Original Article

EGFR mutation tracking predicts survival in advanced *EGFR*-mutated non-small cell lung cancer patients treated with osimertinib

Anna Buder[1#], Maximilian J. Hochmair[2#], Ulrike Setinek[3], Robert Pirker[4], Martin Filipits[1]

[1]Institute of Cancer Research, Department of Medicine I, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria; [2]Karl Landsteiner Institute of Lung Research and Pulmonary Oncology, Department of Respiratory and Critical Care Medicine, Hospital North, Vienna, Austria; [3]Institute for Pathology and Bacteriology, Otto Wagner Hospital, Vienna, Austria; [4]Division of Oncology, Department of Medicine I, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria

Contributions: (I) Conception and design: All authors; (II) Administrative support: A Buder, MJ Hochmair, M Filipits; (III) Provision of study materials or patients: MJ Hochmair, U Setinek; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Prof. Martin Filipits. Institute of Cancer Research, Department of Medicine I, Comprehensive Cancer Center, Medical University of Vienna, Borschkegasse 8a, 1090 Vienna, Austria. Email: martin.filipits@meduniwien.ac.at.

Background: Osimertinib has become standard therapy of advanced epidermal growth factor receptor (*EGFR*)-mutated non-small cell lung cancer (NSCLC) patients and T790M-mediated resistance. We investigated the clinical utility of *EGFR* mutation tracking in plasma-based circulating tumor DNA (ctDNA) after start of osimertinib therapy in metastatic, *EGFR*-mutant NSCLC patients who had progressed on prior therapy with EGFR tyrosine kinase inhibitors (TKIs).

Methods: We enrolled 141 patients with advanced *EGFR*-mutated NSCLC who underwent second-line osimertinib treatment for T790M-positive disease. After initiation of osimertinib, we obtained plasma samples from 108 patients. Plasma ctDNA was tested for *EGFR* mutations by means of droplet digital PCR and was termed positive if any *EGFR* mutation was detected.

Results: Plasma ctDNA was detected in 58 of 108 (54%) patients after osimertinib initiation and was associated with poor progression-free survival (PFS) [hazard ratio (HR) 4.26, 95% confidence interval (CI): 2.55–7.10, P<0.0001] and overall survival (OS) (HR 3.23, 95% CI: 1.80–5.78, P<0.0001). In multivariable analysis, ctDNA status remained significantly associated with PFS and OS (HR 4.87, 95% CI: 2.81–8.44, P<0.0001; HR 3.49, 95% CI: 1.88–6.50, P<0.0001). Patients with persistence of activating EGFR mutations within eight weeks had shorter durations of PFS (HR 6.17, 95% CI: 3.03–12.56, P<0.0001) and OS (HR 4.83, 95% CI: 2.25–10.36, P<0.0001) than patients with total clearance of the activating EGFR mutation. Persistence of activating EGFR mutations in plasma ctDNA remained an independent predictor of poor PFS and OS in multivariable analyses.

Conclusions: Patients with persistence of activating *EGFR* mutations in plasma ctDNA within eight weeks after osimertinib initiation have worse prognosis and may require the addition of chemotherapy or other treatments in order to achieve better outcome.

Keywords: Advanced non-small cell lung cancer (advanced NSCLC); epidermal growth factor receptor mutations (*EGFR* mutations); circulating tumor DNA (ctDNA); droplet digital PCR (ddPCR)

Submitted Jan 10, 2020. Accepted for publication Feb 23, 2020.
doi: 10.21037/tlcr.2020.03.02
View this article at: http://dx.doi.org/10.21037/tlcr.2020.03.02

Introduction

Osimertinib has been established as standard treatment for advanced epidermal growth factor receptor (EGFR) mutated non-small cell lung cancer (NSCLC). The superior efficacy of osimertinib has been shown in two phase III trials (1,2). In the AURA3 trial, osimertinib prolonged progression-free survival (PFS) over platinum-based chemotherapy in pretreated patients with advanced *EGFR*-mutated NSCLC and T790M-mediated resistance (1). The results of this trial led to the approval of second-line osimertinib, for patients with confirmed T790M mutation in tumor tissue or cell-free plasma DNA. In the FLAURA phase III trial, osimertinib showed superior efficacy compared to erlotinib and gefitinib in the first-line treatment of NSCLC with common *EGFR* mutations, irrespective of the T790M status (2). Osimertinib was therefore approved as a first-line treatment for advanced NSCLC with *EGFR* exon 19 deletions or L858R mutations.

