapeutic response and clinical outcomes in NSCLC patients who were treated with osimertinib (n=136).
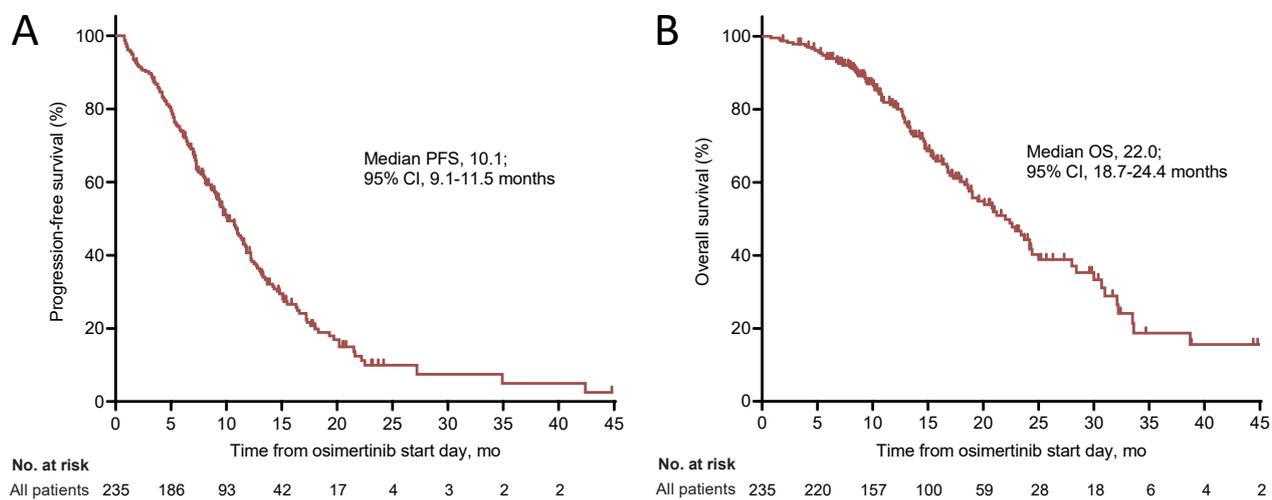

