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ORIGINAL ARTICLE

# The Clinical Impact of Re-biopsies in Lung Adenocarcinoma: a Retrospective Multicenter Study

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**Abstract**
If a patient's cancer progresses while undergoing targeted therapy, a re-biopsy is not mandatory. But when evaluating the benefits and risks on a case-by-case basis (transformation to small cell, assessing for a clinical trial), physicians should inform patients about the possible need for a re-biopsy (5). This was a retrospective and multicentre study. A total of 644 patients with lung adenocarcinoma were reviewed, 625 of whom were ruled eligible. From them, 399 were found to show disease progression, and 126 re-biopsies were performed. Progression status, re-biopsy sites, success of obtaining adequate tissue, molecular patterns after re-biopsy and subsequent treatments were analysed. Survival differences among patients with disease progression were then examined according to re-biopsy status. Overall, 625 patients with adenocarcinoma and a median age of 61.4 were evaluated. Initial tyrosine kinase inhibitor (TKI) usage numbered 37 patients (5.9%). Progression was diagnosed in 399 (63.8%) patients, out of which 26 (31.6%) underwent re-biopsies. The successful number of re-biopsies was 103 (81.7%). No complications were observed after any of the biopsy procedures. Subsequent treatments were changed in 15 patients (11.9%), who began new TKI treatments. Poor performance status was the most common reason for not performing a biopsy (n = 65; 23.8%), followed by the physician's decision (n = 40; 14.6%). Re-biopsies can demonstrate the new characteristics of a tumour and can detect the activation of pre-existing clones, making possible new treatment opportunities for patients. According to the performance status of the patient and the availability of the progressive lesion, we should increase the rate of re-biopsies before the decision to follow up with the best supportive care.

**Keywords** Non-small cell lung cancer · Re-biopsy · Targeted therapy

## Background

Recent molecular developments in cancer biology have yielded a new era of targeted therapy. Among advanced stage non-small cell lung cancer (NSCLC) patients, the existence of targeted mutations, such as epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK) and ROS proto-oncogene 1 (ROS-1), should be investigated at the beginning of their therapy (1). A re-biopsy for patients receiving EGFR-targeted tyrosine kinase inhibitors is strongly recommended when the disease progresses. Detecting any of the secondary resistance mechanisms can guide the selection of second- and third-generation EGFR tyrosine kinase inhibitors (EGFR-TKIs) for treatment in the advanced stages of lung adenocarcinoma [1].

ALK rearrangement is identified in 3–7% of advanced stage NSCLC cases. Until recently, there wasn't sufficient evidence concerning re-biopsies for ALK-rearranged NSCLC patients [2]. Some secondary point mutations have been described as causing acquired resistance to first-line ALK inhibitors (*crizotinib*) e.g., L1152R, C1156Y, F1174L, L1196M, L1198P, D1203N and G1269A [3]. However, many

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studies have reported a wide variety of mutation types; unlike the EGFR mutant NSCLC (T790M), there is no predominant mutation for secondary ALK resistance. Acquired resistance mechanisms need to be better understood for recommending informed therapeutic guidance. Even if there is a good initial response to *crizotinib*, the median duration of response is only 11.3 months, and the transition to the central nervous system (CNS) is insufficient [4]. Due to an inadequate suppression of ALK and an overexpression of alternative point mutations, second-generation ALK inhibitors (alectinib, ceritinib, brigatinib and lorlatinib) were needed. The developed inhibitors were approved by the U.S. Food and Drug Administration (FDA) for the treatment of crizotinib-refractory ALK-rearranged NSCLC patients with no need for testing for secondary mutations [2, 4].

The Second ESMO Consensus Conference on Lung Cancer reported the recommendation (with C grade strength) that performing a re-biopsy is not mandatory when there is a secondary resistance to TKIs and that patient-based risks and benefits should be considered [5]. Therefore, we aimed to investigate attitudes about re-biopsies and the procedure's success rates and safety after disease progression among Turkish patients with lung adenocarcinoma.

