ULUSLARARASI HEMATOLOJI-ONKOLOJI DERGISI ARTICLE International Journal of Hematology and Oncology

The Relationship Between Common EGFR, BRAF, KRAS Mutations and Prognosis in Advanced Stage Non-Small Cell Lung Cancer with Response to the Treatment in Turkey

Mutlu DOGAN[1], Ahmet DEMIRKAZIK[1], Ajlan TUKUN[2,3], Serpil D. SAK[4], Koray CEYHAN[4], Bulent YALCIN[1], Hakan AKBULUT[1], Fikri ICLI[1]

[1] Ankara University Faculty of Medicine, Department of Medical Oncology
[2] Ankara University Faculty of Medicine, Department of Medical Genetics
[3] Duzen Laboratory Groups, Department of Medical Genetics
[4] Ankara University Faculty of Medicine, Department of Pathology, Ankara, TURKEY

ABSTRACT

The aim of the study was to evaluate EGFR (exon 19 deletion, exon 21 L858R point mutation), KRAS and braf mutation rates besides their relationship with survival and response to the treatment in non small cell lung cancer (NSCLC). We evaluated 513 NSCLC patients followed-up between January 2004 and November 2009 according to our registration data, retrospectively. Only 42 advanced stage NSCLC patients had enough tumor tissue material in paraffin blocks for all mutation analysis. The patients were evaluated retrospectively for clinicopathological features, EGFR, KRAS and BRAF mutations, erlotinib treatment, time to progression (TTP) and overall survival (OS). Mutation rates were as 7.14% (two patients) for EGFR exon 19 deletion; 4.76% (one patient)for KRAS codon 61 deletion and 2.38% for BRAF V600E mutation. They had neither EGFR exon 21 point mutation nor different mutations together. Median follow-up was 26 months (5-83) for all patients. It was 43 months (23-83) for the patients who had erlotinib and 23 months (5-61) for those who did not. Ten (23.8%) patients had erlotinib. There was significant survival difference between the patients taking erlotinib and the others (28 ± 3 months versus 15 ± 4 months, p= 0.05). TTP and OS were longer in the patients who had mutations, however the difference was not significant (p= 0.119 and p= 0.06). To our knowledge, this is the first study evaluating EGFR, KRAS and BRAF mutations in advanced stage NSCLC in Turkey .

Keywords: Non-Small cell lung cancer, EGFR, KRAS, BRAF, Erlotinib

ÖZET

Türkiye'de İleri Evre Küçük Hücreli Dışı Akciğer Kanserinde Sık Görülen EGFR, BRAF, KRAS Mutasyonlarının Tedaviye Yanıt ve Prognozla İlişkisi

Çalışmanın amacı, küçük hücreli dışı akciğer kanserinde (KHDAK) EGFR (ekzon 19 delesyonu, ekzon 21 L858R nokta mutasyonu), KRAS ve braf mutasyon oranlarının yanısıra bunların sağkalım ve tedavi yanıtı ile ilişkisini değerlendirmektir. Ocak 2004 - Kasım 2009 arasında merkezimizde takip edilen 513 KHDAK'li hasta retrospektif olarak değerlendirildiğinde sadece 42 ileri evre KHDAK'li hastanın paraffin bloklarında tüm mutasyonların analizi için yeterli tumor dokusu mevcuttu. Hastalar klinikopatolojik özellikler, EGFR, KRAS ve BRAF mutasyonları, erlotinib tedavisi, progresyona kadar geçen süre (TTP) ve toplam sağkalım (OS) açısından retrospektif olarak değerlendirilmiştir. Mutasyon oranları EGFR ekzon 19 delesyonu için %7.4; KRAS kodon 61 delesyonu için %4.76 ve BRAF V600E mutasyonu için %2,38 olarak saptanmıştır. Hiçbirinde EGFR ekzon 21 nokta mutasyonu ve farklı mutasyonların birlikteliği saptanmamıştır. Ortanca takip süresi 26 ay (5-83) olup erlotinib alanlarda 43 ay (23-83), diğerlerinde ise 23 ay (5-61)saptanmıştır. On (23.8%) hasta erlotinib aldı. Erlotinib alanlarla almayanlar arasında sağkalım farkı saptanmıştır (28 ± 3 aya karşılık 15 ± 4 ay, p= 0.05). Mutasyonu olanlarda TTP ve OS daha uzun bulunmasına rağmen fark ististiksel olarak anlamlı bulunmamıştır (p= 0.119 ve p= 0.06). Sonuç olarak, bu çalışma Türkiye'de ileri evre KHDAK'de EGFR, KRAS ve BRAF mutasyonlarının değerlendirildiği ilk çalışmadır.

