Reduced sensitivity for *EGFR* T790M mutations using the Idylla EGFR Mutation Test

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ABSTRACT

Aims Osimertinib is a third-generation *EGFR* (epidermal growth factor receptor) tyrosine kinase inhibitor that is effective in non-small cell lung cancer (NSCLC) harbouring the *EGFR* T790M mutation. The Idylla EGFR Mutation Test is a rapid cartridge-based method for detecting T790M and other *EGFR* mutations. However, false negative T790M results have been reported, and the sensitivity of the assay for this mutation is uncertain.

Methods Eighty NSCLC samples were tested by both Idylla and a next-generation sequencing (NGS) assay; 46 were from patients at disease progression, and 24 of these had known T790M mutations. Droplet digital PCR (ddPCR) was used to confirm NGS findings in samples with the T790M mutation.

Results Of 19 samples with T790M variant allele frequencies (VAF) higher than the stated 5% limit of detection, 14 were detected by Idylla (sensitivity 74%, 95% CI 49% to 90%). Where sufficient sample remained, ddPCR was consistent with NGS findings in all samples. False negative T790M results were associated with higher *EGFR* control Cq values (median 22.8 vs 19.8), presence of the *EGFR* Q787Q polymorphism in cis (80% vs 44%) and presence of an invalid T790M amplification curve. An *EGFR* exon 19 indel with VAF >5% was also not detected by the Idylla assay in two samples.

Conclusions The Idylla EGFR Mutation Test has reduced sensitivity for the T790M mutation compared with NGS and ddPCR methods. The presence of an invalid T790M amplification curve may indicate a possible false negative result that warrants further testing by an orthogonal method.

INTRODUCTION

Sensitising somatic *EGFR* (epidermal growth factor receptor) mutations in non-small cell lung cancer (NSCLC) are predictive biomarkers of response to *EGFR* tyrosine kinase inhibitor (TKI) therapy. However, resistance to TKI therapy is inevitable, and one of the main mechanisms of resistance is acquisition of the *EGFR* T790M mutation. Tumours with this mutation are in turn responsive to the third-generation *EGFR* TKI, osimertinib. Due to the often subclonal nature of the T790M mutation, testing methods with low limits of detection are required;[1] however, because it is a C>T nucleotide change that at low levels may be mimicked by deamination sequence artefacts,[2] this needs to be balanced by sufficient test specificity.

Testing for somatic *EGFR* mutations can be performed by a number of methods. One method that is increasingly used is next-generation sequencing (NGS), which can test hundreds to thousands of genes/targets at one time, detect a range of mutation types, and has low limits of detection suitable for somatic testing applications.[3] However, turnaround times are typically longer compared with other methods due to batching and assay requirements, which can lead to delays in patients receiving therapy. Further, NGS requires expensive sequencing instruments, facilities to accommodate pre-PCR and post-PCR processes, staff with molecular pathology expertise, and may require sample transport to centralised laboratories that have such capabilities.

The Idylla EGFR Mutation Test is a cartridge-based testing solution that tests directly from formalin-fixed paraffin-embedded (FFPE) tissue sections without the need for DNA extraction.[4] This assay detects *EGFR* mutations in exons 18, 19, 20 and 21, including T790M in exon 20, with a limit of detection of '≤5% for most prevalent *EGFR* mutations' (Technical Sheet Idylla EGFR Mutation Test, September 2019). This assay allows laboratories to perform *EGFR* testing without sequencing equipment, staff with extensive molecular pathology experience, or the need to batch samples; it also offers faster turnaround times and may have lower sample requirements compared with other methods.[5]

Many studies have compared the test performance of the Idylla EGFR assay to other methods, including targeted PCR-based assays, mass spectrometry, droplet digital PCR (ddPCR), Sanger sequencing, pyrosequencing and NGS.[5–15] Several of these studies have reported T790M false negative results;[5–7 10 11 15] however, the true sensitivity of the Idylla assay for the T790M mutation remains uncertain, as past studies have reported isolated cases or have used the assay outside of manufacturer instructions (eg, by using extracted DNA as the input instead of FFPE tissue sections).