The analysis of T790M in plasma-based circulating tumor DNA (ctDNA) complemented by tumor tissue biopsies in case of a T790M-negative result in plasma is currently considered the preferred strategy to select *EGFR*-mutated NSCLC patients for second-line therapy with osimertinib (3-8). Continuous monitoring of the tumor genotype could also be important for early identification of emerging changes in tumor biology that negatively affect treatment outcome. In particular, tracking of *EGFR* mutations may be important for response evaluation, real-time assessment of resistance evolution and treatment guidance (9-12). To this end, we investigated the clinical utility of *EGFR* mutation tracking in plasma ctDNA after start of osimertinib therapy in patients who developed resistance to prior treatment with EGFR tyrosine kinase inhibitors (TKIs).

Methods

Patients

Patients with metastatic *EGFR*-mutant NSCLC received second-line osimertinib after detection of a T790M mutation in plasma ctDNA and/or tissue re-biopsy at the Department of Respiratory and Critical Care Medicine, and Ludwig Boltzmann Institute of COPD and Respiratory Epidemiology, Otto Wagner Hospital, Vienna, between February 2016 and August 2017. Diagnostic biopsies were available from each included patient and showed adenocarcinoma histology and *EGFR* mutations in all cases. Blood sampling was performed as part of diagnostic routine procedures. *EGFR* mutation analyses were carried out at the Institute of Cancer Research, Department of Medicine I, Medical University of Vienna. The collection and analysis of blood samples was approved by the local ethics committee (EK No. 1132/2016) and informed consent was obtained from all patients. Forty patients had been included in a previous study (6).

Plasma genotyping

Preparation and storage of blood samples was done as previously described (6). In brief, Cell-Free DNA Blood Collection Tubes (Streck, La Vista, NE, USA) or Cell Free DNA Blood Collection Tubes (Roche, Pleasanton, CA, USA) were used for blood sampling and one blood sample (8 mL) was obtained from all patients at each time point.

For plasma isolation, blood samples were centrifuged at increasing speed (10 minutes at 200 g followed by 10 minutes at 1,600 g). The supernatant was collected and centrifuged again for 10 minutes at 1,900 g.

For ddPCR, we extracted ctDNA from 2 mL plasma using the QIAamp circulating nucleic acid kit (Qiagen, Venlo, The Netherlands) according to manufacturer's instructions.

EGFR deletions in exon 19, L858R, L861Q, S768I, T790M and C797S mutations were assessed by using the QX-200™ ddPCR system (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions.

Custom assays for ddPCR from Life Technologies (Carlsbad, CA, USA) and ddPCR assays from Bio-Rad were used for *EGFR* mutation analysis as previously described (6). We used QuantaSoft analysis software (Bio-Rad) for qualitative and quantitative mutation analysis. All ddPCR assays were performed blinded to the study endpoint and analyzed in triplicate. Finally, the absolute copy-number of mutant alleles per mL of plasma was calculated. We used a threshold of >1 copy/mL for positivity of each mutation analyzed. Plasma ctDNA was termed positive if any *EGFR* mutation was detected.