Anahtar Kelimeler: Küçük hücreli dışı akciğer kanseri, EGFR, KRAS, BRAF, Erlotinib

INTRODUCTION

Lung cancer is the most common and leading cause of cancer deaths all around the world. Despite the many scientific efforts made to this desperate illness, only both performance status (PS) and tumour staging has been accepted as independent prognostic factors for nonsmall cell lung cancer (NSCLC).[1] There is no doubt that new prognostic and predictive factors are needed. Cancer patients with similar histopathologies and clinical features might have different prognosis and response to the treatments. This may be related to the pharmacogenetic variations.Pharmacogenetics explores genetic variations and genetic mutations which might lead different treatment responses. Tailored therapy according to pharmacogenetics may provide less toxic, most effective, as a result cost-effective treatment. Pharmacogenetics may also help to the development of targeted therapies via detecting gene mutations. Epidermal growth factor receptor (EGFR) overexpression in solid tumors such as NSCLC is a well-known process. Nowadays, EGFR monoclonal antibodies (i.e.cetuximab) that bind to the extracellular regions of EGFR and EGFR-tyrosine kinase inhibitors (i.e. erlotinib and gefitinib) which inhibit intracellular phosphorylation are common anti-EGFR strategies.[2] The EGFR somatic mutation rates are 30% for Caucasians and less than 10% for western countries.[3] Exon 19 deletions and exon 21 L858R mutations are the most common EGFR mutations showing anti-EGFR targets.[4] “KRAS” is a well-known oncogene which has a role in some tumors like colorectal and lung cancers. KRAS mutation rate is 20-30% in NSCLC, predominantly in adenocarcinomas.[5] Most of KRAS mutations occur in codon 12 with a rate of 70% as guanine-thymine transversion.[6] KRAS mutation might have an association with de novo platinum resistance.[2,7] We considered that evaluation of KRAS mutation, besides EGFR somatic mutations might contribute to the efficacy of EGFR-TKIs, especially in adenocarcinomas.

The role of BRAF in NSCLC is not as clear as in colorectal carcinoma and malign melanoma.[8,9] V600E (exon 15, T1799A) mutation is the most common BRAF somatic mutation. Evaluation of BRAF V600E mutation may have importance in targetted therapies since its activation has role in intracellular signaling.

Molecular markers and mutation analysis may contribute to predict the clinical outcome of the patients. The aim of the study was to evaluate common EGFR somatic mutations (i.e. exon 19 deletion andexon 21 L858R point mutation) in tumor tissues or cell blocks of advanced stage NSCLC patients, additionally KRAS (codon 12, 13, 61 and 64) and BRAF (V600E) mutations which may contribute to the prognosis and response to the treatment. We also aimed to evaluate mutation rates and probable relations among these mutations.

PATIENTS AND METHOD

Eligibility criteria

We evaluated five hundred and thirteen NSCLC patients who admitted to our center between January 2004 and November 2009 retrospectively, according to our registration database. Only 68 of 513 patients were available after exclusions. The patients who had early stage, histopathologic diagnosis in other centers or cytological diagnosis without cell blocks were excluded. The patients with early stage NSCLC were excluded. It was thought that analysis and follow up of the patients with advanced stage rather than all stages might have been more homogenous. The primary treatment modality is surgery in early stage and more favorable prognosis of these patients might have caused a more heterogenous group analysis if they were included. The patients who had only supportive care and lost to follow-up after first visit were also excluded, besides others who were followed-up in other centers after our chemotherapy plannings.

