

Manuscript type: Original investigation

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

J. Remon¹, C. Caramella², C. Jovelet³, L. Lacroix³, A. Lawson⁴, S. Smalley⁴, K. Howarth⁴, D. Gale^{4,5}, E. Green⁴, V. Plagnol⁴, N. Rosenfeld^{4,5,6}, D. Planchard¹, MV. Bluthgen¹, A. Gazzah¹, C. Pannet¹, C. Nicotra¹, E. Auclin¹, JC. Soria^{1,7}, B. Besse^{1,7}

¹Department of Oncology Medicine, Gustave Roussy, Université Paris-Saclay, Villejuif, France; ²Department of Radiology, Gustave Roussy, Université Paris-Saclay, Villejuif, France; ³ Translational Research Laboratory, AMMICA, INSERM US23/CNRS UNS3655, Gustave Roussy, Villejuif, France; ⁴Inivata Ltd., Cambridge, United Kingdom; ⁵Cancer Research UK Cambridge Institute, University of Cambridge, Cambridge, United Kingdom; ⁶Cambridge Cancer Centre, Cambridge, United Kingdom; ⁷University Paris-Sud and Gustave Roussy Cancer Campus, Villejuif, France;

Word count: 3,399 words

Corresponding author

Prof. Benjamin Besse,

University Paris-Sud and Gustave Roussy Cancer Campus

114 Rue Edouard Vaillant

94805 Villejuif, France

Phone: +33 (0)1 42 11 43 22

Fax: +33 (0)1 42 11 52 19

E-mail: Benjamin.BESSE@gustaveroussy.fr

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 (ctDNA) from blood samples in progressing advanced *EGFR*-mutant NSCLC patients.

Material and methods: ctDNA *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 ctDNA.

Results: The ctDNA *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: ctDNA 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 useful 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 (*ctDNA*) 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 *ctDNA* 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 *ctDNA 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 *ctDNA* 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 *ctDNA* 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 *ctDNA* 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 *ctDNA* was reported in 24 out of 48 (50%) NSCLC patients. (Figure S1)

Activating *EGFR* mutational status in *ctDNA* 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 *ctDNA 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 *ctDNA*, 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 *ctDNA* 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 *ctDNA 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 *ctDNA* 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 *ctDNA* 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 *ctDNA* 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 $\geq 0.06\%$) [17].

Liquid biopsies based on *ctDNA* 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 *ctDNA* (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 *ctDNA* and tumour-derived genotyping, *ctDNA* 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 *ctDNA* 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 *ctDNA* 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, *ctDNA* 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 *ctDNA* 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 *ctDNA 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 *ctDNA* 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]. *ctDNA* 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 *ctDNA*, 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.

Funding: No funding to report

Conflicts of interests: Authors affiliated with Inivata Ltd (AL, SS, KH, DG, NR, EG and VP) are employees, officers and/or share-holders of Inivata Ltd. Inivata Ltd commercialises assays based on the technology described in this paper. Other authors declare no conflicts of interest.

References

1. Midha A, Dearden S, McCormack R. EGFR mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: a systematic review and global map by ethnicity (mutMapII). *Am J Cancer Res* 2015; 5(9):2892–2911.
2. Reguart N, Remon J. Common EGFR-mutated subgroups (Del19/L858R) in advanced non-small-cell lung cancer: chasing better outcomes with tyrosine-kinase inhibitors. *Future Oncol* 2015:1–13.
3. Sequist LV, Waltman BA, Dias-Santagata D et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* 2011; 3(75):75ra26.
4. Yu HA, Arcila ME, Rekhtman N et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin. Cancer Res.* 2013; 19(8):2240–2247.
5. Jänne PA, Yang JC-H, Kim D-W et al. AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. *N. Engl. J. Med.* 2015; 372(18):1689–1699.
6. <http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm>. .
7. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/004124/WC500202022.pdf. .
8. Remon J, Faivre L, Facchinetti F et al. Radiogenomics in 332 metastatic non-small cell lung cancer (NSCLC) patients. *ASCO Meeting Abstracts* 2016; 34(15_suppl):11563.
9. Piotrowska Z, Nierdest MJ, Mino-Kenudson M, Morales-Oyarvide V, Fulton L, Lockerman E, Howe E, Gainor JF. Variation in mechanisms of acquired resistance among EGFR-mutant NSCLC patients with more than 1 postresistance biopsy. *Int J Radiat Oncol* 2014 ; 90:S6–S7.
10. Jovelet C, Ileana E, Le Deley M-C et al. Circulating Cell-Free Tumor DNA Analysis of 50 Genes by Next-Generation Sequencing in the Prospective MOSCATO Trial. *Clin. Cancer Res.* 2016; 22(12):2960–2968.

