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Osimertinib benefit in *EGFR*-mutant NSCLC patients with *T790M*-mutation detected by circulating tumour DNA

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Abstract

Background: Approximately 50% of Epidermal growth factor receptor (*EGFR*) mutant non-small cell lung cancer (NSCLC) patients treated with EGFR tyrosine kinase inhibitors (TKIs) will acquire resistance by the *T790M* mutation. Osimertinib is the standard of care in this situation. The present study assesses the efficacy of osimertinib when T790M status is determined in circulating cell-free tumour DNA (*ct*DNA) from blood samples in progressing advanced *EGFR*-mutant NSCLC patients.

Material and methods: *ct*DNA *T790M* mutational status was assessed by Inivata InVision™ (eTAm-Seq™) assay in 48 *EGFR*-mutant advanced NSCLC patients with acquired resistance to EGFR TKIs without a tissue biopsy between April 2015 and April 2016. Progressing *T790M*-positive NSCLC patients received osimertinib (80 mg daily). The objectives were to assess the response rate to osimertinib according to Response Evaluation Criteria in Solid Tumours (RECIST) 1.1, the progression-free survival (PFS) on osimertinib, and the percentage of *T790M* positive in *ct*DNA.

Results: The *ct*DNA *T790M* mutation was detected in 50% of NSCLC patients. Among evaluable patients osimertinib gave a partial response rate of 62.5% and a stable disease rate of 37.5%. All responses were confirmed responses. After median follow up of 8 months, median PFS by RECIST criteria was not achieved (95% CI: 4-NA), with 6- and 12-months PFS of 66.7% and 52%, respectively.

Conclusions: *ct*DNA from liquid biopsy can be used as a surrogate marker for *T790M* in tumour tissue.

Key words: *EGFR* mutation, *T790M*, osimertinib, lung cancer, ctDNA liquid biopsies

Key message: Liquid biopsies are a usufull tool for personalising treatment in lung cancer patients. Detection of T790M at low levels in plasma samples of EGFR-mutant NSCLC patients predicts efficacy to osimertinib.

Introduction:

The activated epidermal growth factor receptor (*EGFR*) mutation is present in almost 50% of patients with advanced non-small cell lung cancer (NSCLC) who are of Asian ethnicity compared with only 12% in the Caucasian population [1]. These mutations predict sensitivity to first- and second-generation EGFR tyrosine kinase inhibitors (TKIs) such as erlotinib, gefitinib or afatinib. Response rate and progression-free survival with EGFR TKIs are superior to standard first-line platinum doublet chemotherapy, making them the standard of care [2]. However, tumours invariably develop acquired resistance 9 to 13 months after treatment initiation. The substitution of threonine to methionine at amino acid position 790 (*T790M*) in exon 20 of the *EGFR* gene reduces first-generation EGFR TKIs binding, and accounts for over half of acquired resistance mechanisms [3, 4].

Knowledge of acquired resistance mechanisms to EGFR TKIs was one of the triggers behind the development of personalised therapies, with the introduction of the third-generation EGFR-TKIs, which are active against sensitive, as well as resistant *T790M EGFR* mutations, such as osimertinib [5]. Both the FDA and the EMA recently approved osimertinib in patients with acquired EGFR *T790M* mutations tested in a tumour-tissue biopsy or in plasma [6, 7], but noted that osimertinib efficacy has not been prospectively established in patients where *T790M* mutation was determined in plasma with unknown status in the tissue. Lack of available tissue for performing molecular profile (such as when bone metastases are present, as reported in almost 50% of cases [8], requiring decalcification of the samples impairing DNA quality), the location or size of the tumour at progression, and the risk of complications, are serious limitations to re-biopsy NSCLC tumours. Moreover, single site biopsies may not provide a representative profile of the overall predominant resistance mechanisms for a given patient [9].

Liquid biopsies based on circulating cell-free tumour DNA (*ct*DNA) analysis have been described as surrogate samples for molecular analysis replacing solid tumours [10], and may allow real-time sampling of multifocal clonal

evolution [11]. Here we assessed the feasibility of identifying *T790M* mutations in *ct*DNA isolated from blood samples in a cohort of *EGFR*-mutant NSCLC patients with progression under first- or second-generation EGFR TKIs without a tissue biopsy at progression, in order to detect acquired resistance. The efficacy of osimertinib in the *ct*DNA *T790M*-positive NSCLC patients was also assessed.