This study aims to compare the performance of the Conformité Européenne (CE) marked in vitro diagnostic device (IVD) Idylla EGFR Mutation Test to a clinically accredited NGS assay, applied to patients at time of lung cancer diagnosis and in patients with disease progression while on *EGFR* TKI therapy. This study also aims to correlate any identified discordant results with sample-specific factors that may be useful in alerting laboratory users to the possibility of a false negative result.

METHODS

This study included a subset of NSCLC samples referred to the Sullivan Nicolaides Pathology

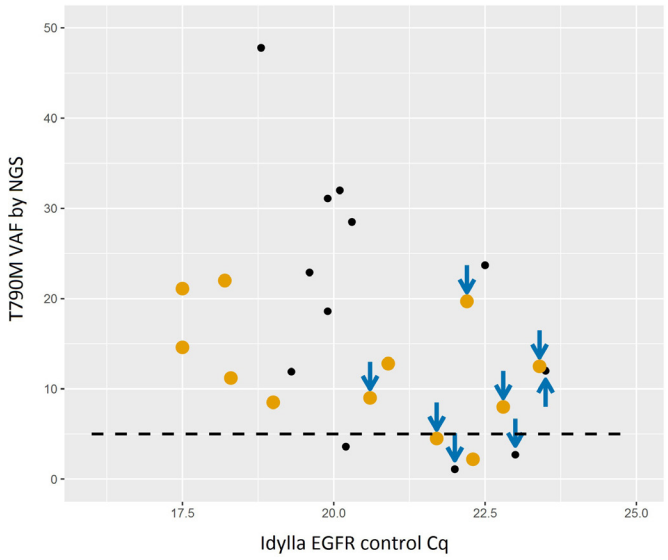

Figure 1 T790M variant allele frequency by NGS versus Idylla EGFR control Cq values. Each dot represents a sample with a known T790M mutation detected by NGS. Larger dots represent samples for which the Q787Q polymorphism is in cis with the T790M mutation. Arrows indicate samples where the T790M mutation was not detected by the Idylla EGFR Mutation Test. The dashed horizontal line represents the stated 5% limit of detection for the Idylla assay. NGS, next-generation sequencing.

laboratory for clinical *EGFR* mutation testing between December 2018 and September 2019. For all samples, H&E-stained tissue sections were reviewed by a histopathologist or senior histopathology scientist for mark-up of the tumour region and estimation of tumour cellularity. A minimum of 10% tumour cellularity and 100 tumour cells was required to be eligible for testing. The Idylla and NGS assays were performed using 4 µm thick unstained FFPE tissue sections cut from the same FFPE block. Scraping of tissue sections for loading onto the Idylla or for DNA extraction occurred on immediately sequential slides (eg, if sections numbered 2–4 were used for loading onto the Idylla, sections 5–7 were used for DNA extraction or vice versa). If a sample required macrodissection to enrich for tumour cells, this was performed for both NGS and Idylla.

The Idylla EGFR Mutation Test (Biocartis, Mechelen, Belgium) was performed according to manufacturer instructions as described previously,[5 7] and analysed using the Idylla Explore analysis software (V.3.2). For NGS testing, DNA was extracted from unstained FFPE tissue sections using the Qiagen GeneRead FFPE DNA Kit (Qiagen, Hilden, Germany), which includes a uracil-N-glycosylase step to reduce the prevalence of deamination artefacts. DNA quantity and amplifiability were assessed using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Massachusetts, USA) and the Illumina FFPE QC Kit (Illumina, San Diego, USA), respectively. NGS library construction was performed using Contextual Genomics FIND IT Cancer Hotspot Panel v4 primers (Contextual Genomics, Vancouver, Canada) and the Nextera XT DNA Library Prep Kit v2 (Illumina, San Diego, USA). Libraries were sequenced on the Illumina MiSeq instrument using v2 sequencing chemistry and 2×150 bp paired-end reads. FASTQ files were analysed using a proprietary bioinformatics pipeline provided by Contextual Genomics. This assay has been validated to ISO 15189 clinical accreditation standards in this laboratory.

