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ORIGINAL ARTICLE

EGFR mutations in patients with non small-cell lung cancer in Bulgaria and treatment with gefitinib

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Summary

Purpose: *To evaluate the* EGFR *mutations in non small cell lung cancer (NSCLC) patients in Bulgaria, as well as to summarize the outcomes of patients with EGFR mutations, treated with gefitinib as first- or subsequent-line therapy.*

Methods: *From January 2010 to March 2012 tumor samples from773 NSCLC patients were evaluated for* EGFR *mutations.*

Results: *Seventy-one mutations were found and 34 patients were treated with gefitinib. Complete remission (CR) was achieved in 2 patients (6.9%), partial remission (PR) in 11 (37.9%), stable disease (SD) in 13 (44.8%), and disease progression (PD) in 3 (10.3%). Higher objective response rate was seen in women and in never-smokers.The mean progression-free survival (PFS) was 11.1 months (95% CI 9.1-13.1), registered in 29 patients (median PFS 10 months ; 95% CI 8.9-11.1).Tolerability to gefitinib was acceptable, with prevalence of skin toxicity, and it did not lead to any significant decline of the patients' quality of life.*

Conclusion: *This is the first study in Bulgaria to evaluate* EGFR *mutations in NSCLC patients,which were encountered in 9.4% of the studied population. The present study confirms the benefits of first- and subsequent-lines of gefitinib for the treatment of this patient group. Our data give grounds for the conclusion that gefitinib is an effective and well-tolerated therapeutic option for patients with locally advanced and metastatic NSCLC harboring* EGFR *mutations.*

Key words: EGFR *mutations, gefitinib, non small cell lung cancer, tyrosine kinase inhibitor*

Introduction

Worldwide, lung cancer is the leading cause of cancer-related deaths in men [1]. Combination chemotherapy with platinum-based doublets has reached its plateau of efficacy for the treatment of patients with unresectable or metastatic disease. Chemotherapy-related toxicity, on the other hand, is not an uncommon cause for treatment discontinuation.The treatment of these patients therefore poses the necessity of new therapies, with new modes of action, ensuring superior efficacy and better tolerability.

Achievements of the current research in relation to the molecular biology of lung cancer and the validation of the prognostic and predictive biomarkers have offered the insight for the development of new therapeutic molecules [2]. The best studied family is the one of the epidermal growth factor receptors (EGFRs), comprising of EGFR/HER1, HER2/*neu*, HER3 and HER4. These receptors take part in a number of cellular processes such as proliferation, apoptosis suppression, cellular mobility and angiogenesis. It has been found that in NSCLC the EGFR regulation is altered through various mechanisms, including overexpression, amplification or mutations [3]. The activation of the EGFR-signaling pathway may contribute to tumor growth and disease progression through stimulation of tumor cells' proliferation, angiogenic factors production, amplification of

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the process of invasion and metastatic spread, and apoptosis suppression [4]. All these factors determined EGFR as a potential therapeutic target in NSCLC, leading to the development of gefitinib – the first oral, selective and reversible *EGFR*-tyrosine kinase inhibitor.

In May 2004 the results of 2 clinical trials were published [5,6], showing that the presence of somatic mutations in the kinase domain of EGFR correlates strongly with increased susceptibility to *EGFR*-tyrosine kinase inhibitors in patients with NSCLC. Studies that followed with the administration of gefitinib as first-line therapy showed much better therapeutic results in NSCLC patients with exon 19 deletion (del746_A750) or exon 21 point mutation (L858R) with objective response rate between 54.8 and 81.6%, and PFS between 9.7 and 13.3 months [7-9].

Until 2010 the first results of 4 phase III randomized clinical trials, comparing gefitinib and platinum-based chemotherapy as first-line therapy in patients with advanced NSCLC, were reported or published [10-13]. In 2 of those trials, the patients' inclusion was based on clinical characteristics suggestive of susceptibility to tyrosine kinase inhibitors and high *EGFR* mutations prevalence [10,11], while in the other 2 all patients included had tumors with proven *EGFR* mutations [12,13]. Analysis of the results of these trials showed that in patients with *EGFR* mutations, compared to standard chemotherapy, the administration of first-line gefitinib yielded longer PFS, higher objective response rates, more favorable toxicity profile and better quality of life.

The main objective of the present study was to assess the prevalence of *EGFR* mutations in NSCLC patients in Bulgaria. The study included patients with locally advanced or metastatic NSCLC, irrespective of age, sex, general condition, histology or implemented first- or subsequent-line chemotherapy.

In patients with proven positive *EGFR* mutation status treated with gefitinib, the therapeutic effect was evaluated through the objective response rate and PFS, and the safety profile of the drug was analyzed.

