Predictors of *EGFR* mutation and factors associated with clinical tumor stage at diagnosis: Experience of the INSIGHT study in Poland

RODRYG RAMLAU[1], PAWEŁ KRAWCZYK[2], RAFAŁ DZIADZIUSZKO[3], IZABELA CHMIELEWSKA[2], JANUSZ MILANOWSKI[2], WŁODZIMIERZ OLSZEWSKI[4], KATARZYNA STENCEL[5], KATARZYNA RAMLAU-PIĄTEK[6], AGNIESZKA SEGIET[7], MICHAŁ SKROŃSKI[8], JACEK GRUDNY[9] and JOANNA CHOROSTOWSKA-WYNIMKO[8]

[1]Department of Oncology, Poznan University of Medical Sciences, 60-569 Poznan; [2]Department of Pneumology, Oncology and Allergology, Medical University of Lublin, 20-954 Lublin; [3]Department of Oncology and Radiotherapy, Medical University of Gdansk, 80-211 Gdansk; [4]The Maria Sklodowska-Curie Institute of Oncology, 02-781 Warsaw; [5]Department of Chemotherapy, Poznan University of Medical Sciences, 60-569 Poznan; [6]Departament of Radiology, Poznan University of Medical Sciences, 61-848 Poznan; [7]First Faculty of Medicine, Medical University of Warsaw, 02-091 Warsaw; [8]Department of Genetics and Clinical Immunology; [9]III Department of Lung Diseases, National Institute of Tuberculosis and Lung Diseases, 01-138 Warsaw, Poland

Received February 24, 2017; Accepted August 3, 2017

DOI: 10.3892/ol.2017.6907

Abstract. Targeted therapy of non-small cell lung cancer (NSCLC) patients with mutations in the epidermal growth factor receptor (*EGFR*) gene has been associated with improved prognosis. However, there is a shortage on data from real-world clinical practice in management of *EGFR*-positive NSCLC patients in Poland. The present study retrospectively analyzed data from the INSIGHT study to evaluate the incidence and clinical management of EGFR-positive NSCLC in Poland. The authors additionally aimed to identify predictors of the *EGFR* mutation and factors associated with clinical stage of the tumor at diagnosis. Incidence of *EGFR* mutations was 11.8% and the most common mutations were a deletion on exon 19 and an L858R substitution on exon 21. Mutations were strongly associated with female gender [male vs. female odds ratio (OR): 0.51; P=0.004] and never having smoked (current/past smoker vs. never smoked OR: 0.16; P<0.001), and advanced clinical stage (stage IV vs. stage I/II OR: 2.89; P=0.029). Patients with *EGFR* mutation were also observed to have a greater propensity to develop bone metastasis (OR: 11.62; P=0.008). Multivariate regression analysis demonstrated that patients with past or current smoking history or a poor performance on the Eastern Cooperative Oncology Group (ECOG) scale were less likely to have the *EGFR* mutation. Furthermore, EGFR-positive patients with greater ECOG scores and a tumor other than adenocarcinoma or squamous cell carcinoma were more likely to present advanced tumors. Early screening for *EGFR* mutation and the use of *EGFR*-targeting therapies as first-line agents may lead to better prognosis and successful clinical management of *EGFR*-positive NSCLC patients.

Correspondence to: Professor Rodryg Ramlau, Department of Oncology, Poznan University of Medical Sciences, Szamarzewskiego 82/84, 60-569 Poznan, Poland
E-mail: rramlau@gmail.com

Key words: non-small cell lung cancer, *EGFR* mutation, tyrosine kinase inhibitors, Poland

Introduction

Treatment of non-small cell lung cancer (NSCLC) requires a complex regimen involving surgery, radiotherapy, and systemic therapy. Molecular status of individual tumors is considered as a predictive factor for response to chemo- or biological therapy or as a prognostic marker for disease progression (1-3). Driver mutations that are known to promote carcinogenesis have been identified in certain genes-mutations in *EGFR* (for epidermal growth factor receptor; *EGFR*), *PI3K* (for phosphatidylinositol 3-kinase; PI3K), *BRAF* (for B-Raf), and *KRAS* (for k-Ras) are considered to have a predictive value in NSCLC patients (4) and the highest incidence of mutations is observed in the *EGFR* and *KRAS* genes (5,6).

Mutations in *EGFR* occur in exons 18-21 which encode the tyrosine kinase domain of EGFR. These cause a loss in autoinhibition of the tyrosine kinase and a continually activated state of its kinase function. Deletion in exon 19 and a L858R (leucine to arginine substitution at position 858) substitution in exon 21 comprise approximately 90% of *EGFR* mutations found in adenocarcinomas of the lung (7-16). Patients with diagnosed mutations in *EGFR* are referred to as EGFR-positive. The incidence of *EGFR* mutation in Caucasian NSCLC patients in Europe is estimated to be 10% (7,9) and is more common in women and non-smokers.

