Detection of EGFR T790M Mutation by Droplet Digital Polymerase Chain Reaction in Lung Carcinoma Cytology Samples

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• Context.—Advanced-stage non–small cell lung carcinoma patients on EGFR-targeted tyrosine kinase inhibitors frequently present with an acquired EGFR T790M resistance mutation. Early detection using a high-sensitivity assay is critical to allow patients to switch to third-generation tyrosine kinase inhibitors. The detection of EGFR T790M mutation is often challenging because of low tumor fraction in posttreatment specimens. Because a large fraction of non–small cell lung carcinoma patients are given a diagnosis by cytology, evaluating a high-sensitivity technique for EGFR T790M detection in these specimens is essential.

Objective.—To evaluate a high-sensitivity droplet digital polymerase chain reaction (ddPCR) assay for EGFR T790M using different cytotologic specimen preparations.

Design.—A total of 42 cytology samples, including smears and cell block preparation, were evaluated for EGFR T790M using ddPCR. The results of the mutation assay were compared to the patient’s known EGFR T790M mutation status.

Results.—The ddPCR assay successfully determined the EGFR T790M mutation status in 36 of 42 samples (86%), including samples with low tumor fraction (<20%). In 4 cases the results of the ddPCR assay could not be compared because the mutation status was unknown at the time of collection of the cytology sample. There was 1 false-positive result, with borderline positivity, and 1 false-negative result. Overall sensitivity and specificity of the ddPCR assay were 93% and 96%, respectively.

Conclusions.—Our results indicate that EGFR T790M ddPCR is a highly sensitive and specific mutational assay that can be used reliably in cytologic specimens, including samples with low tumor fraction.

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The management of non–small cell lung carcinoma (NSCLC), particularly lung adenocarcinoma, has undergone a paradigm shift, with patients harboring sensitizing somatic mutations in EGFR showing a dramatic response to targeted therapy by tyrosine kinase inhibitors (TKIs).1–3 However, a large fraction of these patients eventually develop resistance mechanisms, with an acquired EGFR T790M mutation being the most common.4–6 The early detection of EGFR T790M mutation is critical because third-generation TKIs, such as osimertinib, can be used in these patients.7–11

Advanced-stage NSCLC patients frequently present with resistance to TKI therapy with recurrence and/or progression of disease in the form of a pleural effusion, increasing tumor size, or a new nodule/metastasis. Because these patients are not candidates for surgical resection, small specimens comprising fine-needle aspiration (FNA) or core needle biopsy using minimally invasive sampling techniques are typically used for the diagnosis and molecular testing to evaluate EGFR T790M mutation status. However, posttherapy tumor nodules are often fibrotic and contain large amounts of nonneoplastic stromal tissue with low tumor fraction, making EGFR T790M mutation detection challenging.12–14 The difficulty of detecting EGFR T790M is also true for pleural effusion specimens that frequently contain large numbers of nonneoplastic cells, such as macrophages, mesothelial cells, and lymphocytes, that can lower the tumor fraction in the sample. In addition, the resistance mutation may be present in a subclonal population at a lower allelic frequency (AF) than the sensitizing mutation, making the detection of EGFR T790M in these already low tumor fraction samples extremely difficult.12 This has led to the increasing use of liquid biopsy assays in the setting of
secondary clinical resistance, where circulating tumor DNA isolated from the patient’s plasma is evaluated for EGFR T790M when tissue is inadequate or unavailable.\textsuperscript{12,15–21} Although there is a distinct role for liquid biopsy using plasma cell-free DNA (cfDNA) in such situations, the sensitivity of detection of EGFR T790M is relatively low and often depends on factors such as burden of disease and prevalence of the mutation in cfDNA.\textsuperscript{12,22,23} Therefore, the lung molecular testing guidelines from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology (CAP/IASLC/AMP) recommend that a tissue-based tumor sample testing for EGFR T790M be performed using a high-sensitivity assay (capable of detecting EGFR T790M mutation from a sample containing as little as 5% viable tumor cells), if plasma-based liquid biopsy results are negative.\textsuperscript{12}