Figure S4 Survival outcomes of overall patients. (A) Progression-free survival and (B) overall survival of all patients in our cohort.
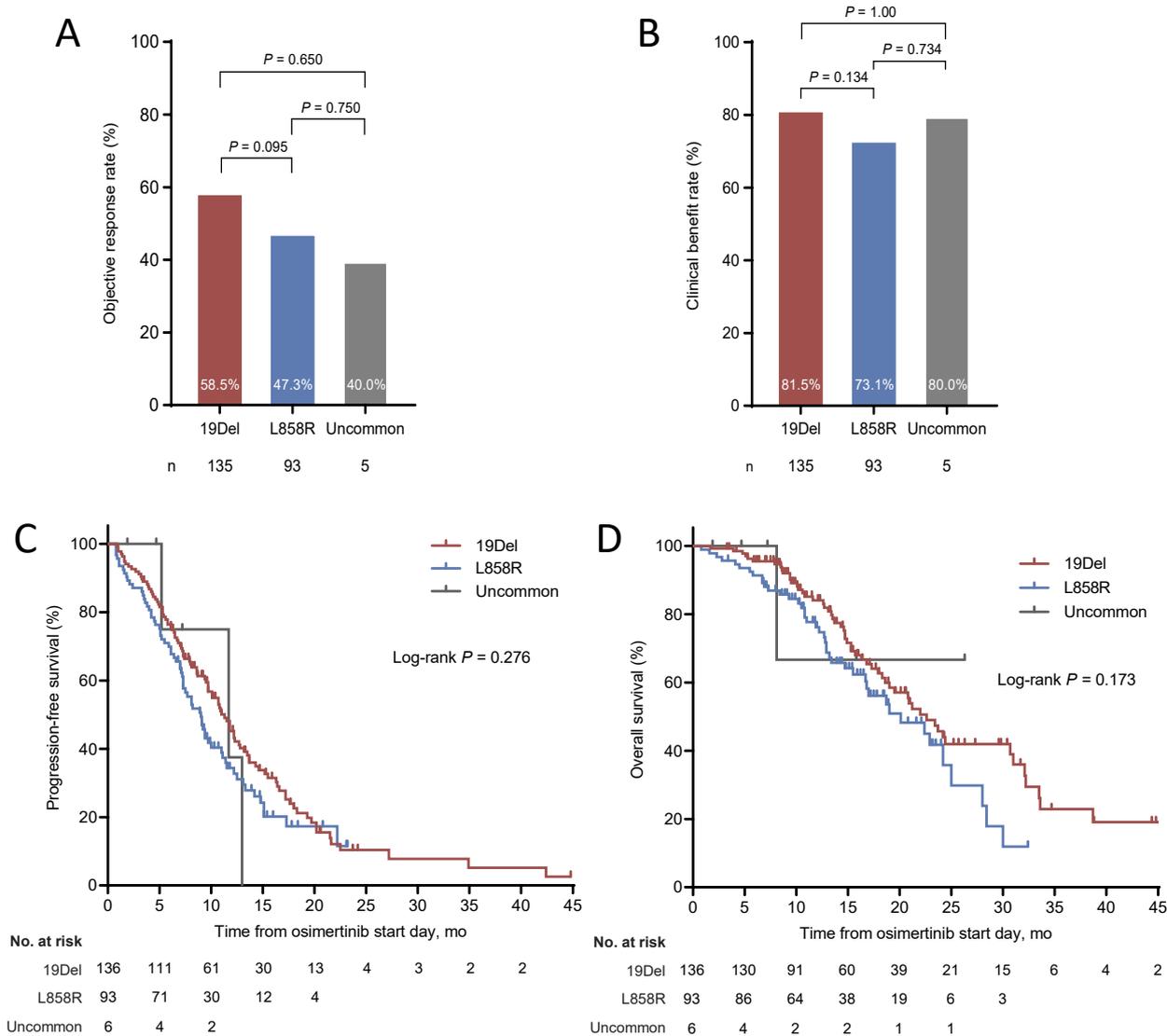
Figure S5 Comparison of clinical outcomes of patients with different *EGFR* mutation types. (A) Overall response rate and (B) Clinical benefit rate in tumors with *EGFR* exon 19 deletions (n=135) or L858R (n=93) or uncommon mutations (n=5). Two patients were not evaluable for response. (C) Progression-free survival in tumors with *EGFR* exon 19 deletions (n=136) or L858R (n=93) or uncommon mutations (n=6), overall log-rank P=0.276. (D) Overall survival in tumors with *EGFR* exon 19 deletions (n=136) or L858R (n=93) or uncommon mutations (n=6), overall log-rank P=0.173.