Samples were only included in this study if they passed quality control criteria for both NGS and Idylla. Provided sufficient sample remained, discordant T790M cases were tested by ddPCR, using the T790M PrimePCR ddPCR Mutation Detection Assay Kit and the QX200 ddPCR system (Bio-Rad, Hercules, USA) as per manufacturer instructions.

RESULTS

Eighty samples were tested by both the Idylla and NGS assays. Thirty-four samples (43%) were from patients referred at time of lung cancer diagnosis, 45 samples (56%) were from patients referred due to disease progression while on *EGFR* TKI therapy, and 1 sample was referred for C797S testing in a patient being treated with osimertinib. Of 34 samples referred at time of diagnosis, 8 were cell blocks (24%), 14 were core biopsies (41%) and 12 were surgical resection samples (35%). Of 46 referred for T790M or C797S testing, 11 samples were cell blocks (24%), 30 were core biopsies (65%) and 5 were surgical resection samples (11%). The Idylla EGFR control Cq value is the average cycle quantification value for the *EGFR* sample processing controls in each of the five multiplex PCR reactions. As a measure of both DNA quantity and amplifiability, this value may be impacted by DNA fragmentation, DNA-protein cross-links or presence of PCR inhibitors. The control Cq value across all samples ranged from 17.3 to 28.3 (median 21.5).

Of 80 samples in total, *EGFR* TKI-sensitising variants were detected by the NGS assay in 58 (73%). Of these 58, 30 were exon 19 in-frame indels (52%), 24 were L858R (41%), 3 were G719 codon mutations (5%) and 1 was S768I (2%). There was concordance between the NGS and Idylla assays for these TKI-sensitising mutations in 53 of 58 samples (91%), at variant allele frequencies (VAF) ranging from 4.1% to 87.1% (median 21.1). Five samples with *EGFR* TKI-sensitising mutations detected by NGS did not have the mutations detected by the Idylla assay; 3 samples had exon 19 indels that are not interrogated by the Idylla assay, while 2 samples had an exon 19 indel (NM_005228.3:c.2237_2255delinsT) that is interrogated by the assay and was detected by NGS at VAF of 8.6% and 8.7%. The control Cq values for these discordant samples were 25.1 and 24.0, respectively.

Forty-six samples were specifically referred to identify the presence of the T790M or C797S mutation, and stated or presumed to be from patients with disease progression while on *EGFR* TKI therapy. Of these, T790M was detected by the NGS assay in 24 samples (54%), at VAF ranging from 1.1% to 47.8% (median 12.7%) (figure 1). All 24 samples harbouring T790M retained concurrent *EGFR* activating mutations; 15 of these were detected with an exon 19 indel, and 9 were detected with L858R. Using the Idylla assay, 16 of these 24 T790M mutations (70%) were detected; the VAF of the concordant T790M mutations ranged from 2.2% to 47.8% (median 19.9%) and *EGFR* control Cq values ranged from 17.5 to 22.5 (median 19.8). Of the 8 samples in which T790M mutations identified by NGS were not detected by Idylla, three had VAF below 5% (1.1%, 2.7% and 4.5%), while five had VAF above the stated 5% limit of detection of the Idylla assay (table 1). T790M was detected by NGS in this latter group at VAF ranging from 8% to 19.7%, and the Idylla *EGFR* control Cq values for these samples ranged from 20.6 to 23.5 (median 22.8). Six of the eight samples discordant for T790M showed VAF comparable to NGS when tested further by ddPCR; two cases had insufficient remaining sample for further testing. In all other samples where sufficient sample remained, confirmatory testing for T790M by ddPCR was consistent with NGS and Idylla findings

Table 1 Samples for which *EGFR* T790M mutation status was discordant between Idylla and NGS