Statistical analyses

We used PFS as assessed by investigators as the primary study endpoint. PFS was defined as the time from first osimertinib dose to disease progression or death from any cause, whichever came first. Overall survival (OS) and response rate (RR) were secondary endpoints. OS was defined as the time from first osimertinib dose to death

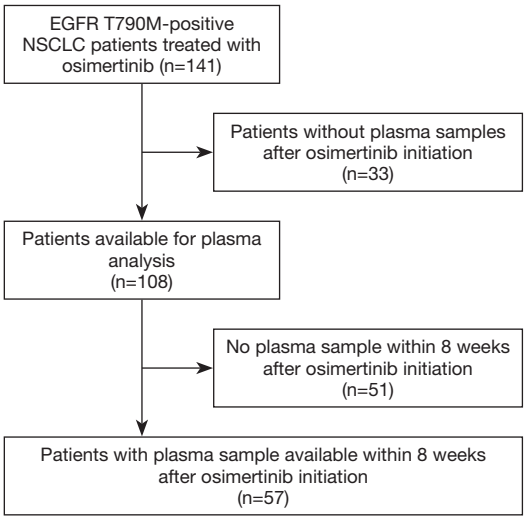

Figure 1 Study flowchart for the process of patient selection.

from any cause. RR was defined as the percentage of patients with response (complete or partial) at restaging after osimertinib initiation. Regular CT scans of the chest and abdomen, usually performed every 6–8 weeks were used to assess tumor response at the medical center of the treating physician according to institutional practice. Additionally, response was confirmed post hoc using Response Evaluation Criteria in Solid Tumors (RECIST) 1.1.

Characteristics of patients included age, gender, presence or absence of extra-thoracic metastases, tissue genotype at diagnosis, and previous EGFR TKI therapy. We used the chi-square test or fisher's exact test to assess associations of plasma genotyping results with clinical parameters and with treatment response. Survival probabilities were calculated with the product limit method according to Kaplan-Meier. Hazard ratios (HRs) and their confidence intervals (CIs) were estimated using univariable and multivariable Cox proportional hazards models. For the multivariable analyses we used full models and stepwise backward selection models that included age (as continuous variable), gender (male, female), presence or absence of extra-thoracic metastases (thoracic, extra-thoracic), tissue genotype at diagnosis (*EGFR* deletions in exon 19, L858R, other *EGFR* mutations), previous EGFR TKI therapy (afatinib, erlotinib, gefitinib, >1 EGFR TKI), and ctDNA status (positive, negative) or status of the activating *EGFR* mutation (detectable, not detectable). All reported P values are two sided. All analyses were performed using IBM SPSS Statistics software, version 25 (SPSS, IBM Corporation, Armonk, NY, USA).

Results

Plasma samples of 141 patients who progressed under first- or second-generation EGFR-TKI therapy were centrally tested for activating *EGFR* mutations (*EGFR* exon 19 deletions, L858R, L861Q, S768I) and the T790M mutation by ddPCR.

At the start of osimertinib, all 141 patients were T790M positive and 122 of 141 (87%) patients were also positive for the corresponding activating *EGFR* mutations. The 19 patients who were T790M positive but in whom the activating *EGFR* mutation was not detectable were also treated with osimertinib.

EGFR mutation tracking in plasma ctDNA was performed in 108 patients (including 15 of 19 T790M-positive but activating *EGFR* mutation-negative patients) undergoing osimertinib treatment for T790M-positive NSCLC after progression under treatment with an EGFR TKI and in whom a plasma sample was available. The study flowchart is shown in *Figure 1*. Blood sampling was performed at several time points during osimertinib treatment starting with the first osimertinib dose. The number of serial samples collected from each patient throughout osimertinib therapy to assess *EGFR* deletions in exon 19, L858R, L861Q, S768I, T790M, and C797S mutations varied between two and thirteen samples. As shown in *Table 1*, only patients with lung adenocarcinoma histology and stage IV disease were enrolled. Prior EGFR TKIs included gefitinib, erlotinib and afatinib and osimertinib was initiated as second-line treatment without exceptions. All patients were T790M mutation-positive assessed by plasma genotyping and/or tissue re-biopsy testing. The tissue genotype at diagnosis included *EGFR* exon 19 deletions in 68 (63%) patients, L858R in 30 (28%), and other *EGFR* mutations in 10 (9%) patients.

