ORIGINAL RESEARCH

Concomitant Mutations in EGFR 19Del/L858R Mutation and Their Association with Response to EGFR-TKIs in NSCLC Patients

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Objective: Differences in efficacy of epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI) have been observed between non-small cell lung cancer (NSCLC) patients with *19 exon deletion (19Del)* and *L858R* mutation. We explored whether the total number or pattern of concomitant mutations of *19Del* and *L858R* may explain their different sensitivities.

Patients and Methods: This study contained the mutational profiles of EGFR-mutated NSCLC patients from two cohorts: Guangzhou (G1) and database (G2). Concomitant mutation status and EGFR-TKI response information were retrieved.

Results: A total of 403 patients covered 283 genes in the G1 and 803 patients with a different gene set in the G2 were included. Similar prevalence of total concomitant mutation number was observed in both G1 (*19Del* 32.48% vs *L858R* 30.45%; *P*=0.68) and G2 (*19Del* 74.9% vs *L858R* 73.2%; *P*=0.65) cohorts. Only *HGF/c-Met* pathway same more related to *L858R* mutation. EGFR-TKI response information was recorded for 134 patients in the G2 cohort. *19Del* showed a higher objective response (OR) rate compared with *L858R*, regardless of concomitant mutations. Compared to patients with OR, non-OR patients had more concomitant mutations, both in *19Del* (53.8% vs 83.3%; *P*=0.021) and *L858R* (51.4% vs 77.8%; *P*=0.029). In particular, total concomitant mutations (OR=0.27; *P*=0.03), sensitive *EGFR* mutations (OR=2.21; *P*=0.04), and *T790M* (OR=0.244; *P*=0.02) significantly affected the TKI response.

Conclusion: Concomitant mutations were widespread in *19Del* and *L858R* and were associated with poorer OR to EGFR-TKIs. However, *19Del* and *L858R* had similar numbers and patterns of concomitant mutations, which might not explain the different sensitivity to EGFR-TKI.

Keywords: epidermal growth factor receptor mutation, 19Del, L858R, non-small cell lung cancer, concomitant mutation

Introduction

Epidermal growth factor receptor (*EGFR*) mutations are a key cancerous driver in non-small cell lung cancer (NSCLC), contributing to nearly half of reported NSCLC cases in Asians and 15% in Caucasians.[1] *EGFR*-tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, are the mainstay of treatment for advanced patients, with an overall response rate of 70% and a median progression free survival (PFS) of 10 months.[2]

19 exon deletion (19Del) and *L858R mutation in 21 exon (L858R)* are two major subtypes of *EGFR* mutations and considered sensitive to *EGFR*-TKIs. We reported

a meta-analysis confirming that *EGFR 19 exon deletion* was associated with better outcomes in treatments with EGFR-TKIs than *L858R* mutation.[3] An updated comprehensive network meta-analysis from our team also revealed that the best treatment strategies differ for these two sensitive mutations.[4]

Hot debate persists regarding the mechanisms for the difference between these two subtypes. Some investigators have explored the structures of *19Del* and *L858R* and suggested different binding affinity of TKIs.[5] We recently reported a higher prevalence of somatic *T790M* in *L858R* (22%) than in *19Del* (19%), this may be one of the mechanisms to explain the discrepancy.[6] With the rapid development of multiple genetic profiling by next generation sequencing (NGS), some recent studies have suggested that other concomitant mutations might also compromise the efficacy of *EGFR*-TKIs.[7]

Thus, it is of interest to explore whether the respective concomitant mutational profiles of *19Del* and *L858R* may explain their different sensitivities to *EGFR*-TKIs. In this study, we obtained individual patient data from two cohorts, the Guangzhou cohort (G1) and the public Database cohort (G2), with NGS on TKI naïve NSCLC patients, to compare the prevalence of the concomitant mutations and analyze their impact on efficacy.

Patients and Methods
Patient Selection of the Guangzhou Cohort (G1)

From May 2018 to July 2019, we consecutively collected patients with NSCLC tumors at the First Affiliated Hospital of Guangzhou Medical University. All procedures performed were in accordance with the ethical standards of the Helsinki Declaration and applicable regulatory requirements. Written informed consent was obtained from all patients to permit genetic analysis of biological samples. Inclusion criteria were: 1) single primary adenocarcinoma; 2) all resected-tissues and lymph nodes were proven by final pathology with paraffin blocks; 3) enough resected-tissue for mutational analyses; and 4) patients with *EGFR* sensitive mutation (*19Del* or *L858R*). Patients were excluded for: 1) multiple lung cancer, 2) non-invasive cancer (eg, adenocarcinoma in situ, minimally invasive adenocarcinoma) or non-adenocarcinoma, 3) preoperative neoadjuvant therapy, 4) local or distant metastasis, 5) patients without sensitive *EGFR* mutation, and 6) patients with both *19Del* and *L858R* mutations.

