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Plasma Cell-Free DNA Testing of Patients With EGFR Mutant Non–Small-Cell Lung Cancer: Droplet Digital PCR Versus Next-Generation Sequencing Compared With Tissue-Based Results

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abstract

PURPOSE To compare the results of plasma cell-free DNA (cfDNA) droplet digital PCR (ddPCR) and next-generation sequencing (NGS) on detection of epidermal growth factor receptor (*EGFR*) primary activating mutations and p.T790M with results of tissue analysis in patients with *EGFR* mutated non–small-cell lung cancer.

METHODS All patients with EGFR mutated non–small cell lung cancer for which a pathology and a plasma specimen were available upon progression between November 2016 and July 2018 were selected. Concordance, Cohen's κ, and intraclass correlation coefficients were calculated.

RESULTS Plasma cfDNA and pathology specimens of 36 patients were analyzed. Agreement between ddPCR and NGS was 86% ($\kappa = 0.63$) for the primary activating mutation and 94% ($\kappa = 0.89$) for the p.T790M detection. Allele ratios were comparable, with an intraclass correlation coefficient of 0.992 and 0.997, respectively. Discrepancies of some degree were found in 15 patients (41.7%). In six patients (16.7%), no mutations were detected in cfDNA. In three patients (8.3%), p.T790M was detected in plasma but not in the pathology specimen, whereas in three other patients (8.3%), p.T790M was demonstrated in the pathology specimen but not in plasma. Concordance of cfDNA and pathology for the primary activating mutation was 69% for ddPCR and 83% for NGS. For the detection of p.T790M, this was 75% ($\kappa = 0.49$) for ddPCR as well as for NGS.

CONCLUSION Mutual agreement is high between NGS and ddPCR in cfDNA on the level of a specific mutation, with comparable ratio results. Plasma testing of *EGFR* primary activating mutations and p.T790M shows high concordance with pathology results, for NGS as well as for ddPCR, depending on the extent of the panel used. In NGS, more genetic aberrations can be investigated at once.

INTRODUCTION

Among cancer deaths worldwide, non–small-cell lung cancer (NSCLC) is the leading cause.[1] The survival of metastasized disease is poor as illustrated by a 1-year survival rate of 23% in the Netherlands between 2010 and 2015.[2] The choice of palliative systemic treatment currently depends on histologic subtype, programmed cell death ligand 1 (PD-L1) expression, and the presence of specific genetic aberrations (also known as driver mutations) for which specific targeted therapies are available.[3] Today, for nonsquamous NSCLC, it is common practice to perform molecular analysis on a tissue biopsy specimen at the time of diagnosis.[3]

Targeted therapies have been developed and registered for treating NSCLC on the basis of the presence of genetic alterations in an expanding number of genes. The most common examples are activating mutations in the genes for epidermal growth factor receptor (*EGFR*) and B-raf proto-oncogene (*BRAF*) and translocations of the anaplastic lymphoma kinase and ROS proto-oncogene 1 genes.[4]

The population with *EGFR* mutated NSCLC is the most comprehensive of these patient groups, with an incidence of at least 10% in the white and up to 35% in the Asian population.[5] Clinical trials have shown high response rates (approximately 70%) and prolonged progression-free survival (PFS) rates up to 1 year on

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CONTEXT

Key Objective

To compare plasma cell-free DNA mutation testing by droplet digital polymerase chain reaction (ddPCR) and next-generation sequencing (NGS) with their concordance with tissue testing.

Knowledge Generated

ddPCR and NGS yield comparable results, with similar sensitivity for the mutations that can be detected by both methods, and the concordance with tissue-based results is high. Discordant cases tend to show intrathoracic and/or CNS progression. The extent of mutations that can be discovered by NGS in one step is larger.