Case	Estimated tumour cellularity (%)	Idylla *EGFR* control cq	Presence of invalid amplification curve	Q787Q in cis	*EGFR* genotype by NGS	T790M VAF by NGS (%)	T790M VAF by ddPCR (%)
1	35	22.2	Y	Y	Exon 19 indel, T790M	19.7	20.1
2	15	23.4	Y	Y	Exon 19 indel, T790M	12.5	13.3
3	25	23.5	Y	N	Exon 19 indel, T790M	12	Insufficient sample
4	10	20.6	Y	Y	Exon 19 indel, T790M	9	8.6
5	15	22.8	Y	Y	Exon 19 indel, T790M	8	7.6
6	10	21.7	Y	Y	L858R, T790M	<5 (4.5)	<5 (3.6)
7	10	23	N	N	L858R, T790M	<5 (2.7)	Insufficient sample
8	35	22	N	N	Exon 19 indel, T790M	<5 (1.1)	<5 (0.9)

ddPCR, droplet digital PCR; NGS, next-generation sequencing; VAF, variant allele frequency.

(table 2). Therefore, out of 19 samples with T790M mutation VAF ≥5%, 14 were detected by Idylla (sensitivity 74%, 95% CI 49% to 90%). There were no false positive T790M results using the Idylla assay, with all 16 T790M mutations identified by the assay being confirmed by NGS.

The Idylla Explore analysis software allows users to view 'invalid' amplification curves, which do not pass the system's threshold for reporting but may indicate the presence of an undetected T790M mutation.[15] Of the eight discordant samples, six had invalid amplification curves for the T790M mutation, while two samples (both with T790M VAF <5%) did not (figure 2). None of the 56 samples which were true negative for T790M (as determined by NGS) showed evidence of an invalid T790M amplification curve.

The *EGFR* Q787Q (p.Gln787Gln) mutation is a common population polymorphism that is present in over 52% of alleles in the gnomAD database. It is known to interfere with the detection of T790M by allele-specific PCR methods.[16 17] Of the 16 samples in which T790M status was concordant between NGS and Idylla, the Q787Q polymorphism was detected in cis with T790M in 7 (44%) of these samples. Of the five samples in which T790M was not detected by Idylla, despite being detected by NGS at VAF>5%, the Q787Q polymorphism was detected in cis with T790M in 4 (80%) of these samples.

DISCUSSION

The Idylla EGFR Mutation Test has reduced sensitivity for the *EGFR* T790M mutation compared with an NGS assay, especially in samples with higher *EGFR* control Cq values (indicating lower quantities of amplifiable DNA) and/or presence of an in cis Q787Q polymorphism. The presence of an invalid T790M amplification curve may be an indicator of a false negative T790M result in this setting.

The Q787Q polymorphism was present in cis in a higher proportion of false negative cases than in true positive cases (80% vs 44%). Although interference of the Idylla assay by this polymorphism is therefore plausible, the primer and probe sequences utilised by the Idylla EGFR Mutation Test are proprietary and interference cannot be conclusively deduced or excluded. Establishing interference would require further study using the primers utilised by the test, and varying the primer sequences to note how this variation affects amplification of the allele with and without the polymorphism in cis. However, even if the polymorphism does affect this assay, it cannot be sufficient on its own to cause a false negative result, as seven cases with the polymorphism in cis had concordant positive T790M results. Similarly, although the median control Cq value in the false negative T790M group was higher

Table 2 Samples for which *EGFR* T790M mutation status was concordant between Idylla and NGS

