



## Research article

## Comparative analysis of QS3D versus droplet digital PCR for quantitative measures of EGFR T790M mutation from identical plasma

Qiaomei Guo<sup>a,b,1</sup>, Lin Wang<sup>a,1</sup>, Xiaohui Liang<sup>a,1</sup>, Mingna Zhao<sup>a</sup>, Xia Huang<sup>b</sup>, Wanxing Xu<sup>a</sup>, Jiatao Lou<sup>a</sup>, Lihua Qiao<sup>a,\*</sup><sup>a</sup> Department of Laboratory Medicine, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, No. 100 Haining Road, Hongkou District, Shanghai, China<sup>b</sup> Department of Laboratory Medicine, Shanghai Chest Hospital, Shanghai Jiao Tong University, No. 241 Huai Hai Road (W), Shanghai, China

## ARTICLE INFO

**Keywords:**  
 cfDNA  
 Concordance  
 Digital PCR  
 Mutation abundance  
 T790M

## ABSTRACT

**Objectives:** The capacity of QuantStudio™ 3D (QS3D) and droplet digital PCR (dPCR) for the detection of plasma Epidermal Growth Factor Receptor (EGFR) mutations have been widely reported. Few comparative studies on the quantitative test of the identical DNA material, however, are carried out. Here we compared the performance of the two methods in detecting EGFR T790M mutation in cell-free DNA (cfDNA) from the same lung cancer patients.

**Methods:** We recruited 72 non-small cell lung cancer (NSCLC) patients who initially respond to tyrosine kinase inhibitor treatment but subsequently developed resistance. Two tubes of 10mL anticoagulant blood were collected and cfDNA was isolated from plasma. Identical cfDNA samples were analyzed for T790M mutation using QS3D and droplet dPCR in parallel.

**Results:** T790M mutation was detected in 15 and 21 cfDNA samples by QS3D and droplet digital PCR, respectively. The 6 discordant samples showed low mutation abundance (~0.1%) and the discrepancy is caused by the stricter threshold settings for QS3D dPCR. The overall agreement between the two methods was 91.7% (66/72). The median allele frequencies for QS3D dPCR and droplet dPCR to detect T790M mutation was 2.01% and 2.62%, respectively. There was no significance in mutation abundance detected by both methods. Both methods are highly correlated with allele frequencies and copy numbers in T790M wild type and mutant, with R<sup>2</sup> of 0.98, 0.92 and 0.95, respectively.

**Conclusion:** Our study demonstrated that QS3D dPCR are highly consistent with droplet PCR for quantitative determination of EGFR T790M mutation in plasma cfDNA.

## 1. Introduction

Molecular targeted therapy has played a pivotal role in the management of non-small cell lung cancer (NSCLC), which account for 85% of lung cancer patients. Genetic testing is critical for confirming the appropriate targeted therapy in NSCLC patients. Those who harbored sensitive Epidermal Growth Factor Receptor (EGFR) mutations benefit from first and/or second-generation EGFR-TKIs. These tumors will finally, however, resistant to EGFR-TKIs through several mechanisms, such as the acquisition of secondary resistance mutations, activation of alternative pathways, aberrance of the downstream pathways and histologic transition [1]. The most common resistance mutation, T790M, accounts for nearly 50% of the group with acquired resistance. Several

studies reported that pretreatment EGFR T790M mutations were detected in TKI-naïve NSCLC patients [2, 3]. Moreover, T790M mutation abundance was shown to correlate with the efficacy of the TKI and prognosis [4, 5]. Therefore, determination of the presence and mutation abundance of T790M is valuable in the clinical setting.

Recent studies have demonstrated plasma cell-free DNA (cfDNA) as a biomarker to identify patients with tumors harboring specific mutations. Moreover, cfDNA shows advantages as it is a minimally invasive approach and minimizes patient exposure to invasive procedures such as tissue biopsy. The traditional gold-standard for T790M detection is q-PCR, which is suitable for tissue gDNA assay with mutation abundance greater than 1%. However, for the analysis of plasma samples, detection goes more difficult due to the lower amounts and lower quality of cfDNA

\* Corresponding author.