Genomic driver gene mutation testing was performed in all included patients and only in resected tumor tissues with NGS. Gene alterations such as *EGFR*, *HER2*, *MET*, *KRAS*, *BRAF*, *ALK*, *ROS1*, and *RET* were tested by a targeted next-generation sequencing method, which was described in other studies.[8,9] Briefly, the assay utilizes a NGS-based target enrichment panel that covers 283 genes and 300k genomic regions. The chips were loaded on a MGISEQ-500 platform and sequenced at a mean depth of over 500x, which achieved genome coverage greater than 99%.

Database Search and Collection Strategy of Database Cohort (G2)

Online electronic databases (PubMed, Medline, Web of Science, and Google Scholar) as well as abstract libraries of the American Society of Clinical Oncology (ASCO), European Society of Medical Oncology (ESMO), and World Conference on Lung Cancer (WCLC) were searched for potentially eligible studies using a combination of the following terms before August 1, 2019: lung cancer, next generation sequencing/NGS/panel, and EGFR mutation. No restriction on publication year or language was set. Reference lists of relevant articles were reviewed for supplementation. Included studies met the following criteria: 1) provided multigene profiling by NGS based on tissue or plasma samples; 2) reported the genetic profiles of both *19Del* and *L858R*; and 3) only included TKI-naïve patients. One of the included studies from G2 is from our center,[8] but the study period did not overlap with G1, avoiding patient duplication.

Data Extraction of Database Cohort (G2)

Data were extracted independently by two investigators (H.R. Liang and C.C. L), and conflicts were adjudicated by a superior investigator (W.H. Liang). For the selected studies, information on all available variables was extracted and entered into a Microsoft Excel database. Mutation status of each gene as well as EGFR-TKI response information (according to RECIST 1.1) were extracted directly from the landscape figure or table. These data were equivalent to individual patient records and, thus, pooled with our data set. Any disagreement was resolved via discussion among the authors.

Statistical Analysis

Student's *t*-test was used to compare the mean of continuous variables, Chi-square test was used to compare the dichotomous variables (Fisher's exact test was used when

expected frequency was less than 5). Odds ratio of concomitant mutations were calculated, and forest plots were conducted by R software (R version 3.6.1). Logistic regression was used to measure the impact of different covariates on binary outcomes. All analyses were performed using SPSS 25.0 (Chicago, IL). Statistical significance was taken as two-sided $P<0.05$.

Results
Cohort Features
In the G1 cohort, 403 patients received NGS and were included in this analysis, 172 (42.68%) carried *19Del* and 231 (57.32%) were *L858R* mutation patients, the demographic characteristics of G1 are summarized in Table 1. All patients were adenocarcinoma without any additional therapy excepted by surgery or biopsy. The average age was 59.97 years old in G1 and 75.19% patients were female. Most patients were stage I to III (73.45%) according to the 8th AJCC, only 107 (26.55%) patients were diagnosed with metastatic adenocarcinoma.

In the G2 cohort, 18 eligible studies,[7,8,10-25] including one publication from our team,[8] were involved in this study (Table 2). A total of 803 patients with *EGFR 19Del* or *L858R* mutation were included in this study. Four hundred and fifteen patients had *19Del* and 388 had *L858R* mutation. Five hundred and seventy-six patients were tested using tissue samples, 131 were based on plasma and 96 patients used both tissue and plasma

samples. We included only TKI-naïve patients in all studies to diminish the co-founding effect after TKI resistance. As different panels were used by different studies, we analyzed *T790M*, *TP53*, and the loci reported by most studies (half of all included).

Mutational Profiles of EGFR 19Del and L858R of G1
The mutational landscapes of G1 are illustrated in Figure 1. The prevalence of total concomitant mutations is similar in both *19Del* and *L858R* mutation (*19Del* 32.48% vs *L858R* 30.45%; $P=0.68$). The distribution of other concomitant mutations between two sensitive mutations were also similar.

Mutational Profiles of EGFR 19Del and L858R of G2
The integrated mutational landscapes of G2 and percentages of *EGFR 19Del* and *L858R* are illustrated in Figure 2. We found similar prevalence of total concomitant mutations (*19Del* 74.9% vs *L858R* 73.2%; $P=0.65$) in *19Del* and *L858R* mutations. The majority of other concomitant mutations were also distributed similarly between two subtypes, except for *CDKN2A* (*19Del* 12.4% vs *L858R* 5.5%; $P=0.01$), *CTNNB1* (*19Del* 13.2% vs *L858R* 2.0%; $P<0.001$), *MET* (*19Del* 3.3% vs *L858R* 8.0%; $P=0.03$), and *HRAS* (*19Del* 7.1% vs *L858R* 0.0%; $P=0.02$). We further divided all concomitant mutations according to signaling pathway distribution and calculated their odds ratios between *19Del* and *L858R* (Figure 3). Only *HGF/c-Met* pathway same more related to *L858R* mutation.