While platinum-based chemotherapy has been the mainstay of NSCLC treatment, advances in molecular diagnostics and targeted therapies have resulted in a paradigm shift with a focus on individualized medicine based on histologic classification, pathologic staging, prognostic markers of survival, and predictive markers of therapeutic response (17). The discovery of *EGFR* mutations in 2004 and subsequent therapeutic response in terms of response rate (RR), progression-free survival (PFS), and quality of life (QoL) elicited in EGFR-positive patients by targeted therapy with EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib have been adequately documented in large-scale clinical trials (12-15,18-24). These results have led to a consensus that the presence of *EGFR* mutations is a strong predictor of TKI treatment response and various associations and working groups, internationally and in Poland, now recommend screening newly diagnosed patients with advanced NSCLC for specific mutations in order to customize the modality of treatment (9,19-32). Despite these, the incidence rates of *EGFR* mutations are found to vary substantially between different regions and countries (8,10,15). Possible reasons for this discrepancy could be lack of clarity in selection criteria for EGFR-positivity testing, differences in molecular methods employed for screening, and issues with sampling and tissue preservation. In addition, improper sample collection, preparation, and storage techniques can render it unamenable for molecular analysis. However, rapid advancement in diagnostics and the use of modern methods such as quantitative polymerase chain reaction (qPCR) allows for detection of *EGFR* mutation in samples containing as low as 1% tumor cells (33) and the use of these techniques as a standard screening procedure in NSCLC categorization could resolve the issue of accurate detection and treatment optimization.

Despite developments in treatment of advanced NSCLC and widely accepted guidelines in place, there is paucity in real-life data on management of EGFR-positive NSCLC in the Central/Eastern European (CEE) region. The main goal of the ImplementatioN of perSonalized medicine In NSCLC in Central Europe: EGFR testing, Histopathology, and clinical feaTures (INSIGHT) registry was to address these shortcomings (34). In this sub-analysis of the INSIGHT study, we report the real-life scenario in clinical management, including diagnosis and treatment, of EGFR-positive advanced NSCLC in Poland. Furthermore, we aimed at identifying predictors of *EGFR* mutation as well as factors associated with the clinical tumor stage at the time of diagnosis.

Patients and methods

Patient enrollment and data collection. The INSIGHT study (34) was a multicenter, observational registry of patients with NSCLC and tested for *EGFR* mutation, conducted between November 2011 and March 2013 in five CEE countries including Poland. Patients were ≥18 years of age, were diagnosed with advanced and/or metastatic NSCLC and had biopsy tissue available for *EGFR* testing, and provided an informed consent to participation in the study. Also included were patients who had already tested positive for *EGFR* mutation and had either commenced or were scheduled to begin treatment with EGFR TKIs.

Data collected included demographics, NSCLC diagnosis, performance status, smoking status, histopathological examination (including *EGFR* mutation), molecular method used for determination of *EGFR* mutation, and prior and current treatment regimens (including EGFR TKIs).

Performance status was assessed according to the Eastern Cooperative Oncology Group (ECOG) scale (35): ECOG=0: Fully active, able to carry on all pre-disease performance without restriction; ECOG=1: Restricted in physically strenuous activity but ambulatory and able to carry our work of a light or sedentary nature; ECOG=2: Ambulatory and capable of all self-care but unable to carry out any work activities; up and approximately more than 50% of waking hours; ECOG=3: Capable of only limited self-care; confined to bed or chair >50% of waking hours; ECOG=4: Completely disabled; cannot carry on any self-care; totally confined to bed or chair; ECOG=5: Dead.

The INSIGHT registry was approved by Ethics Committees and followed all local laws and regulations.

Statistical analysis. Patient characteristics were summarized using descriptive statistics. Categorical variables were compared using Fisher's exact test.

Predictors of presence of EGFR mutation were determined by logistic regression analysis. For each pre-chosen parameter, a univariate model was first developed. Subsequently, these were used to derive a multivariate model through backward elimination. Odds ratio (OR), 95% confidence interval (95% CI) for OR and Wald test P-value were reported.

Factors influencing clinical tumor stage at diagnosis were identified with ordered logistic regression analysis. Clinical tumor stage was divided into four categories-I/II, IIIA, IIIB and IV. A multivariate model was derived as described in the preceding paragraph. Proportional OR, 95% CI for proportional OR (proportional 95% CI) and Wald test P-value were reported.

P<0.05 was considered to indicate a statistically significant difference. Statistical analysis was performed using the R software version 3.1.2 (36).

Results

Patient population. Data from 696 patients collected from four centers in Poland were included in the analysis. The majority of the patients were ≥60 years of age [median: 61.4 years, interquartile range (IQR) 57.0-67.2 years], white Caucasian (n=696, 100%), and male (n=417, 59.9%). Regarding smoking status, 196 (28.2%) patients were currently smoking and 285 (40.9%) were ex-smokers.

Most of the NSCLC cases were advanced and metastases were identified in 140 (20.1%) patients at diagnosis. Common sites of metastases were supraclavicular lymph node (n=36, 25.7%), brain (n=25, 17.9%), lung (n=7, 5.0%), and bone (n=5, 3.6%) and these are classified and compared based on their *EGFR* mutation status in Table I. The primary tumor was identified in 571 (82.0%) patients.

Performance status at the time of diagnosis as defined by ECOG rating was 'good' in a substantial proportion of the patients [ECOG=0 in 16 (3.9%) patients; ECOG=1 in 308 (74.9%) patients] and 'moderate' (ECOG=2) in 81 (19.7%) patients (Table II).

Table I. Metastasis by presence of *EGFR* mutation.