E-mail address: [xqbs23@163.com](mailto:xqbs23@163.com) (L. Qiao).<sup>1</sup> These authors have contributed equally to this work and share first authorship.

extracted from plasma. Therefore, establishing methodologies for accurate evaluation of low-frequency mutations in small amounts of cfDNA is critical.

In the past few years, digital PCR (dPCR) technologies have proven to be an ultra-sensitive technology to detect rare mutations with the capability of absolute quantification. Two representative dPCR platforms including the QuantStudio™ 3D (QS3D) dPCR from Thermo Fisher and QX200 droplet dPCR from Bio-Rad. The two platforms are similar in partition volume (about 0.8 nL) and partition number (approximately 20,000), but different in the partitioning strategies.

Early studies demonstrated that both platforms were able to successfully detect *EGFR* mutations in cfDNA samples [6, 7]. However, no prospective data have been available for directly comparing the absolute quantitative results of the T790M mutation in identical cfDNA samples between QS3D and droplet dPCR system. In this study, we compared the ability of the two platforms to detect T790M mutation in plasma cfDNA from NSCLC patients with drug resistance to identify the more appropriate quantification technology for the similar clinical setting.

## 2. Material and methods

### 2.1. Patients

From August 2018 to November 2019, a total of 89 NSCLC patients with sensitive *EGFR* mutations, such as exon 19 deletion, L858R, G719X and L861Q, were enrolled in our study cohort. All participants received EGFR-TKI treatment and eventually acquired drug resistance. Clinical information including histological subtype, primary mutation type, targeted drugs, and progression free survival (PFS) were recorded. Disease progression status was confirmed by clinicians according to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) [8]. This study was supported by the Institutional Review Board (LS1821) at Shanghai chest hospital. Each subjects has signed informed consent before research.

### 2.2. Sample preparation

Blood samples were collected into two 10-mL PAXgene Blood cfDNA Tubes (Qiagen, Hilden, Germany) at the time of disease progression. Blood samples were processed within 5 days by two sequential steps of centrifugation: first centrifuge at 1600 g for 10 min to remove blood cells and then 16,000g for 10 min to remove additional cellular debris. Plasma cfDNA was extracted with the QIAamp Circulating Nucleic Acid Kit (Qiagen) according to the manufacturer's instructions. The concentrations of cfDNA were quantified by a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

### 2.3. T790M detection by the two digital PCR platforms

The optical input amount of starting material for dPCR is 20–80 ng [9]. In our study, if the extracted cfDNA concentration is too high or too low, dilution or concentration will be performed to obtain proper concentration. Then 6.53 μL cfDNA was input for both of QS3D dPCR and droplet dPCR analysis. The experiments and mutation calling were performed as described previously [7, 9]. In brief, the workflow of dPCR included reaction mixture preparation, generation of partitioning units, thermal cycling and mutation calling. For QS3D digital PCR system, we prepared 14.5 μL of reaction mixture with 6.53 μL cfDNA, 0.72 μL of the EGFR T790M mutation detection assay containing PCR primers and Taqman probes, and 7.25 μL QS3D Digital PCR Master Mix. The automatic chip loader was used to load reaction mixture to a QS3D Digital PCR 20 K Chip. Thermo-cycling were performed following specific amplification conditions: 96 °C pre-denaturation for 10 min; 60 °C for 2 min, 98 °C for 30 s, 60 °C for 2 min, 39 cycles; 60 °C for 2 min. Finally, the chip was taken out and equilibrated to room temperature. Data were processed using a Biochip Reader (Quest genomics, Nanjing, China) [9]. The threshold of positivity for QS3D dPCR was defined as >0.1% AF of

the variant with a minimum of five FAM dye-positive wells. For droplet digital PCR system, the reaction mixture was prepared by mixing 10 μL Supermix for probes (No dUTP), 2 μL ddPCR™ probe assay Kit (Bio-Rad Laboratories, specific for T790M), 6.53 μL of template DNA and 1.47 μL double-distilled water in a total volume of 20 μL. QX200 droplet generator was used to generate droplets, which was transferred to a 96-well clear reaction plates for amplification on a T100 Thermal Cycler (Bio-Rad Laboratories). Thermal cycling profile is: 10 min at 95 °C, followed by 40 cycles of: 94 °C for 30 s, 70 °C for 1 min. These cycles were followed by 98 °C for 10 min and then 4 °C hold. After amplification, PCR products were loaded into the QX200 droplet reader and data was analyzed by Quanta Software (Bio-Rad Laboratories) [7]. AF of >0.1% and a minimum of two FAM dye-positive counts were the threshold for reliable detection of a mutation.