Relevance

When searching for a resistance mechanism, NGS analysis of cell-free DNA in plasma offers a more comprehensive view than ddPCR, with comparable precision at a single mutation level. When no mutations are detected in plasma, tissue-based investigation remains desirable.

average to first-generation tyrosine kinase inhibitors (TKIs).[6] All patients, however, ultimately develop resistance to treatment with TKIs and show progression of disease at some point. There are two main mechanisms of acquired resistance: Pharmacologic (eg, problems with compliance, dose reductions, reduced absorption or increased metabolism, inadequate CNS penetration) and biologic (eg, altered drug target, bypass tracks, phenotypic change, downstream signaling pathways).[7]

The gatekeeper mutation p.T790M in *EGFR* exon 20 is the most common resistance mechanism to first- and second-generation TKIs (erlotinib, gefitinib, and afatinib) in NSCLC with an activating *EGFR* mutation and occurs in more than 50% of patients.[7] Because the availability of osimertinib, a drug that overcomes the p.T790M resistance mechanism that shows high response rates and a substantial median PFS of 8 months, the detection of this gatekeeper mutation has been of utmost importance.[8,9] In addition, there are other known resistance mechanisms for which targeted therapies are available in research or off-label settings. Therefore, it is strongly advised to obtain a new molecular analysis at the time of progression on first-line *EGFR* TKIs.[3]

Although a tissue biopsy is still considered the gold standard for diagnosis of NSCLC, the potential to detect genetic aberrations in the blood, which is often referred to as liquid biopsy, has specific advantages over a tissue biopsy in that it is easier to obtain and has a lower patient burden.[10] Currently, the use of plasma detection of p.T790M at the time of progression on first-line *EGFR* TKIs is widely accepted, and prescription of osimertinib is established on the basis of *EGFR* p.T790M detection in plasma.[11,12]

Several mutation detection techniques are under investigation for application in clinical practice in which a very low detection limit is essential because the amount of circulating cell-free tumor DNA (ctDNA) in the total of cell-free DNA (cfDNA) can be very low. Traditional polymerase chain reaction (PCR) is not sensitive enough to detect these low amounts of tumor DNA. Real-time PCR slightly improves the detection limit (eg, Cobas [Roche, Basel, Switzerland], Therascreen [QIAGEN, Valencia, CA]), but a major improvement in sensitivity was achieved by the development of digital platforms that target specific mutations like droplet digital PCR (ddPCR) and beads, emulsion, amplification, and magnetics digital PCR.[13] This requires a modest amount of time and cfDNA to obtain reliable results. A more broad (untargeted) approach is represented by next-generation sequencing (NGS). A lot of effort was invested in optimizing NGS for use on cfDNA, with adjusted amplicon sizes for amplification of smaller DNA fragments and application of molecular barcodes to recognize the needle in the haystack in low concentrations of ctDNA in the total amount of cfDNA.[14] For optimal results, it is advised to use as much cfDNA in the panel as possible. Depending on the platform used, the lead time requires several working days, which is comparable to NGS on tissue specimens.

This study compares the results of ddPCR (Bio-Rad Laboratories, Hercules, CA) and NGS (Ion Torrent, Thermo Fisher Scientific, Waltham, MA) for detection of primary activating and resistance p.T790M *EGFR* mutations in plasma-derived cfDNA. Outcomes are compared with NGS results of conventional tissue biopsy or cytology.

METHODS

We included all patients with *EGFR* mutated NSCLC with progression on current therapy for which a tissue specimen (histology/cytology) was available in the same time frame and line of treatment as plasma analysis. The study was conducted at Erasmus MC Cancer Institute between November 2016 and July 2018. The maximum time frame between plasma and tissue collection was limited to 3 months.

Plasma samples were collected and cfDNA analyses performed upon progression on current therapy for detection

of primary activating and p.T790M *EGFR* mutations. We prospectively collected all data on requested plasma analyses.

cfDNA Isolation

Blood was collected in 10-mL CellSave Preservative Tubes (CellSearch, Menarini Silicon Biosystems, Castel Maggiore, Italy) and centrifuged for 20 minutes at 1,600 × *g*. Plasma samples were stored at –80°C until cfDNA isolation. Before extraction, samples were centrifuged for 10 minutes at 10,000 × *g*. The cfDNA was extracted using the QIAmp Circulating Nucleic Acid Kit (QIAGEN) from 3 mL of plasma according to the manufacturer's protocol. The DNA was eluted in 50 μL of buffer.

ddPCR Analysis

The actual analysis of *EGFR* activating (exon 19 deletions and p.L858R) and resistance (p.T790M) mutations was performed using ddPCR mutation assays (Bio-Rad Laboratories) as previously described.[15]

