

# Dynamic Changes of EGFR Activating Mutations as an Early Predictor of Progression in Non-Small Cell Lung Cancer Patients Treated with EGFR Tyrosine Kinase Inhibitors

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## Abstract

Serial monitoring of circulating tumor DNA may predict resistance to first-line epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors before CT-confirmed progression. Our objective was to evaluate dynamic changes in plasma EGFR-activating mutations as an early predictor of disease progression before clinical or radiological evidence. For this observational study, 35 patients with advanced, EGFR-positive, non-squamous, non-small cell lung cancer were enrolled. All patients were initiating or receiving EGFR tyrosine kinase inhibitors and had received at least one follow-up CT scan. Peripheral blood samples were collected at each clinical visit, and CT scans were scheduled every 8 to 16 weeks after tyrosine kinase inhibitor initiation and upon clinical/symptom progression. Of the 35 patients, 16 experienced reappearance of EGFR-activating mutations and disease progression. Of these, 12 patients experienced reappearance of EGFR-activating mutations before CT-confirmed progression, 3 had CT-confirmed progression one visit (8 weeks) before detectable EGFR-activating mutations, and 1 had EGFR-activating mutations reappear concurrently with CT-confirmed progression. EGFR-activating mutations reappeared at a mean of 10 weeks (median, 16 weeks) before routinely scheduled CT-confirmed progression. Using study-specific definitions, we observed 5 false positives and no false negatives. This study demonstrates that dynamic changes in circulating tumor DNA can predict resistance to EGFR-TKIs before CT-confirmed progression. Further prospective trials of this promising approach with potential benefits to patient care should be considered.

**Keywords:** Circulating tumor DNA • CT scan • Disease progression • Epidermal growth factor receptor mutation • Lung cancer

## Introduction

Lung cancer is the leading cause of cancer death globally, and the 5-year survival rates of whole populations remain low: 18% in the United States and 13% in Europe [1,2]. However, clinical outcomes improve dramatically when patients are treated with a targeted agent against a molecular alteration, such as epidermal growth factor receptor (EGFR) mutations [3]. Currently, routine testing for EGFR mutations and use of EGFR tyrosine kinase inhibitors (TKIs) are the established standard of care in clinical practice for patients with advanced non-small cell lung cancer (NSCLC) who harbor EGFR-activating mutations (AM) [4-7]. Activating mutations in the EGFR gene are mostly located in the exons 18 through 21, among them deletions in exon 19 and point mutation L858R in exon 21 are the most common ones conferring sensitivity to EGFR TKIs. Central to this treatment approach is the ability to detect such mutations in an easy, reliable, and rapid fashion [8].

EGFR-AMs are predictive of response to EGFR TKIs. Unfortunately, all patients will eventually develop resistance to the initial TKI and experience disease progression approximately 12 months (median) from the start of treatment. In up to 60% of patients, this resistance is caused by a second

single missense mutation within exon 20, known as the T790M mutation, which results in an amino acid change from threonine to methionine [9-11]. Previously, a repeat tissue biopsy at the time of progression was recommended for these patients to characterize the mechanism of resistance. However, obtaining another tissue sample is often limited by the anatomical location of the tumor, patient comorbidities, or poor performance status in advanced disease [12]. Furthermore, EGFR genotyping can fail in tissue samples, and tumor heterogeneity may confound the genotyping results [12].

However, it is possible to overcome these limitations by testing plasma specimens (i.e., a liquid biopsy) for circulating tumor DNA (ctDNA) to enable the detection of tumor EGFR mutation status. Several studies have demonstrated that liquid biopsy testing methods are highly concordant with mutations detected in tumor tissue from patients, which indicates that ctDNA testing of liquid biopsy samples is a feasible, accurate, and minimally invasive alternative to tissue biopsy testing [5,13].

The sensitivity of detecting EGFR-AM, such as the L858R mutation and exon 19 deletions, in liquid biopsy samples using the cobas<sup>®</sup> EGFR Mutation Test (Roche, Pleasanton, CA) is approximately 85% in patients with stage IV disease [14]. Detection of tumor hot-spot mutations (e.g., T790M) in ctDNA at the time of disease progression is now the standard of care, [5,7] but detection of EGFR mutations in ctDNA may also be useful at the time of the primary diagnosis and during treatment [8].