### 2.4. Statistical analysis

The results of the two methods were compared using independent sample t-test. Concordance rates between the different detection platforms were calculated by Cohen's kappa. To investigate the association between the two methods, we used Pearson correlation analysis. P value less than 0.05 (2-sided) was considered to have statistical significance. Prism 8.3.0 (GraphPad Software, USA) or SPSS 24.0 software (IBM Corporation, USA) were used to perform all analyses.

## 3. Results

### 3.1. Patient characteristics

A total of 89 patients with advanced NSCLC and sensitive *EGFR* mutations who achieved resistance to TKIs were enrolled in this study. Among the 89 patients, 17 patients were excluded because of insufficient cfDNA yield, resulting in 72 participants eligible for the study. The patient demographics are listed in Table 1. Among the total patient group, 36 (50%) participants harbored an exon 19 deletion, 27 (37.5%) presented the L858R mutation, and 9 (12.5%) had other uncommon sensitive *EGFR* mutations in tumor samples. As initial treatment, 15 (20.8%)

**Table 1.** Patient characteristics (n = 72).

Characteristic	Number (%)
Age, years	
Median	62
Range	33–87
Sex	
Male	29 (40.3%)
Female	43 (59.7%)
Histology	
Adenocarcinoma	72 (100%)
Original EGFR mutation	
Exon 19 deletion	36 (50%)
L858R	27 (37.5%)
Other uncommon active mutations	9 (12.5%)
Stage	
III	6 (8.3%)
IV	63 (87.5%)
Unkown	3 (4.2%)
Prior EGFR TKI(s)	
Gefitinib	15 (20.8%)
Icotinib	53 (73.6%)
Other TKIs	4 (5.6%)
PFS times, months	
Median	12
Range	2–40

**Table 2.** Results of T790M mutation detection by QS3D and droplet dPCR.

droplet dPCR		QS3D dPCR			
Positive	Negative	Positive	Negative	Kappa	P value
15	0	15	0	0.78	<0.0001
6	51	6	51		

patients received gefitinib, 53 (73.6%) patients received icotinib, and 4 (5.6%) patients received other EGFR-TKIs (erlotinib, afatinib or an in-development EGFR-TKI). The median progression-free survival (PFS) times was 12 months (range, 2–40 months).

### 3.2. Initial qualitative assessment

Cell-free DNA were extracted from 72 plasma samples and undergone the two dPCR assay with the equivalent amount. The mean input amount of cfDNA per sample was 52.32 ng (range: 7.25–156.72 ng). The T790M mutation was detected in 15 and 21 out of 72 cfDNA samples by QS3D and droplet dPCR, respectively (Table 2, Figure 1B). The overall agreement for T790M testing results was 91.7% (66/72, Figure 1A), with a high degree of consistency between QS3D and droplet dPCR (kappa = 0.78,  $p < 0.0001$ , Table 2). Specifically, 15 plasma cfDNA samples were found to be positive and 51 samples were found to be negative by both platforms (Table 1). The positive concordance rate was 71.4% (15/21) and the negative concordance rate was 89.5% (51/57). Six samples were positive by droplet dPCR but negative by QS3D dPCR, whereas no samples were found to be positive by QS3D dPCR but negative by droplet dPCR (Figure 1A).