Methods

The *EGFR* mutations study covered the whole country. Both newly diagnosed NSCLC patients and patients after one or more lines of chemotherapy were included. A mandatory requirement was the histologic confirmation of NSCLC. All patients gave written informed consent for genetic testing in advance.

Genetic testing

For the genetic testing we used genomic DNA isolated from paraffin-embedded tumor tissue from 773 patients with NSCLC. For the DNA extraction we used kits for nucleic acid precipitation from paraffin-embedded tissues (QIAamp DNA FFPE Tissue Kit, Manchester, UK). For the analysis of the first 73 patients we used high resolution real-time polymerase chain reaction (RT PCR) and subsequent sequencing of the aberrant profiles [14]. The high resolution RT PCR analysis *per se* is very sensitive and allows for the detection of mutations with the availability of just 5-10% of tumor cells. The remaining 700 patients with NSCLC were sequenced for activating mutations with Scorpions technology-based RT PCR (therascreen® *EGFR* Mutation Detection Kit RGQ, Manchester, UK). This method allows for the detection of mutations with just 1% tumor cells availability [15].

Gefitinib was administered at a dose of 250 mg once daily until disease progression, manifestation of marked intolerance or patient refusal for further treatment.

The therapeutic effect was evaluated mostly through periodic CT control exams, and, when necessary, with additional studies such as MRI, bone scintigraphy, PET/CT, etc. The objective response was registered as CR, PR, SD and PD according to the standard criteria (RECIST 1.1). The PFS was measured from the start of therapy until disease progression or death. The safety profile was reported according to NCI-CTC (version 4) [16].

Statistics

Statistical analyses were performed through descriptive and graphic analysis of frequencies and cross tabulations. The mean and median PFS were evaluated by Kaplan-Meier analysis with log-rank test. A p-value < 0.05 was considered as statistically significant.

Results

The study was carried out between January 2010 and March 2012. Tumor samples from 773 patients with NSCLC were evaluated for *EGFR* mutations. Of them 545 were male (70.5%) and 228 female (29.5%). The majority (70%) of the patients were aged between 50 and 70 years (Figure 1). All patients were of Caucasian descent. Histological evaluation found adenocarcinoma in 50.9% of the patients, squamous-cell carcinoma in 43.8%, and other histologic types in the remaining 5.4%. The clinical stage distribution was as follows: stage I 3.9%, stage II 6.6%, stage III 31.7%, and stage IV 57.8%. Current smokers were 49.9% of the patients, ex-smokers 32.6%, and never-smokers 17.5%. Of the total of 773 *EGFR* mu-

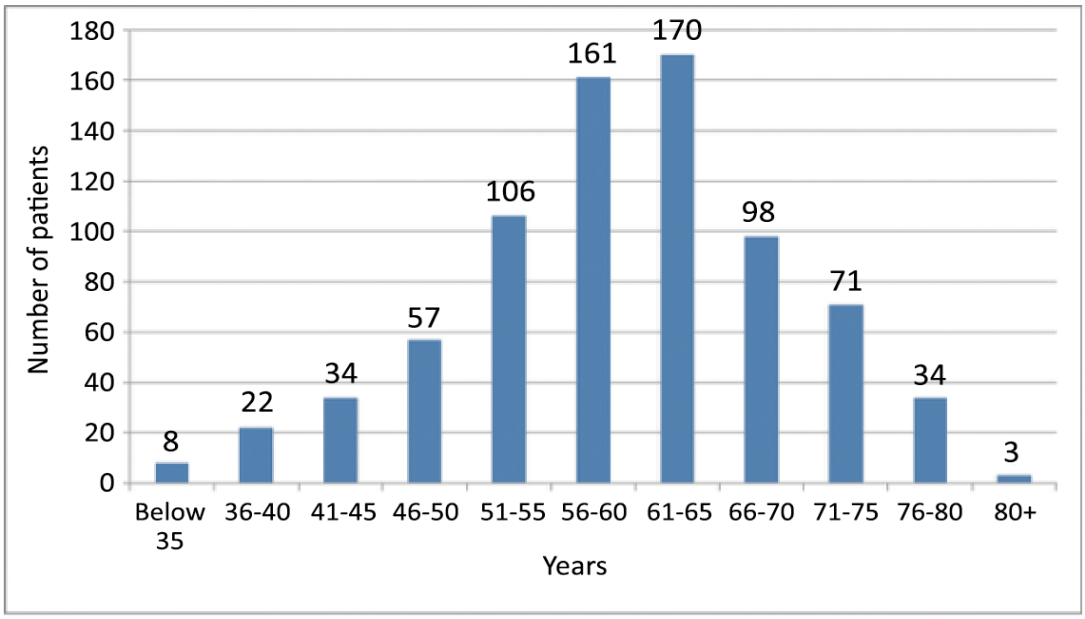

Figure 1. Age distribution of all 773 patients.