NGS on Pathology Specimens and cfDNA

DNA was isolated from formalin-fixed paraffin-embedded tissues enriched for neoplastic cells by manual microdissection as previously described.[16] NGS analysis was performed by semiconductor sequencing with the Ion S5 System (Thermo Fisher Scientific) with the supplier's materials and protocols. Library preparation was performed with 1 to 10 ng of tissue DNA and 4 to 50 ng of cfDNA, depending on the amount of tissue or cfDNA available. Libraries of tissue DNA were prepared with a custom-made primer panel that encompassed, among others, *EGFR* exons 18 to 21, *KRAS* exons 2 to 4, *ERBB2* exons 19 to 21, *BRAF* exons 11 and 15, and the entire coding region of *TP53* using the AmpliSeq Library Kit 2.0-384 LV (Thermo Fisher Scientific); cfDNA library preparation was performed using the Oncomine Lung cfDNA Assay (Thermo Fisher Scientific). Templates were prepared using the Ion 520 & Ion 530 Kit-Chef and sequenced with the Ion S5 Sequencing Kit on an Ion 530 Chip (Thermo Fisher Scientific). Sequence data were analyzed with Variant Caller version 5.6.0.4 (Thermo Fisher Scientific). Variants detected in tissue samples were annotated by SeqNext version 4.2.2 build 503 software (JSI Medical Systems, Kippenheim, Germany). Results are reported as allele ratios (mutated alleles / [mutated + wild-type alleles] × 100%) in the case of at least three positive droplets (ddPCR) or three unique molecules (NGS).

Statistical Analysis

Concordance of ddPCR and NGS with tissue-based results was calculated for the primary activating mutation. Cohen's κ was calculated to evaluate the agreement between ddPCR or NGS and tissue-based results for p.T790M detection and between ddPCR and NGS on cfDNA for the primary activating mutation as well as for p.T790M. Intraclass correlation coefficients were calculated for the ratios of ddPCR and NGS for p.T790M as well as for the primary activating mutation when applicable.

RESULTS

Between November 2016 and July 2018, 162 patients underwent cfDNA analysis on plasma collected in 10-mL CellSave Preservative Tubes. We selected all 36 patients with *EGFR* mutated NSCLC with progression on current treatment of which a histology or cytology specimen was available in the same time frame and line of treatment. Baseline characteristics of the population are listed in Table 1.

Results of Plasma Analyses

Agreement between ddPCR and NGS was 94% (κ = 0.89) for *EGFR* p.T790M detection in plasma and 86% (κ = 0.63) for detection of the primary activating *EGFR* mutation. The quantification in allele ratio (mutant / [mutant + wild type]) proved highly similar for both techniques (Fig 1), with an intraclass correlation coefficient of 0.997 and 0.992, respectively. Discrepant results were found in 15 patients (41.7%). Table 2 lists results of plasma and tissue analyses for all patients.

In six patients (16.7%), the primary activating mutation was a less common variant that was not present in the current ddPCR panel. Therefore, these mutations could not be detected in the plasma by ddPCR. In two of these patients, the primary activating mutation also was not detected by NGS, one of them was not present in the NGS panel. In one other patient, the primary activating mutation was not detected by ddPCR (although present in the panel) but was shown in plasma by NGS detection. At the level of p.T790M detection, better agreement was shown, with only one patient in whom p.T790M was detected by NGS but not by ddPCR.

Comparison With Tissue Results

For NGS, concordance for the primary activating *EGFR* mutation with tissue specimens was 83% (30 of 36 confirmed results). For the mutations that could be detected by the ddPCR panel (shared mutations with the cfDNA NGS panel), 83% were confirmed. However, because of the limitation of checking only p.L858R and exon 19 deletion, the concordance of detection of all *EGFR* activating mutations (also including nonshared mutations) was 69% (25 of 36) compared with NGS-obtained tissue-based results. For *EGFR* p.T790M detection, both ddPCR and NGS showed a concordance of 75% (κ = 0.49) with the tissue-based results.

In six patients (16.7%), all with intrathoracic progression, no mutations were detected in cfDNA (mutation-negative plasma), whereas the tissue analysis showed the presence of *EGFR* p.T790M in three (50%) of these patients. The best response on initiated osimertinib treatment in these three patients was partial response in one and stable disease in two.