## Materials and Methods

We retrospectively reviewed 644 consecutive patients who were diagnosed with adenocarcinoma between January 2011 and October 2018. The observational study included three centres: the Atatürk Chest Disease and Thoracic Surgery Training and Research Hospital, the Gazi University School of Medicine Department of Pulmonology and the Eskisehir Osmangazi University School of Medicine Department of Pulmonology. The study was approved by an institutional review board with the ethical committee number 509-24.11.2017.

Patients' baseline characteristics of age, gender, initial tumour, lymph node and metastasis classifications (TNM staging), treatment modalities, and mutational status were recorded. Their responses to first-line therapy were examined by computed tomography (CT) and evaluated using the Response Evaluation Criteria for Solid Tumours V.1.0 (RESIST V.1.0) [6]. When a progressive disease was identified, the most suitable biopsy procedure according to the lesion and patient's performance status was performed after informed consent was obtained from the patient. If a lesion was deemed to be eligible, various procedures, including computer-guided biopsy, pleural fluid cyto-block investigation, endobronchial ultrasound (EBUS), bronchoscopy (mucosal biopsy/transbronchial biopsy), liquid biopsy and biopsies from extra-pulmonary metastatic sites were performed.

Statistical analyses were performed using IBM SPSS Statistics (IBM Corp., Armonk, NY) 15.0 program. The demographic characteristics of the study group were reported using descriptive statistics (frequencies, proportions, and means or medians). The appropriate chi-squared analysis was performed for comparisons between groups, while differences in time distributions between groups were tested for statistical significance using a log-rank test.

## Results

A total of 644 patients were evaluated in this study. Nineteen patients were excluded due to insufficient data. A total of 625 eligible patients with lung adenocarcinoma, mean age 61.4 ± 9.6 years, were included (Fig. 1). There was a male predominance (n = 480; 76.8%), and most patients were diagnosed to be in an advanced stage (n = 462; 73.9%). There were 37 (5.9%) patients being treated with TKIs. Progression of the disease was identified in 399 (63.8%) patients. Their baseline characteristics are summarized in Table 1.

From the patients diagnosed with progressive disease (PD) (n = 399), 126 (31.6%) underwent re-biopsies. The primary thoracic lesion was the most common site for performing the biopsies, including pleural cyto-block analyses (n = 95; 75.4%). Distant metastatic lesions were sampled in 25 (19.8%) patients, and 6 (4.8%) had liquid biopsies. No complications were observed after any of the biopsy procedures. A total of 103 (81.7%) specimens were suitable for molecular analyses. In 15 patients (11.9%), molecular profiles were found to have changed after their first re-biopsy, making them suitable for new TKI therapy (Table 2). Subsequent treatments for patients with progressive disease according to re-biopsy status are described in Table 2.

There was no difference between the re-biopsy and non-re-biopsy groups in terms of mean age (p = .145) or TNM staging distribution (p = .640). In the non-re-biopsy group, the most common reason for not performing a re-biopsy was patients' poor performance status (n = 65; 23.8%) followed by the physician's decision (n = 40; 14.6%) (Fig. 1). After the first progression, both in the re-biopsy and non-re-biopsy groups, second-line systemic chemotherapy and best supportive care (BSC) comprised most of the subsequent treatments (Table 3). Although the number of patients having subsequent treatment with TKIs was higher than the number of re-biopsy patients, some were continuing initial TKI treatment, while in a small number of patients, first-generation TKI was initiated without any targetable EGFR mutation. The distribution of second-line therapies did not show a significant difference between the two groups (Table 3).