All of the patients had histopathologically documented NSCLC diagnosis either by tissue biopsy or cell block cytology. Paraffin blocks of selected sixty-eight patients were re-evaluated by a pathologist and a cytopathologist. However, paraffin blocks of only 52 patients had enough tumor tissue for next step In their cell blocks. Finally, only 42 advanced stage NSCLC patients’ tumor tissues were available for DNA analysis, since ten patients were excluded because of inadequate material. Treatment modalities and their probable relationships were also discussed.

Smoking status

The patients who had smoking history during diagnosis and smoking cessation within the previous six months of diagnosis were accepted as active smokers.

Survival: Time to progression (TTP) was defined as the interval between the beginning of treatment and progression while overall survival (OS) was defined as the interval between diagnosis and death or the date of last known alive.

DNA isolation: At least five 5 micron sections from these paraffin-embedded tumor tissues were deparaffinized and DNA was isolated from the tissues using QIAamp DNA mini kit, according to manufacturer's instructions.

Mutation analysis: The PCR and sequencing protocol was performed to amplify and sequence exons 19 and 21 of EGFR,exons 1 and 2 of KRAS, and exons 15 of BRAF in order to detect EGFR, KRAS and BRAF mutations using the primers described previously (10-12). Purified PCR products were sequenced on an automatic DNA sequencer (ABI 3130 Genetic Analyzer, Applied Biosystems).

Statistical Analysis: Survival analyses were done according to the Kaplan-Meier method, and the log-rank test was used for survival comparisons. SPSS 10.0 for Windows statistical package was used for all calculations and the patients' data was evaluated by student t test. P< 0.05 was considered to be significant.

RESULTS

Forty-two eligible advanced stage NSCLC patients were enrolled. They were evaluated for EGFR mutations (exon 19 deletion, exon 21 L858R point mutation), KRAS mutations (codon 12, 13, 61 and 64 mutations) and BRAF V600E mutation (T1799A).

Mutation rates were as 7.1% (n= 3) for EGFR exon 19 deletion, 4.7% (n= 2) for KRAS exon 2 codon 61 deletion and 2.3% (n= 1) for BRAF V600E mutation. None of the patients had more than one mutation together.

Median follow-up duration was 26 months (range, 5-83 months) and this was attributed to the enrollment of most patients in 2008 and 2009. Ten patients had erlotinib and median follow-up of these patients was 43 months (range, 23-83 months) whereas it was 23 months (range, 5-61 months) for others. Patient characteristics are summarized in Table 1. Two third of the patients were male with a median age of 59 (range, 37-81). Median overall survival was 17 + 4 months (range, 9-25 months). Nineteen patients had locally advanced disease while 23 patients had metastatic disease. Time-to-progression (TTP) was 13 + 3 (range, 7-19) months for the patients with locally advanced disease and 9 + 2 (months for others with metastatic disease (p= 0.05) (Figure 1a). Overall survival was 26 + 4 (range, 17-35) months for locally advanced disease and 15+ 5 (range, 6-24) months for metastatic disease (p= 0.148) (Figure 1b).

Eastern Cooperative Oncology Group (ECOG)-PS was registered in thirty-two patients at diagnosis. Time-to-progression (11 + 1 months vs 7 + 1) and OS (26 + 6 months vs 17 + 5 months) were similar in the patients with ECOG-PS 0 and 1 (p=0.205 and p=0.848, respectively).

Diagnostic procedures: Bronchoscopic biopsy was the most common preferred diagnostic procedure with a rate of 40.5% (Table 1). Two thirds of the patients (64.2%) had adenocarcinoma with a predominance of bronchioloalveolar carcinoma. Most (56.2%) of the patients had poorly differentiated carcinoma.