11. Murtaza M, Dawson S-J, Pogrebniak K et al. Multifocal clonal evolution characterized using circulating tumour DNA in a case of metastatic breast cancer. *Nat Commun* 2015; 6:8760.
12. Jackman D, Pao W, Riely GJ et al. Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. *J. Clin. Oncol.* 2010; 28(2):357–360.
13. Gale D, Plagnol V, Lawson A, Pugh M, Smalley S, Howarth K, Madi M, Durhman B et al. Analytical performance and validation of an enhanced TAm-Seq circulating tumor DNA sequencing assay. AACR 2016, New Orleans, Abstract 3639 .
14. Forshew T, Murtaza M, Parkinson C et al. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. *Sci Transl Med* 2012; 4(136):136ra68.
15. Therasse P, Arbuck SG, Eisenhauer EA et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J. Natl. Cancer Inst.* 2000; 92(3):205–216.
16. Mok TS, Wu Y-L, Ahn M-J et al. Osimertinib or Platinum-Pemetrexed in EGFR T790M-Positive Lung Cancer. *N. Engl. J. Med.* 2016. doi:10.1056/NEJMoa1612674.
17. Oxnard GR, Thress KS, Alden RS et al. Association Between Plasma Genotyping and Outcomes of Treatment With Osimertinib (AZD9291) in Advanced Non-Small-Cell Lung Cancer. *J. Clin. Oncol.* 2016; 34(28):3375–3382.
18. Marchetti A, Palma JF, Felicioni L et al. Early Prediction of Response to Tyrosine Kinase Inhibitors by Quantification of EGFR Mutations in Plasma of NSCLC Patients. *J Thorac Oncol* 2015; 10(10):1437–1443.
19. Mok T, Wu Y-L, Lee JS et al. Detection and Dynamic Changes of EGFR Mutations from Circulating Tumor DNA as a Predictor of Survival Outcomes in NSCLC Patients Treated with First-line Intercalated Erlotinib and Chemotherapy. *Clin. Cancer Res.* 2015; 21(14):3196–3203.
20. Sorensen BS, Wu L, Wei W et al. Monitoring of epidermal growth factor receptor tyrosine kinase inhibitor-sensitizing and resistance mutations in the plasma DNA of patients with advanced non-small cell lung cancer during treatment with erlotinib. *Cancer* 2014; 120(24):3896–3901.
21. <https://www.qiagen.com/gb/shop/detection-solutions/personalized-healthcare/therascreen-egfr-plasma-rgq-pcr-kit-emea/> .
22. <http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm504540.htm> .

23. Sequist LV, Soria J-C, Goldman JW et al. Rociletinib in EGFR-mutated non-small-cell lung cancer. *N. Engl. J. Med.* 2015; 372(18):1700–1709.
24. Sundaresan TK, Sequist LV, Heymach JV et al. Detection of T790M, the Acquired Resistance EGFR Mutation, by Tumor Biopsy versus Noninvasive Blood-Based Analyses. *Clin. Cancer Res.* 2016; 22(5):1103–1110.
25. Reckamp KL, Melnikova VO, Karlovich C et al. A Highly Sensitive and Quantitative Test Platform for Detection of NSCLC EGFR Mutations in Urine and Plasma. *J Thorac Oncol* 2016; 11(10):1690–1700.
26. Thress KS, Brant R, Carr TH et al. EGFR mutation detection in ctDNA from NSCLC patient plasma: A cross-platform comparison of leading technologies to support the clinical development of AZD9291. *Lung Cancer* 2015; 90(3):509–515.
27. Hata A, Katakami N, Yoshioka H et al. Spatiotemporal T790M Heterogeneity in Individual Patients with EGFR-Mutant Non-Small-Cell Lung Cancer after Acquired Resistance to EGFR-TKI. *J Thorac Oncol* 2015; 10(11):1553–1559.
28. Lim C, Sung M, Shepherd FA et al. Patients with Advanced Non-Small Cell Lung Cancer: Are Research Biopsies a Barrier to Participation in Clinical Trials? *J Thorac Oncol* 2016; 11(1):79–84.
29. Chabon JJ, Simmons A, Newman AM et al. Inter- and intra-patient heterogeneity of resistance mechanisms to the mutant EGFR selective inhibitor rociletinib. *ASCO Meeting Abstracts* 2016; 34(15_suppl):9000.

