EGFR MUTATIONS STATUS FOR NON-SMALL-CELL LUNG CANCER PATIENTS

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Key words: non-small-cell lung cancer (NSCLC), targeted therapies, tyrosine kinase inhibitors, EGFR mutation, cfDNA.

Abbreviations: NSCLC - Non-small cell lung cancer; EGFR - Epidermal growth factor receptor; TKI - Tyrosine kinase inhibitor; FFPE - Formalin-fixed, paraffin-embedded; PFS - Progression-free survival; cfDNA - Circulating cell-free nucleic acids; ctDNA - Circulating tumor nucleic acids

Summary
With the advent of the molecular-targeted therapy, rapid progress has been made in the treatment of advanced or recurrent non-small-cell lung cancer (NSCLC). EGFR mutations detection in tumor is important to determine an appropriate treatment of EGFR TK inhibitors.

We investigated EGFR mutation status for patients with non-small cells lung carcinoma (NSCLC) patients’ tumorous cells from FFPE material in Klaipėda University Hospital, Lithuania (mutation test for the qualitative detection and identification of mutations in exons 18, 19, 20 and 21 of the EGFR gene) and T790M mutation in cfDNA (Liquid biopsy). Mutations of EGFR from FFPE were detected in 24 of the 119 patients (20%). EGFR mutations were more frequently found in women (13 of 36, 36%). cfDNA (Liquid biopsy) testing results show one of the patients had EGFR 20 exon T790M mutation detected which is resistance determining factor for the first and second generation EGFR TK Inhibitors treatment and a predicting marker for the third generation EGFR TK Inhibitors treatment.

Introduction
Lung cancer is the most commonly diagnosed cancer as well as the leading cause of cancer-related deaths in the world [1]. Non-small cell lung cancer (NSCLC) accounts for approximately 80% cases of lung cancer [2]. Most NSCLC patients are diagnosed with advanced or distant stages and they are ineligible for curative surgery and often suffer a poor survival. Identifying biomarkers related to treatment response and prognosis may be helpful to improve the clinical outcome of patients with NSCLC. The developments of molecular pathology methods have become increasingly important in the prediction of chemotherapy sensitivity and mutation analysis to identify driver mutations as important targets of new therapeutic agents. The most significant changes in the treatment of NSCLC revealed in new pathologic classification and in the introduction of molecularly targeted therapies, which include monoclonal antibodies and small molecule tyrosine kinase inhibitors [3]. In the past decade, significant improvements have been made due to the development of targeted therapies, such as EGFR tyrosine kinase inhibitors (TKIs), for advanced NSCLC. Patients with a sensitizing exon 19 deletion or an exon 21 substitution mutation are highly responsive to first-generation EGFR TKIs, such as gefitinib and erlotinib, compared to traditional platinum-based doublet chemotherapy, with a prolonged time to progression or improved progression-free survival (PFS) without serious drug-specific side effects.

The side effects of these agents are generally better tolerated than those of conventional chemotherapy and show higher efficacy. The most important factor follows: histology subtypes, gene mutation status, patients’ selection, drug toxicities and occurrence of drug resistance. A couple years ago a median lung cancer survival rate was 10–12 months. Since specific molecular targets are available, there is a significant increase in median survival rates up to 24–36 months. This gives an opportunity to provide a new standard of care [3]. Detection of EGFR mutations in tumor is very important in determining an appropriate treatment of EGFR TKI. EGFR wild-type tumors have a shorter progression survival rate than those with an EGFR mutation [4].

The use of circulating cell-free nucleic acids (cfDNA), consisting circulating cell-free (tumoral) DNA (cfDNA-ctDNA), as a liquid biopsy in lung cancer offers opportu-
nities to detect resistance mechanisms, such as the EGFR T790M mutation in the case of EGFR TKI use, at an early stage and to detect resistant mutation for EGFR mutated patients acquired resistance to the first- and second-generation EGFR TKI.

**Objective:** to evaluate EGFR gene mutations frequency for Non-Small-Cell Lung Cancer patients in Klaipeda University Hospital.

**Materials and methods**

Samples: formalin-fixed, paraffin-embedded (FFPE) tumor samples were obtained from and diagnosed NSCLC for 119 patients with NSCLC diagnosis from Klaipeda University Hospital.