Variables	EGFR-positive	EGFR-negative	P-value
All metastasis, n (%)	20 (24.4)	120 (19.5)	0.378
OR (95% CI) of *EGFR* mutation in patients with all metastasis vs. no metastasis	1.33 (0.76-2.25)		0.305
Brain, n (%)	2 (2.4)	23 (3.7)	0.757
OR (95% CI) of *EGFR* mutation in patients with brain metastasis vs. no brain metastasis in whole group	0.64 (0.10-2.28)		0.553
Subgroup with metastasis, n (%)	2 (10.0)	23 (19.2)	0.528
OR (95% CI) of *EGFR* mutation in patients with brain metastasis vs. any but brain metastasis	0.47 (0.07-1.78)		0.332
Bone, n (%)	3 (3.7)	2 (0.3)	0.013
OR (95% CI) of *EGFR* mutation in patients with bone metastasis vs. no bone metastasis in whole group	11.62 (1.90-89.24)		0.008
Subgroup with metastasis, n (%)	3 (15.0)	2 (1.7)	0.021
OR (95% CI) of *EGFR* mutation in patients with bone metastasis vs. any but bone metastasis	10.41 (1.62-83.51)		0.014
Lung, n (%)	1 (1.2)	6 (1.0)	0.586
OR (95% CI) of *EGFR* mutation in patients with lung metastasis vs. no lung metastasis in whole group	1.25 (0.07-7.45)		0.837
*S*ubgroup with metastasis, n (%)	1 (5.0)	6 (5.0)	>0.999
OR (95% CI) of *EGFR* mutation in patients with lung metastasis vs. any but lung metastasis	1.00 (0.05-6.32)		>0.999
Liver, n (%)	0 (0.0)	1 (0.2)	>0.999
OR (95% CI) of *EGFR* mutation in patients with liver metastasis vs. no liver metastasis in whole group	NA[a]		NA[a]
Subgroup with metastasis, n (%)	0 (0.0)	1 (0.8)	>0.999
OR (95% CI) of *EGFR* mutation in patients with liver metastasis vs. any but liver metastasis	NA[a]		NA[a]
Adrenal gland, n (%)	0 (0.0)	1 (0.2)	>0.999
OR (95% CI) of *EGFR* mutation in patients with adrenal gland metastasis vs. no adrenal gland metastasis in whole group	NA[a]		NA[a]
*S*ubgroup with metastasis, n (%)	0 (0.0)	1 (0.8)	>0.999
OR (95% CI) of *EGFR* mutation in patients with adrenal gland metastasis vs. any but adrenal gland metastasis	NA[a]		NA[a]
SCL, n (%)	3 (3.7)	33 (5.4)	0.789
OR (95% CI) of *EGFR* mutation in patients with SCL metastasis vs. no SCL metastasis in whole group	0.67 (0.16-1.92)		0.512
Subgroup with metastasis, n (%)	3 (15.0)	33 (27.5)	0.364
OR (95% CI) of *EGFR* mutation in patients with SCL metastasis vs. any but SCL metastasis	0.47 (0.10-1.50)		0.245
Other, n (%)	2 (10.0)	23 (19.2)	0.528
OR (95% CI) of *EGFR* mutation in patients with other metastasis vs. no other metastasis in whole group	1.58 (0.75-3.04)		0.199
Subgroup with metastasis, n (%)	11 (55.0)	55 (45.8)	0.604
OR (95% CI) of *EGFR* mutation in patients with other metastasis vs. any but other metastasis	1.44 (0.56-3.83)		0.449

EGFR, epidermal growth factor receptor; OR, odds ratio; CI, confidence interval; SCL, supraclavicular lymph node. [a]Model parameters estimation impossible because of too few positive observations.

Histopathological diagnosis. Diagnosis of NSCLC was based on evaluation of histological specimen in 82.4% of the patients and of cytological sample in 16.8% of the patients. The most commonly employed methods of sample collection were

Table II. Performance status and clinical stage of tumors by presence of *EGFR* mutation.

	n (%)		
Variables	EGFR negative	EGFR positive	Total
ECOG score			
0	9 (2.5)	7 (13.5)	16 (3.9)
1	275 (76.6)	33 (63.5)	308 (74.9)
2	70 (19.5)	11 (21.2)	81 (19.7)
3	5 (1.4)	1 (1.9)	6 (1.5)
4	0 (0.0)	0 (0.0)	0 (0.0)
Clinical tumor stage			
I/II	67 (13.6)	5 (6.6)	72 (12.7)
IIIA	64 (13.0)	4 (5.3)	68 (12.0)
IIIB	79 (16.0)	6 (7.9)	85 (14.9)
IV	283 (57.4)	61 (80.3)	344 (60.5)

EGFR, epidermal growth factor receptor; ECOG, Eastern Cooperative Oncology Group.

surgical biopsy (32.1%), transbronchial biopsy (25.8%), and endoscopic/endobronchial ultrasound guided needle biopsy (15.5%). Other methods also used were computed tomography guided transpareital biopsy (9.2%), intraluminal biopsy (3.0%), fine needle biopsy without X-ray guidance (2.4%), mediastinoscopy (2.4%), brush cytology (1.7%), computed tomography guided bronchoscopic biopsy (0.9%), bronchial lavage (0.9%), and others (6.0%).

Most of the NSCLC tumors in study patients were of adenocarcinomatous (AC) origin-36.5% of the patients presenting non-mucinous AC, 30.2% presenting non-specified AC, and 3.6% with mucinous AC. A total of 1.3% patients presented with an adenosquamous carcinoma, a tumor type with a mixed histology. A majority of EGFR-TKI treated patients present with adenosquamous carcinomas with a predominance of adenomas.