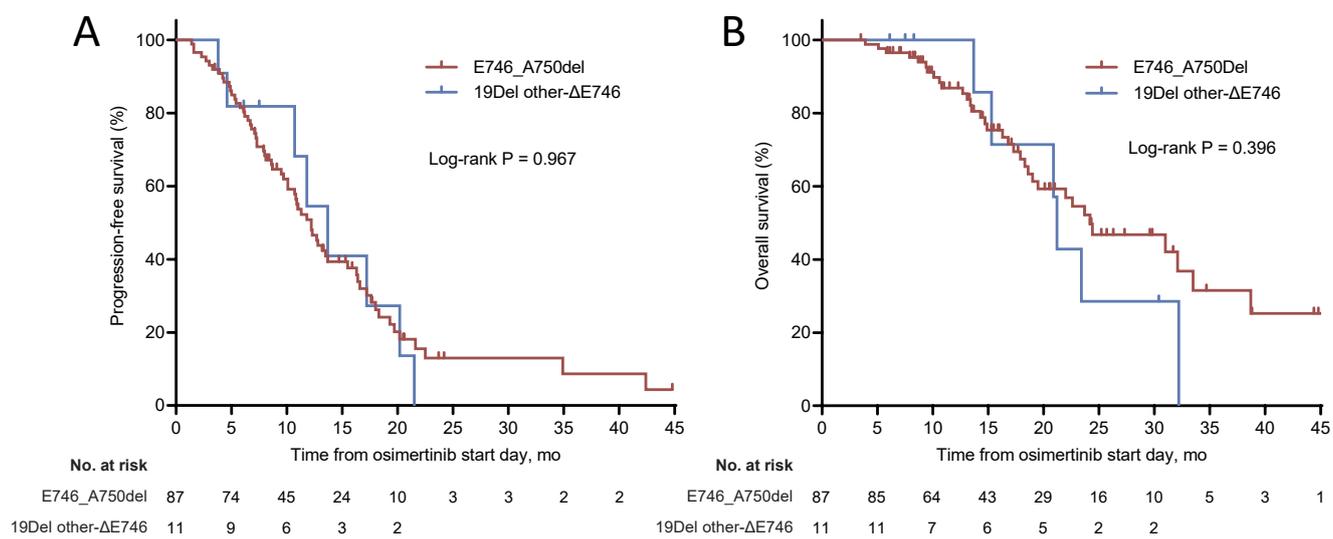**Figure S6** Comparison of p.E746_A750del and other ΔE746 mutations. (A) Progression-free survival in tumors with p.E746_A750del (n=87) or other ΔE746 mutations (n=11) in our cohort (log-rank P=0.967). (B) Overall survival in tumors with p.E746_A750del (n=87) or other ΔE746 mutations (n=11) in our cohort (log-rank P=0.396).
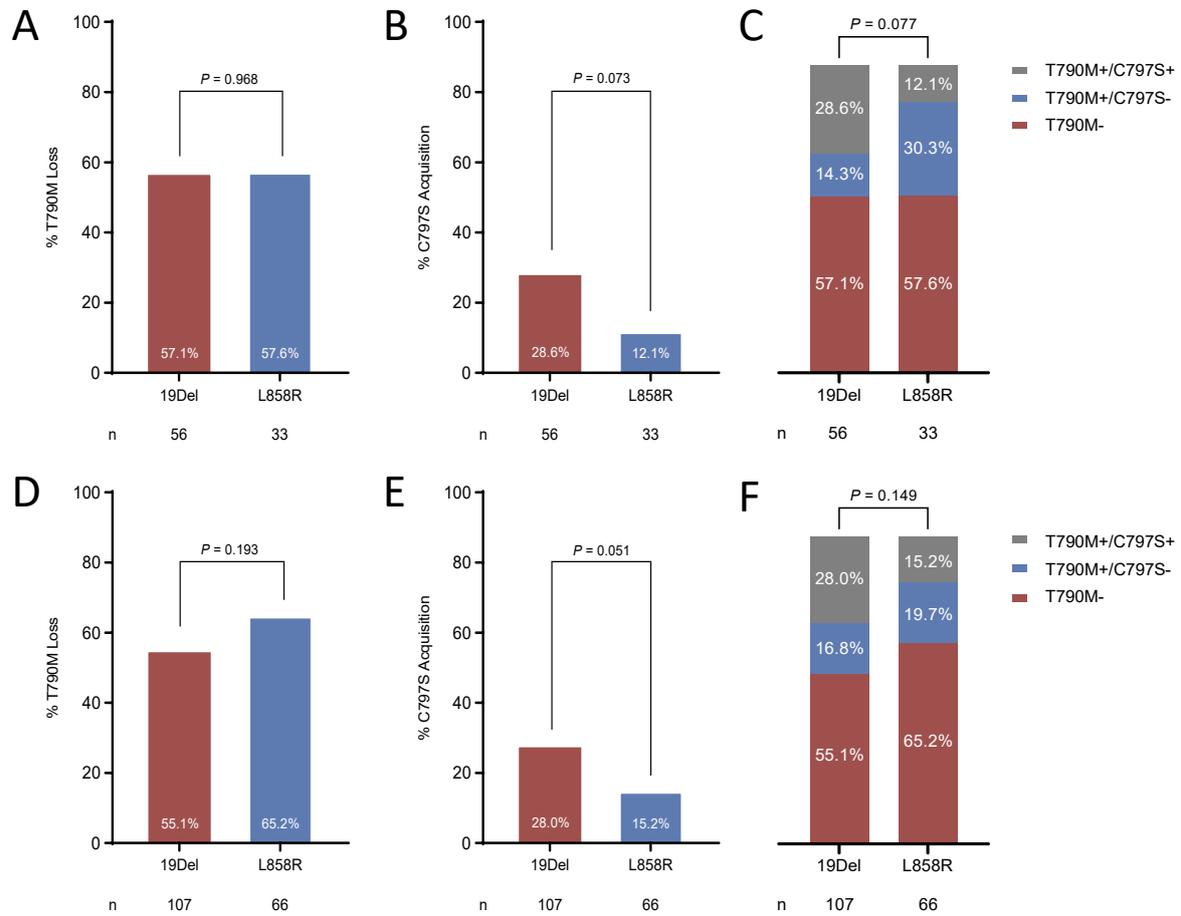
Figure S7 Frequency of T790M loss and C797S acquisition between *EGFR* 19Del and L858R mutations. (A) T790M loss in tumors with *EGFR* exon 19 deletions (19Del) (n=56) and L858R (n=33) (P=0.968) in our cohort. (B) C797S acquisition in tumors with *EGFR* 19Del (n=56) and L858R (n=33) (P=0.073) in our cohort. (C) Distribution of three resistance mechanisms in patients with *EGFR* 19Del (n=56) and L858R (n=33) in our cohort. (D) T790M loss in tumors with *EGFR* 19Del (n=107) and L858R (n=66) (P=0.193) in combined analysis of our cohort and public cohort. (E) C797S acquisition in tumors with *EGFR* 19Del (n=107) and L858R (n=66) (P=0.051) in combined analysis of our cohort and public cohort. (F) Distribution of three resistance mechanisms in patients with *EGFR* 19Del (n=107) and L858R (n=66) in combined analysis of our cohort and public cohort.