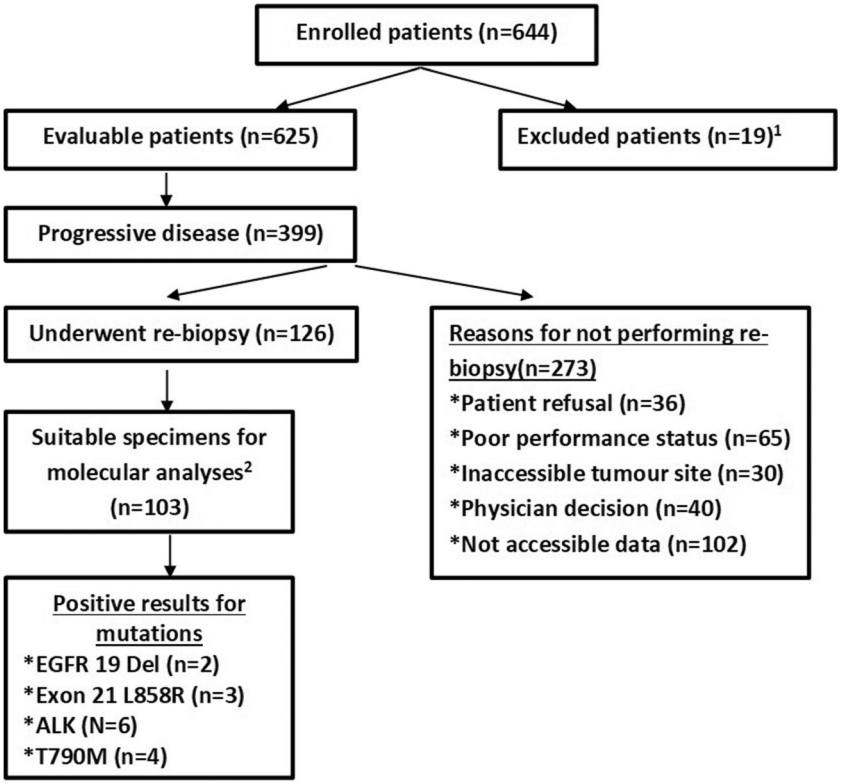


**Fig. 1** Flowchart of study population demonstrating re-biopsy rates, reasons for not performing re-biopsy and new molecular status

**Table 1** General characteristics of study population

| Characteristics | n = 625 |
|---|---|
| Age, mean ± SD, years | 61.4 ± 9.6 |
| Gender, n (%) | 145 (23.2) |
| - Female | 480 (76.8) |
| - Male | |
| Stage, n (%) | 192 (30.8) |
| I – II – III | 433 (69.2) |
| IV | |
| Initial treatment, n (%) | 462 (73.9) |
| - Chemotherapy | 46 (7.4) |
| - Chemoradiotherapy | 5 (0.8) |
| - Palliative radiotherapy | 1 (0.2) |
| - Curative radiotherapy | 37 (5.9) |
| - TKIs | 24 (3.8) |
| - Surgery | 50 (8.0) |
| - BSC | |
| Initial mutational status, n(%) | 36 (5.8) |
| EGFR Exon Del19 | 19 (3.0) |
| EGFR Exon 21 L858R | 34 (5.4) |
| ALK | 0 |
| ROS1 | |
| Progressive disease, n (%) | 399 (63.8) |

SD: Standart deviation

TKIs: Thyrosine kinase inhibitors

BSC: Best supportive care

EGFR: Epidermal growth factor receptor

ALK: Anaplastic lymphoma kinase

ROS1: c-ros oncogene 1

Among all patients (n = 625), the frequency of targetable EGFR mutation (Exon Del19 and EGFR Exon 21 L858R) was 8.8% (n = 55) at the beginning of therapy. Among these cases, 26 patients received EGFR-TKI as a first-line treatment according to TNM stages and performance status. As described in Table 2, only 4 patients harbouring a targetable EGFR mutation revealed the T790M mutation after re-biopsy and continued with second-generation TKI.