Smoking: Seventy percent of the patients had smoking history, most of them were male and in the fifth decade (Table 1). Median smoking rate was 30 pack-years (range, 10-100). We noticed that 51.8% of the smokers were 'ex-smokers' who gave-up smoking before cancer diagnosis. Median smoking cessation duration before diagnosis was estimated as 8 years (1-40). Most (76.9%) of current smokers were male. Median age were as 59 (37-75) and 45 (range, 25-100 pack year) for smoking rate. Adenocarcinoma (46.1%) was almost the most common histopathology in current smokers.

Metastatic sites: Pleura was the most common metastatic site (28.5%). Other sites were bone (25%), brain (21.4%), contralateral lung (21.4%) and surrenal (10.7%). These sites were involved either alone or together. Twenty-six percent of the patients had brain metastasis at diagnosis or during follow-up, in addition most of the brain metastasis (63.6) were as solitary or oligometastasis.

Laboratory values: The patients were also evaluated in terms of anemia, leukocytosis, thrombocytosis,

Table 1. Patient characteristics

Male/female	28/14
Age (median, range)	59 (37-81)
Smoking rate (%)	70
Smoking amount (pack-year; median, range)	30 (10-100)
Smoking cessation before diagnosis (%)	51,8
Smoking cessation duration before diagnosis (year; median, range)	8 (1-40)
ECOG – PS* (n)	
0	10
1	19
Locally advanced stage (n)	19
Stage IV (n)	23
Diagnostic procedure (%)	
Bronchoscopy	40.5
Transthoracic fine needle aspiration biopsy	21.4
Pleural biopsy	9.5
Supraclavicular lymph node biopsy	9.5
Others#	19.1
Histopathologic subtype (%)	
Adenocarcinoma§	64.3
Squamous cell carcinoma	26.2
Othersğ	9.5
Differentiation (%)	
Poorly differentiated	56.3
Moderately differentiated	31.2
Well differentiated	12.5
Epidermal growth factor receptor exon 19 deletion (%)	7.1
KRAS exon 2 codon 61 deletion (%)	4.7
BRAF V600E mutation (%)	2.3
Erlotinib (%)	23.8

*: Eastern Cooperative Oncology Group – performance status, registered in 32 patients
#: Mediastinal lymph node biopsy (7.1%), bronchial lavage (4.8%), brain mass excision (2.4%), lung mass resection (2.4%),'tru-cut'biopsy of bone metastasis (2.4%)
§: Bronchioloalveolar carcinoma (60%)
ğ: Not otherwise specified subtype (7.1%), large cell carcinoma (2.4%)

hypercalcemia and hypoalbuminemia. Median values were in normal ranges for these parameters (i.e. 13.4 g/dl for hemoglobin, 8500/mm^3 for leucocyte, 318000/mm^3 for thrombocyte, 9.4 mg/dl for calcium and 4.1 g/dL for serum albumin level). There were no statistical differences between the patients who had erlotinib or not, in terms of these parameters (p values were as 0.56; 0.26; 0.71; 0.45 and 0.76; respectively).

Patients with epidermal growth factor receptor, KRAS or BRAF mutations

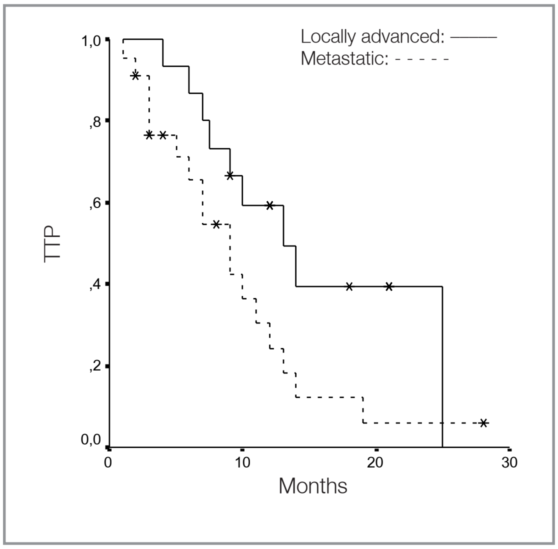

Figure 1a: Time to progression according to stages (p= 0.05)