Factors Associated with Primary Response to EGFR-TKI of G2
One hundred and thirty-four patients in the G2 cohort reported *EGFR*-TKI response information (Figure 4). Compared with patients who had an objective response to TKI, those non-response or progression patients had more concomitant mutations, both in *19Del* (53.8% vs 83.3%; $P=0.021$) and *L858R* (51.4% vs 77.8%; $P=0.029$). According to multivariate logistic regression, total concomitant mutations (Objective response: at least one 17.65% vs none 56.63%; OR=0.27; 95% CI=0.12–0.64; $P=0.03$), *EGFR* mutations (Objective response: *19Del* 74.3% vs *L858* 57.8%, OR=2.21; 95% CI=1.02–4.78; $P=0.04$), and *T790M* (Objective response: *T790M*+ 43.75% vs *T790M*- 73.53%, OR=0.244; 95% CI=0.100–0.590; $P=0.02$) significantly affected the TKI response.

Table 1 Baseline Demographic Features of G1 Cohort

	19 Del (n=172)	SD/%	L858R (n=231)	SD/%	*P*-value
Gender					0.59
Male	45	26.16%	55	23.81%	
Female	127	73.84%	176	76.19%	
Age	57.21	15.16	61.36	11.72	0.32
Stage					0.27
I	86	50.00%	91	39.39%	
II	38	22.09%	53	22.95%	
III	11	6.40%	17	7.36%	
IV	37	21.51%	70	30.30%	
Smoking status					0.20
Smoker	26	15.12%	43	18.62%	
Ex-Smoker	43	25%	21	9.09%	
Non-Smoker/ Unknown	103	59.88%	167	72.29%	