Clinical management of NSCLC. A substantial proportion of study patients (79.6%) received systemic therapy-of these, 85.0% as palliative therapy, 11.3% in neoadjuvant setting, and 3.8% in adjuvant setting (data not shown). In patients not considered for systemic therapy, low performance status and poor compliance were the major individual reasons for the decision. Surgery as a therapeutic intervention was performed in 26.6% of the patients while 13.7% received radiation therapy.

EGFR mutation. A total of 82 (11.8%) study patients were determined to have *EGFR* mutations. Most commonly reported mutations were deletion on exon 19 and L858R substitution on exon 21. Details of distribution of *EGFR* mutations are given in Fig. 1.

Factors associated with presence of EGFR mutation. Patients with *EGFR* mutation were predominantly female [45 of 82

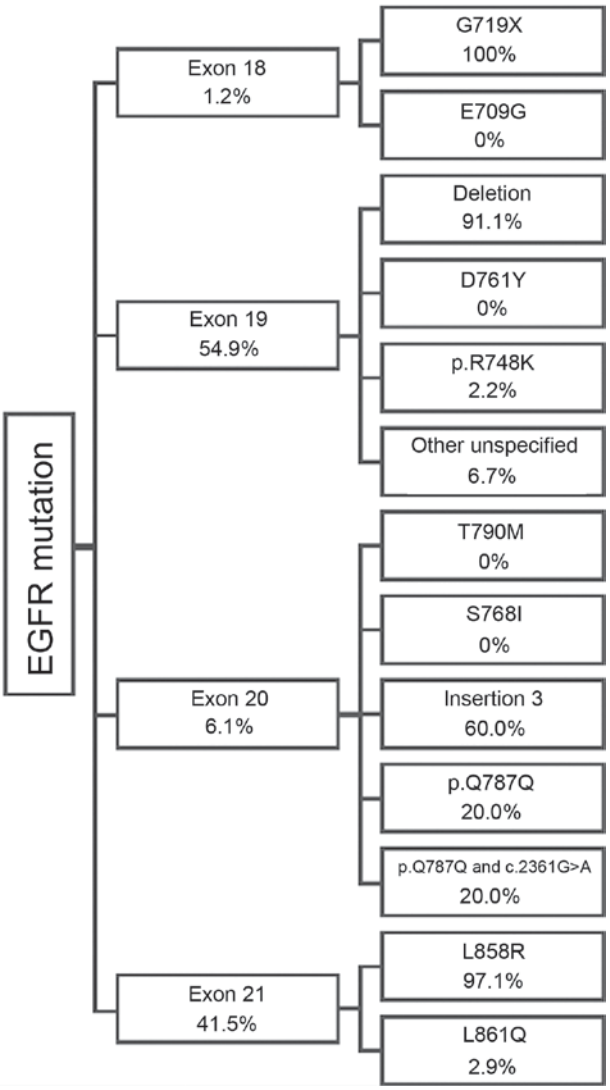

Figure 1. Types of *EGFR* mutations in study population. A patient could have more than one deletion or deletions on more than one chromosome. EGFR, epidermal growth factor receptor.

(54.9%) EGFR-positive patients] in comparison to patients without such mutations (38.1% of EGFR-negative patients) (P=0.005). An EGFR-positive status was strongly associated with gender and males were less likely to be EGFR-positive (male vs. female OR: 0.51; 95% CI: 0.32-0.80; P=0.004). The proportion of never-smokers was higher in EGFR-positive patients (26 of 69 patients, 37.7%; smoking status unknown in 13 patients) than in EGFR-negative patients (6.8%) (P<0.001) and the propensity for EGFR-positive status was lower in patients who were current or past smokers (OR: 0.16; 95% CI: 0.09-0.28; P<0.001). Performance status at the time of diagnosis had a wider distribution and proportion of patients in advanced disease stage was higher in EGFR-positive patients (Table III). Moreover, EGFR-positive patients had a higher predilection to be in clinical stage IV than in stage I/II at the time of diagnosis (OR: 2.89; 95% CI: 1.22-8.50; P=0.029).

EGFR mutation and metastasis. With the exception of metastasis to the bone, no association was observed between the EGFR status and metastatic potential of primary tumors. Incidence of metastasis to the bone was higher (3.7%) in patients with *EGFR*

Table III. Factors associated with advanced cancer stage identified by multivariate analysis.

Variables	Proportional OR (95% CI)	P-value
EGFR mutation status		
EGFR-positive vs. EGFR-negative	2.63 (1.31-5.26)	0.006
Performance on ECOG scale		
ECOG=1 vs. ECOG=0	2.26 (0.80-6.41)	0.125
ECOG=2/3 vs. ECOG=0	5.87 (1.86-18.48)	0.003
Diagnosis		
All other tumour types except squamous cell carcinoma and adenocarcinoma vs. adenocarcinoma	1.92 (1.18-3.14)	0.009
Squamous cell carcinoma vs. adenocarcinoma	2.43 (0.22-26.54)	0.466

EGFR, epidermal growth factor receptor; OR, odds ratio; CI, confidence interval; ECOG, European Cooperative Oncology Group.

mutation in comparison with EGFR-negative patients (0.3%; P=0.013). The odds of *EGFR* mutation in patients with bone metastasis in comparison to the patients without bone metastasis in the entire population or those with any but bone metastasis was also significantly higher (bone metastasis vs. no bone metastasis in the entire group OR=11.62; 95% CI: 1.90-89.23; P=0.008 and bone metastasis vs. any but bone metastasis in metastasis group OR=10.41; 95% CI: 1.62-83.51; P=0.014).