Patients and methods

Patients

Eligible patients treated at the Gustave Roussy (Villejuif, France) between April 2015 and April 2016 were included in this study. Patients had to have advanced NSCLC, the presence of a common activating *EGFR*-mutation in the initial biopsy (*Del19*, *L858R*), clinical or radiological progression to at least one first- or second-generation EGFR TKI [12], and ineligibility for a new tissue biopsy (due to lack of available tissue, localisation and/or patient's refusal) for testing T790M status at the time of progression. There was no upper limit for the number of prior EGFR-inhibitor or systemic therapies. All patients provided written informed consent for biomedical research (CEC-CTC IDRcb2008-AOO585-50) and the institutional ethics committee approved the protocol. Osimertinib at 80 mg daily was prescribed as a part of the French Expanded Access Program in France, which allow its prescription when *T790M* was present in tumour-tissue biopsy or in a liquid biopsy.

Outcomes

The primary endpoint was to determine the overall response rate with osimertinib in patients treated on the basis of a positive *T790M* mutational status from a liquid biopsy results. Secondary endpoints included: the percentage of *T790M* mutation-positive patients identified by *ct*DNA analysis from pretreated *EGFR*-mutant patients with progression to systemic treatment, progression free survival by radiological criteria and investigator's criteria and overall survival on osimertinib.

As an exploratory objective, correlation between RECIST radiological responses with osimertinib and three *ct*DNA predictors was evaluated: (A)

T790M allele fraction, (B) *EGFR* activating mutation allele fraction, and (C) ratio of *T790M* and *EGFR* activating mutation allele fraction.

Progression free survival (PFS) was calculated from the initiation of osimertinib treatment until the date of progression by RECIST 1.1 or death (whichever came first), with censoring at the date of last follow-up if the patient had not progressed. PFS by investigator (time to off-osimertinib progression if osimertinib therapy was extended beyond progression at investigator discretion) was also assessed. Overall survival (OS) was calculated from the initiation of osimertinib treatment until the date of death.

InVision™ (eTAm-Seq™) analysis

10 ml of blood were collected in K2-EDTA tubes and processed at the time of disease progression (clinical or radiological). DNA was extracted from < 5 ml of plasma and analysed by the InVision assay, using enhanced Tagged Amplicon-Sequencing; eTAmSeq™, [13] which was developed from TAm-Seq® assay [14] (Supplement; Appendix 1).

Radiologic assessments

Prior to prescribing osimertinib, all patients underwent tumour imaging, including computed tomography of the chest and abdomen and/or PET-scan. Brain imaging was performed in cases of symptoms. Restaging scans were obtained at least 4-weeks after treatment initiation and then every 6 to 8 weeks. Senior radiologist (C.C.) centrally reviewed the response rate and determined best response to osimertinib according to RECIST v1.1 [15]. The objective response rate was defined as the percentage of patients with response (complete or partial) at first restaging after osimertinib initiation. Confirmed responses were defined as persistent responses (partial or complete) at second radiological assessment. Only evaluable patients who received osimertinib based on positivity for the *T790M* mutation from *ct*DNA liquid biopsies were evaluated for the response rate.

Results

Patient characteristics

Forty-eight advanced *EGFR*-mutant NSCLC patients with radiological or clinical progression on systemic treatment were evaluated for T790M status in a liquid biopsy. Median age was 65 years (range 37-83); 36 (75%) patients were women and 58% were never-smoker. *EGFR* mutation status was *Del19* in 33 (69%) and *L858R* in 15 (31%) NSCLC patients.

T790M status in a liquid biopsy

The *T790M* positivity in *ct*DNA was reported in 24 out of 48 (50%) NSCLC patients. (Figure S1)

Activating *EGFR* mutational status in *ct*DNA analysis confirmed that the original mutation was maintained in 23 out of 24 *T790M*-positive samples. The *T790M* mutation positivity was more frequent among patients with the *EGFR Del19* mutation (20 out of 33 patients, 61%) compared to the EGFR *L858R* mutation (4 out of 15, 27%). Concomitant mutations to *T790M* mutation were reported in three patients (Table 1).

For 9 of the 24 patients with *ct*DNA *T790M*-positivity, the T790M allele fraction (AF) was lower than 0.5% in the liquid biopsy (Table S1).

Osimertinib response rate

Of the 24 NSCLC patients with a *T790M* mutation in the *ct*DNA, 18 received osimertinib at progression and were evaluated for response (Figure S1).