### 3.3. Quantitative analysis comparison

We next compared the AFs of the T790M mutation detected by QS3D and droplet dPCR. The median AFs of the T790M mutations detected by

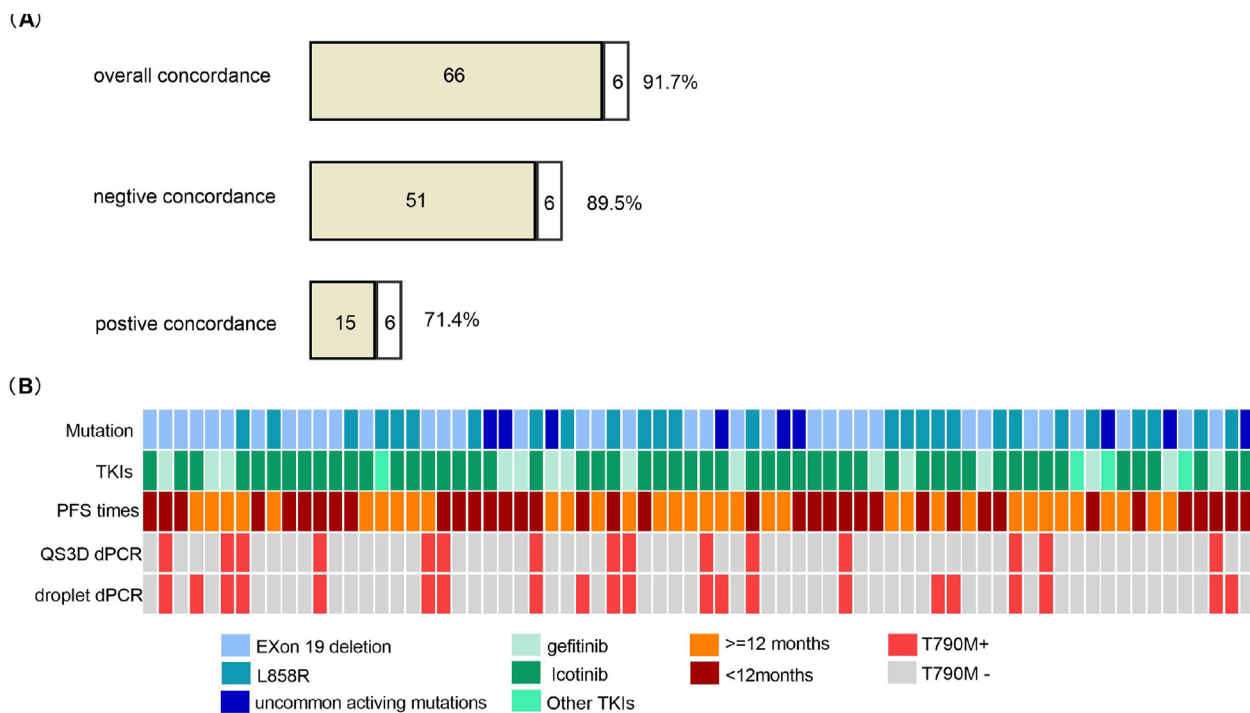
QS3D dPCR was 2.01%, with a range from 0.13% to 9.64%. Similar results were observed in droplet dPCR, with a median AF of 2.62% and range from 0.08% to 10.6% ( $p = 0.7765$ , Figure 2A). Further analysis revealed a strong correlation of mutation abundance (AFs), copy numbers of wild type and mutant of T790M between the QS3D dPCR assay and the droplet dPCR assay ( $R^2 = 0.98, 0.92$  and  $0.95$  respectively) (Figure 2B–D).

