Original Article

Comprehensive analysis of EGFR T790M detection by ddPCR and ARMS-PCR and the effect of mutant abundance on the efficacy of osimertinib in NSCLC patients

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Background: Patients with non-small cell lung cancer (NSCLC) harboring epidermal growth factor receptor (EGFR) mutations often develop systemic disease progression during treatment with EGFR-tyrosine kinase inhibitors (TKIs). Droplet digital polymerase chain reaction (ddPCR) and amplification refractory mutation system (ARMS)-PCR are routinely applied for detection of EGFR mutations, including T790M, which is associated with TKI sensitivity. We compared the efficiency of ddPCR and ARMS-PCR in detecting T790M and explored the association between T790M abundance and osimertinib efficacy.

Methods: Genomic DNA (gDNA) from tissue and cells in hydrothorax and circulating tumor DNA (ctDNA) from peripheral blood (PB), and clinicopathological data were retrospectively collected from 263 patients who visited Sun Yat-sen University Cancer Center for T790M test.

Results: Mean T790M abundance and mutant copy number of cases tested positive by both methods, i.e., the ddPCR+ARMS+ group (19.1%, 636.9), were higher than those in the ddPCR+ARMS– group (0.36%, 12.1), suggesting that ddPCR is more sensitive in detecting samples with low mutant abundance than ARMS-PCR. T790M detection rate was comparable for gDNA and ctDNA samples (44.7% *vs.* 37.6%, P=0.242); however, gDNA sample tended to show more T790M abundance in ddPCR analysis. T790M coexisted with L858R mutation (8/11) more than with deletions in exon 19 (19del) mutation (3/11) in TKI-naive tumors, while 19del co-occurred as often as L858R in post-TKI tumors. T790M+ patients benefited more from osimertinib and showed longer progression-free survival (PFS) (not achieved *vs.* 10.1 months, P=0.0399), while lower T790M abundance (<1.065%) was associated with longer PFS (not achieved *vs.* 8.8 months, P=0.0033).

Conclusions: ddPCR has a higher sensitivity than ARMS-PCR, especially in detecting the less abundant T790M. Although detection rates were comparable for ctDNA and gDNA samples, the mutation abundance was higher in gDNA sample. Finally, low T790M abundance was associated with longer PFS in NSCLC patients receiving osimertinib treatment.

Keywords: T790M abundance; droplet digital polymerase chain reaction (ddPCR); amplification refractory mutation system-polymerase chain reaction (ARMS-PCR); tyrosine kinase inhibitors (TKIs); non-small cell lung cancer (NSCLC)

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Introduction

In recent years, epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) have been largely successful for the clinical treatment of patients with non-small cell lung cancer (NSCLC) harboring activating EGFR mutations. Most of the mutations associated with sensitivity to anilinoquinazoline EGFR inhibitors occur as either multi-nucleotide in-frame deletions in exon 19 (19del), eliminating four amino acids, Leu-Arg-Glu-Ala, or as a single nucleotide variation at nucleotide 2573 (T to G) in exon 21, resulting in substitution of arginine for leucine at position 858 (L858R) (1,2). However, a majority of patients eventually acquire resistance to the drug, with a median time to progression of 10 to 14 months (3,4). Cases where patients with mutations, such as 19del, L858R, G719X, or L861Q (known to be associated with TKI sensitivity), develop systemic progression of disease while on treatment with EGFR-TKIs is defined as acquired resistance to EGFR-TKIs (5).

The T790M mutation leads to threonine-to-methionine amino acid change at position 790 of the EGFR tyrosine kinase domain, causing steric hindrance that may interfere with the binding of TKIs (6). It occurs in 50% to 60% of patients undergoing treatment with EGFR-TKIs and is the most frequent alteration leading to acquired resistance (7-9). In addition, *de novo* T790M mutation is also an important mechanism of primary resistance to EGFR-TKIs (10). The highly sensitive methods of droplet digital polymerase chain reaction (ddPCR) and amplification refractory mutation system (ARMS)-PCR are routinely applied in clinical detection of T790M mutation (11,12). In this study, we compared the detection rates of these two methods and analyzed the associations of T790M status with clinicopathological parameters and progression-free survival (PFS) in patients with NSCLC, providing detailed evidence to better inform clinical decision-making and improve outcomes.