Table 1 summarizes baseline demographic characteristics of NSCLC patients who were *T790M* positive by *ct*DNA and treated with osimertinib. Median age was 63 years, and a total of 78% of patients (14 of 18) were female. All the patients had received at least one prior EGFR TKI. Three or more previous systemic treatment lines were reported in up to 65% of patients and in 70% of cases an EGFR TKI was the last treatment before starting osimertinib.

Two patients were not evaluated for response: one having only bone metastases and the other died due to a treatment-unrelated cerebral haemorrhage. Of the 16 evaluable patients, 10 had a partial response (62.5%), and 6 had stable disease (37.5%). No patients had complete response or disease progression as best response (Table 1 and Figure 1).

Among those patients with partial response (n=10), all had second radiological assessment to confirm response, and the response was confirmed in 90% of

patients (1 patient progressed at the second radiological assessment). Of note, one patient previously treated with rociletinib, received osimertinib as tenth line treatment achieving a partial response.
The median time between the blood draw in which *ct*DNA *T790M* positivity was detected and start of osimertinib treatment was 6 weeks.

Correlation between RECIST and ctDNA predictors
Correlations between RECIST radiological responses with osimertinib and three *ct*DNA predictors: (A) *T790M* allele fraction, (B) *EGFR* activating mutation allele fraction, and (C) ratio of T790M and *EGFR* activating mutation allele fraction were evaluated, however, none showed significance (Figure 2), but a trend (p-value 0.09-0.15) was observed for larger decrease in tumour size for smaller mutant allele fractions of *T790M* or *EGFR* activating mutations. Of the seven cases with best response (decrease of 50% or more in size), 3 cases had T790M detected at <0.25%.

Progression Free Survival and Overall Survival
After a median follow up of 8.5 months, median PFS on osimertinib by RECIST 1.1 criteria was not achieved (95% CI: 4-NA), with a 6- and 12-months PFS of 66.7% and 52%, respectively (Figure 3). By investigator, median PFS was 13 months (95% CI: 8-NA), with 6- and 12-months PFS of 79% and 70%, respectively (Figure S3). At the time of cut-off 4 patients had died; hence overall survival (OS) was not achieved. 1-year OS was 78% (95%CI: 59-97) (Figure S2).

Discussion

Osimertinib is a third-generation oral EGFR TKI developed to treat tumours bearing sensitizing *EGFR* and acquired resistant *T790M*-mutations, that spares the wild type form of the receptor [16]. To the best of our knowledge, our analysis is the first to prospectively test in a real-world setting the efficacy of osimertinib according to *ct*DNA results. In this study, osimertinib achieved a 62.5% response rate and 12-months PFS of 52% among NSCLC patients who

were *T790M*-mutation positive, based on *ct*DNA analysis by a multiplexed deep sequencing [13] assay. These results are comparable to the efficacy reported with osimertinib in patients with *T790M* mutation detected in a tumour tissue biopsy [5, 16]. In the phase 3 AURA3 study, osimertinib provided a 71% of response rate and 12-months PFS of 44% in pre-treated and tissue *T790M*-mutation positive NSCLC patients [16]. However, in the phase I AURA trial, some patients with *T790M*-mutation negative also responded to osimertinib [5] reflecting the inadequacy of tissue-biopsy for catching tumour heterogeneity. In the *post hoc* exploratory analysis of the samples from the phase I AURA trial, which included 216 patients (73% were *T790M*-positive in the tumour) osimertinib gave a response rate of 63% among patients who were *T790M*-mutation positive according to central blood-test genotyping by the BEAMing method (allelic fraction for positive results for *T790M* mutation ≥ 0.06%) [17].

Liquid biopsies based on *ct*DNA analysis are described as surrogate samples for tumour molecular analysis [10], and also as potential dynamic markers for monitoring the efficacy of EGFR TKI [18, 19] and early detection of resistance mutations [20]. Liquid biopsy assays have been developed for analysis of hot-spot mutations and gene panels. Hot-spot assays can offer lower complexity and some PCR-based assays for detection of mutations in *EGFR* (including activating mutations and the T790M mutation) have received CE-mark [21] and approval by the FDA for in-vitro use [22]. Several commercial laboratories now offer sensitive assays for ctDNA using targeted deep sequencing of gene panels that include *EGFR*. In our study, the rate of *T790M* mutation positivity in a liquid biopsy among *EGFR*-mutant patients progressing on systemic treatment was 50%, which is consistent with previous biopsy series [3, 4] and clinical trials [5, 23]. In a recent prospective exploratory analysis, the resistance-associated mutation in *ct*DNA (tested by cobas *EGFR* Mutation Testv2) among *EGFR*-mutant NSCLC patients was detected in 50% of patients, and concordance with tumour biopsy-derived genotyping was 61% [24]. Among patients with sufficient material for concurrent *ct*DNA and tumour-derived genotyping, *ct*DNA identified the *T790M* mutation in 5 of 25 (20%) in whom the concurrent study biopsy was negative. Similarly, in the phase I AURA trial, *T790M* was detected in plasma of 30% of patients with *T790M*-negative tumours [17]. Discrepancies between tumour biopsy and *ct*DNA genotyping