Case	Estimated tumour cellularity (%)	Idylla *EGFR* control cq	Presence of invalid amplification curve	Q787Q in cis	*EGFR* genotype by NGS	T790M VAF by NGS (%)	T790M VAF by ddPCR (%)
9	50	18.8	N/A	N	L858R, T790M	47.8	48.5
10	15	20.1	N/A	N	Exon 19 indel, T790M	32.0	32.5
11	40	19.9	N/A	N	Exon 19 indel, T790M	31.1	Insufficient sample
12	40	20.3	N/A	N	L858R, T790M	28.5	28.0
13	15	22.5	N/A	N	Exon 19 indel, T790M	23.7	Insufficient sample
14	60	19.6	N/A	N	Exon 19 indel, T790M	22.9	22.5
15	25	18.2	N/A	Y	L858R, T790M	22.0	22.5
16	70	17.5	N/A	Y	Exon 19 indel, T790M	21.1	21.3
17	50	19.9	N/A	N	Exon 19 indel, T790M	18.6	16.2
18	20	17.5	N/A	Y	L858R, T790M	14.6	Insufficient sample
19	20	20.9	N/A	Y	Exon 19 indel, T790M	12.8	13.1
20	60	19.3	N/A	N	L858R, T790M	11.9	11.9
21	50	18.3	N/A	Y	Exon 19 indel, T790M	11.2	10.4
22	40	19	N/A	Y	L858R, T790M	8.5	Insufficient sample
23	35	20.2	N/A	N	Exon 19 indel, T790M	3.6	3.0
24	15	22.3	N/A	Y	L858R, T790M	2.2	Insufficient sample

ddPCR, droplet digital PCR; N/A, not applicable; NGS, next-generation sequencing; VAF, variant allele frequency.