DNA was extracted from sections of tissues FFPE tumour cells. DNA was extracted using Qiagen DNA FFPE tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions.

The EGFR mutation qualitative detection and identification of mutations were accomplished using therascreen EGFR Pyro Kit in exons 18, 19, 20 and 21 of the EGFR gene Kit, (Qiagen, Hilden, Germany).

Circulation tumor cells from plasma were extracted with QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) and these cells EGFR mutation (19 exon’s deletions, 20 exon’s T790M and 21 exon’s L858R) were detected using therascreen EGFR Plasma RGQ PCR Kit (Qiagen, Manchester, UK).

**Results**

EGFR mutations were detected in 24 (20%) out of 119 samples, while the rest of the samples did not have EGFR mutations.

<table>
<thead>
<tr>
<th>Mutation +</th>
<th>Mutation -</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>13</td>
<td>23</td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>72</td>
</tr>
<tr>
<td>Total</td>
<td>24 (20%)</td>
<td>95 (80%)</td>
</tr>
</tbody>
</table>

Table 1. DNA samples from NSCLC patients were tested for EGFR mutations

<table>
<thead>
<tr>
<th>G719X (Exon 18)</th>
<th>G719A (Exon 18)</th>
<th>Exon 19 deletion</th>
<th>S768I (Exon 20)</th>
<th>L858R (Exon 21)</th>
<th>L861Q (Exon 21)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>1 (4,2%)</td>
<td>3 (12,5%)</td>
<td>7 (29,2%)</td>
<td>2 (8,3%)</td>
<td>8 (33,3%)</td>
<td>3 (12,5%)</td>
</tr>
</tbody>
</table>

Table 2. EGFR mutations distribution

30% female and 70% male patients’ DNA were tested. EGFR mutations detected more frequently in females compared to males (36% female and 13% male were EGFR positive) (Table 1.)

The EGFR mutation frequency in Caucasian NSCLC patients was reported to be 10-15% [5]. A recent study reviewed EGFR mutation incidence in European countries [6,7]. EGFR mutation frequency in Europe ranged from 6% (Switzerland) to a maximum of 37.5% (Germany), depending on ethnicity and patient’s characteristics.

Hatson I et al. accomplished research results were different compared to our results – we have detected more mutations in female and male population (approximately 3 times more female and 4 times male population), however, identified mutations frequency was similar in both researches (the most common mutations were 19 exon deletion and 21 exon L861Q mutation) [8].

De Grève J et al. accomplished research was similar compared to our results – EGFR mutations detected in approx. of 20% in both investigate populations [9].

The most frequent mutations were 19 exon deletion and 21 exon L681Q mutations. These two mutations constitute 62,5% out of all detected mutations (Table 2.). These patients had Second Generation EGFR TKI treatment.

Two patients were testing for T790M mutation status (NSCLC heterogeneous tumor had been suspected), because progression of the disease during treatment with the EGFR TK Inhibitors. Circulating tumor DNA (ctDNA) is the cell-free DNA released from dying cancer cells [10], and represents an emerging field of cancer research [11].

One of the patients had EGFR 20 exon T790M mutation detected which is resistance determining factor for the EGFR TKI treatment and a predicting marker for the Third Generation EGFR TKI treatment. T790M is a frequently reported secondary mutation of the EGFR gene and is detected in 60% of patients with acquired resistance to EGFR TKI therapy [12].

**Discussions**

Nowadays, EGFR-TKIs are the most successful example of targeted therapy in NSCLC. EGFR gene mutations are the standard biomarkers for selecting NSCLC patients to receive EGFR-TKIs treatment. EGFR mutations can be
hological diagnosis, driver mutations detection from FFPE, future lung cancer treatment sequence will be as follows: patient, based on molecular profile knowledge, we are planning to adopt in treatment (clinical testing stages [9,13], some of the drugs are already adopted in treatment – TKI therapy (Second Generation EGFR TKI), while EGFR mutation G719M is resistance mutation to Second Generation EGFR TKI. Patients whose biopsies tested negative for T790M and had no response to treatment, may have been due to tumor heterogeneity [13]. T790X mutation is sensitive to Third Generation EGFR TKIs, but this has to be proved by a circulation tumor cells test.