may result from technological differences, or sampling of different tumour cell populations in a heterogeneous setting [24]. Studies focusing on the discrepancy of T790M mutation between tissue and plasma samples are underway using amplification-refractory mutation system (ARMS) and droplet digital PCR methods (NCT02418234). Moreover, recent data suggest that *ctDNA T790M* mutation derived from NSCLC tumours can be detected with high sensitivity in urine as well as in plasma, enabling complementary modes of tissue and liquid biopsies in EGFR TKI resistant NSCLC [25]. Although sensitivity and specificity of *ct*DNA varies across different technology platforms [26], the establishment of robust and standardised protocols for blood sampling, processing, storage, DNA extraction and analysis will support liquid biopsies as new standard tests in the near future for tumour genotyping as well as predictive biomarkers [26].

In this setting, the relatively low number of patients, the heavy degree of pre-treatment population included in our analysis (median of four previous treatment lines, 33% with at least two EGFR TKIs before osimertinib initiation and two patients previously pre-treated with T790M-inhibitors), the lack of corresponding tumour sample for all patients, and the heterogeneity in terms of lines of treatment are all considered as potential limitations. Moreover, *ct*DNA cut-off points to define the clinical relevance of the findings specifically based on functional consequences, namely their ability to predict therapeutic responsiveness, are required before *ct*DNA can be routinely implemented in clinical practice. Interestingly, the observation in our cohort that the *T790M* allele fraction was not significantly correlated with clinical response suggests that any level of *T790M* positivity may be clinically relevant, independent of the allele fraction threshold. However, the relatively long time delay between establishment of *ct*DNA T790M positivity and osimertinib initiation may mean that the allele fraction at the moment of treatment initiation may be higher than the reported results. Our data suggests a possible importance for detection of *T790M* at low allele fractions, but additional studies are needed to confirm the minimum biological threshold with clinical relevance.

Testing tumour tissue is so far the recommended method for detecting the presence of the resistant *T790M* mutation among *EGFR*-mutant NSCLC patients and tailoring treatment [6, 7], Prior biopsy-based studies have reported

multiple acquired resistance mechanisms in approximately 5% to 15% of NSCLC patients with EGFR TKIs [3, 4]. However, up to 23% of tumour tissue specimens available at the time of acquired resistance have been reported as providing limited, low quality material for tumour genotyping [4, 24], and may not be representative of the entire genomic landscape of the tumour [9, 27]. In addition, not all patients are suitable for new tissue biopsy at progression, which can thereby delay treatment initiation [28]. Recently, mechanisms of acquired resistance after first-line EGFR TKI were analysed in *ct*DNA by CAPP-Seq in 41 *EGFR*-mutant NSCLC patients. At least 46% of these tumours had developed another mechanism of acquired resistance in addition to *T790M* mutation, and these multiple resistance mechanisms were associated with poorer outcome to third generation EGFR TKIs [29]. In our analysis, blood samples from three patients reported concomitant mutations with no clear correlation with outcome: one *PIK3CA* mutation, previously reported as mechanism of acquired resistance [3]; and two other mutations, *STK11* and *NRAS* mutation, not previously described as acquired resistance mechanisms to first- or second-generation EGFR TKI. However, *NRAS* mutation has been recently reported as an acquired mechanism of resistance to osimertinib in preclinical models [30]. *ct*DNA analysis may allow the development of rational trials for personalised selection of combined therapies to address intratumoural heterogeneity, however, a risk-benefit assessment should be performed to avoid substantial increases in toxicity.

Conclusion:

In this analysis of liquid biopsies in a small cohort of *EGFR*-mutant NSCLC patients with acquired resistance to systemic treatment, our results provide relevant clinical data about the efficacy of osimertinib in a real-world setting among patients where *T790M*-positivity was detected in *ct*DNA, supporting the use of such liquid biopsies for personalising treatment in lung cancer patients. Our results suggest a possible clinical importance for detection of *T790M* at low levels in plasma samples.