Table 2 The Characteristics of Included Studies

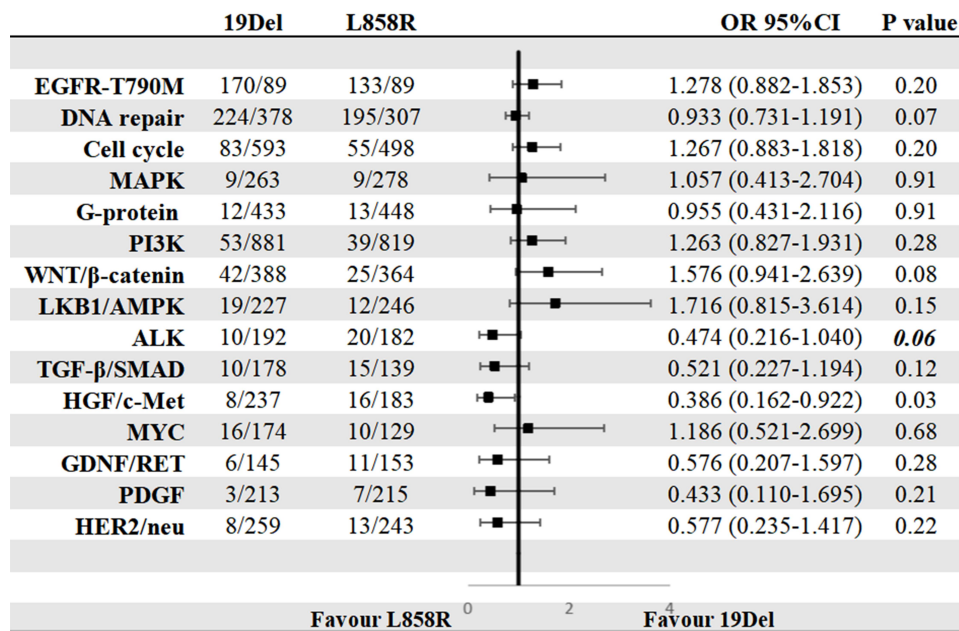| Author	Journal	19Del Patients No.	L858R Patients No.	Treatment Outcomes	Specimen	Race	Disease Stage	Detection	Depth	Sequencing
Hong et al.[7]	JAMA Oncol 2018	29	29	Yes	Plasma	Asian	IV	NGS	15,370X	49 gene
Yu et al.[10]	Chin J Cancer 2016	12	12	No	Tissue	Asian	IIIB-IV	NGS	469X	483 gene
Sato et al.[11]	Sci Rep 2018	23	20	No	Tissue	Asian	I-IV (III-IV 45%)	NGS	500X	415 gene
Li et al.[12]	Cancer lett 2018	7	17	No	Tissue	Asian	Not given	NGS	>10,000X	318 gene
Ku et al.[13]	J Pathol Transl Med 2018	32	37	No	Tissue	Asian	I-IV (III-IV 96%)	NGS	>500X	50 gene
Blakely et al.[14]	Nat Genet	28	20	Yes	Plasma	Caucasian	III-IV	NGS	Not given	Not given
Liu et al.[8]	Cancer Sci 2017	62	88	No	Tissue	Asian	I-IV (III-IV 30%)	NGS	>500X	145 gene
Jakobsen et al.[15]	Oncotarget 2018	11	8	No	Tissue	Caucasian	I-IV (advanced 55%)	NGS	Not given	22 gene
Cao et al.[16]	OncoTargets and Therapy 2018	1	1	No	Both	Asian	I-IV (III-IV 68%)	NGS	1214X for tissue; 2628X for plasma	Not given
Jin et al.[17]	Lung Cancer	36	30	No	Both	Asian	Advanced	NGS	600X for tissue; 2000X for plasma	416 gene
Liao et al.[18]	FEBS Open Bio 2018	6	0	No	Tissue	Asian	IIA-IV (III-IV 66.7%)	NGS	678X	390 gene
Vanderlaan et al.[19]	Lung Cancer 2017	11	4	No	Tissue	Caucasian	Not given	NGS	Not given	Not given
Yu et al.[20]	Clin Cancer Res 2018	97	71	No	Tissue	Caucasian	IV	NGS	Not given	Not given
Xu et al.[21]	Transl Oncol 2018	13	15	Yes	Both	Asian	Advanced	NGS	Not given	Not given
Tian et al.[22]	Lung Cancer 2018	16	5	No	Tissue	Asian	I-IV (III-IV 90%)	NGS	1855X	168 gene
Martorell et al.[23]	Clin Lung Cancer 2017	5	1	No	Tissue	Caucasian	I-IV (III-IV 71%)	NGS	1000X	Not given
Hou et al.[24]	Sci Rep 2017	12	13	No	Plasma	Asian	IIIB-IV	NGS	950X	508 gene
Nahar et al.[25]	Nat Commun 2018	14	17	No	Tissue	Asian	I-II	NGS	114X	Whole-exome sequencing