EGFR mutation and histopathological tumor staging. An association between histopathological diagnoses of NSCLC and presence of *EGFR* mutation is given in Table IV. The most striking observation is decreased probability of *EGFR* mutation in not otherwise specified (NOS) type NSCLC in comparison to adenocarcinoma (OR=0.23; 95% CI: 0.06-0.64; P=0.015). Patients without *EGFR* mutation were noted to undergo surgical treatment more frequently than EGFR-positive patients but this difference could not be statistically validated (27.9 vs. 19.5%; P=0.151). We also observed that more patients with *EGFR* mutation received radiotherapy in comparison to EGFR-negative patients (24.4 vs. 11.7%; P=0.004).

Factors associated with presence of EGFR mutation. As shown in Table V, multivariate regression analysis revealed that NSCLC patients who were past or current smokers or had lower performance as per ECOG scale were less likely to carry a *EGFR* mutation.

Factors associated with clinical tumor stage at diagnosis. The proportional OR between stages I/II, IIIA, IIIB, and IV for ECOG=1 vs. ECOG=0 was 1.515 (95% CI: 0.580-3.958; P=0.397). In contrast, the proportional OR between stages when comparing ECOG=2-3 and ECOG=0 was 4.076 (95% CI: 1.394-11.923; P=0.010) thereby indicating that performance status substantially diminished with progression in clinical stages.

The NOS type NSCLC was associated with greater odds of more advanced clinical tumor than adenocarcinoma. Odds of more advanced clinical tumor in bronchoalveolar carcinoma, large cell carcinoma, mixed carcinoma, or squamous cell carcinoma did not differ significantly from odds in adenocarcinoma diagnosis. In contrast, the mixed type carcinoma had lower odds of more advance clinical stage than adenocarcinoma.

The results of multivariate regression modelling (Table III) confirm previous findings that patients with poor ECOG scores, *EGFR* mutation, and diagnosed with NSCLC of a histopathological type other than adenocarcinoma or squamous cell carcinoma have a higher likelihood of being diagnosed with advanced clinical tumors.

Discussion

We found that the incidence of *EGFR* mutation in the Polish subpopulation of the INSIGHT study is similar that reported for the general European population (5). We also reiterate previous findings that EGFR-positive status is associated with female gender and never-smoker status. The clinical stage of NSCLC at the time of diagnosis was usually more advanced in EGFR-positive patients thereby precluding radical surgery and promoting radiotherapy and systemic therapy in these patients. However, one must take note that the presentation of advanced stage in NSCLC patients with *EGFR* mutations is not always associated with an increased probability of bearing such a mutation; the late diagnosis could simply be because patients in Stage I or II are not routinely tested for mutations. We also discovered that the propensity to develop bone metastasis, usually associated with a poor prognosis (37), was almost 12-fold higher in EGFR-positive NSCLC patients in comparison to their EGFR-negative counterparts. However, this statistic may not be an accurate reflection of the actual risk when we take in consideration the small patient number in the reported study.

The choice of appropriate molecular methods is important for reliable detection of *EGFR* mutations, especially in samples with low tumor cell count. The effectiveness of these methods could be jeopardized by suboptimal procedures of tumor sampling and preservation techniques. Nevertheless, results from a multicenter, retrospective study designed to evaluate effectiveness of various methods for *EGFR* mutation testing showed no substantial difference in detected frequency of mutations between cytological and histological samples (33). This implies that the low tumor cellularity evidenced with

Table IV. *EGFR* mutation and clinical stage of tumor by histopathological diagnosis.

OR (95% CI) of EGFR-positive status	P-value	Proportional OR (95% CI) of clinical stage of tumor	P-value
Bronchoalveolar carcinoma vs. adenocarcinoma			
3.51 (0.48-18.38)	0.151	2.38 (0.25-22.21)	0.446
Large-cell carcinoma vs. adenocarcinoma			
0.88 (0.05-4.90)	0.904	1.18 (0.29-4.80)	0.818
Mixed cell carcinoma vs. adenocarcinoma			
2.11 (0.46-7.11)	0.266	0.13 (0.03-0.57)	0.007
NOS carcinoma vs. adenocarcinoma			
0.23 (0.06-0.64)	0.015	2.07 (1.25-3.42)	0.005
Other carcinoma vs. adenocarcinoma			
2.64 (0.92-6.67)	0.051	1.48 (0.60-3.65)	0.399
Squamous cell carcinoma vs. adenocarcinoma			
1.08 (0.17-4.03)	0.919	1.97 (0.40-9.82)	0.406

EGFR, epidermal growth factor receptor; OR, odds ratio; CI, confidence interval; NOS, not otherwise specified.

Table V. Factors associated with presence of *EGFR* mutation identified by multivariate analysis.