### 3.4. Analysis of discordant samples

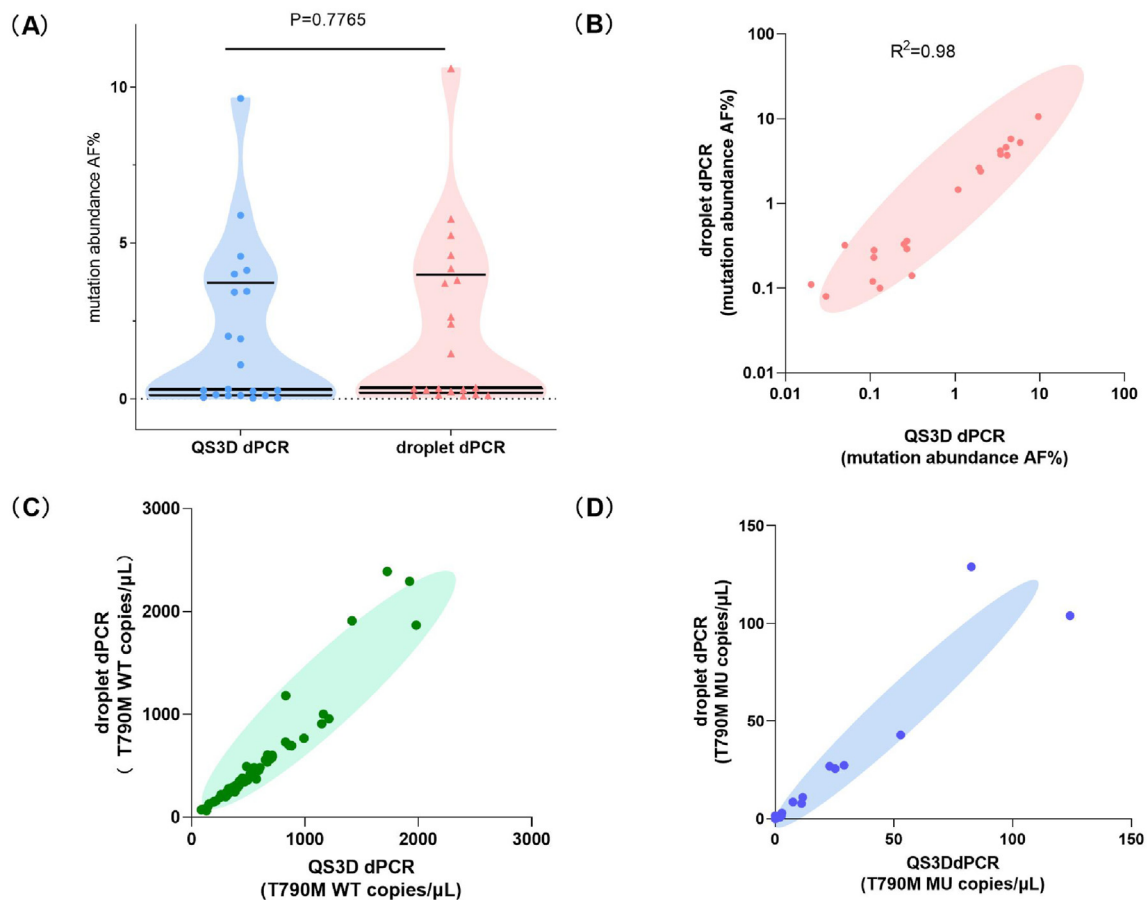
Six plasma samples were scored negative by QS3D dPCR but positive by droplet dPCR, and the samples were found to contain low T790M abundance (Table 3). The AF values detected using droplet dPCR in the discordant samples were close to 0.10%. Despite being below the positive threshold, the T790M mutation abundance detected by QS3D dPCR still showed a good correlation with that by droplet dPCR (Figure 2B and D). This result revealed that the discrepancy is probably because of stricter threshold settings for QS3D dPCR.

## 4. Discussion

The past decade has seen the prosperity of precision medicine, leading to the expansion of dPCR technologies in the molecular diagnostic industry. Compared with conventional real-time PCR, dPCR is superior in its extreme sensitivity, unprecedented quantification capability and tolerance to PCR inhibitors. Today, the applications surrounding dPCR are mounting, and in oncology, dPCR is widely used for quantification and tracking of tumor-specific mutations during the course of cancer treatments [10, 11]. Here, we conducted a comparison study of two dPCR platforms, QS3D dPCR and droplet dPCR, to determine their clinical ability to monitor variants in cfDNA at disease progression status using EGFR T790M mutation as an example. Previous studies have demonstrated that the two digital PCR platforms are of comparable effectiveness in quantifying genome DNA and virus DNA [12, 13, 14], the similar



**Figure 1.** Initial qualitative comparison of T790M detection results between QS3D dPCR and droplet dPCR. The cfDNA extracted from 72 plasma samples were assessed the EGFR T790M mutation status by the two methods, respectively. (A) The two platform showed high overall, positive and negative concordance of T790M detection. The light yellow column represents the number of samples with consistent results, and the white column represents the number of samples with inconsistent results. (B) Heatmap representing the primary sensitive EGFR mutation, EGFR-TKIs type, PFS times and T790M results test by QS3D and droplet dPCR.