Several clinical trials have explored the value of sequential testing of EGFR mutations in plasma to predict response to therapy and/or identify the development of resistance to EGFR TKIs [15-22]. These studies have demonstrated those dynamic changes in levels of EGFR-AM, rather than the presence of specific mutations (e.g., T790M), mirror response and resistance to EGFR TKIs. Serial monitoring of ctDNA may predict the loss of efficacy

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for first-line EGFR TKIs by detecting the reappearance of an EGFR-AM and/or the appearance of a T790M mutation in blood weeks before radiologic Response Evaluation Criteria in Solid Tumors (RECIST)-defined progression is confirmed [15,19-22].

In routine clinical practice, response and progression with first-line EGFR TKIs are assessed clinically and radiologically using RECIST version 1.1 [23] but there are no strict guidelines to address the frequency of computed tomography (CT) in routine clinical practice [6,7]. CT remains the gold standard to confirm response and progression, but the optimal use of CT during treatment has yet to be defined [6]. Overutilization of CT can result in increased costs and added burden to the health care system, unnecessary exposure to radiation and contrast dye, and inconvenience to the patient. However, increasing the interval between CT scans could delay a diagnosis of disease progression [24]. Therefore, we posit that serial monitoring of dynamic changes of EGFR-AM using liquid biopsy samples might help clinicians identify progressive disease well before clinical or radiologic progression and allow for more efficient use of CT scans.

We initiated a single-center, prospective, observational study to monitor dynamic changes in EGFR-AM levels via ctDNA in patients with advanced EGFR-AM positive NSCLC who received first-line EGFR TKI therapy. The study was conducted under the Slovenian Research Agency Grant Protocol J3-4076. The primary objective was to evaluate the utility of measuring dynamic changes in plasma EGFR-AM as an early predictor of disease progression before the detection of clinical or radiologic progression. The secondary objective was to determine whether the frequency of routinely performed CT scans could be reduced with the use of liquid biopsy samples.

## Materials and Methods

### Patient selection and examination

The eligibility criteria required patients to have cytologically or histologically confirmed NSCLC harboring an EGFR-AM, as determined by standard reflex testing with the cobasEGFR Mutation Test at the time of diagnosis. In addition, all patients were required to have advanced NSCLC (stage III or IV) that was measurable by RECIST version 1.1 and an ECOG performance status of 0-2. Patients who were initiating standard first-line therapy with EGFR TKIs (i.e., erlotinib, gefitinib, or afatinib) or were already receiving EGFR TKI therapy (but in remission) were included. All patients must have had at least one follow-up CT scan.

Routine follow-up visits, including a clinical evaluation and laboratory testing, were required every 8 to 16 weeks. CT scans of the affected organs (i.e., thorax, abdomen, or central nervous system) were mandatory at week 8 after the start of EGFR TKI therapy, and recommended every 16 weeks thereafter or upon any evidence of clinical or symptom progression. The radiologic evaluation adhered strictly to RECIST version 1.1.

Peripheral blood samples for EGFR plasma testing were obtained at each scheduled visit until disease progression or discontinuation of the first-line EGFR TKI therapy, whichever occurred last. Written informed consent was obtained from all patients, ethics approval was obtained from the Slovenian National Ethics Committee (approval number 40/04/12), and the study was conducted according to the revised and amended principles of the Declaration of Helsinki.

### ctDNA EGFR testing

Peripheral blood samples (5 mL) were collected in K2-EDTA tubes (Vacutainer, BD, Franklin Lakes, NJ) or special cell-free tubes (Roche Cell-Free DNA Collection Tube, Roche, Pleasanton, CA), and transported to the laboratory within 30 minutes after collection. Plasma was separated from blood within 2 hours after collection by two-step centrifugation (first at 2189g for 10 minutes at 4°C, and then at 16,000g for 10 minutes at 4°C) and stored at -80°C. Circulating-free DNA (cfDNA) was isolated from a minimum of 2 mL of plasma using the cobas cfDNA Sample Preparation Kit (Roche, Pleasanton, CA). Both version 1 and 2 of the cobasEGFR Mutation Test (Roche, Pleasanton, CA)

were used for plasma testing to detect mutations in exons 18 through 21 of the EGFR gene.