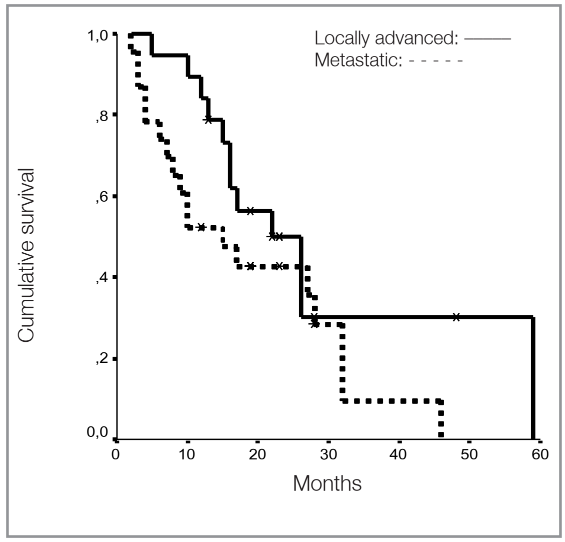

Figure 1b: Overall survival rates according to stages (p= 0.148)

Two patients with EGFR exon 19 deletions and one-patient with KRAS exon 2 codon 61 deletion had erlotinib among six patients with any mutations.

Epidermal growth factor receptor exon 19 deletion

Two patients with mutation had erlotinib while the third one did not since she refused other treatments after first line chemotherapy. Two patients who had erlotinib had locally advanced disease. The first patient was a 70-year old woman without smoking history. She had 4 cycles of cisplatinum and gemcitabine for relapsed disease after postoperative 25 months. Stable disease was achieved with chemotherapy, however she had progression after 2 months. She had partial response with grade 2 skin rash after 2 months of erlotinib treatment. Her disease was controlled with 16 months of erlotinib. Shehaslived for additional 11 months after erlotinib was stopped without any serious events. Her OS was 59 months. The other patient was a 65-year old man. He was a current smoker at diagnosis with 50 pack-year smoking history. He had complete remission after 4 cycles of neoadjuvant cisplatinum and vinorelbine, however he had relapse after 9 months. He had stable disease with 6 cycles of carboplatinum and paclitaxel. He was followed-up with supportive care for 6 months until progression. Erlotinib was started for progressive disease and he had complete response with 2 months of erlotinib. He had grade 2 skin rash during 8 months of erlotinib therapy. He had an OS of 28 months.Overall survival of the third patient with EGFR mutation was estimated as 32 months without documented any other treatments.

Time-to-progression was 25+5 (range, 10-31) months in the patients with any mutation and 9+1 (range, 6-12) months in the other patients without any mutations (p= 0.119) (Figure 2a). However, OS seemed to be higher in mutant patients. It was 32+3 months (range, 26-38) in the mutant patients and 16 + 2 months in the others (p= 0.053) (Figure 2b).

Two patients had KRAS exon 2 codon 61 deletions. The first one was a 57-year old male patient with locally advanced disease. He was an ex-smoker with 25-pack year smoking history. He had stopped smoking 8 years ago. Partial remission was achieved with chemo radiotherapy (radiotherapy with weekly cisplatinum) followed by 3 cycles of neoadjuvant cisplatinum and gemcitabine. He was given cisplatinum and docetaxel as second line chemotherapy since he had progression after 9 months. He was followed-up for 10 months with stable disease, eventually he died because of disease progression. His OS was estimated as 19 months. The other patient was a 53-year old woman with metastatic disease. She had partial response with 6 cycles of cisplatinum and gemcitabine as first line therapy. Erlotinib was started as second line treatment because of progressive disease after 11 months. She had stable disease with grade 2 skin rash-