Methods

Patients

From August 2017 to February 2019, 263 cases that consulted for T790M mutation test by ddPCR in the department of molecular diagnostics of Sun Yat-sen University Cancer Center were retrospectively collected. All patients were diagnosed with NSCLC by pathological examination and the last follow-up was done on 26th February 2019. Objective tumor responses were evaluated every 6–8 weeks in accordance with the Response Evaluation Criteria in Solid Tumors guidelines (version 1.1) (13). Patients with sensitive EGFR mutation had received erlotinib, gefitinib or icotinib orally at a recommended dose, and some patients had received osimertinib treatment. The current study was approved by the Ethics Committee of Sun Yat-sen University Cancer Center, and all patients provided signed informed consent.

DNA extraction

Genomic DNA (gDNA) was extracted from formalin fixed paraffin-embedded (FFPE) tumor tissue and cell pellet centrifuged from hydrothorax using a QIAGEN DNA FFPE Kit (Qiagen, Dusseldorf, Germany) according to the manufacturer's instructions and quantified with a NanoDrop2000 (NanoDrop Technologies, Wilmington, DE, USA). From 10 mL of whole blood, 5 mL plasma was collected and used to isolate and purify circulating tumor DNA (ctDNA) using a QIAamp Circulating Nucleic Acid Kit (Qiagen), following the manufacturer's instructions.

ARMS-PCR and ddPCR

ARMS assay (AmoyDx, Xiamen, China) was conducted using ABI 7500 (Applied Biosystems, Foster City, CA, USA), while ddPCR assay (YUANQI BIO, Shanghai, China) was performed by QX200 Droplet Digital PCR (ddPCR™) (BIO-RAD, Hercules, CA, USA) system. The result was interpreted as positive when the mutant copy number ≥3 in ddPCR, and the T790M abundance was calculated as 100%× (mutant copy number/total copy number).

Statistical analysis

PFS1 was defined as the time from the start of the first-generation EGFR-TKI treatment to the first documentation of progressive disease (PD) or the last follow-up, and PFS2 was defined as the time from the beginning of osimertinib treatment to the second PD or the last follow-up. All time-to-event outcomes were estimated using the Kaplan-Meier method and compared across groups using the log-rank test. The associations between T790M and clinical characteristics were analyzed using the Chi-squared test. Differences between groups were assessed by Student's *t*-test or one-way analysis of variance. The cut-off value of

T790M mutant abundance was decided using the receiver operating characteristic curve. Two-tailed P values <0.05 were considered statistically significant. SPSS 23.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 6 (La Jolla, CA, USA) software were used for statistical analyses and graphical representations.

Results

Clinicopathological characteristics of patients

As shown in *Table 1*, samples of 115 males and 148 females were included in our study, and most of them were diagnosed as adenocarcinoma in TNM stage IV. Sample types included tissue, hydrothorax, and peripheral blood (PB). The average age of the patients was 59.5 (ranging from 26 to 87). Eighty-eight patients had 19del, 87 patients had L858R, 53 patients had mutations of other types, and 35 patients were classified as wild type (WT). Among all cases, 203 patients had received first-generation TKIs as first-line treatment, and 68 patients had received osimertinib after the first PD.

Comparison of detection efficacy of ddPCR and ARMS PCR

In our study, the total positive rate of T790M in 263 samples tested by ddPCR was 40.7% (107/263), the mean mutant abundance and copy number were 8.1% (0.06% to 76.3%) and 241 (3 to 2,916), respectively. Along with ddPCR, 53 samples were also subjected to ARMS-PCR at the same time, of which there were 18 samples judged negative by both ddPCR and ARMS-PCR. However, only 21 samples were recognized as positive by ARMS-PCR, as opposed to 35 positive samples detected by ddPCR (*Table 2*). The average T790M mutant abundance and copy number in the ddPCR+ARMS+ group (19.1%, 636.9) were both significantly higher than that in the ddPCR+ARMS– group (0.36%, 12.1) (*Figure 1A*,*B*). Noticeably, the sensitivity and the concordant rate (true negative and true positive) of ARMS-PCR were only 27.8% and 63.9% of ddPCR in samples with mutant abundance of <1%, and they gradually added up to 60.0% and 73.6%, respectively, when the mutant abundance range was enlarged to <100% (*Figure 1C*,*D*).

Together, it demonstrated that both ddPCR and ARMS-PCR exhibited a high specificity in detection of negative samples. However, ddPCR performed better than ARMS-PCR in identifying positive samples and was especially sensitive in detecting less abundant T790M mutation.

