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Assessment of *EGFR* gene mutations in cf-DNA in monitoring of response to EGFR TKIs in patients with lung adenocarcinoma

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INTRODUCTION

Molecular analysis of cf-DNA in NSCLC patients enables detection and monitoring of *EGFR* mutations. It allows for detection of the acquired resistance for 1st and 2nd generation of EGFR-TKIs caused by Thr790Met substitution. The third generation of EGFR-TKIs (osimertinib) could overcome the resistance in patients with Thr790Met mutation.

METHODS

The studied group included 23 Caucasian patients (8 male and 15 female, median age 71±9) with diagnosed lung adenocarcinoma and with *EGFR* mutations detected in tumor samples. Blood samples were collected before administration of EGFR-TKIs in all patients, and re-collected repeatedly from 10 patients during therapy. *EGFR* mutations and content of mutated cf-DNA were analyzed using ctEGFR Mutation Analysis Kit (Entrogen, USA) in Rotor-Gene Real-Time PCR device (Qiagen, Germany).

RESULTS

We showed 11 deletions in exon 19 and 12 Leu858Arg substitution in exon 21 of *EGFR* gene in FFPE tissue and cellblocks materials. Mean content of mutated DNA was 34.54% (range: 1.66%-95.26%). There were no significant differences between mutated DNA content and type of analyzed materials. Based on these results, patients were qualified for EGFR-TKIs therapy: 10 patients with erlotinib, 10 – afatinib, 2 – gefitinib and one with chemotherapy. 15 patients showed partial response, 7 – disease stabilization. PFS and OS median has not been reached.

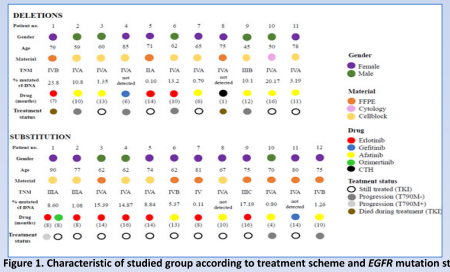

Figure 1. Characteristic of studied group according to treatment scheme and *EGFR* mutation status.

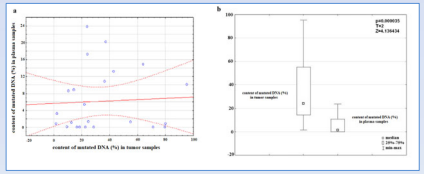

Figure 2. Correlation between concentration of mutated DNA in tumor material and in plasma samples (a). Differences between content of mutant DNA in tumor and plasma samples (b).

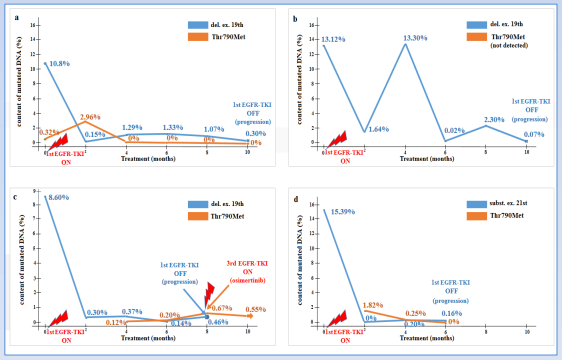

Figure 3. Changes in concentration of mutant DNA in plasma samples during EGFR-TKIs treatment in four exemplary cases (a-d). Detailed explanation in text.

Analysis of plasma samples

EGFR mutations in plasma samples were confirmed in 19 patients – overall concordance/sensitivity 82.61% (19/23). Mean content of mutated cf-DNA was 7.44% (range: 0.02% – 23.8%). The weak correlation between content of mutated DNA in plasma and tumor materials was observed (Figure 2a). The content of mutated DNA was significantly lower in cf-DNA than in materials from tumor biopsy (Wilcoxon test: $p<0.000035$; Figure 2b).

We re-obtained plasma samples from 10 patients every two months during routine controls. We observed severe decrease of mutated DNA content (exon 19 deletions or Leu858Arg substitution) within first two months of therapy and stabilization of mutated DNA content within next months which also correlated with clinical response to EGFR-TKIs. The content of mutated DNA remained low at the moment of clinical and radiological progression. The changes in concentration of mutated DNA in exemplary four cases are presented on Figure 3.

Monitoring of Thr790Met substitution in plasma samples during therapy

Initial content of mutated DNA with Thr790Met substitution was undetectable in most of the patients. The cf-DNA with this mutation appeared during therapy, when the response to EGFR-TKIs was observed. At the moment of progression, the content of mutated Thr790Met DNA was low (Fig. 3a and 3d). Disease progression related to increased content of cf-DNA with Thr790Med was observed only in one case (Fig. 3c). In this patient, the concentration of mutated DNA grew gradually during erlotinib administration and at the moment of progression, it exceeded the content of DNA with Leu858Arg mutation. Therefore, the patient was qualified to third EGFR-TKI generation (osimertinib), that resulted in partial remission and stabilization of cf-DNA with Thr790Met mutation content.

Correlation between content of mutated DNA and lung cancer staging (8th ed. Of TNM classification)

Patients with metastatic NSCLC had significantly higher content of mutated cf-DNA than patients with earlier stages of NSCLC (U-Mann Whitney test: $p=0.02$). In patients treated with EGFR-TKIs, the initial content of mutated cf-DNA had no effect on the risk of progression and death calculated with Kaplan-Meier and Cox logistic regression methods.

CONCLUSION

Analysis of *EGFR* mutations in cf-DNA shows lower sensitivity compared with DNA isolated from tumor samples. However, multiple evaluations of *EGFR* status may be useful in monitoring of therapy effectiveness and allows for early detection of acquired resistance to 1st and 2nd generation of EGFR-TKIs. In near future, liquid biopsy molecular testing could replace genetic examination of tumor material obtained by invasive procedures in patient' qualification to molecularly targeted therapies.