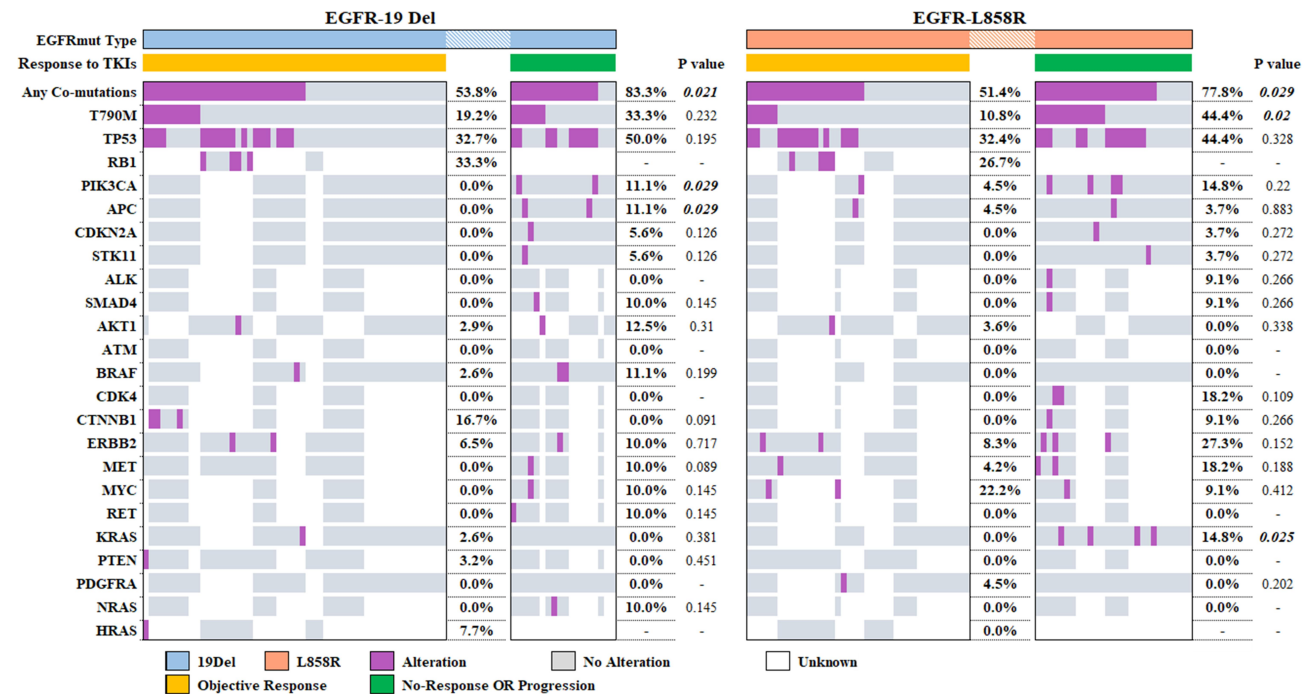**Abbreviation:** NGS, next generation sequencing.
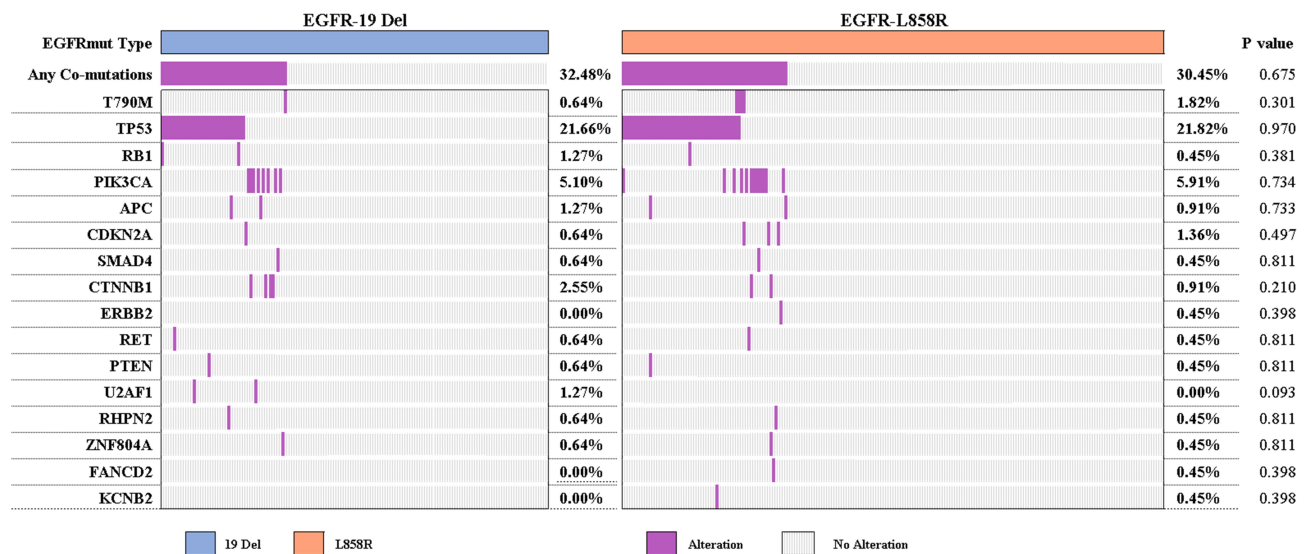
Figure 1 Genetic mutations landscape identified by targeted next-generation sequencing of G1: Alteration map of 403 patients with NSCLC harboring EGFR sensitive mutation.
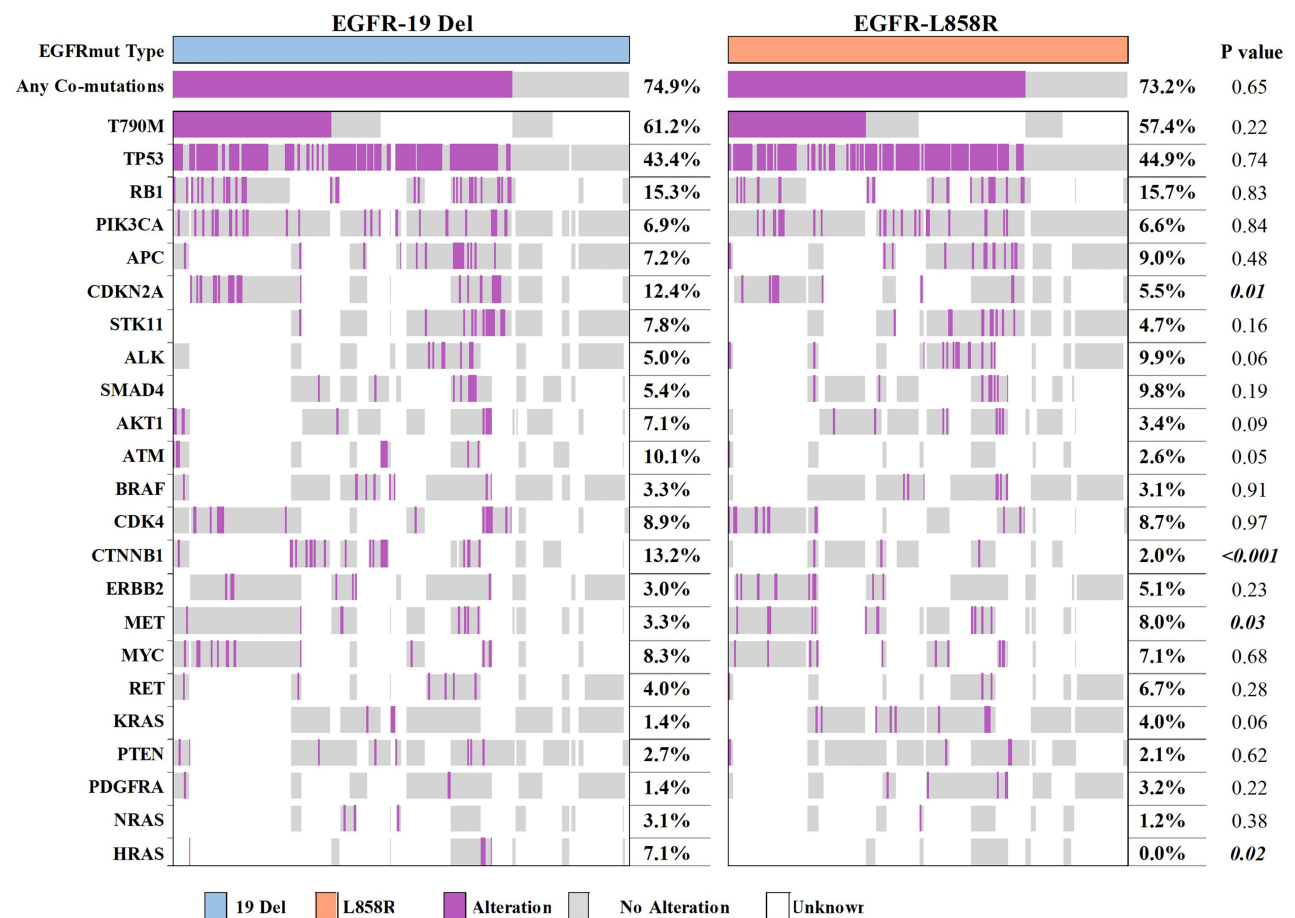
Figure 2 Individual patient data of genetic mutations identified by targeted next-generation sequencing of G2: Alteration map of 803 patients with NSCLC harboring EGFR sensitive mutation.
Note: Bold and italic formatting indicates *P*-value with significant difference.