Influence of sample type on detection rate of T790M by ddPCR

According to our analysis, the T790M positive rates were 40.2% (33/82), 56.3% (18/32), and 37.6% (56/149) in tissue, hydrothorax, and PB sample groups, respectively. In terms of DNA source, the T790M detection rate in ctDNA from plasma of PB was similar to that in gDNA from tissue and cells of hydrothorax (37.6% *vs.* 44.7%, P=0.242). The average mutant abundance in T790M+ gDNA samples was statistically higher than that in ctDNA samples (11.1% *vs.* 5.3%, P=0.0325). Nevertheless, the average mutant copy number in T790M+ gDNA samples was numerically but not statistically higher than that in ctDNA samples (323.8 *vs.* 165.3, P=0.0930) (*Figure 2A*,*B*). The ddPCR+ARMS+/ddPCR+ARMS– ratio in gDNA samples (15/8) was also numerically, but not statistically higher than that in ctDNA samples (6/6, P=0.383) (*Figure 2C*,*D*).

Interestingly, among three patients (A, B and C) who had submitted both tissue and PB samples to be tested by ddPCR, patient A got consistent negative results, patient B got consistent positive results, and it is worth noticing that the mutant abundance in tissue sample (58.2%) was much higher than that in PB sample (10.8%). Patient C tested positive on the basis of the tissue sample (14.9%), but negative on the basis of the PB sample (0%). In addition, another two patients (D and E) had submitted both hydrothorax and PB samples to be measured by ddPCR; patient D was judged as T790M– in both samples and patient E was judged as T790M+ in both samples, but with different mutant abundances—6.9% in hydrothorax and 1.1% in PB (*Table 3*).

Accompanying sensitive mutation composition in pre- and post-TKI subset

T790M mutation is one of the most important causes of primary TKI resistance (10). In the current study, T790M+ was detected in 11 patients before they received any TKI therapies. These patients comprised six males and five females, and they submitted eight tissue samples and three hydrothorax samples. We observed that primary T790M mutation occurred with L858R in eight samples and 19del in three samples in the pre-TKI subset, while acquired T790M coexisted with 19del in 37 samples and L858R in

Table 1 The clinicopathological characteristics of enrolled patients

Parameters	T790M+ (n=107)	T790M− (n=156)	P
Sex			0.100
Male	40	75	
Female	67	81	
Age			<0.001
<60	37	116	
≥60	70	40	
Sample type			0.149
Tissue	33	49	
Hydrothorax	18	14	
Peripheral blood	56	93	
TNM stage			0.408
III	6	12	
IV	84	111	
Uncertain	17	33	
Pathology			0.088
Adenocarcinoma	100	136	
Squamous carcinoma	0	6	
Others	7	14	
Differentiation			1.000
High	2	3	
Moderate	24	35	
Low	54	78	
Uncertain	27	40	
Type of TKI			0.119
Erlotinib	23	48	
Gefitinib	35	35	
Icotinib	28	34	
None	21	39	
Response to TKI			0.068
CR+PR	0	2	
SD	18	40	
PD	72	81	
Uncertain	17	33	
EGFR mutation			0.026
19del	40	48	
21L858R	44	43	
Other mutation	16	37	
Wild type	7	28	

TKI, tyrosine kinase inhibitor; EGFR, epidermal growth factor receptor; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Table 2 The results for 35 T790M+ samples detected by ddPCR and ARMS-PCR

Sample number	Sex	Sample type	ddPCRT790M	ARMST790M	Mutant copy number	Mutant abundance (%)
1	M	PB	+	+	2,916	76.30
2	M	T	+	+	2,119	58.20
3	F	T	+	+	2,068	56.29
4	M	T	+	+	1,624	53.00
5	F	T	+	+	208	44.83
6	M	H	+	+	1,624	25.90
7	M	T	+	+	860	25.24
8	F	T	+	+	734	21.90
9	M	T	+	+	469	17.30
10	M	T	+	+	281	6.81
11	F	T	+	+	197	4.23
12	F	PB	+	+	38	2.58
13	M	T	+	+	26	1.80
14	M	T	+	+	23	1.70
15	M	PB	+	+	40	1.11
16	F	PB	+	+	51	1.11
17	F	PB	+	+	26	0.85
18	F	PB	+	+	31	0.64
19	F	H	+	+	18	0.38
20	M	T	+	+	15	0.30
21	F	H	+	+	7	0.14
22	F	PB	+	–	56	1.76
23	F	PB	+	–	28	0.97
24	M	H	+	–	21	0.51
25	M	H	+	–	24	0.29
26	F	PB	+	–	7	0.27
27	F	T	+	–	3	0.20
28	F	T	+	–	3	0.19
29	F	PB	+	–	6	0.17
30	F	PB	+	–	4	0.17
31	F	PB	+	–	4	0.16
32	F	T	+	–	3	0.12
33	M	T	+	–	3	0.10
34	F	H	+	–	5	0.09
35	M	H	+	–	4	0.06