TABLE 1. Baseline Characteristics

Characteristic	Patients, No. (%)
No. of patients	36
Age, mean, years (range)	66 (45-85)
Sex	
Male	12 (33.3)
Female	24 (66.7)
Smoking status	
Never	15 (41.7)
Former	9 (25)
Current	3 (8.3)
Unknown	9 (25)
Pack-years	
0	15 (41.7)
1-15	4 (11.1)
15-30	2 (5.6)
> 30	2 (5.6)
Unknown	13 (36)
Activating EGFR mutation	
Exon 18	2 (5.6)
Exon 19	24 (66.7)
Exon 21 p.L858R	9 (25)
Exon 21 other	1 (2.8)
Lines of therapy	
Mean before PA specimen (range)	1.6 (1-5)
1	25 (69.4)
2	5 (13.9)
3	3 (8.3)
4	1 (2.8)
5	2 (5.6)
Previous chemotherapy	
Yes	8 (22.2)
No	28 (77.8)
Current therapy at time of PA specimen	
Erlotinib	24 (66.7)
Gefitinib	4 (11.1)
Osimertinib	6 (16.7)
Chemotherapy	1 (2.8)
PD-1 inhibitor	1 (2.8)
Type of PA specimen	
Histology	29 (80.6)
Cytology	7 (19.4)

Abbreviations: EGFR, epidermal growth factor receptor; PA, primary activating; PD-1, programmed cell death 1.

In three patients (8.3%), cfDNA analysis detected p.T790M, which was not demonstrated in the tissue specimen (ddPCR positive in two patients, NGS positive in three patients). In two of these patients, there was an evident extrathoracic progression site. Upon treatment with osimertinib, there was progressive disease as best response in two of these patients (one of whom had an additional *PIK3CA* mutation that was detected in the corresponding tumor tissue); the other patient had already received osimertinib treatment, and the additional clinical course was unknown because treatment was coordinated in another hospital.

In three other patients (8.3%), p.T790M was detected in tumor tissue but not in cfDNA, whereas the primary activating *EGFR* mutation was detected in the plasma. These patients all had intrathoracic progression and/or a new CNS localization. One patient showed stable disease as best response to osimertinib, another died before subsequent therapy could be initiated, and the clinical course of the third patient remained unknown because he was treated elsewhere.

DISCUSSION

This study compared two accurate and promising techniques for detection of targetable genetic aberrations in NSCLC in the same plasma samples and confirms the high concordance of cfDNA with tumor tissue analysis as published earlier.[13,17] We did not calculate negative predictive value or positive predictive value because we did not consider the tissue analysis as the gold standard; it is a known phenomenon that tumor heterogeneity might result in a mutation-negative biopsy specimen while a mutation can be present in another (metastasized) region. Our findings support this because three patients had p.T790M detected in plasma but not in tissue. We do not believe that they had false-positive findings but, rather, that the biopsy specimen did not represent the whole spectrum of genetic aberrations of the disease.

ddPCR is considered a highly sensitive PCR platform that, next to high concordance with tissue-based results, also offers high sensitivity and specificity compared with earlier PCR assays (Taqman PCR with peptide nucleic acid; Therascreen; Cobas; and beads, emulsion, amplification, and magnetics digital PCR).[12,18] NGS has proven its qualities in molecular analysis of tissue and has promising evolving capabilities for mutation analysis of cfDNA.[19-21] Both techniques yield results as a percent of the total (ratio), which is considered a benefit over methods that merely indicate positive or negative.