Next-generation sequencing (NGS) serves as a highly multiplexed multigene platform for performing comprehensive mutational analyses of NSCLC-related genes, including EGFR. The analytic sensitivity of an NGS assay is 5% to 10%, and it may not always provide the analytic sensitivity necessary to detect EGFR T790M in patients presenting with secondary resistance to TKI therapy, especially in tissue samples with low tumor fraction. In addition, the cost and turnaround time of performing NGS analysis in scenarios where the clinical question specifically revolves around the presence of an EGFR T790M mutation argue for the need for a rapid, cost-effective, and high-sensitivity assay that can interrogate these samples for EGFR T790M. Previous studies have shown that a high-sensitivity droplet digital polymerase chain reaction (ddPCR) assay can rapidly and accurately detect EGFR T790M mutation in formalin-fixed, paraffin-embedded tissue blocks (unpublished data). Because a large fraction of NSCLC samples that are sent for EGFR T790M testing are cytology samples that may not necessarily have formalin-fixed, paraffin-embedded blocks, in the present study we evaluate different cytologic specimen preparations, including direct smears and formalin-fixed, paraffin-embedded cell blocks, for determining EGFR T790M mutation status using ddPCR.

**MATERIALS AND METHODS**

**Patient Samples**

A total of 42 cytology samples from 36 patients were included in this study. The patient demographics are summarized in Table 1. The samples were selected as follows: (1) a validation set of consecutive in-house cytologic samples (n = 21) from NSCLC patients that had EGFR mutational analysis by NGS between May 2016 and April 2017 and had leftover DNA to perform EGFR T790M ddPCR; (2) 5 non-NSCLC samples with EGFR mutational analysis by NGS and leftover DNA, included as a negative control; and (3) an additional 16 cytologic samples of metastatic lung adenocarcinoma with a known EGFR T790M mutation status based on mutation testing of a prior/subsequent tissue sample, included as the test set. All samples had a known cytologic diagnosis, estimated tumor percentage, and known EGFR T790M mutation status based on sequencing of a concurrent/prior/subsequent tissue sample (Table 2 and Supplementary Table 1 [see supplemental digital content, containing 1 table and 1 figure at www.archivesofpathology.org in the August 2020 table of contents]). Tumor fraction in specimens was estimated as the proportion of tumor cells relative to the total number of nucleated cells (benign and malignant cells). Samples with 3000 cells or more on smears and/or cell block sections were considered adequate for testing.

**Table 1. Patient Demographics and Clinicopathologic Correlates**

| Value                      |
|---------------------------|
| Age, y, median (range)    | 66.5 (34–91) |
| Sex, No. (n = 36)         |              |
| Male                      | 22           |
| Female                    | 14           |
| Cytology specimen source  |              |
| (n = 42)                  |              |
| Pleural effusion          | 13           |
| Lymph node FNA            | 10           |
| Lung FNA                  | 8            |
| Soft tissue FNA           | 6            |
| Bronchoalveolar lavage    | 2            |
| Kidney FNA                | 1            |
| Thyroid gland FNA         | 1            |
| Bronchial brushing        | 1            |

**Abbreviation:** FNA, fine-needle aspiration.

**Extraction.**—DNA was extracted from patient samples and cell lines using the PicoPure DNA extraction kit (Arcturus, Mountain View, California) and the Agencourt AMPure XP kit (Agencourt Biosciences, Beverly, Massachusetts), respectively, as per the manufacturer’s instructions. DNA was quantified by a Qubit 2.0 fluorometer using the dsDNA HS Assay kit (Thermo Fisher Scientific, Waltham, Massachusetts).

**Droplet Digital PCR Assay.**—The ddPCR assay was performed using a total of 10 ng of genomic DNA on the QX100 Droplet Digital PCR System (Bio-Rad, Hercules, California), as per the manufacturer’s protocol. The ddPCR assay was optimized using serially diluted T790M-positive cell lines (1% and 0.5% dilutions; see Supplementary Material and Supplementary Figure 1). Two cell lines, H1975 (ATCC CRL-5908) and DLD1 (ATCC CCL-221) ATCC (Manassas, Virginia), were used as positive and negative controls, respectively. All samples were run in duplicate. A threshold of more than 10 000 total droplets was set for any samples to be included in the analysis. Two-dimensional amplitude plots on control wells (ie, negative control) were used to establish the threshold for the x-axis and y-axis. Significant amplitude differences and concentration differences between replicates were used as quality indicators for poor handling or mixing of samples. Based on our prior experience using formalin-fixed, paraffin-embedded tissue samples, a set cutoff threshold of more than 10 000 total droplets, 5 or more positive droplets, and AF of 0.1 or more was used to determine positivity.