|	19Del	L858R		OR 95%CI	P value
EGFR-T790M	170/89	133/89		1.278 (0.882-1.853)	0.20
DNA repair	224/378	195/307		0.933 (0.731-1.191)	0.07
Cell cycle	83/593	55/498		1.267 (0.883-1.818)	0.20
MAPK	9/263	9/278		1.057 (0.413-2.704)	0.91
G-protein	12/433	13/448		0.955 (0.431-2.116)	0.91
PI3K	53/881	39/819		1.263 (0.827-1.931)	0.28
WNT/β-catenin	42/388	25/364		1.576 (0.941-2.639)	0.08
LKB1/AMPK	19/227	12/246		1.716 (0.815-3.614)	0.15
ALK	10/192	20/182		0.474 (0.216-1.040)	*0.06*
TGF-β/SMAD	10/178	15/139		0.521 (0.227-1.194)	0.12
HGF/c-Met	8/237	16/183		0.386 (0.162-0.922)	0.03
MYC	16/174	10/129		1.186 (0.521-2.699)	0.68
GDNF/RET	6/145	11/153		0.576 (0.207-1.597)	0.28
PDGF	3/213	7/215		0.433 (0.110-1.695)	0.21
HER2/neu	8/259	13/243		0.577 (0.235-1.417)	0.22

Favour L858R   0   2   4   Favour 19Del

Figure 3 Forest map of concomitant mutation in NSCLC harboring EGFR sensitive mutations according to signaling pathway.
Note: Bold and italic formatting indicates *P*-value with significant difference.

Figure 4 Individual patient data of genetic mutations identified by targeted next-generation sequencing of G2: Alteration map of 134 NSCLC EGFR sensitive mutated patients underwent TKI therapy.
Note: Bold and italic formatting indicates *P*-value with significant difference.