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Patient	Gender	Age (years)	Pack-yrs	EGFR mutation	T790M AF (%)	Previous systemic treatments	Previous EGFR TKI	Other mutations (#)	Last treatment before Osimertinib	RECIST Osimertinib
1	M	51	0	Del19	0.41	3	1	TP53 (P151X, R273H)	Erlotinib	NE
2	F	56	0	Del19	15.96	3	2	TP53 (Q331*, V225A)	Erlotinib	SD (-10%)
3	M	54	6	Del19	0.86	3	1	TP53 (R337C) STK11 (P179L)	Erlotinib -BVZ	PR (-50%)
4	F	37	0	Del19	1.06	3	2	TP53 (Q165*)	Erlotinib	PR (-84%)
5	M	67	6	Del19	1.60	4	2	CTNBB1 (S37S)	Pem / Cis	PR (-50%)
6	F	83	10	Del19	6.96	2	2	CTNNB1 (S33C)	Erlotinib	SD (0%)
7	F	67	0	Del19	19.60	1	1	CDKN2A (frameshift) TP53 (frameshift)	Erlotinib	PR (-50%)
8	F	70	0	L858R	0.25	2	1	NRAS (A59G)	Pem	SD (-26%)
9	F	66	5	L858R	0.07	10	3 [+]	-	Erlotinib	PR (-65%)
10	F	81	0	L858R	5.38	4	3	TP53 (P60X, splice) PIK3CA (E545K)	Pem	PR (-33%)
11	F	70	0	Del19	0.31	3	1	TP53 (R282W)	Pem	NE
12	F	58	0	Del19	0.24	6	2	-	Erlotinib	PR (-68%)
13	F	54	0	Del19	2.24	3	2	-	Pem / Cb	SD (9%)
14	F	59	10	Del19	0.14	2	1	TP53 (I232S)	Gefitinib	PR (-50%)
15	F	67	2	L858R	0.30	3	1	EGFR (K860I)	Erlotinib	SD (-20%)
16	M	61	20	Del19	0.70	5	3 [++]	TP53 (E343*, C238Y, C135X)	Afatinib	SD (-18%)
17	F	54	3	Del19	3.95	2	1	TP53 (R249S)	Gefitinib	PR (-32%)
18	F	65	0	Del19	0.68	1	1	CTNNB1 (S37C)	Gefitinib	PR (-32%)

M: Male. F: Female. AF: Allelic Fraction. BVZ: Bevacizumab. Pem / Cis: Pemetrexed / Cisplatin. Pem / Cb: Pemetrexed / Carboplatin.
NE: Not evaluable. SD: Stable disease. PR: Partial Response
other mutation at the moment of *T790M* positive in the liquid biopsy (all patients had the common *EGFR* mutation at the time of *T790M* mutation positive, except patient number 15 whom original *EGFR Del19* mutation was not found at acquired resistance).
[+]This patient had already received rociletinib. [++]This patient has already been treated with osimertinib.

Table 1. Patients' characteristics with *T790M* mutation positive in a liquid biopsy who received osimertinib

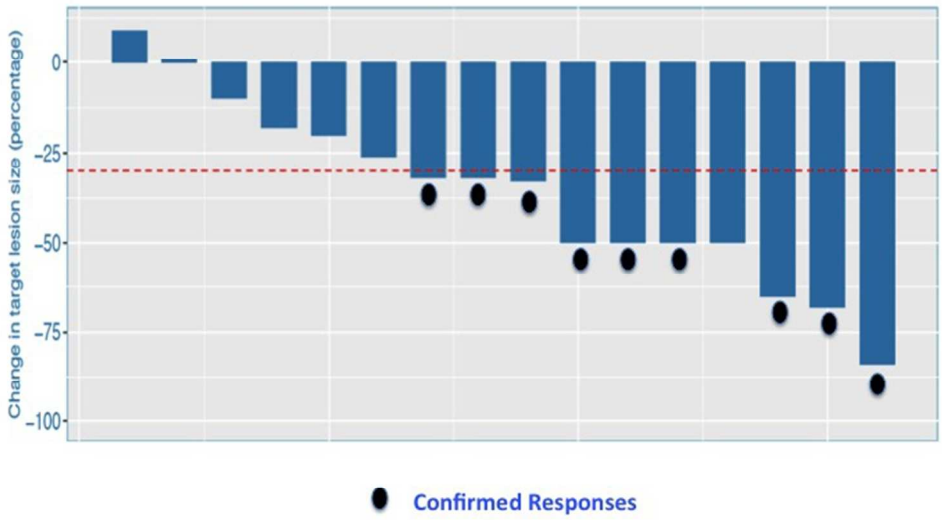

Figure 1. Best percentage change in target-lesion size
(Waterfall plot of *T790M* positive NSCLC patients in a liquid biopsy treated with osimertinib)