**RESULTS**

The 42 samples analyzed in this study included FNA samples (n = 26) from lymph node (n = 10), lung (n = 8), soft tissue (n = 6), kidney (n = 1), and thyroid gland (n = 1), and pleural effusions (n = 13), bronchoalveolar lavage (n = 2), and bronchial brushing (n = 1; Table 1). Of these, 37 samples were from lung carcinoma patients with diagnoses of lung adenocarcinoma (n = 36) and NSCLC not otherwise specified (n = 1); 5 additional samples of nonlung origin, included as negative control, had diagnoses of gastric adenocarcinoma, breast adenocarcinoma, high-grade serous carcinoma, oropharyngeal squamous cell carcinoma, and small cell carcinoma of gastrointestinal origin (Table 2).

The median estimated tumor fraction in these 42 cases was 30% (range, 1%–90%). Of these, 28 cases (66.7%) had direct smears that were used for DNA extraction, 12 cases (28.6%) had cell block sections, and 2 cases (4.7%) had a combination of smears and cell block sections that were used. In cases where direct smears were employed for
extraction, the median number of slides used was 1 smear (range, 1–5), with a combination of both Diff Quik–stained and Papanicolaou-stained smears. The median DNA concentration in these samples was 17.6 ng/L (range, 2.1–136 ng/L). No difference in DNA yield was seen between cases that used direct smears versus those that used cell block sections.

Of the 26 samples in the validation set that had a concurrent NGS analysis on the same specimen, 8 samples (31%) had a known EGFR T790M mutation, in addition to an EGFR sensitizing mutation, 3 samples (11%) had an EGFR sensitizing mutation but were negative for EGFR T790M, and the remaining 15 samples (58%) were wild-type for EGFR mutations (Table 2). Of the 8 cases with the EGFR T790M mutation, 7 had prior documentation of an EGFR sensitizing mutation and a history of TKI therapy with clinical suspicion for secondary resistance to therapy. The ddPCR assay was able to successfully detect the EGFR T790M mutation in all 8 samples (100% concordance). Of the remaining 18 samples that were wild-type for EGFR T790M mutation by NGS, 17 (94%) were wild-type by the ddPCR assay, with a single sample (case 23) showing a borderline positive result for EGFR T790M mutation (AF, 0.12; 6 positive droplets), just above the cutoff threshold (ie, 5 positive droplets) for the assay. This was an FNA sample of a high-grade serous carcinoma that did not have any known EGFR sensitizing mutations or a history of TKI therapy, and the corresponding NGS analysis was negative for the EGFR T790M mutation. The ddPCR assay could not be repeated or confirmed on an orthogonal platform because of insufficient DNA for further evaluation.

The 16 samples included in the test set for this study were all cases of metastatic lung adenocarcinoma with known EGFR sensitizing mutations that were on TKI therapy and subsequently developed acquired resistance with the EGFR T790M mutation. Priority was given to cases with borderline or relatively low tumor fraction, with 67% of the samples showing an estimated tumor fraction of 20% or less. Of these, 4 cases had a tumor fraction of 10%, 3 cases had 5%, and 2 cases had 1%. A single Diff-Quik/Papanicolaou-stained smear from all 16 samples yielded adequate amounts of DNA for the ddPCR assay. The results from the ddPCR assay were compared to those of the patients with known EGFR T790M status at the time of the cytologic procedure. Of these, 14 cases (88%) had a known EGFR sensitizing mutation, with 7 cases (44%) harboring a known EGFR T790M mutation. The ddPCR assay was able to successfully detect the EGFR T790M mutation in 6 of these cases (86%), with a single case (case 31) that failed to detect the known EGFR T790M mutation (Table 2).

Five cases that were negative for EGFR T790M mutation (by sequencing of a prior sample) were also negative by ddPCR, whereas the EGFR T790M mutation status was unknown at the time of collection in 4 samples. Of these, 2 cases (cases 27 and 40) had a prior sample that showed only the EGFR sensitizing mutation, but testing on subsequent tissue samples revealed an acquired EGFR T790M mutation.