In addition, EGFR mutations in the plasma samples were expressed as a semi-quantitative index (SQI). The SQI value correlates with the amount of EGFR-mutant ctDNA in the sample in copies per mL, and is independent of the amount of background wild-type DNA. The SQI is determined by using the observed PCR cycle threshold and a proprietary, unique algorithm for the specific EGFR mutation. If tested on sequentially collected plasma samples, the SQI value can identify a trend that reflects tumor response or progression [25].

### Data analysis

Patients were divided into 3 groups to facilitate the analysis of the different research questions: Group 1 consisted of patients whose EGFR-AM disappeared (reached zero) during treatment and who had CT-confirmed disease progression after week 24; Group 2 of patients with early progression (< 24 weeks) and/or no EGFR-AM clearance during treatment; and Group 3 of patients without observed disease progression during the observation period. Data from Group 1 were used for all analyses, and data from Groups 2 and 3 only to analyze dynamic changes of EGFR-AM over time.

### Methodology to determine false positivity/negativity

All samples that showed a reappearance of EGFR-AM after week 24 that subsequently disappeared (i.e., dropped back to zero) were considered false positives. The cut off of week 24 was chosen for two reasons: (1) Fluctuations/oscillations in EGFR-AM levels after the start of treatment were expected prior to AM clearance, and (2) disease progression during this period would be considered early progression, thus negating the need for continued EGFR-AM monitoring. False negatives were defined as samples associated with CT-confirmed disease progression without a corresponding prior or concurrent reappearance of EGFR-AM.

## Results

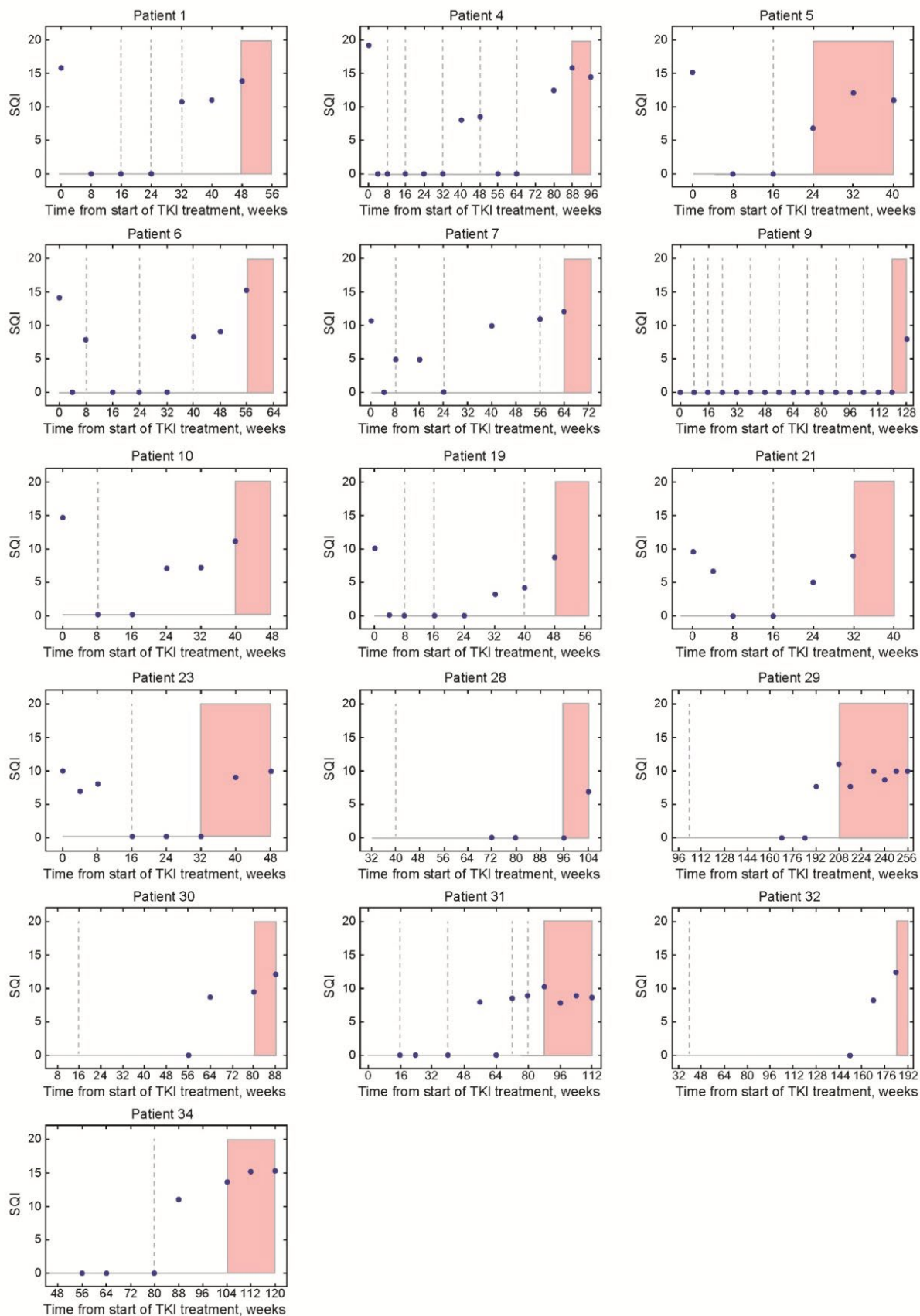
Between May 2014 and March 2017, 35 patients were enrolled in the study at a single study site in Slovenia, of whom 27 patients were newly diagnosed and TKI naïve and 8 patients (No. 27- No. 34) were enrolled while on first-line EGFR TKI therapy. The patient characteristics are shown in Table 1.