254x190mm (72 x 72 DPI)

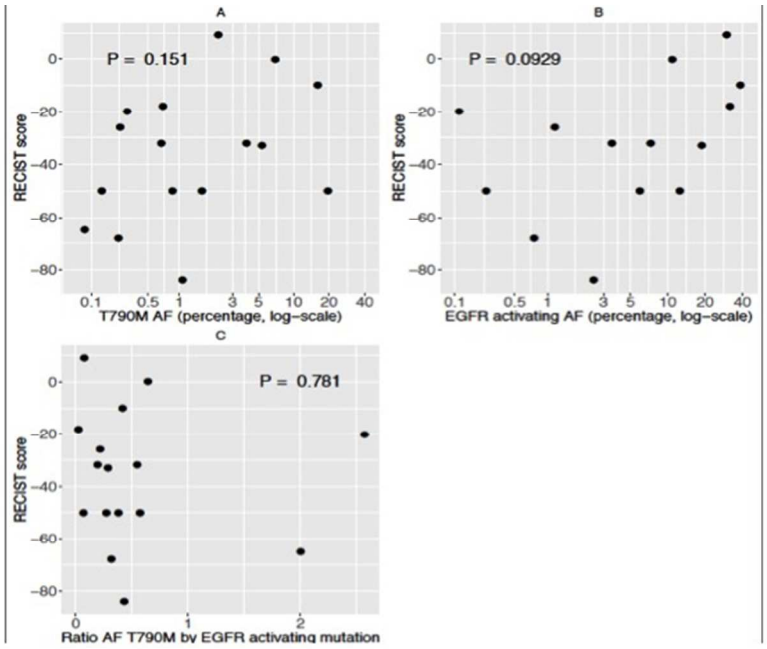

Figure 2. Correlation between RECIST radiological responses with osimertinib and three *ct*DNA predictors: (A) *T790M* allele fraction, (B) *EGFR* activating mutation allele fraction, and (C) T790M by *EGFR* activating mutation allele fraction ratio

254x190mm (72 x 72 DPI)

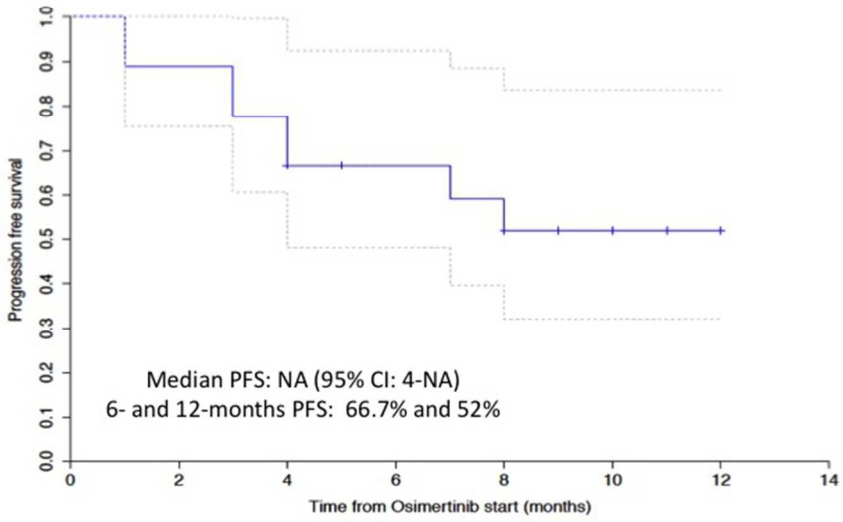

Figure 3. Progression Free Survival (PFS) by RECIST 1.1 criteria
NA: not achieved

254x190mm (72 x 72 DPI)

Appendix 1. Supplement.

The InVision assay, sequences regions in 35 genes including a panel of "hotspot" regions of interest and full gene coverage of selected genes. Sequences were generated using Illumina sequencing and molecular analysis, including status for T790M mutation assessed based on Inivata's validated ISoMA analytical pipeline. In this study, samples were analysed using a combination of Inivata core gene panels version 1.2 for the first 15 patients and version 1.4 for the remaining 33 patients [16].