The moderate agreement between cfDNA and tissue-based results on p.T790M detection is partly the result of mutation-negative plasma samples. The six patients (16.7%) in whom no mutations were detected in plasma could reflect the limited sensitivity of cfDNA analysis in NSCLC, which was earlier reported to be approximately 60% to 80%.[22,23] This is defined not only by the platform limitations but also by the lack of shedding of tumor DNA in the circulation in some patients (eg, the patients in our

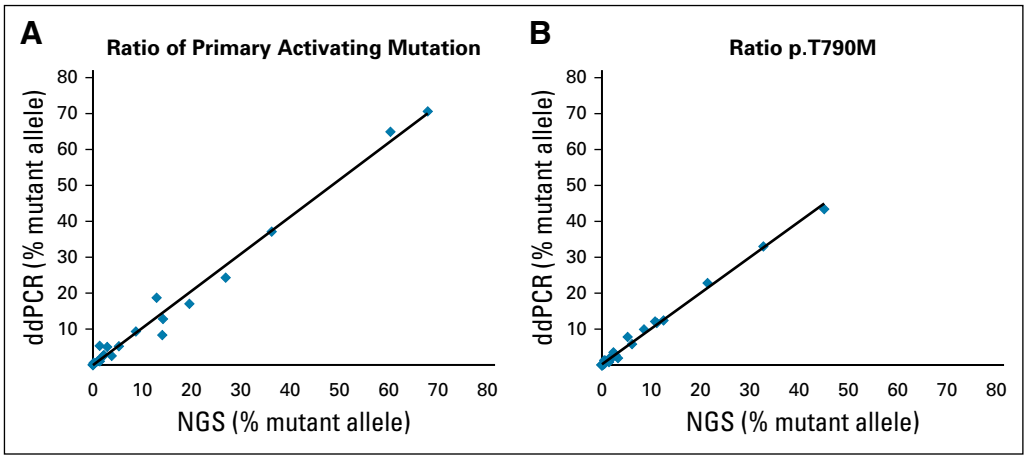

FIG 1. Correlation between the ratio of mutant versus total cell-free DNA of next-generation sequencing (NGS) and droplet digital polymerase chain reaction (ddPCR) results. (A) The primary activation mutation (n = 26 because of missing mutations in the ddPCR panel and nonquantifiable NGS results). (B) p.T790M (n = 36).

study were all found to have intrathoracic progression) and the limitation of the volume taken for plasma analysis.[24,25] If the primary activating *EGFR* mutation is not detectable in the plasma and p.T790M is also not detected, the result is not conclusive and of no clinical use for determining additional treatment options. Therefore, tissue biopsy specimens are desirable to detect the resistance mechanism in such patients. The mutation-negative plasma subset has a substantial negative effect on the concordance between cfDNA and tissue biopsy results.

On the other hand, the three patients (8.3%) in whom p.T790M was detected in cfDNA but not in tumor tissue also contribute to the limitation of agreement. These three patients might represent the concept of tumor heterogeneity, where the location of the biopsy does not represent the full spectrum of genetic aberrations of the disease.[24,26] The p.T790M-positive cells might represent a subclone of limited extent, or another (not yet detected) alternative resistance mechanism might be of greater influence (illustrated by the patient with a *PIK3CA* mutation in the pathology specimen). The patients who showed p.T790M in tissue analysis but not in plasma all had intrathoracic and/or CNS progression, which again supports the theory that those sites are associated with a lesser rate of shedding of tumor DNA into blood.[25]

The concordance between NGS and ddPCR in cfDNA of a specific genetic aberration that is targetable for ddPCR, like p.T790M, is higher than for the broader and heterogeneous group of activating EGFR mutations because not all activating EGFR mutations were present in the current ddPCR panel and, thus, will be missed. In such cases, it is unclear whether plasma is false negative for p.T790M because of limited sensitivity of ddPCR or true negative because of absence of ctDNA, as the primary activating *EGFR* mutation also could not be detected.

Because osimertinib showed improved PFS when used in first-line treatment in the AZD9291 Versus Gefitinib or Erlotinib in Patients With Locally Advanced or Metastatic NSCLC (FLAURA) trial[27] and registration for this indication by the Food and Drug Administration and European Medicines Agency is a fact, the expectation is that in the near future, most patients will be treated with osimertinib upfront, and p.T790M detection will be of lesser importance. However, mechanisms of acquired resistance on first-line osimertinib presented at the European Society for Medical Oncology 2018 Congress showed a shift toward more mesenchymal-epithelial transition factor amplifications (14%); some secondary *EGFR* mutations, like p.C797S (7%); and human epidermal growth factor receptor 2 amplifications (2%).[28] In this light, a broader approach to investigate the resistance mechanism upon progression seems desirable.