**Figure 2.** The quantitative comparison analysis of T790M detection in cfDNA. Quantification of the cfDNA derived from clinical samples by QS3D dPCR was compared with the droplet dPCR. (A) The median allele frequency detected by QS3D dPCR was 2.01% and while the median allele frequency for droplet dPCR was 2.62%. The solid line represents the median and quartiles. The linear relationship for quantification of (B) T790M mutation allele frequencies, (C) T790M wild type, and (D) T790M mutant between the QS3D and droplet dPCR was demonstrated by the scatter plots. Each data point represents detection value derived from one cfDNA samples. Correlation coefficients were 0.98, 0.92, 0.95 for allele frequencies, wild type copy numbers and mutant copy numbers, respectively. Abbreviations, AF, allele frequency; WT, wild type; MU, mutant.

**Table 3.** Samples with different T790M mutation results in the two dPCR assays.

Sample ID	QS3D dPCR	droplet dPCR
09004	Neg (0.05% <sup>a</sup> , 1/1886 <sup>b</sup> )	Pos (0.32%, 4/1118)
09036	Neg (0.11%, 4/3628)	Pos (0.23%, 4/2473)
09048	Neg (0.03%, 3/8796)	Pos (0.08%, 3/6731)
09066	Neg (0.02%, 1/3657)	Pos (0.11%, 2/2540)
09067	Neg (0.11%, 3/2796)	Pos (0.12%, 2/2300)
09089	Neg (0.11%, 4/3606)	Pos (0.28%, 5/2981)

<sup>a</sup> Allele frequency.

<sup>b</sup> FAM + counts/VIC + counts.

results were also observed in our plasma cfDNA assay. The overall consistency for T790M mutation detection is 91.7% between the two digital PCR platforms ( $\kappa = 0.78$ ,  $p < 0.0001$ ). However, due to the limited case numbers in our study, further prospective study should be conducted to confirm our conclusion.

In further analysis, we found that the six discordant samples all showed positive FAM fluorescence signals, which are very close to the positive threshold of QS3D dPCR. These results indicate that the stricter threshold setting is likely to contribute to the lower detection rate of QS3D dPCR.

Previous studies have reported that approximately 50% of patients treated with EGFR-TKIs harbor T790M mutation at disease progression [15, 16, 17]. However, the detection rate of T790M in our study was less

than 30%. A real-world study based on 525 Asian NSCLC patients showed that T790M was detected in 37.1% and 28.8% patients in the first tissue biopsy and liquid biopsy, respectively [18], which is consistent with our data. These results indicate that repeat testing in cfDNA is useful in detecting low frequency mutations [18].

We further confirmed that a similar AFs were acquired by both platforms. Furthermore, a strong correlation of AFs and absolute copy numbers for WT and Mu were demonstrated between the QS3D and droplet dPCR assay for T790M detection from cfDNA. These results indicated the high resilience of different dPCR platforms. The little discrepancy of mutation abundance between the two methods might be because of subsampling errors and repeated tests could improve the accuracy of quantification.

This study had several limitations. First, the detection results of T790M from our plasma samples could not be confirmed by tissue biopsy or other reliable commercial platforms limited by the Low yields of extracted cfDNA from plasma samples. However, both the QS3D and droplet dPCR platforms have shown perfect performance in the detection of plasma T790M mutations [2, 7, 9, 19, 20]. We were also unable to track and evaluate the effect of osimertinib in T790M positive participants, especially in six discordant cases. Only three of the six patients treated with osimertinib, and all of them harbored unsatisfactory effects and very short PFS. Some studies suggested that the abundance of EGFR mutation predicts benefit from TKI treatment for advanced NSCLC patients [5, 21]. Indeed, the mutation abundance of T790M is represented in a relatively low AF in the six discordant samples with AF near around

0.10%, which is consistent with the above conclusions. In real-world clinical settings, such patients should be encouraged to undertake the repeat test and to keep track the T790M status to determine Whether the individual is suitable for targeted therapy.