Factor	OR (95% CI)	P-value
Smoking status		
Ex/current smoker vs. never smoked	0.12 (0.05-0.25)	<0.001
Performance on ECOG scale		
ECOG=1 vs. ECOG=0	0.19 (0.06-0.70)	0.009
ECOG=2 vs. ECOG=0	0.25 (0.06-1.01)	0.048
ECOG=3 vs. ECOG=0	0.54 (0.02-4.98)	0.624

EGFR, epidermal growth factor receptor; OR, odds ratio; CI, confidence interval; ECOG, European Cooperative Oncology Group.

cytological and small biopsy samples did not hinder the sensitivity of the real-time PCR assay employed in the study for detecting *EGFR* mutations. This offers a substantial benefit in screening NSCLC patients with poorer performance status or those in whom invasive procedures are contraindicated. Despite the demonstrated benefits of TKI therapy in EGFR-positive NSCLC and guidelines recommending timely screening and treating at-risk patients with TKI as first-line chemotherapy (38), these are not widely applied in clinical practice in Poland. While we cannot identify any particular reason for this, it has been demonstrated that routine nationwide molecular profiling of NSCLC patients is feasible and provides immense benefit in terms of frequency of driver mutations and their specific type (39). Of special interest are NSCLC with uncommon *EGFR* mutations that occur in approximately 1.0% of NSCLC cases (40), or those with poor prognosis such as adenosquamous carcinoma (41), detected in 1.3% of our study patients, and EGFR-positive NSCLC metastasizing to the bone (42). The heterogeneous molecular profiles presented in these types need to be studied in detail in order to not only study the occurrence and pathological differences arising from various mutations but also to design specific therapies aimed at molecular targets (40).

The database of the INSIGHT registry is the first of its kind for Poland in our knowledge and it provides robust data on sampling methods, molecular testing, frequency and types of *EGFR* mutations, treatment modalities, and prognostic factors related to Polish patients with EGFR-positive NSCLC. In comparison to conventional chemotherapy, EGFR TKI therapy has been demonstrated to be more effective and safer in NSCLC patients with *EGFR* mutations. A notable conclusion of a meta-analysis of 13 phase III trials was that targeted therapy with EGFR TKIs noticeably improved PFS [Hazard ratio (HR): 0.43; 95% CI: 0.38-0.49] but had no such effect on overall survival (OS) (HR: 1.01; 95% CI: 0.87-1.18) (43). A plausible reason for this lack of improvement in OS could be that EGFR TKIs were used as second or further lines of therapy, once conventional chemotherapy was found to lack therapeutic effect. Results from the OPTIMAL study have shown that erlotinib as a first-line treatment enhances PFS and has a better safety profile in comparison with conventional chemotherapy (24). Similar beneficial effects on PFS have been reported from two landmark phase III trials on the irreversible EGFR TKI, afatinib (21,22). In addition, while no statistically significant differences were evident in these studies between afatinib and cisplatin (latter in combination with either permetrexed or gemcitabine) as first-line treatment in EGFR-positive stage IIIB and IV lung adenocarcinoma patients, subgroup analysis has shown that OS of patients with del19 *EGFR* mutation who were administered afatinib was substantially improved (23). In the LUX-Lung 3 trial, afatinib-treated patients presenting del 19 mutation had median OS of 33.3 months (95% CI: 26.8-41.5) in comparison to 21.1 months (95% CI: 16.3-30.7) in those who received conventional chemotherapy (HR: 0.54; 95% CI: 0.36-0.79; P=0.0015). Similarly, in the LUX-Lung 6 trial, patients with del 19 mutations who received

afatinib had median OS of 31.4 months (95% CI: 24.2-35.3) vs. OS of 18.4 months (95% CI: 14.6-25.6) in those on conventional therapy (HR: 0.64; 95% CI: 0.44-0.94; P=0.020).

Despite being the first of its kind disease registry that gathered information on clinical management of EGFR-positive NSCLC in the CEE region, the INSIGHT study and our sub-analysis have some inherent limitations. Being an observational study, we could only capture a snapshot of current practices at predetermined locations in Poland and the nationwide situation could differ. One way to rectify this could be drafting and implementing guidelines that would require participation from all tertiary cancer centers in Poland in a national NSCLC registry. Another drawback of the INSIGHT registry is that since it was not prospective by nature, we could not assess the long-term benefits and effectiveness of current strategies in the therapeutics of EGFR-positive NSCLC in Poland and the CEE region. These questions can only be answered by elaborate prospective studies and we hope that our registry serves as an impetus to such investigations.

In conclusion, EGFR TKIs are effective agents against EGFR-positive NSCLC and should be routinely considered as first-line treatment in these patients; therefore *EGFR* testing should be performed at the earliest. Data from the INSIGHT registry could be used to improve guidelines, standardize screening techniques, as well as create a predictor algorithm for example a nomogram (44). Such an approach that will integrate individual risk factor analysis and improve EGFR-targeted therapies could lead to personalized gene-directed therapies for EGFR-positive NSCLC patients.

Acknowledgements

The editorial help with this manuscript was provided by Mateusz Spalek, Satyen Shenoy and Agnieszka Linkiewicz-Zegan.