Fifty-eight out of 108 (54%) patients treated with osimertinib were ctDNA positive. Samples were classified as positive if any *EGFR* activating or resistance mutation was detected. ctDNA was more frequently detected in males than in females (P=0.009) but no other correlation between ctDNA status and clinical variables was seen (*Table 1*). Eighty-two percent of the patients responded to osimertinib.

At a median follow-up of 32.3 months (95% CI: 29.1–35.6 months), 75 of 108 (69%), patients had progressed and 57 of 108 (53%) had died. Median PFS was 12.0 months (95% CI: 9.1–14.9 months) and median OS was 21.1 months (95% CI: 11.4–30.7 months). In univariate

Table 1 Patient characteristics

Characteristics	No. of patients, N=108	ctDNA not detected, N=50	ctDNA detected, N=58	P value
Age (years)				0.06
Median [range]	69 [38–86]	71 [38–86]	66 [45–83]	
<65 years	38 (35%)	13 (26%)	25 (43%)	
≥65 years	70 (65%)	37 (74%)	33 (57%)	
Gender				0.009
Male	28 (26%)	7 (14%)	21 (36%)	
Female	80 (74%)	43 (86%)	37 (64%)	
Metastases				0.12
Thoracic	31 (29%)	18 (36%)	13 (22%)	
Extra-thoracic	77 (71%)	32 (64%)	45 (78%)	
EGFR tissue genotype				0.53
Exon 19 deletion	68 (63%)	31 (62%)	37 (64%)	
L858R	30 (28%)	14 (28%)	16 (28%)	
L861Q	4 (4%)	1 (2%)	3 (5%)	
G719X	1 (1%)	0 (0%)	1 (2%)	
Exon 20 insertion	1 (1%)	1 (2%)	0 (0%)	
L858R/L861Q	1 (1%)	1 (2%)	0 (0%)	
L858R/S768I	1 (1%)	1 (2%)	0 (0%)	
L858R/Exon 18 mutation	1 (1%)	0 (0%)	1 (2%)	
G719X/S768I	1 (1%)	1 (2%)	0 (0%)	
Previous EGFR TKI therapy				0.79
Afatinib	47 (44%)	20 (40%)	27 (47%)	
Erlotinib	10 (9%)	6 (12%)	4 (7%)	
Gefitinib	34 (32%)	16 (32%)	18 (31%)	
>1 EGFR TKI	17 (16%)	8 (16%)	9 (16%)	

Percentages may not total 100 because of rounding. ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

analyses, PFS and OS were independent of age, gender, tissue genotype at diagnosis, and previous EGFR TKI therapy but correlated with presence of extra-thoracic metastases (*Table 2*). Detectable plasma-based ctDNA was associated with shorter PFS (median 8.4 versus 29.4 months, HR 4.26, 95% CI: 2.55–7.10, P<0.0001) (*Table 2* and *Figure 2A*) and OS (median 15.3 versus not reached, HR 3.23, 95% CI: 1.80–5.78, P<0.0001) (*Table 2* and *Figure 2B*). Multivariate analysis revealed that ctDNA status was independently associated with PFS and OS (HR 4.87, 95% CI: 2.81–8.44, P<0.0001; HR 3.49, 95% CI: 1.88–6.50, P<0.0001) (*Table 2*).

Within eight weeks after osimertinib initiation, activating EGFR mutations and the T790M mutation were detected in plasma ctDNA of 19/57 (33%) and 8/57 (14%) patients, respectively. The C797S mutation was first detected 5.7 months after osimertinib initiation and was found in 6 of 57 (11%) patients.

We observed no association between clinical features and presence or absence of activating EGFR mutations (data not shown). Patients with persistence of the activating EGFR mutation had a significantly lower RR than patients without detectable activating EGFR mutations (24.5% versus 75.5%, P=0.001) (*Table 3*). Presence or absence of the T790M mutation had no impact on response to osimertinib (*Table 3*).