Interestingly, in patients without any concomitant mutation, *19Del* consistently showed better treatment outcomes (Objective response: *19Del* 88.9% vs *L858R* 75.0%). However, the response rates in both mutation types decreased significantly when contaminated with at least one other mutation (Objective response: *19Del* 65.1% vs *L858R* 47.5%) (Figure 5).
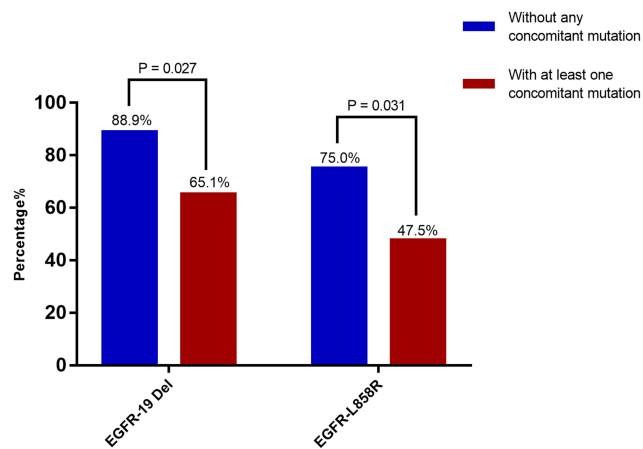
Figure 5 The effect on response to EGFR-TKI of concomitant mutations in 19Del and L858R NSCLC patients of G2.

Discussion

This study involved two cohorts based on NGS individual patient data and comprehensively compared the concomitant mutational profiles of *EGFR 19Del* and *L858R* in TKI naïve patients. Similar to recent studies, we observed that concomitant mutations were widespread both in *19Del* and *L858R* mutated NSCLC patients from two cohorts, which indicated *EGFR*-mutant NSCLC is not a single oncogene-driven disease.[8,14] We also found concomitant mutation was associated with poorer treatment outcomes both in *19Del* and *L858R*; patients with *L858R* showed significantly less response to TKIs than those with *19Del* regardless of the concomitant mutation status. However, we failed to conclude that the distribution of concomitant mutations was different in *19Del* and *L858R* mutations.

Previously, it has been believed that both *EGFR 19Del* and *L858R* are sensitive to EGFR-TKIs. However, a pooled analysis of Lux-Lung 3 and Lux-Lung 6 suggested that *19Del* patients might benefit more from the second-generation EGFR-TKI Afatinib than *L858R*.[26] We also performed a meta-analysis showing a similar trend observed among first-generation TKIs, erlotinib, and gefitinib.[3] A recent study found *L858R* had a significantly higher incidence of concomitant mutation than *19Del*, and indicated the inferior treatment outcome associated with *L858R* could be attributed to the higher incidence of concomitant mutations.[7] However, according to the present large-scale individual data in both G1 and G2, we found similar prevalence of total concomitant mutations and the majority of mutational spectrum between *19Del* and *L858R* are the same. When dividing these concomitant mutations according to signaling pathway, no difference was found between *19Del* and *L858R*. Furthermore,

analysis of TKI treatment related outcomes suggested that both *19Del* and *L858R* had generally similar concomitant mutation patterns, and fewer *L858R* patients showed an objective response to TKIs than *19Del* patients, regardless of the concomitant mutation status. Thus, we claimed that the efficacy discrepancy between *19Del* and *L858R* might not be attributed to their respective concomitant mutation status.

There are several well-known resistance markers, somatic *T790M*, *TP53*, *KRAS*, and so forth, which indicated a lower response rate and shorter PFS in *EGFR* mutant patients using TKIs.[19,27] Among these alterations, somatic *T790M* has attracted the most attention because it is the main reason for the acquired resistance to first-generation TKIs. Our previous research showed that the presence of somatic *T790m* at baseline is higher in *L858R* than in *19Del*.[6] The absolute concomitant rate is 22% (*L858R*) and 14% (*19Del*), respectively, the majority of biopsy is detected by polymerase chain reaction (PCR). However, in the G2 cohort from the present study, according to pooled individual data based on NGS, concomitant somatic *T790M* were similar in *19Del* (73.4%) and *L858R* (72.4%) mutations. Furthermore, the total detection rate of *T790M* is significantly higher than the former report and G1.