In conclusion, our data support that both QS3D and droplet dPCR may offer absolute quantification with highly accurate evaluation of T790M mutation abundance. Digital PCR may serve as a powerful tool to meet the ever more stringent demand for accuracy and provide precise results in liquid biopsy.

## Declarations

### Author contribution statement

Qiaomei Guo: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Lin Wang, Xiaohui Liang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mingna Zhao: Analyzed and interpreted the data; Wrote the paper.

Xia Huang, Jiatao Lou: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Wanxing Xu: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Lihua Qiao - Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

### Funding statement

Mrs Qiaomei Guo was supported by National Natural Science Foundation of China [82002224].

Lin Wang was supported by National Natural Science Foundation of China [81802938] and Shanghai Youth Medical Talents [SHWRS (2020)\_087].

Jiatao Lou, Mrs Qiaomei Guo were supported by Shanghai Municipal Health Bureau [2019CXJQ03 & 20204Y0202].

### Data availability statement

Data included in article/supp. material/referenced in article.

### Declaration of interest's statement

The authors declare no conflict of interest.

### Additional information

The clinical trial described in this paper was registered at Shanghai chest hospital under the registration number LS1821.

### Acknowledgements

We thank Gabrielle White Wolf, PhD, from Liwen Bianji, Edanz Editing China ([www.liwenbianji.cn/ac](http://www.liwenbianji.cn/ac)), for providing writing assistance and proof reading the article of this manuscript.