Patients with persisting activating EGFR mutations in ctDNA within eight weeks after the first dose of osimertinib had a significantly shorter PFS (median 3.4 versus 26.9 months; HR 6.17, 95% CI: 3.03–12.56, P<0.0001) (*Figure 2C* and *Table S1*) and shorter OS (median 9.4 versus

Table 2 Univariable and multivariable Cox proportional hazards models

Variable	Progression-free survival		Overall survival	
	Univariable	Multivariable	Univariable	Multivariable
	HR (95% CI); P value	HR (95% CI); P value	HR (95% CI); P value	HR (95% CI); P value
Age	1.00 (0.98–1.02); 0.87	1.01 (0.98–1.03); 0.48	1.01 (0.98–1.03); 0.73	1.02 (0.99–1.05); 0.23
Gender	0.94 (0.56–1.56); 0.81	1.56 (0.89–2.73); 0.12	0.84 (0.48–1.48); 0.55	1.17 (0.64–2.12); 0.61
Metastases	1.92 (1.07–3.44); 0.03	1.63 (0.89–2.98); 0.11	2.11 (1.06–4.17); 0.03	1.88 (0.94–3.76); 0.08
EGFR tissue genotype	1.29 (0.90–1.85); 0.17	1.44 (0.96–2.17); 0.08	1.39 (0.93–2.09); 0.11	1.62 (1.04–2.52); 0.03
Previous EGFR TKI therapy	1.00 (0.82–1.22); 0.99	1.04 (0.84–1.29); 0.70	1.16 (0.93–1.46); 0.19	1.26 (0.99–1.61); 0.06
ctDNA	4.26 (2.55–7.10); <0.0001	4.87 (2.81–8.44); <0.0001	3.23 (1.80–5.78); <0.0001	3.49 (1.88–6.50); <0.0001

ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor; HR, hazard ratio; 95% CI, 95% confidence interval.