Table S1 Characteristics of patients grouped by EGFR mutation types

Characteristics	19Del (n=136)	L858R (n=93)	Uncommon (n=6)
Age at osimertinib treatment			
Median [range], years	53.5 [27–81]	57 [31–79]	51 [45–65]
≤60 years	96 (70.6)	52 (55.9)	5 (83.3)
>60 years	40 (29.4)	41 (44.1)	1 (16.7)
Sex			
Female	84 (61.8)	58 (62.4)	1 (16.7)
Male	52 (38.2)	35 (37.6)	5 (83.3)
Smoking history			
Never	100 (73.5)	59 (63.4)	5 (83.3)
Current/ever	24 (17.7)	22 (23.7)	1 (16.7)
Unknown	12 (8.8)	12 (12.9)	0 (0.0)
Brain metastasis			
Yes	62 (45.6)	47 (50.5)	3 (50.0)
None	74 (54.4)	46 (49.5)	3 (50.0)
Histological type			
Adenocarcinoma	132 (97.1)	89 (95.7)	6 (100.0)
Others	4 (2.9)	4 (4.3)	0 (0.0)
Type of previous TKI			
Gefitinib	74 (54.4)	39 (41.9)	0 (0.0)
Erlotinib	36 (26.5)	26 (28.0)	3 (50.0)
Icotinib	25 (18.4)	19 (20.4)	1 (16.7)
Afatinib	0 (0.0)	0 (0.0)	1 (16.7)
None[†]	1 (0.7)	9 (9.7)	1 (16.7)
PFS of previous TKI[†]			
<6 months	8 (5.9)	15 (17.9)	2 (40.0)
≥6 months	127 (94.1)	69 (82.1)	3 (60.0)
Line of osimertinib treatment*			
1st line	1 (0.7)	9 (9.7)	1 (16.7)
2nd line	85 (62.5)	60 (64.5)	3 (50.0)
3rd or more line	50 (36.8)	24 (25.8)	2 (33.3)

[†], Eleven patients received osimertinib as first-line treatment since harboring de novo T790M mutation. *, Line of osimertinib treatment refers to the treatment line of osimertinib administration. For example, a patient who received osimertinib as third-line therapy will have previously received two treatment regimens in metastatic setting, which can include prior chemotherapy. TKI, tyrosine kinase inhibitor; PFS, progression-free survival; EGFR, epidermal growth factor receptor.