References

1. Coate LE, John T, Tsao MS and Shepherd FA: Molecular predictive and prognostic markers in non-small-cell lung cancer. Lancet Oncol 10: 1001-1010, 2009.
2. Kerr KM, Bubendorf L, Edelman MJ, Marchetti A, Mok T, Novello S, O'Byrne K, Stahel R, Peters S, Felip E, *et al*: Second ESMO consensus conference on lung cancer: Pathology and molecular biomarkers for non-small-cell lung cancer. Ann Oncol 25: 1681-1690, 2014.
3. O'Byrne KJ, Gatzemeier U, Bondarenko I, Barrios C, Eschbach C, Martens UM, Hotko Y, Kortsik C, Paz-Ares L, Pereira JR, *et al*: Molecular biomarkers in non-small-cell lung cancer: A retrospective analysis of data from the phase 3 FLEX study. Lancet Oncol 12: 795-805, 2011.
4. Chatziandreou I, Tsioli P, Sakellariou S, Mourkioti I, Giannopoulou I, Levidou G, Korkolopoulou P, Patsouris E and Saetta AA: Comprehensive molecular analysis of NSCLC; Clinicopathological Associations. PLoS One 10: e0133859, 2015.
5. Dearden S, Stevens J, Wu YL and Blowers D: Mutation incidence and coincidence in non small-cell lung cancer: Meta-analyses by ethnicity and histology (mutMap). Ann Oncol 24: 2371-2376, 2013.
6. D'Arcangelo M and Cappuzzo F: K-Ras mutations in non-small-cell lung cancer: Prognostic and predictive value. ISRN Mol Biol 2012: 837306, 2012.
7. Aisner DL and Marshall CB: Molecular pathology of non-small cell lung cancer: A practical guide. Am J Clin Pathol 138: 332-346, 2012.
8. Boch C, Kollmeier J, Roth A, Stephan-Falkenau S, Misch D, Grüning W, Bauer TT and Mairinger T: The frequency of EGFR and KRAS mutations in non-small cell lung cancer (NSCLC): Routine screening data for central Europe from a cohort study. BMJ Open 3: pii:e002560, 2013.
9. Eberhard DA, Giaccone G and Johnson BE; Non-Small-Cell lung Cancer Working Group: Biomarkers of response to epidermal growth factor receptor inhibitors in Non-Small-Cell Lung Cancer Working Group: Standardization for use in the clinical trial setting. J Clin Oncol 26: 983-994, 2008.
10. Gahr S, Stoehr R, Geissinger E, Ficker JH, Brueckl WM, Gschwendtner A, Gattenloehner S, Fuchs FS, Schulz C, Rieker RJ, *et al*: EGFR mutational status in a large series of Caucasian European NSCLC patients: Data from daily practice. Br J Cancer 109: 1821-1828, 2013.
11. Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T and Mitsudomi T: Mutations of the epidermal growth factor receptor gene in lung cancer: Biological and clinical implications. Cancer Res 64: 8919-8923, 2004.
12. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, *et al*: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Eng J Med 350: 2129-2139, 2004.
13. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, *et al*: EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. Science 304: 1497-1500, 2004.
14. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, Singh B, Heelan R, Rusch V, Fulton L, *et al*: EGF receptor gene mutations are common in lung cancers from 'never smokers' and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci USA 101: 13306-13311, 2004.
15. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, Palmero R, Garcia-Gomez R, Pallares C, Sanchez JM, *et al*: Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): A multicentre, open-label, randomised phase 3 trial. Lancet Oncol 13: 239-246, 2012.
16. Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, Fong KM, Lee H, Toyooka S, Shimizu N, *et al*: Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 97: 339-346, 2005.
17. Souglakos J: Customizing chemotherapy in non-small cell lung cancer: The promise is still unmet. Trans Lung Cancer Res 4: 653-655, 2015.
18. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, *et al*: Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Eng J Med 362: 2380-2388, 2010.
19. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, Seto T, Satouchi M, Tada H, Hirashima T, *et al*: Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): An open label, randomised phase 3 trial. Lancet Oncol 11: 121-128, 2010.
20. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, *et al*: Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Eng J Med 361: 947-957, 2009.
21. Sequist LV, Yang JC, Yamamoto N, O'Byrne K, Hirsh V, Mok T, Geater SL, Orlov S, Tsai CM, Boyer M, *et al*: Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol 31: 3327-3334, 2013.
22. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, Li W, Hou M, Shi JH, Lee KY, *et al*: Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): An open-label, randomised phase 3 trial. Lancet Oncol 15: 213-222, 2014.
23. Yang JC, Wu YL, Schuler M, Sebastian M, Popat S, Yamamoto N, Zhou C, Hu CP, O'Byrne K, Feng J, *et al*: Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): Analysis of overall survival data from two randomised, phase 3 trials. Lancet Oncol 16: 141-151, 2015.
24. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, Zhang S, Wang J, Zhou S, Ren S, *et al*: Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): A multicentre, open-label, randomised, phase 3 study. Lancet Oncol 12: 735-742, 2011.