F, female; H, hydrothorax; M, male; T, tissue; PB, peripheral blood; ddPCR, droplet digital polymerase chain reaction; ARMS, amplification refractory mutation system; PCR, polymerase chain reaction.

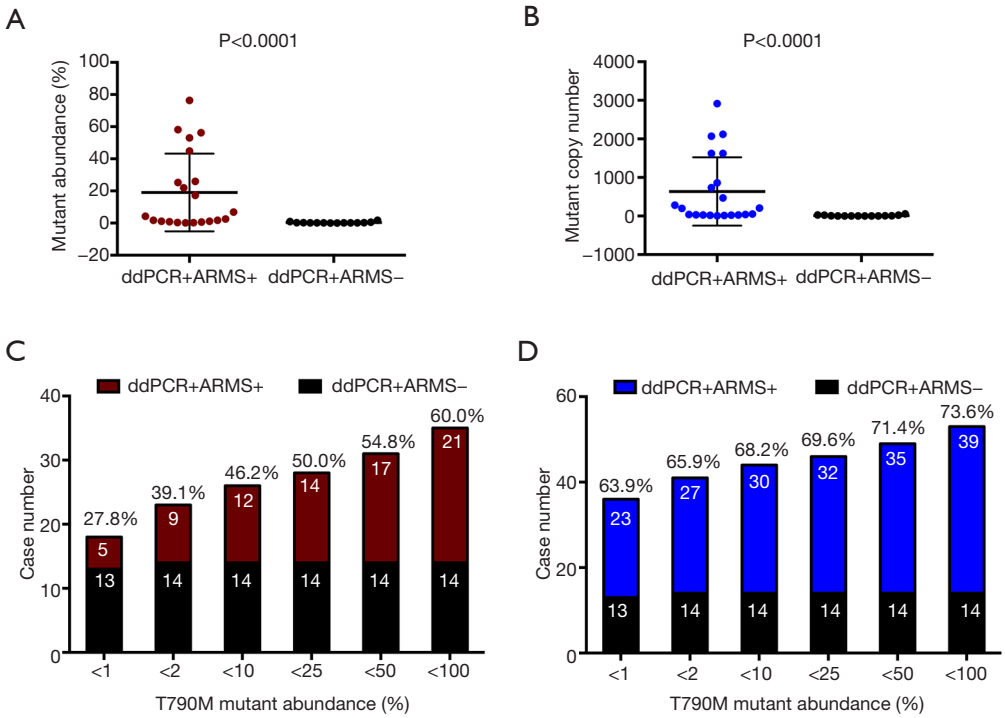

Figure 1 Comparison of detection efficacy of ddPCR and ARMS-PCR. T790M mutant abundance (A) and the mutant copy number (B) in ddPCR+ARMS+ group were significantly higher than that of ddPCR+ARMS− group. The relative sensitivity (C) and concordant rate (D) of ARMS-PCR compared with ddPCR in different groups with mutant abundance of less than 1%, 2%, 10%, 25%, 50% and 100%. ddPCR, droplet digital polymerase chain reaction; ARMS, amplification refractory mutation system; PCR, polymerase chain reaction.

36 samples in the post-TKI subset (*Figure 3A,B*, *Table S1*).

PFS1 of patients with sensitizing EGFR mutation receiving first-generation TKIs

PFS1 was assessed in 203 patients receiving the first generation of TKIs based on TKI type and activating mutation type. According to the Kaplan-Meier survival analysis, the PFS1 of erlotinib-group was markedly longer than that of the gefitinib-group (31.0 *vs.* 17.5 months, P=0.0355) and that of icotinib-group (31.0 *vs.* 13.2 months, P=0.0004) (*Figure 3C*), while the PFS1 was similar in patients with 19del versus L858R mutations (21.9 *vs.* 15.2 months, P=0.1746) (*Figure 3D*).