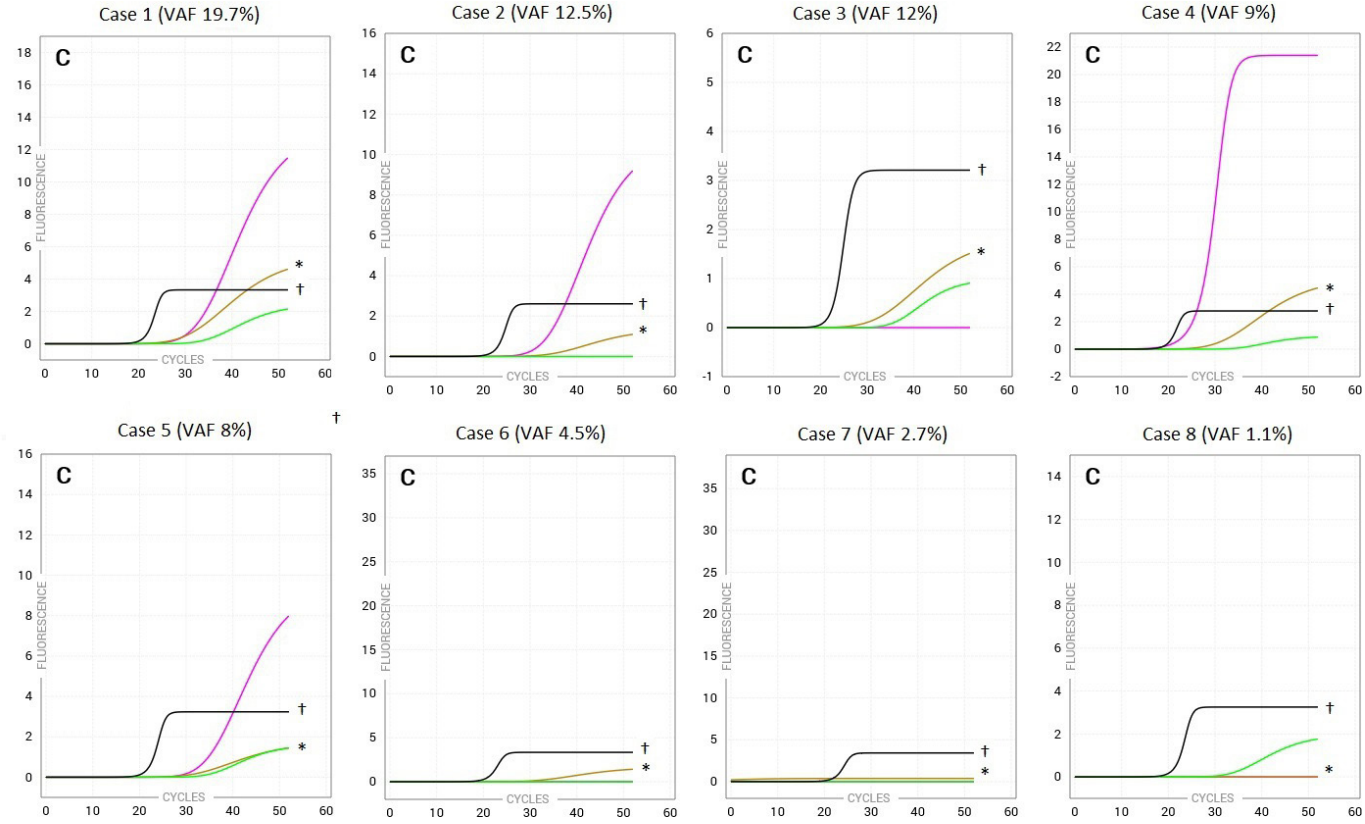

Figure 2 Invalid T790M amplification curves in samples discordant for T790M. *Invalid T790M-specific amplification curve. †A sample processing control curve that reflects the amount of amplifiable DNA in the sample. VAF, variant allele frequency.

than that of the true positive T790M group (22.8 vs 19.8), T790M was detected in samples with control Cq values as high as 22.5, showing that we could not establish a clear control Cq threshold for predicting potential false negative results.

An invalid T790M amplification curve was observed in all discordant samples with T790M present at VAF >5%, and in one sample in which the VAF was 4.5%. However, it was not observed in two discordant samples with T790M present below VAF <5%, nor was it observed in 56 other samples known to be true negative for T790M on NGS. These results suggest that an invalid T790M amplification curve may be a sensitive and specific predictor of the presence of an undetected T790M mutation. However, these results do not speak to the specificity of invalid amplification curves for non-T790M mutations on this platform, as invalid curves were observed in many samples for exon 19 indels despite being absent on NGS.

Past studies have examined the performance of the Idylla EGFR Mutation Test in patients with known T790M mutations, or in patients tested at time of disease progression (who may harbour a T790M mutation).[5–7 9–14] Many of these studies have similarly reported discordant T790M results using the Idylla assay, as well as for other actionable *EGFR* mutations, in comparison to reference methods.[5–7 10 11 15] These studies have reported on use of the Research Use Only assay[5 10 11] as well as the CE-marked IVD assay.[6 7 15] Explanations provided for these discordances have included insufficient tumour material, tumour heterogeneity, or mutations being present below the stated 5% limit of detection. Patients with known T790M mutations were typically a small fraction of these study cohorts, and the few discordant T790M results observed in each study may not have been sufficient to arouse suspicion of reduced sensitivity. The exception to this is the study by Bocciarelli *et al*, which tested 29 samples with known T790M mutations, and similarly reported a test sensitivity of 65.5% for the T790M mutation as compared with an NGS method. However, this study directly deposited extracted DNA into the Idylla cartridges, rather than tissue sections (as per manufacturer instructions), thereby limiting its external validity for the majority of users. The primary finding of our study is therefore supported by the frequency and consistency of discordant T790M results reported in the literature.

There has been conjecture about the clinical relevance of somatic mutations present at low VAF,[18] which may be due to the presence of minor subclones or low tumour cellularity in the sample being tested. Given that five out of eight discordant samples had estimated tumour cellularity of 10%–15%, it is likely that the low VAF observed are due to low tumour cellularity in the biopsy specimen. In other words, it is likely to be present in a significant proportion of cells in the tumour proper, and therefore of clinical significance to therapy selection.