The 35 patients were categorized into three groups: 16 patients in Group 1 (EGFR-AM reappearance and disease progression; Figure 1); 5 in Group 2 (early progression and/or no EGFR-AM disappearance; Figure 2); and 14 in Group 3 (no disease progression during observation period; Figure 3).

In the main group (Group 1), 12 of 16 patients (75%) experienced reappearance of EGFR-AM before disease progression confirmed by a CT scan (Figure 1). Interestingly, 5 of these 12 patients (Numbers 1, 6, 7, 19, and 31) had a concurrent CT scan that did not yet show disease progression. One

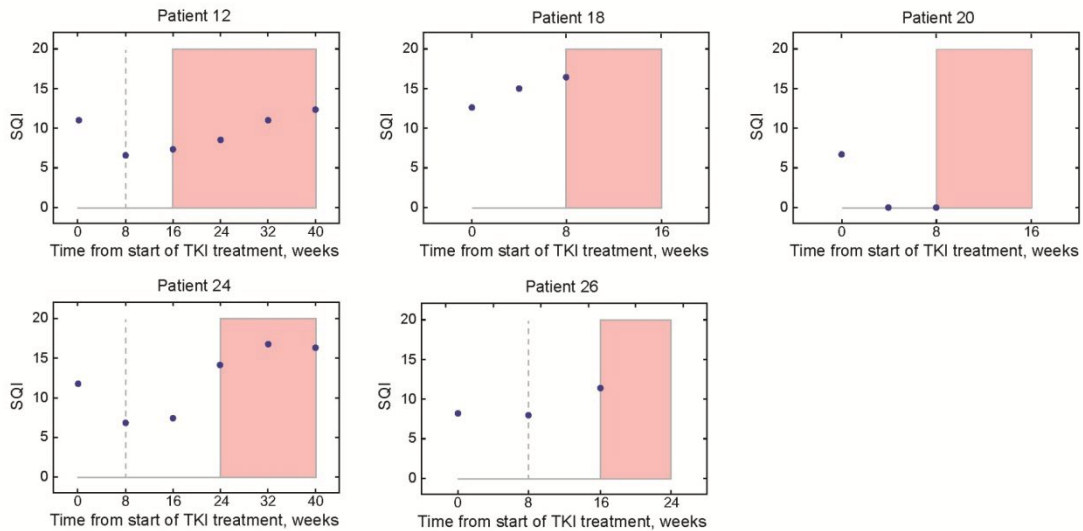
**Table 1.** Patient characteristics.