Figure S1. Flowchart presenting T790M status and osimertinib treatment. PD: progressive disease. CT: chemotherapy

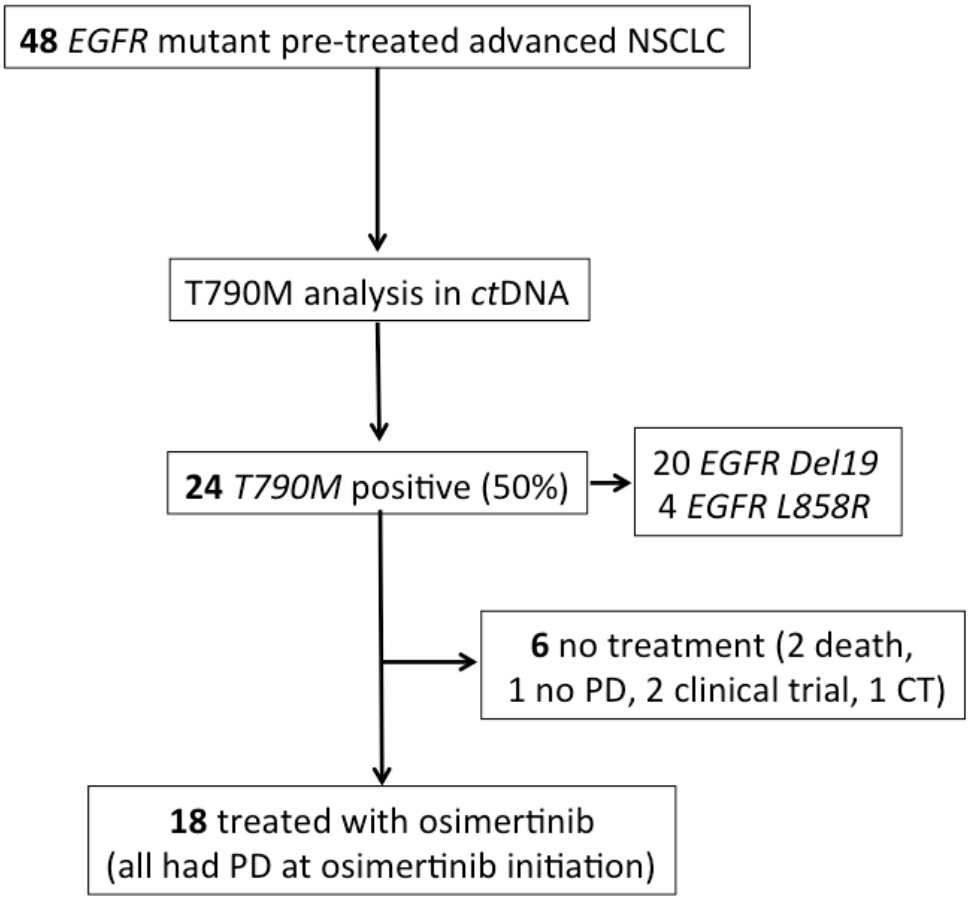

Figure S2. Progression Free Survival (PFS) by investigator. NA: not achieved

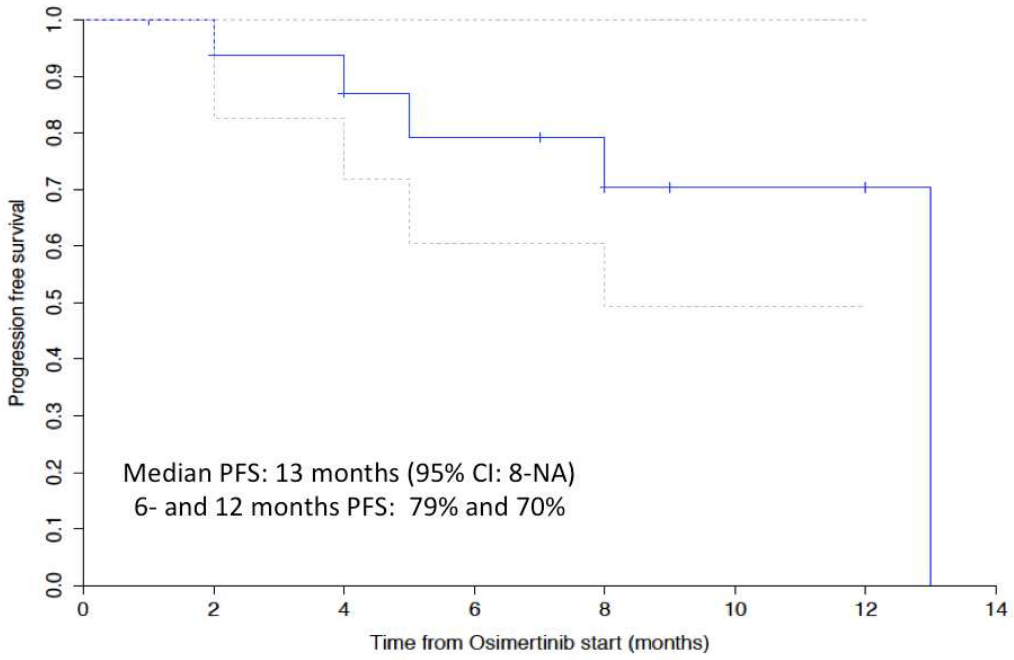

Figure S3. Overall survival (OS) on osimertinib. NA: not achieved

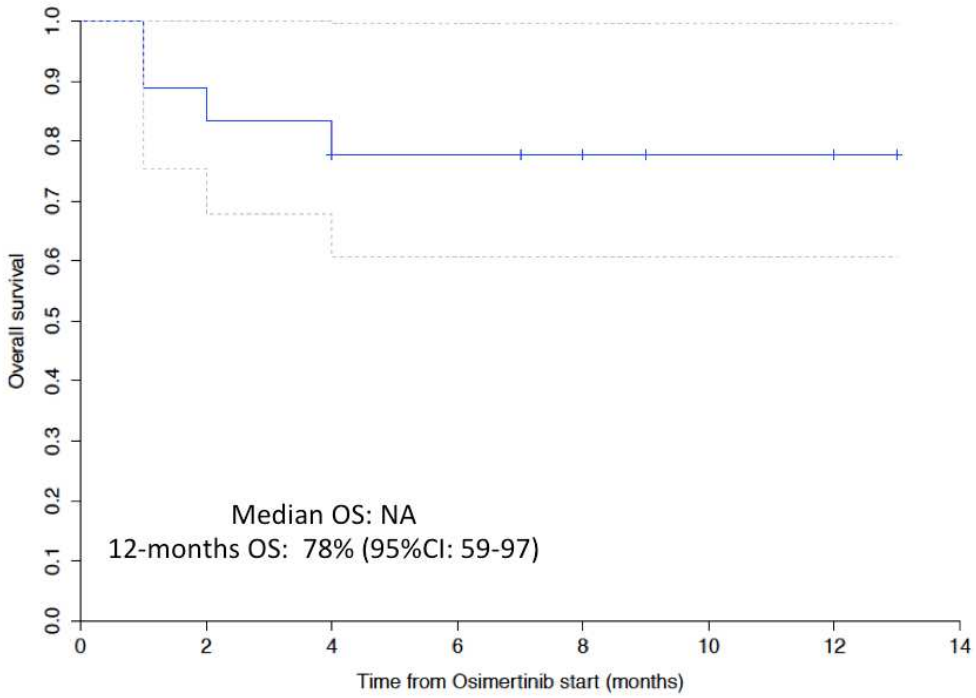
Overall survival
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
2
4
6
8
10
12
14
Time from Osimertinib start (months)
Median OS: NA
12-months OS: 78% (95%CI: 59-97)

Table S1. Allele fraction of *EGFR* activating mutation and *T790M* mutation positive.

Patient	Gender	Age (years)	*EGFR* Mutation subtype	AF of *T790M (%)*	AF of EGFR mutation (%)
1	M	51	Del19	0.41	0.93
2	F	56	Del19	15.96	38.46
3	M	54	Del19	0.86	12.45
4	F	37	Del19	1.06	2.48
5	M	67	Del19	1.60	5.93
6	F	83	Del19	6.96	10.84
7	F	67	Del19	19.60	51.61
8	F	70	L858R	0.25	1.15
9	F	66	L858R	0.07	0.03
10	F	81	L858R	5.38	18.8
11	F	70	Del19	0.31	1.51
12	F	58	Del19	0.24	0.75
13	F	54	Del19	2.24	29.76
14	F	59	Del19	0.14	0.25
15	F	67	L858R	0.30	0.11
16	M	61	Del19	0.70	31.73
17	F	54	Del19	3.95	7.25
18	F	65	Del19	0.68	3.50
19	M	67	Del19	4.60	25.25
20	F	70	Del19	7.54	37.39
21	M	54	Del19	0.10	18.05
22*	M	55	Del19	4.02	25.01
23	F	48	Del19	0.11	0.31
24*	F	67	Del19	0.59	2.36

M: Male. F: Female. AF: Allelic Fraction. *: Patients with *C797S* mutation (both currently treated with osimertinib)