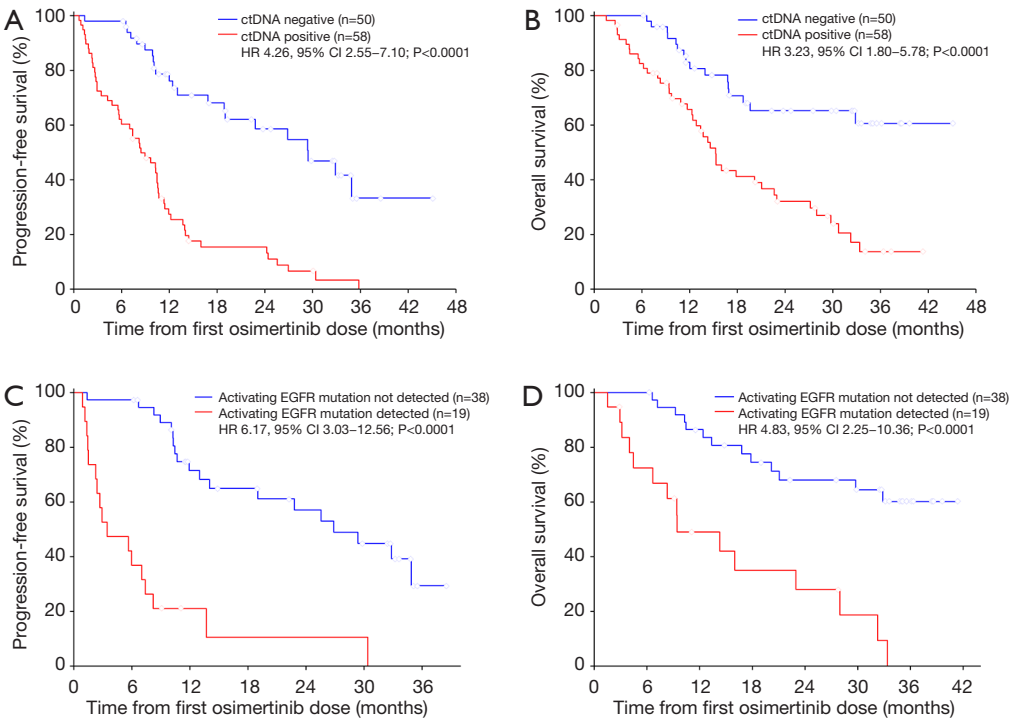

Figure 2 Kaplan-Meier curves for progression-free survival (A,C) and overall survival (B,D) according to plasma ctDNA status or persistence of the activating *EGFR* mutation in plasma ctDNA within eight weeks after osimertinib initiation. ctDNA, circulating tumor DNA; *EGFR*, epidermal growth factor receptor.

not reached; HR 4.83, 95% CI: 2.25–10.36, P<0.0001) (*Figure 2D* and *Table S1*) compared to patients with total clearance of the activating EGFR mutation in plasma. Similarly, presence of the T790M mutation in ctDNA correlated with shorter PFS (median 7.0 versus 19.0 months; HR 2.32, 95% CI: 1.00–5.37, P=0.05) and OS (median 16.0 versus 33.4 months; HR 2.76, 95% CI: 1.10–6.93, P=0.03) (*Table S1*). Multivariable analyses using stepwise backward elimination models showed that the persistence of activating *EGFR* mutations in plasma ctDNA was the only parameter that independently predicted shorter PFS (HR 7.83, 95% CI: 3.53–17.34, P<0.0001) and OS (HR 4.90, 95% CI: 2.25–

Table 3 Response to osimertinib

Mutation	CR/PR	SD/PD	P value
Activating EGFR mutation			0.001
Not detectable	37 (75.5%)	1 (12.5%)	
Detectable	12 (24.5%)	7 (87.5%)	
T790M mutation			1.0
Not detectable	42 (86%)	7 (87.5%)	
Detectable	7 (14%)	1 (12.5%)	

EGFR, epidermal growth factor receptor; CR, complete response; PR, partial response; SD, stable disease; PD, disease progression.

10.69, P<0.0001) of patients (*Table S1*).

Discussion

The findings of our present study suggest that tracking of *EGFR* mutations in plasma ctDNA by means of ddPCR during second-line therapy with osimertinib is clinically relevant in patients with advanced *EGFR*-mutated NSCLC. If activating *EGFR* mutations persist in plasma ctDNA within eight weeks after start of osimertinib, a shorter PFS and OS can be expected in the respective patients.

Similar findings have been reported by others (9-11). In an exploratory analysis of the FLAURA trial (11), persistence of activating *EGFR* mutations in ctDNA at three weeks and six weeks after start of osimertinib therapy was associated with shorter PFS (11). In an exploratory analysis of the FASTACT-2 study, patients with *EGFR* mutation-negative plasma samples at cycle 3 had longer PFS and OS than patients whose samples were still *EGFR* mutation positive at cycle 3 (9). In another study, patients with a higher allele frequency of the activating *EGFR* mutation or a higher activating *EGFR* mutation/T790M ratio had a shorter PFS compared to those with a lower allele frequency or a lower ratio (10).

Our results suggest that *EGFR* mutation tracking could be useful for guiding treatment in the future. Patients in whom the activating *EGFR* mutations in ctDNA are not found within eight weeks after osimertinib initiation, should continue with osimertinib. In these patients, a median PFS of 26.9 months was observed and median OS survival was not reached at a follow-up of 34.8 months. However, patients with persisting activating *EGFR* mutations in plasma ctDNA may require a change in treatment because of their poor outcome. They may benefit from the addition of chemotherapy to osimertinib or other treatments.

This possibility is supported by findings of phase III trials in the first-line setting, in which the combination of gefitinib with chemotherapy resulted in longer PFS and OS compared to gefitinib alone (13,14). Therefore, these treatment strategies warrant further investigation within clinical trials among patients with persistence of activating *EGFR* mutations.