Characteristics	
Age, mean (range), y	66.2 (42-85)
<b>Sex, n (%)</b>	
Female	29 (82.9)
Male	6 (17.1)
<b>EGFR mutation before EGFR TKI, n (%)</b>	
Exon 19 deletions	23 (65.7)
L858R point mutation	8 (22.8)
L858R point mutation + T790M	1 (2.9)
Other activating mutations	3 (8.6)
<b>EGFR TKI, n (%)</b>	
Erlotinib	15 (42)
Gefitinib	10 (29)
Afatinib	10 (29)



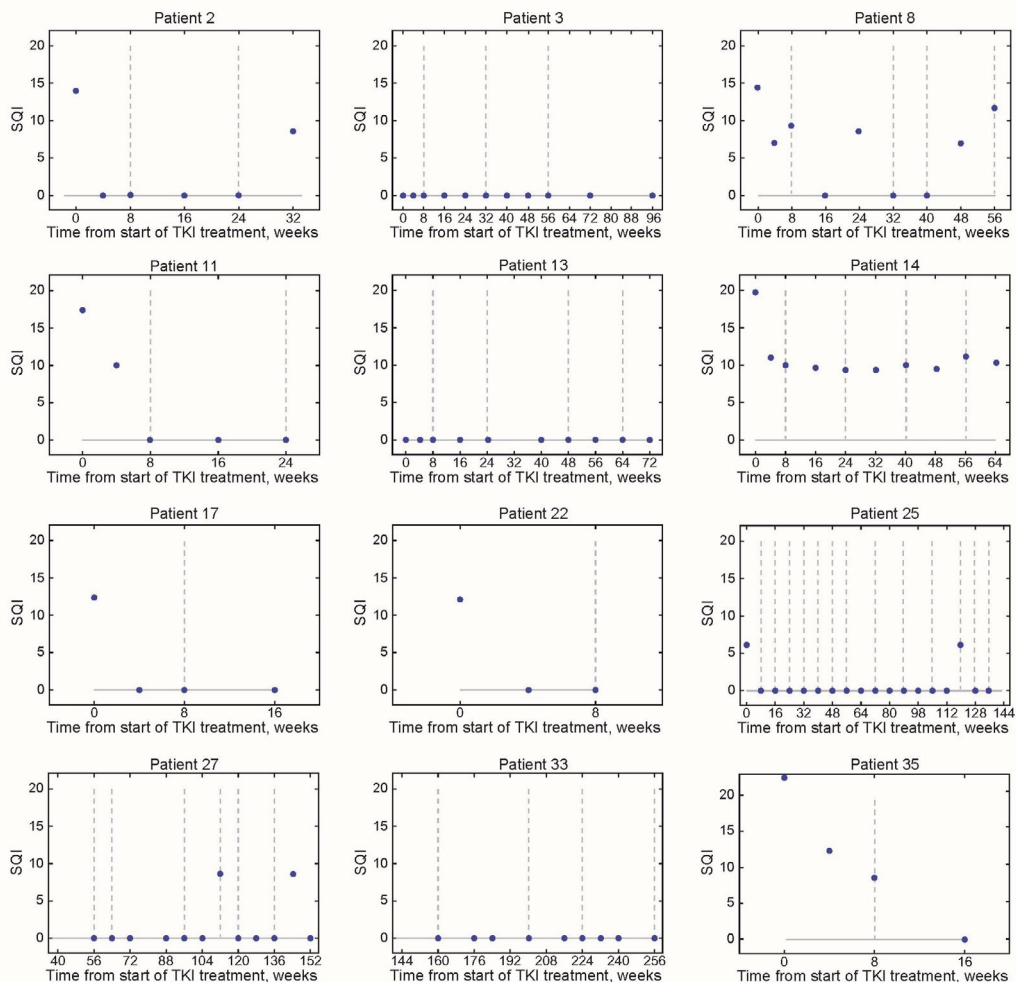
**Abbreviations:** SQI: Semi-Quantitative Index; TKI: Tyrosine Kinase Inhibitor.

**Figure 1. Test results in Group 1:** Patients who had epidermal growth factor receptor–activating mutations (EGFR-AM) that disappeared during treatment and computed tomography (CT)–confirmed disease progression. Patients and their corresponding measurements (EGFR-AM levels expressed as semi-quantitative index and CT scans) are visualized over time from the start of treatment (or start of observation) until disease progression. Patients who were not monitored for EGFR-AM from the start of tyrosine kinase inhibitor treatment (Numbers 28, 29, 30, 31, 32, and 34) can be easily recognized because their plots do not contain EGFR-AM at week zero, and their timelines generally do not begin at zero either. Red zone, progressive disease confirmed by CT; grey dotted lines, CT scan (without progression if before red zone); blue dots, EGFR-AM.