| 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

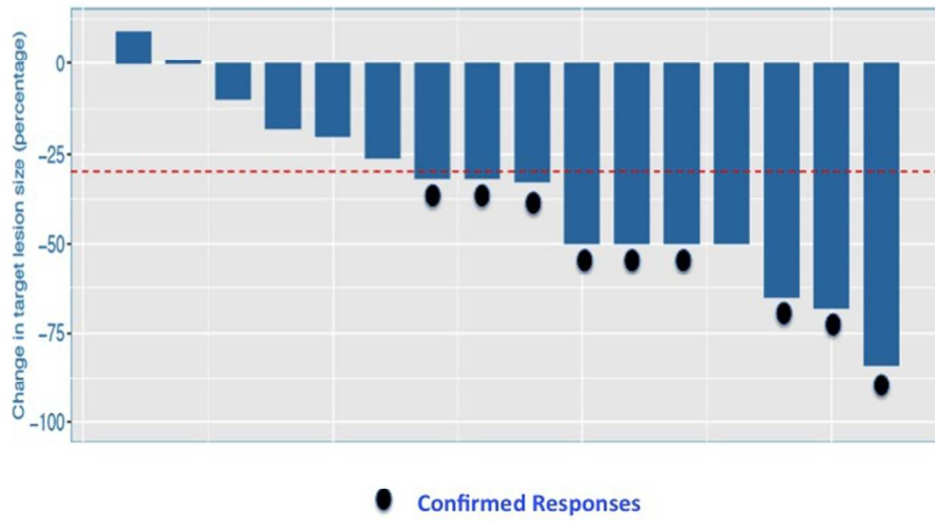


Figure 1. Best percentage change in target-lesion size (Waterfall plot of *T790M* positive NSCLC patients in a liquid biopsy treated with osimertinib)

254x190mm (72 x 72 DPI)

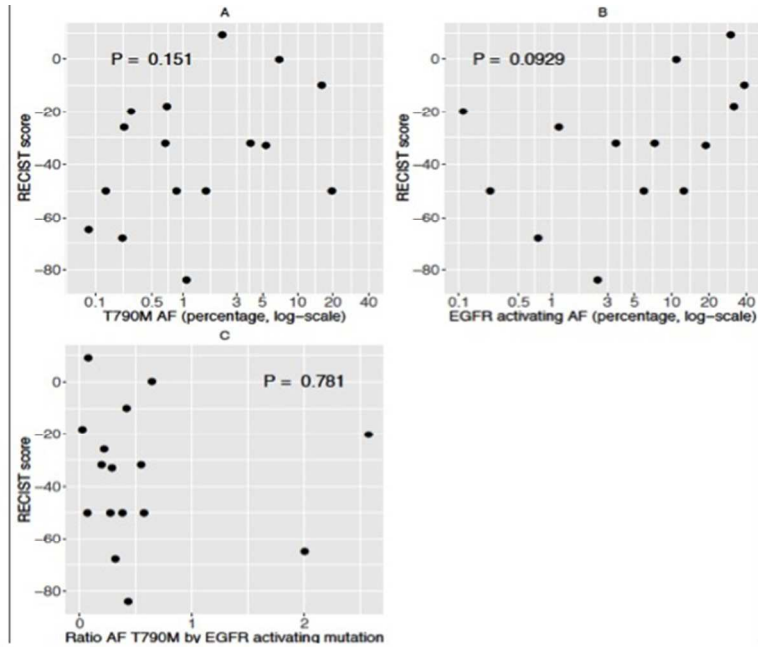


Figure 2. Correlation between RECIST radiological responses with osimertinib and three *ctDNA* predictors: (A) *T790M* allele fraction, (B) *EGFR* activating mutation allele fraction, and (C) *T790M* by *EGFR* activating mutation allele fraction ratio

254x190mm (72 x 72 DPI)