## References

- [1] L. Huang, L. Fu, Mechanisms of resistance to EGFR tyrosine kinase inhibitors, *Acta Pharm. Sin. B* 5 (5) (2015) 390–401.
- [2] M. Watanabe, T. Kawaguchi, S. Isa, M. Ando, A. Tamiya, A. Kubo, et al., Ultra-sensitive detection of the pretreatment EGFR T790M mutation in non-small cell lung cancer patients with an EGFR-activating mutation using droplet digital PCR, *Clin. Cancer Res.* 21 (15) (2015) 3552–3560.
- [3] Y. Matsumoto, K. Sawa, M. Fukui, J. Oyanagi, N. Yoshimoto, T. Suzumura, et al., Predictive impact of low-frequency pretreatment T790M mutation in patients with EGFR-mutated non-small cell lung cancer treated with EGFR tyrosine kinase inhibitors, *Lung Cancer* 139 (2020) 80–88.
- [4] M. Beau-Faller, E. Pencreach, C. Leduc, H. Blons, J.P. Merlio, P.P. Bringuier, et al., Independent prognostic value of ultra-sensitive quantification of tumor pretreatment T790M subclones in EGFR mutated non-small cell lung cancer (NSCLC) treated by first/second generation TKI, depends on variant allele frequency (VAF): results of the French cooperative thoracic intergroup (IFCT) biomarkers France project, *Lung Cancer* 140 (2020) 19–26.
- [5] Q. Zheng, S. Hong, Y. Huang, H. Zhao, Y. Yang, X. Hou, et al., EGFR T790M relative mutation purity predicts osimertinib treatment efficacy in non-small cell lung cancer patients, *Clin. Transl. Med.* 9 (1) (2020) 17.
- [6] J. Xu, W. Wu, C. Wu, Y. Mao, X. Qi, L. Guo, et al., A large-scale, multicentered trial evaluating the sensitivity and specificity of digital PCR versus ARMS-PCR for detecting ctDNA-based EGFR p.T790M in non-small-cell lung cancer patients, *Transl. Lung Cancer Res.* 10 (10) (2021) 3888–3901.
- [7] Q.M. Guo, L. Wang, W.J. Yu, L.H. Qiao, M.N. Zhao, X.M. Hu, et al., Detection of plasma EGFR mutations in NSCLC patients with a validated ddPCR lung cfDNA assay, *J. Cancer* 10 (18) (2019) 4341–4349.
- [8] E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, et al., New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1), *Eur. J. Cancer* 45 (2) (2009) 228–247.
- [9] R. Zhou, Y. Cai, Z. Li, S. Shen, M. Sha, S.R. Head, et al., A digital PCR assay development to detect EGFR T790M mutation in NSCLC patients, *Front. Lab. Med.* 2 (3) (2018) 89–96.
- [10] A. Moreno-Manuel, S. Calabuig-Farinas, A. Obrador-Hevia, A. Blasco, A. Fernandez-Diaz, R. Sirera, et al., dPCR application in liquid biopsies: divide and conquer, *Expert Rev. Mol. Diagn.* (2020) 1–13.
- [11] G. Perkins, H. Lu, F. Garlan, V. Taly, Droplet-based digital PCR: application in cancer research, *Adv. Clin. Chem.* 79 (2017) 43–91.
- [12] R.T. Hayden, Z. Gu, S.S. Sam, Y. Sun, L. Tang, S. Pounds, et al., Comparative performance of reagents and platforms for quantitation of cytomegalovirus DNA by digital PCR, *J. Clin. Microbiol.* 54 (10) (2016) 2602–2608.
- [13] C. Fava, S. Bernardi, E.M. Gottardi, R. Lorenzatti, L. Galeotti, F. Ceccherini, et al., Alignment of Qx100/Qx200 droplet digital (Bio-Rad) and QuantStudio 3D (ThermoFisher) digital PCR for quantification of BCR-ABL1 in Ph+ chronic myeloid leukemia, *Diseases* 9 (2) (2021).
- [14] J. Pavsic, A. Devonshire, A. Blejec, C.A. Foy, F. Van Heuverswyn, G.M. Jones, et al., Inter-laboratory assessment of different digital PCR platforms for quantification of human cytomegalovirus DNA, *Anal. Bioanal. Chem.* 409 (10) (2017) 2601–2614.
- [15] L.V. Sequist, B.A. Waltman, D. Dias-Santagata, S. Digumarthy, A.B. Turke, P. Fidias, et al., Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors, *Sci. Transl. Med.* 3 (75) (2011), 75ra26.
- [16] D.R. Camidge, W. Pao, L.V. Sequist, Acquired resistance to TKIs in solid tumours: learning from lung cancer, *Nat. Rev. Clin. Oncol.* 11 (8) (2014) 473–481.
- [17] V.A. Papadimitrakopoulou, J.Y. Han, M.J. Ahn, S.S. Ramalingam, A. Delmonte, T.C. Hsia, et al., Epidermal growth factor receptor mutation analysis in tissue and plasma from the AURA3 trial: osimertinib versus platinum-pemetrexed for T790M mutation-positive advanced non-small cell lung cancer, *Cancer* 126 (2) (2020) 373–380.
- [18] F. Imamura, M. Kimura, Y. Yano, M. Mori, H. Suzuki, T. Hirashima, et al., Real-world osimertinib for EGFR mutation-positive non-small-cell lung cancer with acquired T790M mutation, *Future Oncol.* 16 (21) (2020) 1537–1547.
- [19] X. Wang, X. Li, H. Guo, L. Zhu, Z. Peng, J. Wang, et al., Highly Sensitive Droplet Digital PCR Method for Detection of de novo EGFR T790M Mutation in Patients with Non-Small Cell Lung Cancer, *OncoTargets Ther.* 13 (2020) 10621–10630.
- [20] L. Lettig, N. Sahnane, F. Pepe, R. Cerutti, C. Albeni, F. Franzi, et al., EGFR T790M detection rate in lung adenocarcinomas at baseline using droplet digital PCR and validation by ultra-deep next generation sequencing, *Transl. Lung Cancer Res.* 8 (5) (2019) 584–592.
- [21] X. Li, W. Cai, G. Yang, C. Su, S. Ren, C. Zhao, et al., Comprehensive analysis of EGFR-mutant abundance and its effect on efficacy of EGFR TKIs in advanced NSCLC with EGFR mutations, *J. Thorac. Oncol.* 12 (9) (2017) 1388–1397.