**Abbreviations:** SQI: Semi-Quantitative Index; TKI: Tyrosine Kinase Inhibitor.

**Figure 2. Test results in Group 2:** Patients who had either early progression (< 24 weeks) and/or no epidermal growth factor receptor-activating mutations (EGFR-AM) clearance during treatment. Patients and their corresponding measurements (EGFR mutation levels expressed as a semi-quantitative index and computed tomography [CT] scans) are visualized over time from the start of tyrosine kinase inhibitor treatment (or start of observation) until disease progression. Red zone, progressive disease confirmed by CT; grey dotted lines, CT scan (without progression if before red zone); blue dots, EGFR-AM.



**Abbreviations:** SQI: Semi-Quantitative Index; TKI: Tyrosine Kinase Inhibitor.

**Figure 3. Test results in Group 3:** Patients without observed disease progression. Patients and their corresponding measurements (epidermal growth factor receptor [EGFR] mutation levels expressed as semi-quantitative index and computed tomography [CT] scans) are visualized over time from the start of tyrosine kinase inhibitor treatment (or start of observation) until disease progression. Patients who were not monitored for EGFR-activating mutations (AM) from the start of tyrosine kinase inhibitor treatment (Patients 27 and 33) can be easily recognized because their plots do not contain EGFR-AM at week zero, and their timelines do not begin at zero either. Red zone, progressive disease confirmed by CT; grey dotted lines, CT scan (without progression if before red zone); blue dots, EGFR-AM.



patient (No. 5) had EGFR-AM reappear at the same time as CT-confirmed disease progression. Only 3 patients (Numbers 9, 23, and 28) had CT-confirmed disease progression without concurrently detectable EGFR-AM. However, EGFR-AM reappearance was observed at the next visit (8 weeks later) in all 3 patients. In Group 2, EGFR ctDNA failed to clear in 4 of 5 patients, and these patients progressed prior to week 24 (Figure 2).

## False positives and negatives

False positives were observed in Group 1 (Figure 1) for Patient No. 4 (weeks 40 and 48, consecutively) and Patient No. 31 (week 56). In Group 3 (Figure 3), false positives were observed for Patient No. 8 (week 24), Patient No. 25 (week 120), and twice for Patient No. 27 (weeks 112 and 144). No false positives were observed for patients in Group 2. When examining the potential false-positive data, we noted that the increase in EGFR-AM levels observed in Patient No. 8 (week 24; Figure 3) could still be part of early EGFR-AM negativization, which occurred within 24 weeks after treatment initiation.

Separately, Patient No. 31 had a true positive (week 72) almost immediately after the false positive (week 56; Figure 1), so these data might not be interpreted as real false positives. However, to be consistent, both were counted as false positives. Altogether, false positives were observed in 14% of patients (5 of 35 patients: 4 patients with a single false positive and 1 with two false positives). The changes in EGFR-AM levels observed in Patients No. 6 (Week 8) and No. 7 (Weeks 8 and 16) were not considered false positives, because they occurred before the week 24 cut off.

Importantly, there were no patients with false negatives at any of the 16-week intervals.

## EGFR-AM reappearance and disease progression

All cases of disease progression in patients from Group 1 were accompanied by the reappearance of EGFR-AM, either prior to or, in the minority of cases, just after CT-confirmed disease progression (Figure 4). EGFR-AM reappeared before or at the time of CT-confirmed disease progression in 13 of 16 cases, with a delay of 8 weeks (i.e., at the next scheduled visit) in the remaining three cases. Overall, EGFR-AM reappeared at a mean of 10 weeks (median, 16 weeks) prior to CT-confirmed disease progression.