In practice, frequently, only a limited amount of material for DNA investigation is available, and this is also the case for blood samples. Because every primer combination in the ddPCR panel needs a new input of specimen, this is a disadvantage when looking for a resistance mechanism with a wide view. An advantage is the fast lead time because ddPCR can generate a quick answer for the clinician (ie, within 1 working day when needed).

NGS can explore a broad spectrum of genetic aberrations in a single run, and the possibilities to detect translocations and amplifications are expanding quickly. Thus, with the expanding knowledge of resistance mechanisms and possible targeted treatments (in development) for these, the detection of a broad set of genetic aberrations seems desirable. For example, a *BRAF* V600E mutation can appear next to the primary activating *EGFR* mutation for which a dabrafenib and trametinib combination can be added to the current treatment. On the other hand, NGS is more time consuming and still much more expensive than ddPCR. Both plasma-based approaches are limited by the fact that some resistance mechanisms need a tissue-based diagnosis (eg, transformation to small-cell lung cancer).

In conclusion, our study demonstrates that results of *EGFR* mutation detection in cfDNA by NGS and ddPCR are comparable, with a high agreement when the ratio of *EGFR* mutant alleles to wild-type alleles is compared. NGS was

TABLE 2. Patients and Results

Patient	Age, Years	Smoking Status	Activating Mutation *EGFR*	Drug During Biopsy	Activating Mutation *EGFR*, %			p.T790M *EGFR* Exon 21, %			Other Findings, %		
					PA NGS	cfDNA ddPCR	cfDNA NGS	PA NGS	cfDNA ddPCR	cfDNA NGS	PA NGS	cfDNA ddPCR	cfDNA NGS
1	78	Unk	Ex19del	Erlotinib	51	9.3	8.7	—	—	—	*TP53* p.R273C 51, *MET* amplification	—	*TP53* p.R273C 3.59
2	64	Never	Ex21 p.L858R	Gefitinib	38	5.3	1.36	13	2	2.07	*SMAD4* p.R361H 12	—	—
3	61	Never	Ex19del	Osimertinib	82	13.3	+(nq)	75	12.1	10.77	*EGFR* p.C797S 76, *TP53* p.R213X 42	*EGFR* p.C797S 9	*EGFR* p.C797S 10.57
4	53	Former	Ex19del	Osimertinib	52	8.3	14.05	11	1.9	3.25	*EGFR* p.848L 41, *TP53* p.278R 39	*EGFR* p.C797S 1.9	*EGFR* p.C797S 0.41
5	52	Never	Ex18 p.E709A + Ex18 p.G719S	Erlotinib	36	—†	1.87	—‡	1.3	0.46	*TP53* p.R175H 43, *PIK3CA* p.E542K 28	—	*TP53* p.R175H 1.22
					36	—†	1.87						
6	64	Former	Ex19del	Erlotinib	80	3.8	+(nq)	61	2.7	2.78	—	—	—
7	64	Unk	Ex19del	Osimertinib	77	2.5	3.80	—	—	—	*TP53* p.Q165X 29, *FGFR1* p.N546K 48	—	—
8	53	Former	Ex19del	Erlotinib	57	2.6	2.10	19	1.2	0.69	—	—	—
9	76	Former	Ex19del	Osimertinib	35	0.21	0.06	—‡	—‡	0.69	—	—	—
10	66	Unk	Ex18 p.G719S + Ex20 p.S768I	Osimertinib	58	—†	51.69	14	33	32.73	*TP53* p.R249M 76	—	*TP53* p.R249M 51.60
					59	—†	52.39						
11	82	Never	Ex19del	Erlotinib	37	48.9	+(nq)	15	12.4	12.48	*TP53* c.673-1G>A 60	—	—
12	52	Current	Ex21 p.L858R	Erlotinib	97	18.7	12.91	0.10	7.8	5.21	—	—	—
13	66	Former	Ex21 p.L858R	Erlotinib	53	5.2	5.26	5	1.8	1.49	—	—	—
14	59	Former	Ex19del	Erlotinib	38	—§	—§	—	—	—	*TP53* p.K132M 60	—	—
15	61	Former	Ex19del	Erlotinib	46	—§	—§	13	—‡	—‡	*TP53* c.980_981dup 60	—	—
16	64	Never	Ex19del	Erlotinib	40	—§	—§	—	—	—	*HER2* amplification	—	—
17	65	Never	Ex19del	Erlotinib	96	18	+(nq)	8	—‡	—‡	*EGFR* amplification	—	—
18	62	Never	Ex19del	Erlotinib	80	—§	—§	26	—‡	—‡	—	—	—
19	73	Former	Ex19delins	Erlotinib	58	—†	—§	28	—‡	—‡	—	—	—
20	79	Never	Ex19del	Erlotinib	25	22.1	+(nq)	9	9.9	8.52	—	—	—
21	73	Never	Ex19del	Erlotinib	36	1	1.39	—	—	—	*TP53* p.Q317* 33	—	—
22	45	Unk	Ex19del	Erlotinib	86	5	2.88	41	3.5	2.33	*TP53* p.P177H 68	—	—
23	76	Unk	Ex21 p.L861Q	Erlotinib	68	—†	0.89	—	—	—	*TP53* c.96+1G>C 90	—	—
24	77	Unk	Ex19del	Gefitinib	45	—‡	0.23	24	—‡	—‡	—	—	—
25	76	Never	Ex19del	PD-1 inhibitor	89	37.1	36.25	—	—	—	*TP53* p.R273H 65	—	*TP53* p.R273H 2.40