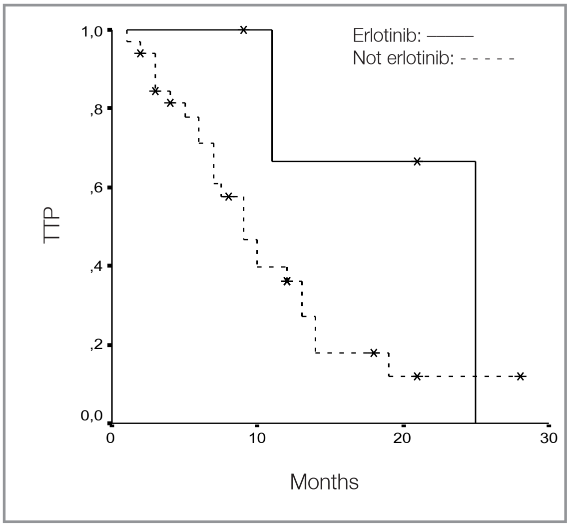

Figure 2a. Time-to-progression of the patients who had any mutation or not (p= 0.119).

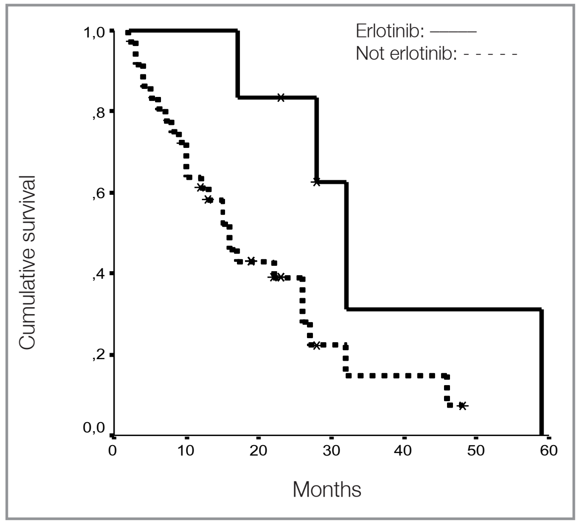

Figure 2b. Overall survival of the patients who had any mutation or not (p= 0.053).

es and grade 1 diarrhea. She was followed-up with supportive care after progression on seven months of erlotinib treatment and her OS was 28 months.

The patient with BRAF V600E mutation was a 62-year old man. He was a current smoker with 50-pack year smoking history. He had pleural nodules without effusion at diagnosis. The pleural nodules were considered as nonmetastatic since they were stable although primary lesion regressed with 3 cycles of cisplatinum and gemcitabine. So, he was operated. He is still in remission with an OS of 22 months.

Characteristics and treatment results of the patients taking erlotinib

Ten patients had erlotinib. Patient characteristics are summarized in Table 2. Male/female ratio was 0.42 (3/7). All of them had adenocarcinoma and 75% of those who could have been subtyped had bronchioloalveolar carcinoma.Two patients had smoking history. Median age was 58 (41-81). Erlotinib was used as a median second line therapy (range, 1-4) with a median duration of 7.5 (1-16) months. Overall survival was 28 + 3 months in the patients who had erlotinib whereas it was estimated as 15 + 2 months in the others (p= 0.068) (Figure 3). A patient had complete remission (CR), 2 patients had partial response (PR), and 4 patients had stable disease (SD) however 3 patients had progressive disease (PD). The patient with CR and a patient with PR had EGFR exon 19 deletions while a patient with SD had KRAS exon 2 codon 61 deletions. None of the patients in the erlotinibgroup had BRAF V600E mutation. However, the patient who had CR with EGFR exon 19 deletion was a current smoker with 50 pack-year smoking history.