Cases 37 to 40 were samples collected from the same patient within 3 months of each other. The first 2 samples (cases 37 and 38) were collected prior to any mutation testing and were negative for EGFR T790M mutation by ddPCR. Sample 39, negative for EGFR T790M mutation by ddPCR, was collected concurrently at the time of a tissue biopsy which showed only an EGFR L858R mutation by NGS analysis. The patient was started on TKI therapy; however, after an initial response the patient showed progression of disease, and a bronchial brushing specimen (case 40) was collected. The tumor fraction of this bronchial brushing specimen was low (20%), thus precluding NGS analysis; however, the ddPCR assay was able to successfully detect the EGFR T790M mutation from this specimen (Table 2). The overall sensitivity and specificity of the ddPCR assay for detecting EGFR T790M mutation were 93% and 96%, respectively (Figure).
| Case | Diagnosis | Site | Tumor, % | EGFR Sensitizing Mutation | TKI | EGFR T790M Status (Cytology/Tissue) | T790M Status | T790M FA | No. of Positive Droplets | No. of Total Droplets | Concurrent Specimen | Concordance |
|------|-----------|------|----------|---------------------------|-----|---------------------------------|-------------|--------|------------------------|---------------------|---------------------|-------------|
| 1    | Metastatic lung ADC | ST 70 | Exon 19 del | Yes | Mutant | Mutant | 30.45 | 1605 | 18 074 | Yes | TP |
| 2    | Metastatic lung ADC | Kidney 85 | Exon 19 del | Yes | Mutant | Mutant | 32.65 | 292 | 18 808 | Yes | TP |
| 3    | Lung ADC | Lung 90 | Exon 19 del | Yes | Mutant | Mutant | 18.25 | 274 | 17 566 | Yes | TP |
| 4    | Metastatic lung ADC | ST 30 | Exon 19 del | No | WT | WT | 0.08 | 3 | 17 859 | Yes | TN |
| 5    | Metastatic lung ADC | Lung 40 | WT | No | WT | WT | 0.18 | 4 | 18 514 | Yes | TN |
| 6    | Lung ADC | LN 60 | WT | No | WT | WT | 0.1 | 4 | 17 449 | Yes | TN |
| 7    | Metastatic lung ADC | LN 30 | L858R | No | Mutant | Mutant | 14.25 | 541 | 16 629 | Yes | TP |
| 8    | Lung ADC | Lung 60 | Exon 19 del | Yes | Mutant | Mutant | 16.4 | 802 | 17 925 | Yes | TP |
| 9    | Lung ADC | Lung 65 | L858R | Yes | Mutant | Mutant | 7.75 | 424 | 16 298 | Yes | TP |
| 10   | Lung ADC | Lung 70 | Exon 19 del | Yes | Mutant | Mutant | 25.35 | 1254 | 10 748 | Yes | TP |
| 11   | Metastatic lung ADC | ST 70 | Exon 19 del | Yes | Mutant | Mutant | 10.6 | 626 | 17 680 | Yes | TP |
| 12   | Lung ADC | Lung 21 | WT | No | WT | WT | 0.12 | 2 | 17 016 | Yes | TN |
| 13   | Lung ADC | Lung 30 | WT | No | WT | WT | 3.95 | 2 | 15 282 | Yes | TN |
| 14   | Metastatic lung ADC | LN 50 | E709K; G719A | No | WT | WT | 0.14 | 1 | 19 247 | Yes | TN |
| 15   | Metastatic lung ADC | PL fluid 50 | WT | No | WT | WT | 21.75 | 0 | 14 936 | Yes | TN |
| 16   | Metastatic lung ADC | LN 60 | WT | No | WT | WT | 0.49 | 2 | 15 896 | Yes | TN |
| 17   | Lung ADC | Lung 80 | WT | No | WT | WT | 18.3 | 3 | 15 485 | Yes | TN |
| 18   | Metastatic lung ADC | ST 80 | WT | No | WT | WT | 0.19 | 3 | 10 150 | Yes | TN |
| 19   | Metastatic lung ADC | LN 90 | WT | No | WT | WT | 0.6 | 2 | 13 546 | Yes | TN |
| 20   | Metastatic lung ADC | LN 50 | WT | No | WT | WT | 0.45 | 5 | 16 430 | No | - |
| 21   | Metastatic NSCLC | LN 70 | Exon 19 del | No | WT | WT | 0.12 | 6 | 19 132 | Yes | FP |
| 22   | Metastatic gastric ADC | LN 30 | WT | No | WT | WT | 0.15 | 1 | 17 531 | No | - |
| 23   | Metastatic HG Serous CA | ST 25 | WT | No | WT | WT | 0.05 | 2 | 18 683 | Yes | TN |
| 24   | Metastatic SCC | LN 80 | WT | No | WT | WT | 0.34 | 2 | 17 630 | Yes | TN |
| 25   | Metastatic breast CA | ST 90 | WT | No | WT | WT | 0.04 | 1605 | 18 683 | Yes | TN |
| 26   | Metastatic SQCell CA | Thyroid 90 | WT | No | WT | WT | 0.15 | 15 171 | No | TN |
| 27   | Metastatic lung ADC | PL fluid 5 | Exon 19 del | Yes | Unknown | Unknown | 0.45 | 5 | 16 430 | No | - |
| 28a  | Metastatic lung ADC | PL fluid 20 | L858R | No | WT | WT | 0.25 | 1 | 16 285 | Yes | TN |
| 29a  | Metastatic lung ADC | PL fluid 60 | L858R | No | WT | WT | 0.7 | 2 | 18 070 | Yes | TN |
| 30   | Metastatic lung ADC | PL fluid 1 | Exon 19 del | No | WT | WT | 0.32 | 2 | 12 830 | No | TN |
| 31   | Metastatic lung ADC | PL fluid 1 | Exon 19 del | Yes | Mutant | WT | 1.15 | 1 | 15 171 | No | FN |
| 32a  | Metastatic lung ADC | PL fluid 60 | Exon 19 del | Yes | Mutant | Mutant | 26 | 60 | 18 343 | Yes | TP |
| 33a  | Metastatic lung ADC | PL fluid 5 | Exon 19 del | Yes | Mutant | Mutant | 36 | 135 | 18 356 | No | TN |
| 33   | Metastatic lung ADC | BAL 10 | Exon 19 del | Yes | Mutant | Mutant | 7 | 40 | 14 829 | Yes | TP |
| 35a  | Metastatic lung ADC | LN 5 | G719S | No | Mutant | Mutant | 56 | 485 | 17 635 | No | TP |
| 36a  | Metastatic lung ADC | BAL 10 | G719S | No | Mutant | Mutant | 52 | 617 | 16 330 | No | TP |
| 37a  | Metastatic lung ADC | PL fluid 30 | unknown | No | Unknown | WT | 0.15 | 1 | 17 531 | No | - |
| 38a  | Metastatic lung ADC | PL fluid 40 | unknown | No | Unknown | WT | 0.15 | 2 | 17 510 | No | - |
| 39a  | Metastatic lung ADC | PL fluid 10 | L858R | No | WT | WT | 0 | 0 | 18 005 | Yes | TN |
| Case | Site | Diagnosis | EGFR Sensitizing Mutation | T790M Status | Concurrent Specimen | No. of Positive Droplets | No. of Total Droplets | No. of Concurrent Specimen | Sensitizing T790M | T790M | Total Specimen Concordance |
|------|------|-----------|---------------------------|--------------|---------------------|-------------------------|------------------------|-------------------------|-----------------|--------|---------------------------|
| 41   | BB   | Metastatic lung ADC | Exon 19 del | Yes | Unknown | 0.7 | 5 | 3 | False positive; FA functional abundance. | 14.868 | 14.469 | 15.620 |
| 42   | BB   | Metastatic lung ADC | Exon 19 del | Yes | Mutant | 22.8 | 286 | 15 | True positive; FA functional abundance. | 14.868 | 14.469 | 15.620 |