Another treatment strategy in patients with persisting activating *EGFR* mutations could be chemo-immunotherapy (15). This treatment option is supported by an exploratory analysis of the IMpower150 trial, which indicated longer OS for the addition of atezolizumab to chemotherapy plus bevacizumab compared to chemotherapy plus bevacizumab in patients with advanced non-squamous *EGFR* mutant NSCLC (15).

Our findings indicate that *EGFR* mutation tracking during second-line osimertinib therapy provides clinically relevant information. Patients with persistence of activating *EGFR* mutations in plasma ctDNA eight weeks after the first dose of osimertinib have worse survival and may require other treatments such as the combination of osimertinib with chemotherapy or chemo-immunotherapy. All these treatment options should be explored within clinical trials in the future and may further improve the outcome of patients with advanced *EGFR*-mutated NSCLC.

Acknowledgments

Funding: The study was funded by AstraZeneca Austria.

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tlcr.2020.03.02). AB reports personal fees from AstraZeneca, outside the submitted work; MJH reports personal fees from AstraZeneca, personal fees from Boehringer Ingelheim, personal fees from Bristol-Myers Squibb, personal fees from Merck Sharp & Dohme, personal fees from Novartis, personal fees from Pfizer, personal fees from Roche, outside the submitted work; RP reports personal fees from AstraZeneca, personal fees from Boehringer Ingelheim, personal fees from Gedeon Richter, personal fees from Genmab, personal fees from Merck Sharp & Dohme, personal fees from Regeneron, personal fees from Roche, outside the submitted work; RP serves as the unpaid editorial board member of *Translational Lung Cancer Research* from Dec 2019 to Nov 2020. MF reports personal fees from AstraZeneca, personal fees from

Bayer, personal fees from Biomedica, personal fees from Boehringer Ingelheim, personal fees from Eli Lilly, personal fees from Merck Sharp & Dohme, personal fees from Myriad Genetics Inc., personal fees from Pfizer, personal fees from Roche, outside the submitted work; US has no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The collection and analysis of blood samples was approved by the local ethics committee (EK No. 1132/2016) and informed consent was obtained from all patients.

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Cite this article as: Buder A, Hochmair MJ, Setinek U, Pirker R, Filipits M. *EGFR* mutation tracking predicts survival in advanced *EGFR*-mutated non-small cell lung cancer patients treated with osimertinib. Transl Lung Cancer Res 2020;9(2):239-245. doi: 10.21037/tlcr.2020.03.02

Supplementary

Table S1 Univariable and multivariable Cox proportional hazards models

Variable	Progression-free survival		Overall survival	
	Univariable	Multivariable*	Univariable	Multivariable*
	HR (95% CI); P value	HR (95% CI); P value	HR (95% CI); P value	HR (95% CI); P value
Age	1.01 (0.98–1.05); 0.46	1.04 (0.997–1.08); 0.07	1.00 (0.97–1.04); 0.84	
Gender	0.76 (0.36–1.58); 0.45		0.71 (0.31–1.61); 0.41	
Metastases	2.64 (0.93–7.49); 0.07	2.84 (0.98–8.21); 0.054	2.80 (0.85–9.29); 0.09	2.77 (0.84–9.22); 0.1
EGFR tissue genotype	1.02 (0.60–1.75); 0.94		1.30 (0.73–2.29); 0.38	
Previous EGFR TKI therapy	0.91 (0.68–1.22); 0.54		1.09 (0.79–1.50); 0.61	
T790M	2.32 (1.00–5.37); 0.05		2.76 (1.10–6.93); 0.03	
Activating EGFR mutation	6.17 (3.03–12.56); <0.0001	7.83 (3.53–17.34); <0.0001	4.83 (2.25–10–36); <0.0001	4.90 (2.25–10.69); <0.0001

*, stepwise backward elimination mode. EGFR, epidermal growth factor receptor; HR, hazard ratio; 95% CI, 95% confidence interval.