The initial ALK positivity was 5.4% (n = 34 patients); 11 patients were using ALK inhibitors as a first-line treatment. Twenty-two of the ALK + patients had progressive disease, and only 1 patient underwent a re-biopsy resulting in showing ALK positivity again. The patient continued to receive first-generation ALK inhibitor treatment. The most common reasons for not performing re-biopsies in the remaining 21 patients was physician's decision (n = 13). In 3 patients, the lesion was not accessible, 4 patients had poor Eastern Cooperative Oncology Group (ECOG) performance status and 1 patient refused the biopsy procedure. Subsequent treatments for these 21 patients were chemotherapy (n = 8), BSC (n = 8) and palliative radiotherapy (n = 5).

## Discussion

Currently, morphological and immune-histochemical diagnoses of lung adenocarcinoma can define the predominant

**Table 2** Characteristic of patients with positive targeted mutation after first re-biopsy

| Stage | First-line treatment | Initial mutational status | Biopsy localisation | Mutational status after re-biopsy | Treatment after re-biopsy |
|---|---|---|---|---|---|
| IV | Chemotherapy | EGFR 21 exon (+) | primary lesion | T790M (+) | TKI |
| IV | Chemotherapy | Negative | primary lesion | ALK (+) | TKI |
| IV | Chemotherapy | Negative | primary lesion | ALK (+) | TKI |
| IV | Chemotherapy | Negative | primary lesion | EGFR exon 21(+) | TKI |
| III | Chemo-radiotherapy | Negative | primary lesion | ALK (+) | TKI |
| IV | Chemotherapy | Negative | primary lesion | ALK (+) | TKI |
| IV | Chemotherapy | Negative | primary lesion | EGFR 19 deletion | TKI |
| III | Chemo-radiotherapy | Negative | primary lesion | ALK (+) | TKI |
| IV | Chemotherapy | Negative | primary lesion | ALK (+) | TKI |
| IV | TKI | EGFR 19 deletion (+) | primary lesion | T790M (+) | TKI |
| IV | Chemotherapy | Negative | Distant metastasis | EGFR 21 exon (+) | TKI |
| III | TKI | EGFR 19 deletion (+) | primary lesion | T790M (+) | TKI |
| IV | TKI | EGFR 19 deletion (+) | primary lesion | T790M (+) | TKI |
| IV | Chemotherapy | Negative | primary lesion | EGFR 21 exon (+) | TKI |
| IV | Chemotherapy | Negative | primary lesion | EGFR 21 exon (+) | TKI |

TKI: Thyrosine kinase inhibitor

EGFR: Epidermal growth factor receptor

ALK: Anaplastic lymphoma kinase

C*risotinib* was the subsequent TKI for ALK positive patients

pattern according to differentiation (lepidic growth, acinar, papillary and micropapillary patterns and solid pattern with mucin); hence, detailed molecular analyses should be conducted at the beginning of lung cancer treatment [7] for utilizing adequate small biopsy samples to choose the most suitable therapeutic options for patients. Such analyses are all the more important because different morphological patterns produce differences in survival rates. For example, TKIs are novel therapeutic agents that are widely used in personalized medicine in lung adenocarcinoma with positive driver mutation [8].

Resistance to targeted therapy is the leading problem in adenocarcinomas. Unresponsiveness to TKIs is expected to occur approximately 1 year after starting them [9], at which time major problems begin. Even though there are some pathways explaining acquired resistance to first-line EGFR-TKIs, to demonstrate the new histopathological and molecular status of a tumour by acquiring a new biopsy specimen is a novel proposal in thoracic oncology. Among 139 lung cancer patients diagnosed with progressive disease while receiving EGFR-TKI treatment, re-biopsies were performed on 75 [9]. Their continuing rates of therapy with third-generation EGFR-TKI or other approved TKIs were significantly higher than for patients who did not have a re-biopsy. The most common reasons for not undergoing a repeat biopsy were poor medical condition, ineligible tumour site or physician's decision [9]. Similarly, in current study continuing rates of therapy with