Eight patients (80%) who had erlotinib died after a median follow-up of 27 (12-59) months.Apatient with CR and EGFR exon 19 deletion and another one with PR and without any mutations are alive. The first patient had relapse after 8 months of CR and his OS was estimated as 28 months. The second one had cisplatinum and pemetrexed since he had progression after 9 months of follow-up. His OS was estimated as 22 months.Three patients with PD did not have any mutations. Two of them had locally advanced disease while the thirdone had surrenal metastasis. The first patient with locally advanced disease was a 63-year old man who had smoked rarely before diagnosis. He had chemo radiotherapy after achievement of SD with 3 cycles of neoadjuvant cisplatinum and gemcitabine. However, he had PD after 7 months of follow-up. He had erlotinib as a second line treatment and his OS was estimated as 12 months. The second patient with locally advanced disease was a 46-year old female patient without smoking history. She had second line docetaxel treatment after she had stable disease with neoadjuvant cisplatinum and vinorelbine. She had erlotinib as a third line therapy since she had PD after 14 months. Her OS was 26 months. The third patient with surrenal metastasis was a 67-year old man with 10 pack-year smoking history. He gave up smoking 40 years ago. He had three lines of chemotherapy including platinum, taxane, gemcitabine and

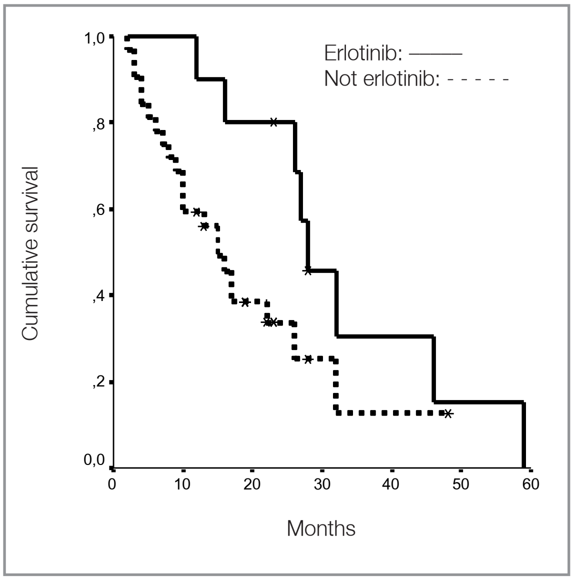

Figure 3. Overall survival of the patients who had erlotinib or not (p= 0.068).

vinorelbine before erlotinib. His OS was 32 months.

None of the patients had severe (grade 3-4) toxicity. Two patients [CR (n= 1), PR (n= 1)] with EGFR exon 19 deletion had grade 2 skin rash and a patient (SD) with KRAS V600E mutation had grade 2 skin rash and grade 1 diarrhea. None of patients without any mutation had erlotinib toxicity.

Characteristics and treatment responses of the patients who had monoclonal antibodies and other tyrosine kinase inhibitors

Three patients had monoclonal antibody with chemotherapy and two patients had sorafenib, a multi-tyrosine kinase inhibitor.

The first patient who had monoclonal antibody was a 57-year old man. He had 3 cycles of neoadjuvant cisplatinum, pemetrexed and bevacizumab. He was operated after he had partial response with neoadjuvant chemotherapy and he was given 3 cycles of adjuvant pemetrexed and docetaxel.But,surrenal metastasis occurred after 7 months.He had thoracic paravertebral mass after second line vinorelbine and gemcitabine. He had brain metastasis afterwards. He died because of progression and his OS was estimated as 14 months.

Other two patients were 59-year old men who had cetuximab, an EGFR monoclonal antibody with chemotherapy. One of them had metastasis and he died because of febrile neutropenia after 2 cycles of cisplatinum, vinorelbine and cetuximab. The other one had cisplatinum, gemcitabine and cetuximab for locally advanced disease. He had stable disease after 4 cycles of therapy and had maintenance cetuximab for 10 months. He is alive without progression and his OS was estimated as 13 months.