A limitation of this study and others that compare the Idylla to other platforms is that the Idylla consumes the tissue sections used to perform the test. As such, different tissue sections are used for each method, and these may differ in their genotype or VAF due to tumour heterogeneity. However, our study used immediately sequential tissue sections for Idylla and DNA extraction to minimise this possibility. Further, our primary finding of recurrent false negatives for the T790M mutation has been replicated in multiple other studies, including Bocciarelli *et al*, in which the same DNA extract was used for both NGS and Idylla testing.[15]

There are a number of broader implications stemming from this study. First, there is a significant risk of a false negative result for the T790M mutation if one relies only on the proprietary pass/fail quality metrics inherent to the Idylla system. Bocciarelli *et al* have proposed a testing algorithm in which all samples with control Cq

values>25 are retested by another method; however, given that all of our discordant samples had control Cq values<25, we disagree with this recommendation. Our study did not establish a clear control Cq threshold below which a false negative T790M result could be excluded. Further, the presence of the Q787Q polymorphism and its phase can only be established if a sequencing assay such as NGS is used, and therefore, it also cannot be used as a predictive factor. However, the presence of an invalid amplification curve may be a predictor of a false negative result requiring further testing. An even more conservative approach would be for all samples from patients tested at disease progression to be retested by another method such as NGS or ddPCR, if no T790M mutation is identified. Provided sufficient sample is available for multiple testing methods, this would still allow laboratories to take advantage of the rapid turnaround afforded by the Idylla assay while minimising the potential for false negative results.

Our findings suggest a need for manufacturers to accurately characterise assay performance in 'real world' samples with poorer or more variable quality. In the current Idylla IFU, the highest *EGFR* control Cq value for which limits of detection have been characterised is 21.3. However, 42 of 80 (53%) samples tested in our cohort, referred from a variety of pathology laboratories, had a control Cq value higher than 21.3. The expected test performance data applicable to such samples is therefore not available.

In the USA, the third-generation *EGFR* TKI osimertinib has been approved by the Food and Drug Administration for first-line therapy in patients with NSCLC and a sensitising *EGFR* mutation.[19] It has been shown to have superior efficacy as first-line therapy compared with older TKIs, as well as having efficacy against central nervous system metastases.[19 20] The sensitivity of assays for T790M, which typically arise in response to first-generation and second-generation *EGFR* TKI therapy, may therefore become less clinically relevant. However, the two false negative exon 19 indel cases identified in this study may indicate that the issue is not limited to T790M. We, therefore, suggest that further manufacturer validation data and/or continued research to explore the analytical limits of the Idylla platforms are warranted. Of particular interest is the impact of control Cq values on the limit of detection for other genes/mutations targeted by these assays, and whether Cq values corresponding to various levels of sensitivity can be defined.

The Idylla EGFR Mutation Test has lower sensitivity than NGS for the T790M mutation, especially in samples with higher Idylla EGFR control Cq values and/or the Q787Q polymorphism in cis, and an invalid amplification curve may be a sensitive and specific predictor of the presence of an undetected T790M mutation. In patients with clinical progression on first-generation or second-generation *EGFR* TKI therapy, the absence of an *EGFR* T790M mutation on the Idylla EGFR Mutation Test may warrant further testing by orthogonal methods.

Take home messages

- Osimertinib is a third-generation *EGFR* tyrosine kinase inhibitor that is effective in non-small cell lung cancer with the *EGFR* T790M mutation.
- The Idylla EGFR Mutation Test has reduced sensitivity for detecting the T790M mutation compared with next-generation sequencing and droplet digital PCR methods, especially in samples with higher Idylla EGFR control Cq values and/or the Q787Q polymorphism in cis.
- The presence of an invalid T790M amplification curve may indicate a possible false negative result that warrants further testing by an orthogonal method.