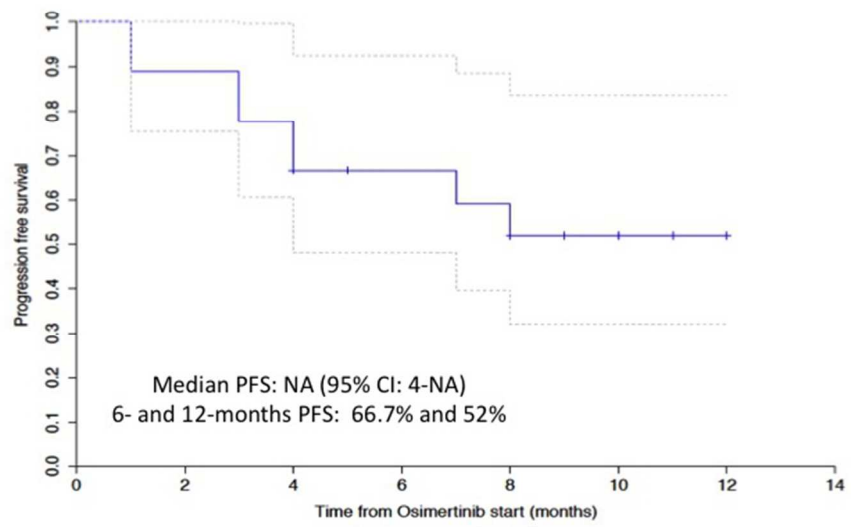


Figure 3. Progression Free Survival (PFS) by RECIST 1.1 criteria
 NA: not achieved