25. Ettinger DS, Akerley W, Bepler G, Blum MG, Chang A, Cheney RT, Chirieac LR, D'Amico TA, Demmy TL, Ganti AK, *et al*: Non-small cell lung cancer. J Natl Compr Canc Netw 8: 740-801, 2010.
26. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, Jenkins RB, Kwiatkowski DJ, Saldivar JS, Squire J, *et al*: Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: Guideline from the College of American pathologists, international association for the study of lung cancer and Association for Molecular Pathology. Arch Pathol Lab Med 137: 828-860, 2013.
27. Marchetti A, Normanno N; AIOM-SIAPEC-IAP, Pinto C, Taddei GL, Adamo V, Ardizzoni A, Botti G, Bardelli A, Comin C, *et al*: Recommendations for mutational analysis of EGFR in lung carcinoma. Pathologica 102: 119-126, 2010 (In English, Italian).
28. Pirker R, Herth FJ, Kerr KM, Filipits M, Taron M, Gandara D, Hirsch FR, Grunenwald D, Popper H, Smit E, *et al*: Consensus for EGFR mutation testing in non-small cell lung cancer: Results from a European workshop. J Thorac Oncol 5: 1706-1713, 2010.
29. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, Beer DG, Powell CA, Riely GJ, Van Schil PE, *et al*: International association for the study of lung cancer/American thoracic society/European respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 6: 244-285, 2011.
30. Westwood M, Joore M, Whiting P, van Asselt T, Ramaekers B, Armstrong N, Misso K, Severens J and Kleijnen J: Epidermal growth factor receptor tyrosine kinase (EGFR-TK) mutation testing in adults with locally advanced or metastatic non-small cell lung cancer: A systematic review and cost-effectiveness analysis. Health Technol Assess 18: 1-166, 2014.
31. Keedy VL, Temin S, Somerfield MR, Beasley MB, Johnson DH, McShane LM, Milton DT, Strawn JR, Wakelee HA and Giaccone G: American Society of Clinical Oncology provisional clinical opinion: Epidermal growth factor receptor (EGFR) Mutation testing for patients with advanced non-small-cell lung cancer considering first-line EGFR tyrosine kinase inhibitor therapy. J Clin Oncol 29: 2121-2127, 2011.
32. Krawczyk P, Chorostowska-Wynimko J, Dziadziuszko R, Jassem J, Krzakowski M, Langfort R, Puacz E, Wasąg B and Wojas-Krawczyk K: Methodological recommendations for the diagnostics of EGFR gene mutations and ALK gene rearrangement in the selection of non-small-cell lung cancer patients to molecularly targeted therapies. Pneumonol Alergol Pol 82: 437-444, 2014.
33. Krawczyk P, Ramlau R, Chorostowska-Wynimko J, Powrózek T, Lewandowska MA, Limon J, Wasąg B, Pankowski J, Kozielski J, Kalinka-Warzocha E, *et al*: The efficacy of EGFR gene mutation testing in various samples from non-small cell lung cancer patients: A multicenter retrospective study. J Cancer Res Clin Oncol 141: 61-68, 2015.
34. Ramlau R, Cufer T, Berzinec P, Dziadziuszko R, Olszewski W, Popper H, Bajcic P, Dušek L, Zbozinkova Z and Pirker R; INSIGHT study team: Epidermal growth factor receptor mutation-positive non-small-cell lung cancer in the real-world setting in central Europe: The INSIGHT Study. J Thorac Oncol 10: 1370-1374, 2015.
35. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET and Carbone PP: Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5: 649-655, 1982.
36. R Development Core Team: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, 2014.
37. Santini D, Barni S, Intagliata S, Falcone A, Ferraù F, Galetta D, Moscetti L, La Verde N, Ibrahim T, Petrelli F, *et al*: Natural history of non-small-cell lung cancer with bone metastases. Sci Rep 5: 18670, 2015.
38. Petrelli F, Borgonovo K, Cabiddu M and Barni S: Efficacy of EGFR tyrosine kinase inhibitors in patients with EGFR-mutated non-small-cell lung cancer: A meta-analysis of 13 randomized trials. Clin Lung Cancer 13: 107-114, 2012.
39. Barlesi F, Mazieres J, Merlio JP, Debieuvre D, Mosser J, Lena H, Ouafik L, Besse B, Rouquette I, Westeel V, *et al*: Routine molecular profiling of patients with advanced non-small-cell lung cancer: Results of a 1-year nationwide programme of the French Cooperative Thoracic Intergroup (IFCT). Lancet 387: 1415-1426, 2016.
40. Beau-Faller M, Prim N, Ruppert AM, Nanni-Metéllus I, Lacave R, Lacroix L, Escande F, Lizard S, Pretet JL, Rouquette I, *et al*: Rare EGFR exon 18 and exon 20 mutations in non-small-cell lung cancer on 10 117 patients: A multicentre observational study by the French ERMETIC-IFCT network. Ann Oncol 25: 126-131, 2014.
41. Powrózek T, Krawczyk P, Ramlau R, Sura S, Wojas-Krawczyk K, Kucharczyk T, Walczyna B, Szumiło J, Szyszka-Barth K, Milecki P, *et al*: EGFR gene mutations in patients with adenosquamous lung carcinoma. Asia Pac J Clin Oncol 10: 340-345, 2014.
42. Krawczyk P, Nicoś M, Ramlau R, Powrózek T, Wojas-Krawczyk K, Sura S, Jarosz B, Szumiło J, Warda E, Mazurkiewicz T, *et al*: The incidence of EGFR-activating mutations in bone metastases of lung adenocarcinoma. Pathol Oncol Res 20: 107-112, 2014.
43. Lee CK, Brown C, Gralla RJ, Hirsh V, Thongprasert S, Tsai CM, Tan EH, Ho JC, Chu da T, Zaatar A, *et al*: Impact of EGFR inhibitor in non-small cell lung cancer on progression-free and overall survival: A meta-analysis. J Natl Cancer Inst 105: 595-605, 2013.
44. Girard N, Sima CS, Jackman DM, Sequist LV, Chen H, Yang JC, Ji H, Waltman B, Rosell R, Taron M, *et al*: Nomogram to predict the presence of EGFR activating mutation in lung adenocarcinoma. Eur Respir J 39: 366-372, 2012.