(Continued on following page)

TABLE 2. Patients and Results (Continued)

Patient	Age, Years	Smoking Status	Activating Mutation *EGFR*	Drug During Biopsy	Activating Mutation *EGFR*, %			p.T790M *EGFR* Exon 21, %			Other Findings, %		
					PA NGS	cfDNA ddPCR	cfDNA NGS	PA NGS	cfDNA ddPCR	cfDNA NGS	PA NGS	cfDNA ddPCR	cfDNA NGS
26	66	Never	Ex21 p.L858R	Chemo	34	1.05	0.97	18	0.29	0.48	*TP53* p.C135G 88	—	—
27	73	Unk	Ex19delins	Gefitinib	49	—†	0.20	—	—	—	—	—	*KRAS* p.G13C 0.23
28	62	Former	Ex19del	Erlotinib	77	12.8	14.20	51	11.7	11.13	*TP53* c.560delG 79	—	—
29	75	Unk	Ex19del	Osimertinib	79	64.9	60.33	65	43.4	45.07	—	—	—
30	73	Current	Ex21 p.L858R	Gefitinib	44	0.6	0.16	20	—‡	—‡	—	—	—
31	59	Never	Ex21 p.L858R	Erlotinib	65	24.3	26.91	16	5.8	6.10	*TP53* c.673-1G>T 71	—	—
32	85	Never	Ex21 p.L858R	Erlotinib	40	2.70	2.24	—‡	0.80	1.43	*TP53* p.H214D 60	—	—
33	50	Current	Ex21 p.L858R	Erlotinib	29	1.20	1.13	—	—	—	*EGFR* ex20 p.S768I 38, *CTNNB1* p.G34V 26	—	*EGFR* ex20 p.S768I 2.72
34	71	Never	Ex19delins	Erlotinib	26	—†	—†	—	—	—	—	—	—
35	63	Unk	Ex21 p.L858R	Erlotinib	93	17	19.55	—	—	—	*TP53* p.R158P 61, *MET* amplification	—	*TP53* p.R158P 0.68
36	58	Never	Ex19del	Erlotinib	+(nq)	70.60	67.89	+(nq)	22.8	21.41	—	—	—

Abbreviations: cfDNA, cell-free DNA; Chemo, chemotherapy; ddPCR, droplet digital polymerase chain reaction; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; MET, mesenchymal-epithelial transition factor; +(nq), not quantifiable; NGS, next-generation sequencing; PA, pathology specimen; PD-1, programmed cell death 1; Unk, unknown

†Mutation not in panel.

‡Discrepancy in results.

§No mutations detected in liquid biopsy.

comparable with ddPCR in sensitivity for p.T790M detection. NGS performed better in detecting specific, sometimes previously unknown, genetic alterations because of the broader panel but at a higher cost. Our results confirm the ability to detect targetable aberrations in blood, which provides possibilities for new lines of targeted treatments in daily practice without the necessity of tissue procurement in many patients.

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