Abbreviations: ADC, adenocarcinoma; BAL, bronchoalveolar lavage; BB, bronchial brushing; CA, carcinoma; del, deletion; FA, fractional abundance; FN, false negative; FP, false positive; HG, high grade; ILN, inguinal lymph node; NSCLC, non–small cell lung carcinoma; PCR, polymerase chain reaction; Pl, pleural; SCC, squamous cell; SqCell, squamous cell; ST, soft tissue; TN, true negative; TP, true positive.

**DISCUSSION**

Advanced-stage NSCLC patients with EGFR sensitizing mutations who are being treated with EGFR-targeted TKI therapy frequently present with resistance to TKI therapy, with recurrence and/or progression of disease. The EGFR T790M mutation in exon 20 is the most commonly detected acquired resistance mechanism in these patients, and the revised CAP/IASLC/AMP lung cancer molecular testing guideline strongly recommend testing specifically for this mutation using a high-sensitivity mutational assay to select patients for third-generation EGFR-targeted TKIs. However, low tumor fractions in the posttreatment biopsies and effusion specimens frequently make the detection of the EGFR T790M mutation quite challenging. Therefore, in this study we evaluated the validity and feasibility of using a high-sensitivity orthogonal ddPCR assay to interrogate different cytologic specimen preparations for EGFR T790M mutation.