**Table 3** Subsequent treatments for patients with progressive disease according to re-biopsy status

| Subsequent regimen | Re-biopsy group (n = 126) | Non re-biopsy group (n = 273) | P value |
|---|---|---|---|
| Second line systemic chemotherapy | 69 (54.7%) | 141 (51.6%) | 0.563 |
| Third generation TKIs* | 3 (2.3%) | - | - |
| TKIs (gefitinib, erlotinib, afatinib, crizotinib) | 15 (11.9%) | 19 (6.9%) | 0.10 |
| Best supportive care | 37 (29.3%) | 100 (36.6%) | 0.155 |
| Immunotherapy[1] | 2 (1.5%) | 1 (0.3%) | 0.236 |
| Palliative thorax radiotherapy* | - | 12 (4.3%) | - |

[1] Drugs were available within international-multicentre study

TKIs: Thyrosine kinase inhibitors

*: not suitable for statistical analyses

TKIs was higher in re-biopsy group and poor performance status and patient refusal were the common reasons not to perform biopsy procedure.

Re-biopsies for the ALK-positive patients did not reveal any clinically significant histopathological results. If at that time second-generation ALK inhibitors were available, it would have been a great opportunity for the ALK-resistant patients.

Only 4 EGFR-mutant patients revealed the T790M mutation and were subsequently treated with third-generation TKI. Overall, 11.9% (n = 15) patients continued with new targeted drugs in re-biopsy group. Similarly, a study including 65 lung adenocarcinoma patients demonstrated that, by having re-biopsies, 15% of the patients were given the chance to receive third-generation TKI, emphasizing the importance of performing re-biopsies and preserving extra frozen samples from the first diagnosis [10].

Not only for patients using first-line TKIs, but also for patients with a prior history of systemic chemotherapy (without any ALK or EGFR mutations), re-biopsies can offer guidance for new drug choices [11]. In our study, 11 patients with prior histories of no targetable mutations were subsequently treated with new targeted therapies after having re-biopsies (Table 2).

Due to its retrospective methodology, the absence of reasons for not performing re-biopsies in 102 patients should be considered a limitation of our study. In some cases, although the patients' performance status was good, the clinician's decision may have been that a biopsy was not required.

Tumour heterogeneity is a major point to consider. In a small specimen with a low mutated signal and higher tumour burden, the detection of targetable mutations can be difficult. Good clinical responses to EGFR-TKIs are possible if patients have targetable EGFR mutations in the bulk of the tumour cells. Additionally, DNA quality is another important factor when detecting mutations [12]. There are reports demonstrating a heterogeneity of EGFR mutations within the primary tumour and between primary tumour and metastases [13]. Regardless of the initial treatment, acquired resistance to first-line therapy should be re-assessed by re-sampling from either the primary tumour or an accessible metastatic site to determine the genotype of NSCLCs [12].

Although re-biopsies are not supported by a high level of evidence in guidelines, the repeat biopsy rate in this study, 126 patients (31.5%), is lower than expected. Designing the study retrospectively, starting to perform molecular analyses in the beginning of 2013, difficulties in accessing new drugs and different attitudes of health centres are the major factors for the low rate. According to a literature review article on re-biopsies in lung cancer, only 14 studies were available in the PubMed database. Despite that sometimes a patient with lung cancer is unsuitable for undergoing multiple re-biopsies, there is an obvious need for more research on re-biopsies in lung cancer [14].

Clinicians possess a variety of diagnostic tools to sample lung, pleura or mediastinal lymph nodes under current conditions. For example, a study comparing re-biopsy procedures in pre-treated lung cancer patients found an overall success rate of 90% for thoracic and extra-thoracic biopsies without any major complications [15].

## Conclusions

This study demonstrated that re-biopsy is feasible and acceptable in NSCLC patients with advanced or metastatic disease. It is important to obtain adequate tissue to improve the success rates of molecular re-analyses. Demonstrating changed tumour behaviour and new molecular stratification can direct clinicians to initiate treatment with second- and third-generation TKIs.

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### Compliance with Ethical Standards

**Conflicts of Interest** The authors they have no conflicts of interest or received financial support for this study.

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