T790M mutation and its mutant abundance predicts osimertinib therapeutic effect

Among the assessable patients, 47 T790M+ patients and 21 T790M− patients had received osimertinib treatment after PD. Among them, 43 patients were evaluated as stable disease (SD) and 25 patients were evaluated as PD until the last follow-up. The data showed that the PFS2 in T790M+ patients was significantly longer than that in the T790M− patients on osimertinib (not achieved *vs.* 10.1 months, P=0.0399) (*Figure 4A*). Moreover, patients with T790M abundance lower than 1.065% had a more prolonged PFS2 than those with higher T790M abundance (not achieved *vs.* 8.8 months, P=0.0033, *Figure 4B*).

Discussion

Newly acquired resistance in NSCLC patients with activating EGFR mutation after first-generation TKI therapy is a serious problem leading to poor outcome of the disease. The secondary T790M mutation in the catalytic cleft of the EGFR tyrosine kinase domain introduces a bulkier amino acid side chain in the ATP-kinase-binding pocket, which alters the activity of erlotinib and gefitinib by increasing the ATP affinity at the binding pocket, thereby minimizing the efficacies of the EGFR-TKIs (6,14). According to our analysis, the total detection rate of T790M

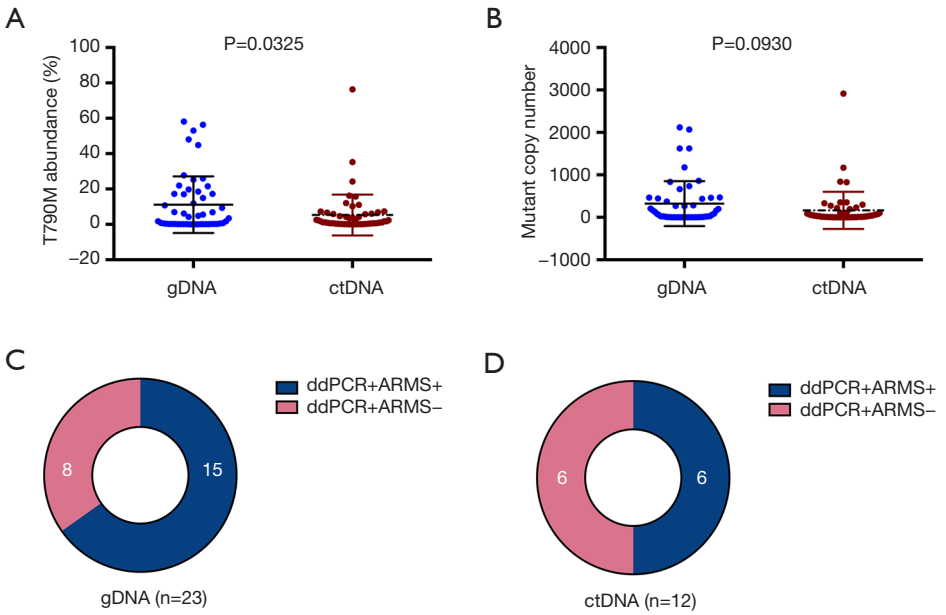

Figure 2 Influence of DNA source on detection rate of ddPCR and ARMS-PCR. The T790M mutant abundance (A), but not T790M mutant copy number (B), was significantly higher in gDNA samples than in ctDNA samples. (C,D) The ddPCR+ARMS+/ddPCR+ARMS− ratio in gDNA samples (15/8) was numerically, but not statistically higher than that in ctDNA samples (6/6, P=0.383). ddPCR, droplet digital polymerase chain reaction; ARMS, amplification refractory mutation system; PCR, polymerase chain reaction.

Table 3 T790M mutant abundance of different samples from the same patient by ddPCR

Patient	T790M mutant abundance (%)		
	T	H	PB
A	0	–	0
B	58.2	–	10.8
C	14.9	–	0
D	–	0	0
E	–	6.9	1.1

H, hydrothorax; PB, peripheral blood; T, tissue; ddPCR, droplet digital polymerase chain reaction.

in all types of samples was 40.7%, which was similar to the detection rate of about 50% reported in re-biopsy tumor tissue from NSCLC patients with acquired resistance to EGFR-TKI therapy (9).