Our samples included direct smears and cell blocks from a variety of FNA samples, pleural effusions, bronchial brushing, and lavages. All samples yielded adequate amounts of DNA. The validation set of samples, which had NGS analysis performed from the same cytologic specimen, had adequate DNA for both NGS analysis as well as the ddPCR assay. The EGFR T790M mutation status detected by ddPCR showed high concordance (96%) with that of the NGS analysis. A single false-positive result with borderline positivity was detected in one of the non-NSCLC cases, indicating the need to exert some caution in samples with borderline positivity, including low AF and low numbers of positive droplets. Repeat analysis or evaluation on a high sensitivity orthogonal platform may be needed in such cases.

The EGFR T790M status by ddPCR in the test cases showed 94% concordance with that of the NGS analysis results, with a single sample resulting in a false-negative result. The false-negative result was likely due to the low tumor fraction (estimated at 1%). Although the EGFR T790M mutation status in the remaining low–tumor fraction samples, including a second sample with 1% tumor fraction, were concordant with the results of the NGS assay, these results would indicate that one needs to exert some caution in very low tumor fraction samples (<5%) to avoid risking a false-negative result. This caveat has been demonstrated in other studies and recently highlighted in the CAP/IASLC/AMP guidelines that the absence of a mutation in very low tumor fraction samples does not necessarily exclude the possibility of a mutation.

The ddPCR assay also successfully detected an EGFR T790M mutation in a low tumor (20%) bronchial brushing specimen (case 40), deemed inadequate for NGS analysis, in a patient with suspected secondary resistance to TKI therapy. The ability of the ddPCR assay to successfully detect the EGFR T790M mutation from this specimen highlights the utility of having a high-sensitivity assay for low–tumor fraction samples that can identify patients with acquired resistance and select those eligible for third-generation EGFR-targeted TKIs. This can avoid the need for additional liquid biopsy testing or an invasive repeat biopsy (as done in the case of this patient), thus improving patient morbidity by allowing the patient to rapidly switch to a third-generation TKI.

In addition, the ddPCR assay identified 1 patient (cases 35 and 36) with tumor fractions of 5% and 10% in 2 samples collected within 8 months of each other that demonstrated
EGFR T790M mutations at AFs of 56% and 52%, respectively, raising suspicion for a de novo mutation. A corresponding surgical biopsy evaluated by NGS confirmed the germ-line EGFR T790M mutation. Germ-line EGFR T790M mutations have been reported in the literature as a cause of possible familial predisposition for the development of lung carcinoma and may prompt the patient to be referred for genetic counseling.25-27

In advanced-stage EGFR-mutated NSCLC patients who demonstrate progression or relapse after an initial response to EGFR-TKI therapy, detecting a low subclonal EGFR T790M mutation from a posttreatment low-tumor-fraction specimen can be challenging. Therefore, it is imperative that molecular laboratories validate high-sensitivity assays for the detection of EGFR T790M mutation from these samples. Several studies have reported the utility of ultrasensitive assays, such as ddPCR, using circulating tumor DNA (ctDNA) and cfDNA from the patient’s plasma.15,16,18,28 The CAP/IASLC/AMP guidelines recommend evaluating for EGFR T790M mutation from plasma in the setting of suspected EGFR-TKI resistance when a tissue sample is unavailable or insufficient, because a positive result can guide therapy with osimertinib.12 However, plasma-based cfDNA analysis for EGFR T790M has intermediate sensitivity (0.4–0.78), and a tissue biopsy is recommended if the results are negative.12 Our results indicate that ddPCR for the detection of EGFR T790M mutation is a highly sensitive and specific (sensitivity and specificity of 93% and 96%, respectively) assay that can be used reliably in cytologic specimens, including samples with low tumor fraction, thus providing a higher-sensitivity alternative to plasma-based cfDNA analysis. However, some caution needs to be exerted in samples with very low tumor fraction (<5%) and with borderline positivity results (low AF and/or low numbers of positive droplets) to avoid the risk of false-negative and false-positive results, respectively. Future studies with a larger sample size will be needed to support the findings of this study. The turnaround time of EGFR T790M ddPCR assay is significantly lower than that of NGS analysis (2–3 days versus 5–7 days, respectively), thus allowing the patient to rapidly switch to third-generation TKIs, if positive for acquired resistance mutation. The integration of EGFR T790M detection by ddPCR into clinical practice will likely reduce the inadequate rate for low-tumor-fraction samples, without the need for a repeat biopsy, and provide a rapid and cost-effective way to select patients for third-generation EGFR-targeted TKIs.

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