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REFERENCES

1 Uguen A, Troncone G. A review on the Idylla platform: towards the assessment of actionable genomic alterations in one day. *J Clin Pathol* 2018;71:757–62.
2 Ye X, Zhu Z-Z, Zhong L, *et al*. High T790M detection rate in TKI-naive NSCLC with EGFR sensitive mutation: truth or artifact? *J Thorac Oncol* 2013;8:1118–20.
3 Jennings LJ, Arcila ME, Corless C, *et al*. Guidelines for validation of next-generation sequencing-based oncology panels: a joint consensus recommendation of the association for molecular pathology and College of American pathologists. *J Mol Diagn* 2017;19:341–65.
4 Tan LY, Walker SM, Lonergan T, *et al*. Superior multiplexing capacity of PlexPrimers enables sensitive and specific detection of SNPs and clustered mutations in qPCR. *PLoS One* 2017;12:e0170087.
5 Evrard SM, Taranchon-Clermont E, Rouquette I, *et al*. Multicenter evaluation of the fully automated PCR-based Idylla EGFR mutation assay on formalin-fixed, paraffin-embedded tissue of human lung cancer. *J Mol Diagn* 2019;21:1010–24.
6 Colling R, Bancroft H, Langman G, *et al*. Fully automated real-time PCR for EGFR testing in non-small cell lung carcinoma. *Virchows Arch* 2019;474:187–92.
7 Huang H, Springborn S, Haug K, *et al*. Evaluation, validation, and implementation of the Idylla system as rapid molecular testing for precision medicine. *J Mol Diagn* 2019;21:862–72.
8 De Luca C, Gragnano G, Pisapia P, *et al*. *EGFR* mutation detection on lung cancer cytological specimens by the novel fully automated PCR-based Idylla *EGFR* Mutation Assay. *J Clin Pathol* 2017;70:295–300.
9 Van Haele M, Vander Borght S, Ceulemans A, *et al*. Rapid clinical mutational testing of *KRAS*, *BRAF* and *EGFR*: a prospective comparative analysis of the Idylla technique with high-throughput next-generation sequencing. *J Clin Pathol* 2020;73:35–41.
10 Ghigna M-R, Crutu A, Florea V, *et al*. Endobronchial ultrasound-guided fine-needle aspiration for pulmonary carcinomas genotyping: experience with 398 cases including rapid *EGFR/KRAS* analysis in 43 cases. *J Thorac Dis* 2018;10:4653–8.
11 Lambros L, Caumont C, Guibourg B, *et al*. Evaluation of a fast and fully automated platform to diagnose *EGFR* and *KRAS* mutations in formalin-fixed and paraffin-embedded non-small cell lung cancer samples in less than one day. *J Clin Pathol* 2017;70:544–9.
12 De Luca C, Rappa AG, Gragnano G, *et al*. Idylla assay and next generation sequencing: an integrated EGFR mutational testing algorithm. *J Clin Pathol* 2018;71:745–50.
13 Thomas De Montpréville V, Ghigna M-R, Lacroix L, *et al*. Egfr and KRAS molecular genotyping for pulmonary carcinomas: feasibility of a simple and rapid technique implementable in any department of pathology. *Pathol Res Pract* 2017;213:793–8.
14 Ilie M, Butori C, Lassalle S, *et al*. Optimization of *EGFR* mutation detection by the fully-automated qPCR-based Idylla system on tumor tissue from patients with non-small cell lung cancer. *Oncotarget* 2017;8:103055–62.
15 Bocciarelli C, Cohen J, Pelletier R, *et al*. Evaluation of the Idylla system to detect the $EGFR^{T790M}$ mutation using extracted DNA. *Pathol Res Pract* 2020;216:152773.
16 Xu S, Duan Y, Lou L, *et al*. Exploring the impact of *EGFR* T790M neighboring SNPs on ARMS-based T790M mutation assay. *Oncol Lett* 2016;12:4238–44.
17 Chen Y-L, Lin C-C, Yang S-C, *et al*. Five Technologies for Detecting the *EGFR* T790M Mutation in the Circulating Cell-Free DNA of Patients With Non-small Cell Lung Cancer: A Comparison. *Front Oncol* 2019;9:631.
18 Shin H-T, Choi Y-L, Yun JW, *et al*. Prevalence and detection of low-allele-fraction variants in clinical cancer samples. *Nat Commun* 2017;8:1377.
19 Soria J-C, Ohe Y, Vansteenkiste J, *et al*. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N Engl J Med* 2018;378:113–25.
20 Goss G, Tsai C-M, Shepherd FA, *et al*. Cns response to osimertinib in patients with T790M-positive advanced NSCLC: pooled data from two phase II trials. *Ann Oncol* 2018;29:687–93.