Our data showed that ddPCR was extremely sensitive in detecting T790M with low mutant abundance, and this advantage was supported by several other studies. Wang *et al.* demonstrated that the T790M detection rates were 46.7% (35/75) and 25.3% (19/75) in 75 patient plasma samples by ddPCR and ARMS-PCR, respectively (12). Feng *et al.* showed that only 3 out of 17 T790M+ samples were identified as positive by ARMS-PCR, and none of the T790M+ samples with mutant allele frequency <0.5% was correctly confirmed by ARMS-PCR (15). Additionally, Zhang *et al.* reported that the results for 9 out of 10 clinical samples were consistent by ARMS-PCR and ddPCR; however, one sample indicated to be EGFR WT by ARMS-PCR was confirmed as T790M+ with 7 mutant copies in the background of 6,000 WT copies (abundance 0.12%) by ddPCR (11). In the present study, 18 T790M− samples were all classified as negative by both methods; however, 14 T790M+ samples with abundance <2.0% were inconsistently recognized as EGFR WT by ARMS-PCR, illustrating that ddPCR has a parallel specificity with and a much higher sensitivity than ARMS-PCR. Therefore, for potential beneficiaries of osimertinib with low level of T790M mutation, which is difficult to be recognized by ARMS-PCR, ddPCR would be a more advisable choice to avoid false negative results.

The choice of sample type is always an important concern faced by clinicians. Currently, tissue sample remained the optimized choice when available, considering

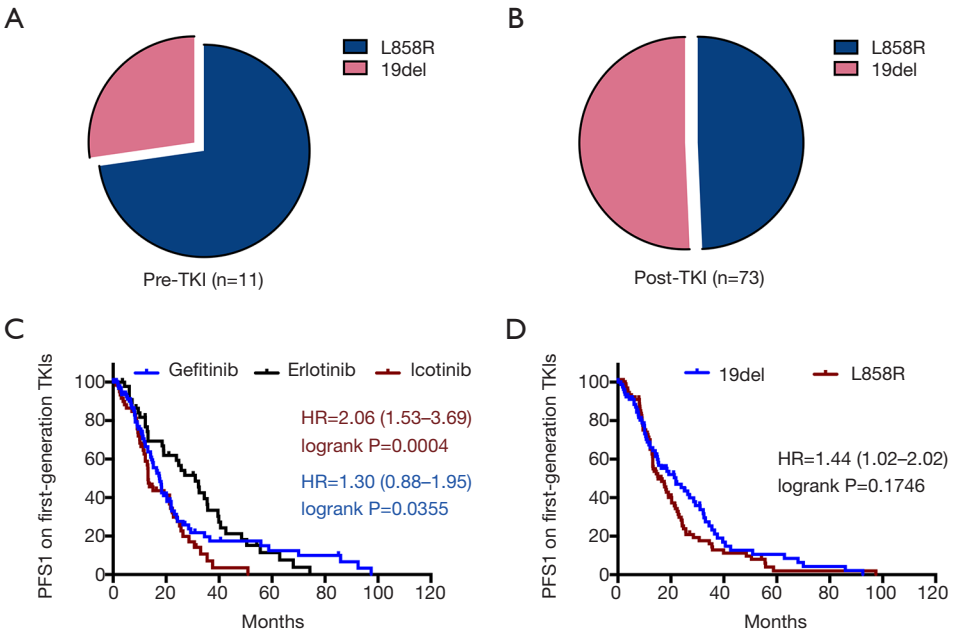

Figure 3 The impact of different accompanying mutations and TKIs on PFS1 in NSCLC patients. (A,B) The coexistence distribution of 19del and L858R with T790M mutation pre- and post-TKI therapy. (C) PFS1 of erlotinib-group was markedly longer than that of gefitinib-group (31.0 *vs.* 17.5 months, P=0.0355) and that of icotinib-group (31.0 *vs.* 13.2 months, P=0.0004). (D) The PFS1 was similar in patients with 19del versus L858R mutations (21.9 *vs.* 15.2 months, P=0.1746). TKI, tyrosine kinase inhibitor; PFS, progression-free survival; NSCLC, non-small cell lung cancer.