Somatic *T790M* variant is an indication of primary TKI resistance, the ability to identify low *VAF* (Variant Allele Fraction) at this locus is important in determining drug administration.[28] NGS has been rapidly adopted in molecular diagnosis, and the capability to detect specific low-level mutations is important.[29] Gene mutations may be detected at less than 5% *VAF* when performing deep sequencing of targeted NGS panels. A study showed an in-house developed cancer hotspot NGS assay at read depth of 2672X can detect *T790M* variant at near 2% *VAF*, but the real time PCR assay failed to show a positive result in the same patient,[30] which indicated the NGS might had better capacity to recognize low *VAF* mutation. Using NGS, the current study found more somatic concomitant *T790M* mutations than a formal study at the baseline of *EGFR* mutant NSCLC patients.[6] The present co-existing rate of somatic *T790M* in *19Del* and *L858R* is also different compared with the former study,[6] we believe the reason for the inconsistency may also be the detection method. With the use of NGS, more somatic *T790M* mutations of low frequency in *19Del* than *L858R* were detected. Different concomitant mutation associated mechanisms affect the treatment response in these two sensitive mutations. The specific underlying mechanism for primary resistance and

relative countermeasures in *L858R* and *19Del* mutant patients warrants further investigation.

The total concomitant mutation rate was similar in *EGFR 19Del* and *L858R* according to G1 and G2 cohorts, however, the spectrum of concomitant mutation was different in the two sensitive mutations or different cohorts. The total concomitant mutation rate was 35% in G1 and more than 70% in G2. One of the reasons for this finding may be different screening panels and depth between G1 and G2. Another reason would be that the two sensitive mutations do not share the same concomitant mutation, but under the same screening condition, the total rate of concomitant mutation is the same. This assumption can be supported by different original studies from G2, their respective concomitant mutation spectrums were different, but the total rate was the same between *19Del* and *L858R*. This evidence also confirmed that *19Del* and *L858R* owned similar total numbers and patterns of concomitant mutations, which might not explain the different sensitivity to *EGFR*-TKI of two sub-types.

There is a concern that treatment strategies might affect the objective response, which is one of the main evaluated indices in this study. However, not all studies offered drug information. Besides, some studies included more than one *EGFR* TKIs, without labeled individual information of each patient. It is difficult to analyze the influence of TKI on therapeutic outcomes. We have published a Bayesian network meta-analysis, comparing every strategy that used in first-line therapy for *EGFR*-mutated NSCLC patients based on all published RCTs and conference abstracts.[4] We found that osimertinib and gefitinib plus pemetrexed based chemotherapy were associated with the best progression free survival and overall survival benefits for patients with advanced *EGFR*-mutated NSCLC, compared with other first line treatments. The treatments resulting in the best progression free survival for patients with the *exon 19 deletion* and *L858R* mutations were osimertinib and gefitinib plus pemetrexed based chemotherapy, respectively, which strongly supports that the two sensitive mutations have different responses to *EGFR*-TKI.

There are some limitations to our study. Firstly, some heterogeneity and missing data (presented as unknown) existed in the sequencing platform from G2, which is because of not all NGS based screening panels in G2 covers the same hotspot. Furthermore, the sequencing depths are different among the included studies, which could lead to a potential bias. Secondly, the absolute number of gene mutation frequencies might affect the result, however, from the limit information of original studies that were included in our study, mutation frequencies could not be got. We only get the information of whether a patient had a mutation, thus the false discovery rates (FDRs) could not be calculated. Further large-scale study is needed to demonstrate the association between the number of samples and the mutation frequencies. Thirdly, because of the raw data limitation (Supplementary Appendix), we used objective response to evaluate the therapeutic effect rather than PFS or OS, which may not reflect the real survival situation, and G1 did not offer any therapeutic or follow-up information. Moreover, not all patients in G2 had response data, and, therefore, may not reflect the overall information for the G2 cohort. Finally, this study lacked the abundance of each mutation, thus quantitative analysis is not available.

Conclusion

Our study demonstrated that concomitant mutations were widespread both in *19Del* and *L858R* mutated NSCLC patients and the prevalence of total concomitant mutations is similar between *19Del* and *L858R*. Patients with *L858R* showed significantly less response than those with *19Del*, regardless of the concomitant mutation status, indicating that some intrinsic factors, rather than concomitant mutations, might underly the varied sensitivity of *19Del* and *L858R* to EGFR-TKIs.

Data Sharing Statement

For original deidentified individual patient data please contact drjianxing.he@gmail.com. Data will be made available for a period of 5 years after the publication date.

Ethics Approval and Consent to Participate

The study protocol has been approved by the Research Ethics Committee of the First Affiliated Hospital of Guangzhou Medical university (S-201525).

Author Contributions

All authors were involved in conception and design, provision of study materials, collection and assembly of data,

data analysis and interpretation, manuscript writing, and approval. All authors agree to take responsibility and be accountable for the contents of the article. Wenhua Liang and Jianxing He provided the administrate support.

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Disclosure

The authors report no conflicts of interest for this work.

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