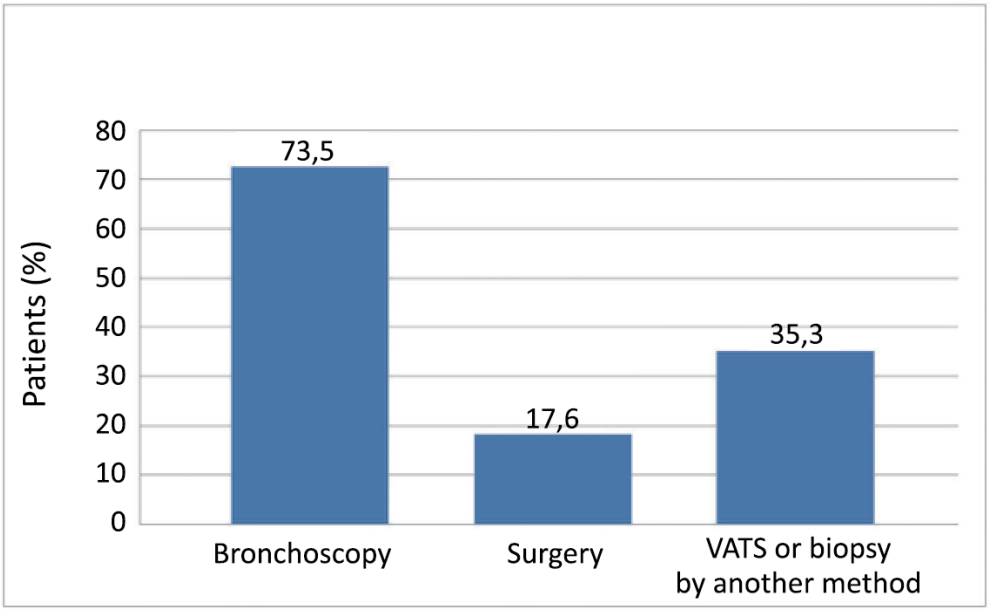

Figure 2. Procedures used for the diagnosis of the gefitinib-treated patients.

tation tests performed, 21 were unsuccessful for various reasons. Of the remaining 752 samples, mutations were found in 71 (9.4%). In the remaining 681 (90.6%) no mutations were found. This small number of positive patients is probably due to the low sensitivity of sequencing (35-50% tumor cells).

Analysis of the subgroup of patients with EGFR *mutations*

In the patient group with *EGFR* mutations (N=71), women prevailed over men (N=43 and N=28, respectively). The largest age group, with 75% of the patients, was the one between 50 and 70 years of age. In 59.7% of the patients the histologic type of the tumor was adenocarcinoma, in 33.9% squamous-cell carcinoma, and in the remaining 6.5% another type (bronchioalveolar or mixed). Stage IA disease had 1.6% of the patients, stage IIB 3.3%, stage IIIA 21.3%, stage IIIB

Table 1. Demographic characteristics of 34 patients with positive EGFR mutations, treated with gefitinib

Characteristics	*N*	*%*
Sex		
Male	12	35.3
Female	22	64.7
Age, years (mean 59.24; 95% CI 56.26 - 62.22)		
36-40	1	2.9
41-45	2	5.9
46-50	4	11.8
51-55	4	11.8
56-60	6	17.6
61-65	8	23.5
66-70	8	23.5
76-80	1	2.9
Histology		
Squamous-cell	8	23.5
Adenocarcinoma	24	70.6
Mixed adenocarcinoma and squamous-cell carcinoma	1	2.9
Mixed adenocarcinoma	1	2.9
History of smoking		
Current smoker	7	20.6
Never-smoker	14	41.2
Ex-smoker	13	38.2
Line of therapy with gefitinib		
First	16	47.1
Second	12	35.3
Third	4	11.8
Fourth	2	5.9

Table 2. Adverse events with gefitinib treatment

Adverse events	*Patients, %*
None	55.9
Skin	29.4
Liver	6.0
Flu-like	2.9
Fatigue	2.9
Total	100

Figure 3. Patient distribution according to the duration of gefitinib treatment.

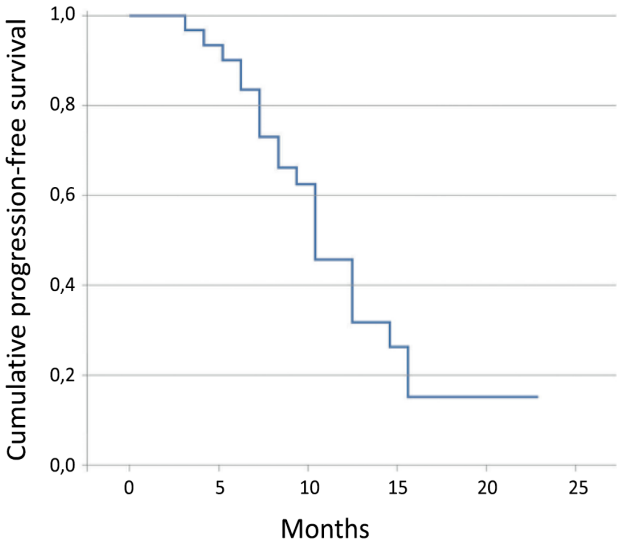

Figure 4. Progression-free survival (median 10 months, range 8.9-11.0).