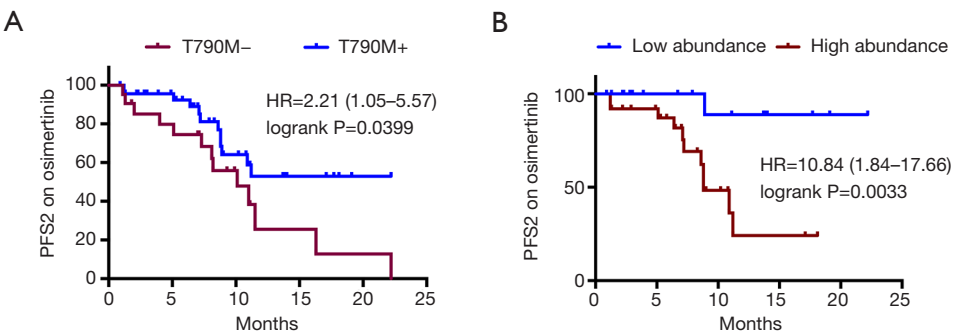

Figure 4 Influence of T790M and mutant abundance on PFS2 in patients receiving osimertinib. (A) The PFS2 in T790M+ osimertinib-group was significantly longer than that of T790M– osimertinib-group (not achieved *vs.* 10.1 months, P=0.0399). (B) The patients with lower T790M abundance (<1.065%) had a more prolonged PFS2 than those with higher T790M abundance (not achieved *vs.* 8.8 months, P=0.0033). PFS, progression-free survival.

its higher DNA yield and mutation content. However, re-biopsy cannot be routinely obtained in the clinical practice. Previous studies have shown the feasibility of investigating EGFR mutation status in ctDNA using different technologies; the detection rate ranged from 16.7% to 34.3%, and concordance rate ranged from 66.3% to 92.9% (16-18). In our study, the detection rate in ctDNA using ddPCR was 37.6%, which was comparable with that in gDNA (44.7%). Besides, PB sample is relatively easy to get during the whole treatment process and ctDNA could reflect the dynamic change of disease progression. As more molecularly tailored treatment options become available

for NSCLC patients, ctDNA in PB sample should become a generally accepted source of DNA, providing a potential alternative to tumor-derived samples for EGFR mutation analysis.

T790M was reported to always occur concurrently with sensitizing EGFR mutations, and we found 92.5% (99/107) T790M+ patients harbored sensitizing EGFR mutations. It has been established that acquired T790M was more prevalent to coexist with 19del, while primary T790M mutation was more likely to coexist with L858R. Some rare second-site mutations implicated in acquired resistance other than T790M, such as D761Y, L747S, and T854A, were reported to always coexist with L858R (7,19-21). Wang *et al.* reported that 31 patients with 19del and 14 patients with L858R harbored acquired T790M (12). Li *et al.* reported that 44 patients with 19del and 25 patients with L858R harbored acquired T790M, while 10 patients with 19del and 30 patients with L858R harbored primary T790M (22). A meta-analysis showed that T790M was more frequent in 19del than in L858R among patients with acquired resistance to TKIs (53% *vs.* 36%; OR 1.87; P<0.001) (23). Another meta-analysis suggested that primary T790M is less frequent in patients harboring 19del compared with those carrying L858R (14% *vs.* 22%; OR 0.59; P<0.001) (24). In this study, we found that 37 patients with 19del and 36 patients with L858R carried acquired T790M, 3 patients with 19del and 8 patients with L858R carried primary T790M (*Figure 3A,B*, *Table S1*), which is roughly consistent with previous data. However, the ratio of 19del to L858R in acquired T790M group was not that high in this study compared with the results reported in the above-mentioned studies. Zhuo *et al.* reported that 21.4% patients were aged 65 years or above in the 19del group, whereas this proportion was markedly higher in the L858R group (38.4%, P=0.015) (25), suggesting that the mutation type might be associated with age. In light of this report, we analyzed our data and found that the average age of patients with L858R was significantly higher than that of patients with 19del (60.8 *vs.* 55.3, P=0.0029, *Figure S1A*). Moreover, there were considerably more patients older than 70 years old and less patients younger than 50 years old in the L858R group, compared with the 19del group (*Figure S1B*), suggesting that the difference in age distribution might be one of the reasons why acquired T790M tended to frequently coexist with L858R in this study.