## Discussion

Measuring the dynamic changes in plasma EGFR mutations has been used to monitor disease progression in patients with NSCLC treated with TKI therapy. However, we do not know whether monitoring EGFR-AM, specifically in serially collected plasma, can predict response to therapy or disease progression compared with concomitant CT imaging [15,17,19,21,26]. Herein, we conducted a single-center, prospective, observational study to determine whether dynamic changes in plasma EGFR-AM could be used as an early indicator of response to therapy and subsequent disease progression before a regularly scheduled CT scan. The focus of this study was on detecting response to therapy and disease progression using EGFR-AM, instead of monitoring for the T790M resistance mutation alone.

We found that EGFR-AM appeared before or at the time of CT-confirmed disease progression in a majority of cases (13 of 16). Overall, EGFR-AM reappearance occurred several weeks (mean, 10 weeks; median, 16 weeks) prior to radiologic disease progression. In the remaining 3 cases, EGFR-AM appeared at the next scheduled clinic visit after disease progression. The delay was never longer than 8 weeks (i.e., one visit later), which suggests there were no real false negatives.

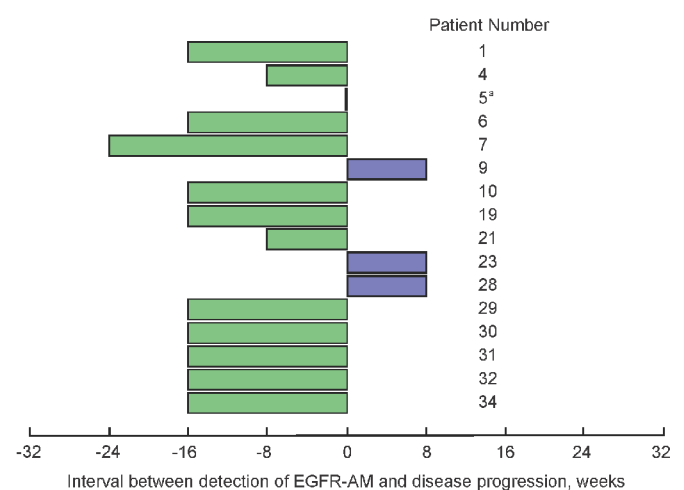
The presence of false-positive results was rather small. False positives were observed in 5 of 35 cases, once each in 4 patients and twice in 1 patient. The presence of false positives might result in additional CT scans. However, if a confirmatory second positive ctDNA result for EGFR-AM were required prior to a scan, the false positive rate can be kept to a minimum. In addition, false positives are unlikely to lead to an over diagnosis, as the number of true positives far outweighs the number of false positives. An instance of a positive result with EGFR-AM testing without concordant CT-confirmed disease

progression may also arise when tumor masses are too small to be detected radiologically, and such cases would not be false positives [27]. However, this does not seem to be the case in our patients with false-positive test results because 4 of these 5 patients already had 2 or 3 CT scans performed after ctDNA EGFR-AM positivity without the detection of radiologic progression.

Importantly, we observed no false negatives at any of the 16-week intervals. CT scans were always performed when clinical symptoms appeared, which suggests that the risk of significantly delaying the detection of disease progression with liquid biopsy samples is unlikely. These data are in line with those from other studies that used the cobasEGFR test [12,28,29].

Our data suggest that in the majority of cases, monitoring dynamic changes in EGFR-AM using regular liquid biopsy samples could complement traditional CT scan-based detection of disease progression and may allow for more efficient use of CT scans. Because there were no false negatives, the evaluation of EGFR-AM alone would likely detect disease progression within a shorter time frame and enhance clinicians' ability to evaluate for disease progression and accurately counsel patients [8,24,28]. Furthermore, ctDNA can reflect tumor progression anywhere within the body, whereas follow-up CT scans often focus only on specific parts of the body already affected with metastatic growth [24,30] and can miss progression in areas not scanned. Therefore, the former can provide a more comprehensive picture of tumor growth and distant metastases and allow for early medical evaluation, intervention, and treatment adaptation.

Even if CT scans are scheduled only after the reappearance of EGFR-AM, our data show that disease progression would not be missed for more than 8 weeks in any patient. In the hypothetical situation where a follow-up CT scan would be performed every 8 weeks (or an even shorter interval), the temporal interval between reappearance of EGFR-AM and CT-confirmed disease progression (mean, 10 weeks; median, 16 weeks) would likely be smaller, and perhaps EGFR-AM reappearance could occur after CT-confirmed progress. However, this is an unlikely scenario given the current safety, logistical, and financial limitations to conducting frequent follow-up CT scans in routine clinical practice. Although we do not propose eliminating follow-up CT scans, we believe that if EGFR-AM are continuously undetectable, it would give physicians confidence that the current therapy is still effective and aid the physician with the timing of ongoing imaging. In addition, CT scans should always be performed on the basis of clinical symptomatology or after EGFR-AM reappearance. To eliminate the possibility of a false positive EGFR-AM reappearance, consideration should also be made to perform a confirmatory plasma test, especially if the patient is clinically stable.