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

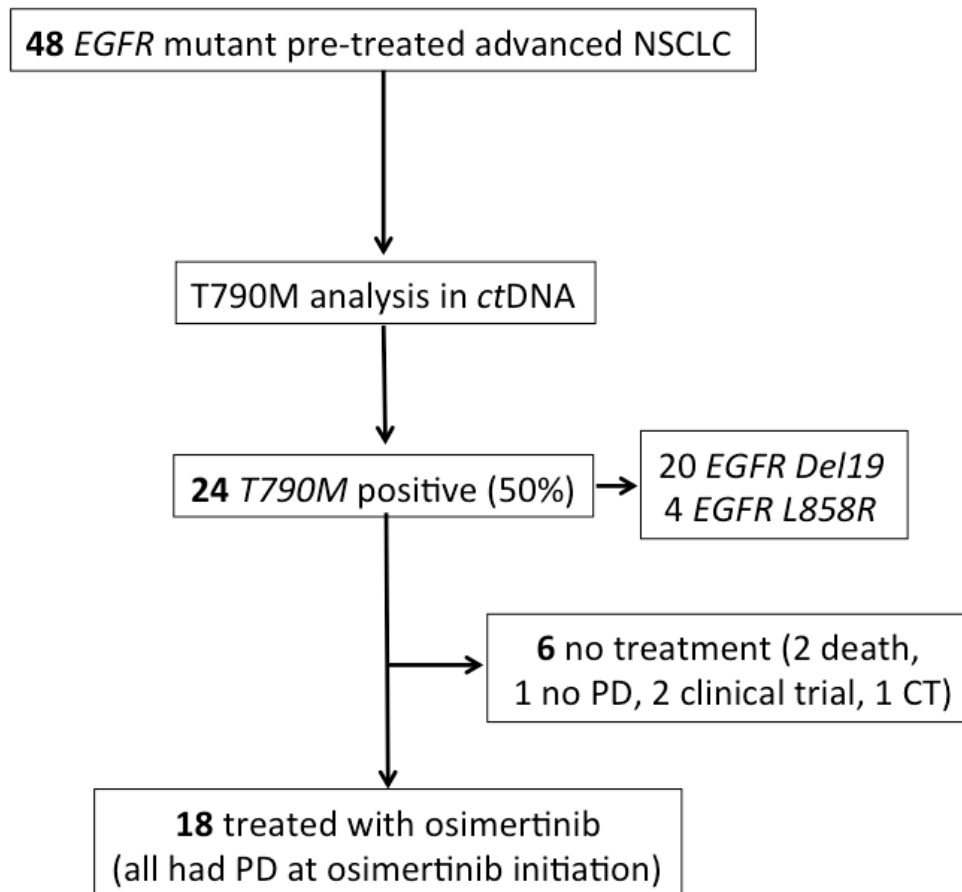


Figure S2. Progression Free Survival (PFS) by investigator. NA: not achieved

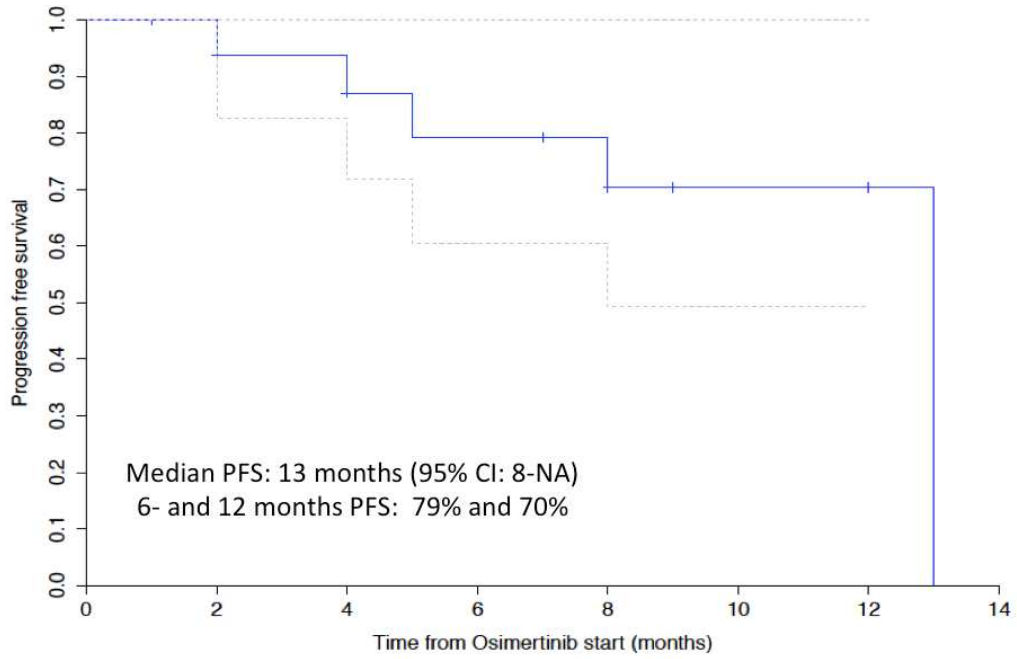


Figure S3. Overall survival (OS) on osimertinib. NA: not achieved

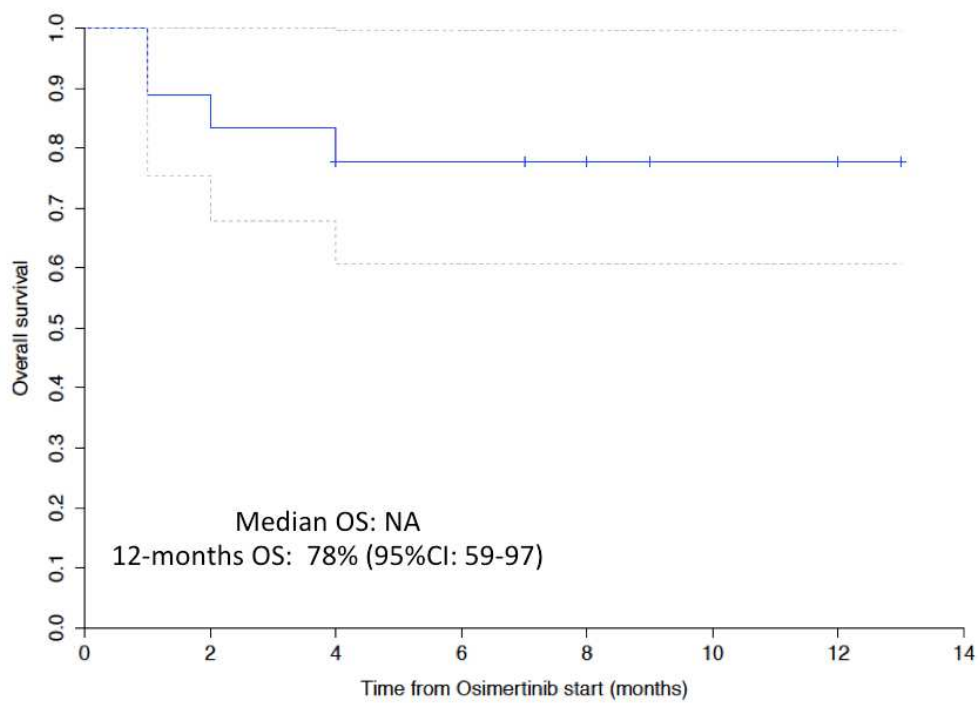


Table S1. Allele fraction of *EGFR* activating mutation and *T790M* mutation positive.

| Patient | Gender | Age (years) | <i>EGFR</i> Mutation subtype | AF of <i>T790M</i> (%) | AF of <i>EGFR</i> 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)