9.8%, and stage IV 63.9%. Most of the patients were either never-smokers (39.7%), or ex-smokers (33.3%), the current smokers being only 27%.

Of a total of 71 patients with *EGFR* mutations, 34 were treated with gefitinib, and the remaining 37 did not receive this treatment for various reasons. Gefitinib was administered to 22 women and 12 men, of whom 24 were diagnosed with adenocarcinoma, 8 with squamous-cell carcinoma, and 2 with mixed histology tumors. The stage distribution was as follows: stage IIIA6 patients, stage IIIB 4 patients, and stage IV 24 patients. Seven patients were current smokers, 13 were ex-smokers, and 14 were never-smokers. The patients with exon 19 mutations (N=19) prevailed. The most common diagnostic procedure was bronchoscopy, followed by VATS and thoracotomy (Figure 2).

Gefitinib was administered as first-line therapy to 16 patients, as second-line therapy to 12, as third-line therapy to 4, and as fourth-line to 2 patients. In 8 patients the therapy lasted less than 6 months, in 20 from 6 to 12 months, and in the remaining 4 patients – more than a year (Table 1, Figure 3).

CR was achieved in 2 patients (6.9%), PR in 11 (37.9%), SD in 13 (44.8%), and PD in 3 (10.3%). No difference regarding the therapeutic results was found in relation to age or histologic type of the tumor.

The mean PFS was 11.1 months (95% CI 9.1 to 13.1), registered in 29 patients (Figure 4), while the median PFS was 10 months (95% CI 8.9 to 11.1).

As far as the reported adverse reactions are concerned, the most common ones were skin rash, seen in 29.4% of the patients, diarrhea in 2.9%, elevated liver function tests in 6.0%, flu-like symptoms in 2.9%, and fatigue in 2.9% (Table 2).

Discussion

In this study we found that 9.4% of the studied population had *EGFR* mutations. This rate is similar to the results of Marchetti et al. [17], who found 10% rate of *EGFR* mutation in a study of 375 tumor samples from lung adenocarcinoma. Three years ago the Spanish Lung Cancer Group published the results of a prospective study that screened *EGFR* mutations in 2105 patients with metastatic NSCLC [8]. *EGFR* mutations were found in 350 of them (16.6%) – a significantly higher rate compared to our results. Nevertheless, our study is ongoing and in the future, with the increased number of tested samples, the rate of the *EGFR* mutations may change.

In the group with *EGFR* mutations (71 patients),women were almost twice as much as men (43 vs 28), with prevalence of adenocarcinoma over squamous-cell or other types of cancers (59.7% vs 33.9% vs 6.5%, respectively), as well as prevalence of never-smokers or ex-smokers over current smokers (39.7% vs 33.3% vs 27.0%, respectively). These data correspond to the literature data [5-7,10-13].

Gefitinib, administered as both first- and subsequent-line therapy, has, in a number of clinical trials, proven its advantages with regards to efficacy, safety profile and quality of life [7,9-13].

In this study gefitinib produced 2 CRs (6.9%) and 11 PRs (37.9%), i.e. the objective response rate was 44.8%. SD was seen in 13 (44.8%) patients, and PD in 3 (10.3%) patients. Higher objective response rate was found in women and never-smokers. There was no difference in the therapeutic results in relation with age and tumor histology. The mean PFS was 11.1 months for all cases, and 9.5 months when gefitinib was administered as first-line treatment.

Toxicity of gefitinib was acceptable, with prevalence of skin toxicity, and did not lead to any significant deterioration of the patients' quality of life.

The latest guidelines advocate the use of gefitinib as first-line therapy in NSCLC patients with *EGFR* mutations, outmatching conventional cytotoxic therapy with regards to PFS and safety, which are both reliably more favorable.

The relation between these mutations and gefitinib's more favorable therapeutic activity can improve the prognosis of the disease, and has to be investigated further and taken into consideration when choosing the therapeutic strategy.

In our study the rate of the *EGFR* mutations in NSCLC patients was 9.4%.

This study also confirmed the benefits of first- and subsequent-lines of gefitinib administration in this patient group.

Our data give grounds to conlude that gefitinib is an effective and well-tolerated therapeutic option for patients with locally advanced and metastatic NSCLC with cells positive for *EGFR* mutations.

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