**Figure 4.** Comparison between epidermal growth factor receptor-activating mutation (EGFR-AM) reappearance and computed tomography-confirmed disease progression. Positive values (blue) correspond to situations where progression was confirmed before an increase in EGFR-AM levels was observed. Negative values (green) correspond to situations where progression was confirmed after an increase in EGFR-AM levels. An increase in EGFR-AM occurred together with computed tomography-confirmed progressive disease.

In routine clinical practice, liquid biopsy samples have many advantages over CT scans. They are easy to perform, pose little to no risk, and are less burdensome to the health care system and to the patient [12,24-30]. Although this pilot observation study involved only 16 patients (35 for the purpose of studying false positivity), we believe that the findings indicate that the use of liquid biopsy samples is a promising approach with potentially clinically relevant benefits. This study was designed around routinely used CT follow-up schedules. The weakness of this approach is that follow-up CT scans were not performed at each EGFR-AM determination, and not always performed every 16 weeks for each patient. However, the strength of this approach is its representation of a real-world scenario. Thus, the comparison between the timing of CT- or clinically detected progression and EGFR-AM-detected progression (reappearance) in this study is likely to be representative of real-world situations.

The case of Patient No. 9 (EGFR-AM appeared during progression, even though undetectable at the beginning of the study; Figure 1) suggests that there could be a correlation between quantitative levels of EGFR-AM and burden of disease. However, in most cases, the level of EGFR-AM appears not to be relevant. Our data are in line with other observations that show that only the reappearance of the EGFR-AM is informative [31,32]. Despite the limited sensitivity of ctDNA EGFR detection, this test may be more accurate to predict disease progression in real-world practice than CT scans, which are performed every 16 weeks or less frequently. In addition, failure to clear ctDNA by week 24 may identify a group of patients with early progression who can be transitioned to other therapies in a timely manner. The question of whether earlier detection of progressive disease is clinically meaningful in terms of overall survival or quality of life remains unanswered. Theoretically, earlier detection of progression would prevent the patient from continuing on ineffective therapy, with related toxicity, and possibly promote changing to a potentially effective therapy. However, a large prospective clinical trial comparing changing therapy at the time of molecular progression to changing therapy at the time of radiographic/clinical progression is required to answer this question.

Based on this work, we propose that such a prospective controlled clinical trial to evaluate the timing of changing therapy as well as the scheduling of follow-up CT scans based on routine monitoring for the presence or absence of EGFR A Min blood should be considered. In such a trial, in addition to evaluating when to change therapy, if EGFR-AM is not detected, a CT scan would not be performed (or could be performed less frequently than the default schedule). Conversely, if EGFR-AM is detected or if the patient is symptomatic, an immediate CT scan should be performed. The results from such a trial might be of great value to countries with limited resources and access to CT scans because this new strategy would still allow for a timely determination of disease progression, rapid determination of some specific resistant mutations, such as T790M, and treatment tailoring.

## Conclusion

In conclusion, we have demonstrated that it is feasible to follow EGFR-AM in ctDNA in an Eastern European population. This study demonstrates that dynamic changes in ctDNA are highly predictive of disease progression and are seen an average of 2 to 3 months before radiographic progression. There was a low false-positive rate and no false negatives. We believe that routine use of ctDNA for the detection of EGFR-AM in patients with EGFR-mutated NSCLC can provide valuable adjunctive information and may aid clinical management. Prospective trials of this promising approach would clarify the benefits to patient care and should be considered.

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## Data Sharing Statement

The General Data Protection Regulations prevent the investigators from sharing data derived from clinical studies that involve real patients, contains personal health information, and might be linked directly to those patients. In addition, the informed consent document did not specifically allow for the dissemination of the data used in this paper.

## Funding Disclosures

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