In addition, some researchers have speculated that 19del might play a distinct biological function considering its higher coincidence with acquired T790M. Preclinical data have demonstrated that the EGFR T790M/19del mutant consistently shows increased activity compared with the T790M/L858R mutant, as measured by morphologic transformation, soft agar colony formation, and tumorigenicity assays (26). Yu *et al.* reported that the patients with 19del presented a longer PFS after first-line EGFR-TKI treatment (14.4 *vs.* 11.4 months, P=0.034) compared with those with L858R, although no statistically significant difference in OS was observed (27). Jackman *et al.* found that patients with 19del had a significantly longer OS (38 *vs.* 17 months, P=0.04) and improved PFS (24 *vs.* 10 months), although not independently significant in a multivariate analysis, compared with patients with L858R following treatment with gefitinib or erlotinib (28). However, Yu *et al.* reported that the PFS after EGFR-TKIs was similar for patients with 19del *vs.* L858R mutations (15 *vs.* 17 months, P=0.99) (8). Zhuo *et al.* also demonstrated that no significant difference was detected in PFS or OS between 19del and L858R groups after eliminating potential imbalanced factors, such as sex, age, histological type, clinical stage, brain metastases, mutation frequency and therapy line, through a propensity score matching method (25). In the present study, the results showed statistically significant difference neither in the proportion of 19del and L858R coexistence with acquired T790M nor in PFS1 between 19del group and L858R group. So at least for now, whether patients carrying 19del and L858R mutations exhibit different responsiveness to EGFR-TKIs and the potential mechanism for such difference remains controversial.

We demonstrated that higher mutant abundance of T790M was associated with a shorter PFS, which was supported by previous research that high mutant copy number of T790M (≥105 per mL of plasma) was correlated with shorter PFS (5.5 months *vs.* not achieved) and shorter OS (9.1 months *vs.* not achieved) (29). A high T790M mutant abundance might be indicative of a higher proportion of cells harboring the T790M mutation, leaving the tumor lesions more vulnerable to T790M-dependent resistance. Moreover, higher mutant abundance may also reflect higher tumor burden and higher aggressiveness.

We acknowledge several limitations to our study. First, the nature of retrospective and single-institution study might cause some statistical bias. Second, matched tissue and liquid biopsy results were not available for most of the patients; therefore, the concordance rate of T790M in gDNA and ctDNA could not be evaluated. Third, the follow-up time was not long enough to draw any conclusion

on OS in these NSCLC patients.

Conclusions

In conclusion, ddPCR is a better choice of method to detect EGFR T790M mutation due to its higher sensitivity and ability to provide quantification of mutant abundance and copy number. Moreover, our study revealed that ctDNA was comparable with gDNA in detecting T790M by ddPCR, and patients with T790M mutation, especially those with low abundance, could benefit more from osimertinib.

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Footnote

Conflicts of Interest: 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. This study was approved by the Ethics Committee of Sun Yat-sen University Cancer Center (No. B2018-157-01), all patients provided signed informed consent, and the research was carried out in accordance with the Helsinki Declaration. The study outcomes will not affect the future management of the patients.

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Supplementary

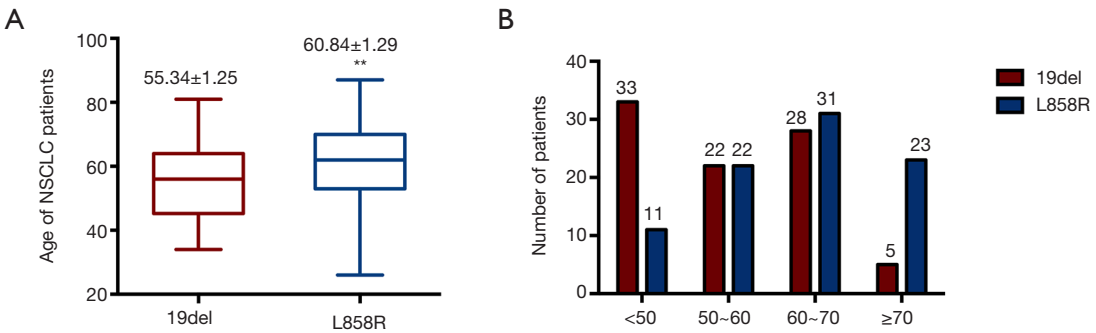

Figure S1 The average age and age distribution of patients carrying 19del and L858R mutations. (A) The average age of patients with L858R was significantly higher than that of patients with 19del (60.8 *vs.* 55.3, P=0.0029); (B) the numbers of patients in different age groups (age <50, 50≤ age <60, 60≤ age <70, age ≥70 years old) carrying 19del and L858R. **, P<0.01.

Table S1 The coexistence of 19del and L858R with acquired and primary T790M

Sensitive mutation	T790M+		
	Acquired	Primary	Total
19del	3	37	40
L858R	8	36	44