Table S2 Univariate and multivariate analysis for progression-free survival

Characteristics	Univariate analyses		Multivariate analyses	
	HR (95% CI)	P value	HR (95% CI)	P value
Age at osimertinib treatment, years				
≤60	1.00 (Reference)		1.00 (Reference)	
>60	0.78 (0.56–1.09)	0.148	0.72 (0.51–1.02)	0.061
Sex				
Female	1.00 (Reference)		1.00 (Reference)	
Male	1.29 (0.94–1.75)	0.110	1.15 (0.79–1.67)	0.458
Smoker				
Never	1.00 (Reference)		1.00 (Reference)	
Ever	1.23 (0.86–1.78)	0.261	1.05 (0.68–1.63)	0.827
Unknown	0.60 (0.29–1.24)	0.171	0.64 (0.31–1.35)	0.243
Brain metastases				
Yes	1.00 (Reference)		1.00 (Reference)	
None	0.78 (0.58–1.06)	0.117	0.77 (0.56–1.06)	0.108
Histological type				
Adenocarcinoma	1.00 (Reference)		1.00 (Reference)	
Others	2.62 (1.22–5.62)	0.013	2.56 (1.15–5.68)	0.021
Line of osimertinib treatment*				
1st line[†]	0.61 (0.27–1.40)	0.245	0.44 (0.19–1.04)	0.061
2nd line	1.00 (Reference)		1.00 (Reference)	
3rd or more line	1.42 (1.04–1.95)	0.028	1.32 (0.94–1.84)	0.105
EGFR subtype				
19Del ΔE746	1.00 (Reference)		1.00 (Reference)	
19Del non-ΔE746	1.73 (1.11–2.69)	0.016	1.70 (1.08–2.69)	0.022
L858R	1.48 (1.05–2.07)	0.025	1.70 (1.19–2.43)	0.004
Uncommon	1.36 (0.43–4.35)	0.603	1.43 (0.43–4.73)	0.557

[†], Eleven patients received osimertinib as first-line treatment since harboring de novo T790M mutation. *, Line of osimertinib treatment refers to the treatment line of osimertinib administration. For example, a patient who received osimertinib as third-line therapy will have previously received two treatment regimens in metastatic setting, which can include prior chemotherapy. HR, hazard ratio; CI, confidence interval; *EGFR*, epidermal growth factor receptor; 19Del, exon 19 deletion.

Table S3 Univariate and multivariate analysis for overall survival

Characteristics	Univariate analyses		Multivariate analyses	
	HR (95% CI)	P value	HR (95% CI)	P value
Age at osimertinib treatment, years				
≤60	1.00 (Reference)		1.00 (Reference)	
>60	1.19 (0.78–1.82)	0.419	1.20 (0.77–1.87)	0.413
Sex				
Female	1.00 (Reference)		1.00 (Reference)	
Male	1.44 (0.96–2.15)	0.076	1.46 (0.89–2.39)	0.131
Smoker				
Never	1.00 (Reference)		1.00 (Reference)	
Ever	1.29 (0.80–2.07)	0.291	1.01 (0.57–1.80)	0.978
Unknown	1.83 (0.77–4.34)	0.172	1.66 (0.65–4.20)	0.287
Brain metastases				
Yes	1.00 (Reference)		1.00 (Reference)	
None	0.91 (0.61–1.35)	0.629	0.83 (0.54–1.27)	0.383
Histological type				
Adenocarcinoma	1.00 (Reference)		1.00 (Reference)	
Others	4.83 (2.08–11.24)	<0.001	3.92 (1.49–10.27)	0.006
Line of osimertinib treatment*				
1st line[†]	1.15 (0.41–3.18)	0.794	1.24 (0.43–3.52)	0.692
2nd line	1.00 (Reference)		1.00 (Reference)	
3rd or more line	1.42 (0.94–2.13)	0.092	1.59 (1.03–2.46)	0.035
EGFR subtype				
19Del ΔE746	1.00 (Reference)		1.00 (Reference)	
19Del non-ΔE746	1.67 (0.93–2.99)	0.085	1.36 (0.72–2.55)	0.341
L858R	1.65 (1.06–2.58)	0.027	1.70 (1.06–2.73)	0.026
Uncommon	0.83 (0.11–6.08)	0.857	0.70 (0.09–5.34)	0.730

[†], Eleven patients received osimertinib as first-line treatment since harboring de novo T790M mutation. *, Line of osimertinib treatment refers to the treatment line of osimertinib administration. For example, a patient who received osimertinib as third-line therapy will have previously received two treatment regimens in metastatic setting, which can include prior chemotherapy. HR, hazard ratio; CI, confidence interval; EGFR, epidermal growth factor receptor; 19Del, exon 19 deletion.