In Lithuania all patients who are diagnosed with NSCLC are tested for EGFR gene mutations, which are linked to the EGFR TKIs treatment. College of American Pathologists (CAP), International Association for Lung Cancer (IASLC) and the Association for Molecular Pathology (AMP) guide-lines are similar to Lithuanian, but they recommend additionally doing molecular marker for NSCLC treatment - ALK rearrangement.

EGFR mutation testing and ALK rearrangement status are the only two molecular markers considered standard of care for NSCLC treatment and are the subject of CAP, International IASLC and AMP Guide-lines published in 2013 [14]. EGFR mutation and ALK rearrangement testing are also a feature of the Version 1.2014 National Comprehensive Cancer Network (NCCN) clinical practice guidelines for Non-Small Cell Lung Carcinoma [15] and KRAS mutation testing as a potential prognostic marker for NSCLC [16,17].

It makes assumptions for better patients’ treatment and optimizes treatment options – personalized medicine. Molecular lung cancer profile knowledge allows developing new diagnostic methods and targeting therapy instruments. One of the newest target therapy instruments is the third generation EGFR TKI treatment form assigned to occurring EGFR gene 20 exon T790M resistance mutations to second generation EGFR TKI treatment. Therefore, if patients presently treated with the second generation EGFR TKI treatment and it treatment was unsuccessful are tested for resistance mutations, because tumor can be heterogeneous.

Various driver mutations blocking drugs for lung cancer treatment are being developed - research is in different clinical testing stages [9,13], some of the drugs are already adopted in treatment (EGFR, ALH, KRAS, BRAF), therefore, based on molecular profile knowledge, we are planning to test 20-56 oncogenes for driver mutations. In the future lung cancer treatment sequence will be as follows: pathological diagnosis, driver mutations detection from FFPE, new generation sequencing (20-56 oncogenes panel), molecular profile analysis and then results will determine the best method of lung cancer treatment.

Targeted therapy based on molecular characterizations has greatly influenced the treatment strategies in NSCLC. Gene mutation analyses are the commonly used predictive biomarkers for selecting NSCLC patients to receive targeted agents. However, the current mutation analyses are often based on tumor tissues and have many limitations [18]. First, the accessibility of tumor tissues is not always satisfactory as most NSCLC patients are diagnosed with advanced stages and unsuitable to provide tissues through invasive surgery or biopsy. Second, surgery and biopsy are not without clinical complications. The adverse events rate for thoracic biopsy was reported to be approximately 20% [18]. Furthermore, some percentages of NSCLC patients will develop resistance to molecular-targeted agents [20].

Reference

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**EGFR MUTACIJŲ STATUSAS TARP PACIENTŲ, SERGANČIŲ NESMULKIALASTELINE PLAUČIŲ KARCINOMA**

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Raktažodžiai: nesmulkialastelė plaučių karcinoma, taikininė terapija, tirozinkinazės inhibitoriai, EGFR geno mutacijos.

Santrauka

Tikslas: Įvertinti EGFR geno mutacijų dažnius pacientams, sergančiams nesmulkialaštelė plaučių karcinoma Klaipėdos universiteto ligoninėje ligoninėje.

Molekulinės-taikininės terapijos atsiradimas padarė proveržį gydant nesmulkialaštelę plaučių vėžį. Naviko EGFR mutacijų nustatymas yra svarbus parenkant taikininę EGFR terapiją.

Klaipėdos universiteto ligoninėje ligoninėje iš FFPI ėminų mes nustatome pacientų, sergančių nesmulkialaštelė plaučių vėžiu, mutacijų statusą (tiriame 18, 19, 20 ir 21 egzono mutacijas), bei 20 egzono T790M mutaciją cirkuliująčiose DNR (skysta biopsija).

FFPI ėminėse EGFR mutacijos buvo nustatytos 24 pacientams iš 119 (20%). EGFR mutacijos dažniausiai nustatytos moterims (13 moterų iš 36, tai sudaro 36%). Taip pat nustatytą vieną iš dviejų pacientų EGFR 20 egzono T790M mutaciją cirkuliująčiose DNR (skysta biopsijoje). Ši mutacija yra resistentiškumo faktorius pirmos ir antros kartos EGFR taikinėne terapijai, taip pat predikcinis markeris trečiosios kartos EGFR taikinėne terapijai.

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