Table 2. The characteristics of the patients who had erlotinib

Male / female	3/7
Age (median, range)	58 (41-81)
Erlotinib treatment line (median, range)	2 (1-4)
Follow-up duration (months; median, range)	27 (12-59)
Smoking rate (%)	20
ECOG – PS* (n)	
0 / 1	1 / 6
Locally advanced stage (n)	5
Stage IV (n)	5
Histopathologic subtype (n)	
Adenocarcinoma**	10
Epidermal growth factor receptor exon 19 deletion (n)	2
KRAS exon 2 codon 61 deletion (n)	1
BRAF V600E mutation (n)	0
Erlotinib treatment line (median, range)	2 (1-4)
Erlotinib duration (months; median, range)	7.5 (1-16)
Remission rates with erlotinib (n/%)	
Complete remission / Partial remission	1 (10) / 2 (20)
Stable disease / Progressive disease	4 (40) / 3 (30)
Response in epidermal growth factor receptor exon 19 deletion (n)	
Complete remission / Partial remission	1 / 1
Response in patient with KRAS exon 2 codon 61 deletion (n)	
Stable disease	1
Toxicity (n)	
Grade 2 skin rash***	2
Grade 1 diarrhea and grade 2 skin rash****	1

*: Eastern Cooperative Oncology Group– performance status; registered in 7 patients
**: Adenocarcinoma subtype was documented in 4 patients; bronchioloalveolar carcinoma (n=3), mucinous adenocarcinoma (n= 1)
***: With EGFR exon 19 deletion; complete response (n= 1) and partial response (n= 1)
****: With KRAS exon 2 codon 61 deletion; stable disease (n= 1)

Two patients with adenocarcinoma had sorafenib, a VEGFR tyrosine kinase inhibitor. The first patient was a 64-year old man who had had sorafenib as a second line therapy for locally advanced disease. He had progression after 4.5 months although he had stable disease within 2 months of therapy. The second patient was a 44-year old woman. She had progression with sorafenib as a third line therapy for metastatic disease. Grade 2 skin rash was observed in both of them. They are alive with OS of 23 and 14 months, respectively.

Other Treatments

Eight patients with locally advanced disease were operated. Three of them were operated at diagnosis while 5 patients could have been operated after neoadjuvant chemotherapy. Adjuvant chemotherapy was given to six patients who were operated and onepatient had chemo radiotherapy as adjuvant chemotherapy. The other patient who had neoadjuvant treatment has been followed up without any adjuvant treatment. He had BRAF V600E mutation and he is still alive without any progression.Six of these eight patients had also adjuvant radiotherapy.

The most common preferred chemotherapy regimen was cisplatinum and gemcitabine.

DISCUSSION

Epidermal growth factor receptor somatic mutation rate in NSCLC is unclear in Turkey. We consider that our study has importance despite small number of patients, because it is the first study evaluating EGFR mutations, besides KRAS and BRAF mutations in NSCLC in our country. Epidermal growth factor receptor activating somatic mutations at regions exon 18-21 encode intracellular phosphorylation and they are well-known mutations with clinical significance.[13-15] We determined EGFR mutation rate as 7.1% in our study and all of them were exon 19 deletion. Epidermal growth factor receptor exon 19 deletion was also more common as EGFR mutation in Spain.[16] In NSCLC, EGFR mutation rate was reported as 16.6% by Rosell et al in Spain whereas it was reported as 12.1% in ISEL ('Iressa Survival Evaluation in Lung Cancer') study.[16,17] None of our patients had EGFR exon 21 L858R point mutation although it is also a common EGFR mutation. But, this might have been related to the limited number of our patients. In addition, none of the patients had more mutations together similar to other studies in the literature.[17,18] All of our patients with EGFR mutation (n= 3) had adenocarcinoma in parallel to the literature and two of them had no smoking history.[16,17,19,20]

It was reported that passive smoking ('environmental tobacco smoke exposure') might have been inversely related to EGFR mutations, even in the people who had never smoked actively.[20] It was not easy to document 'real' passive smokers among non-smokers in our study since the criteria for second-hand smoke 'intensity' are not so clear almost in the literature. So, its effect on EGFR mutation rates could not have been clarified.

Epidermal growth factor receptor, KRAS and BRAF mutations have prognostic significance. Epidermal growth factor receptor exon 19 deletion is a good prognostic factor while others are poor prognostic factors. Survival of our patients with any mutations seems to be better. However, this might have been related to the extensiveness of the disease rather than mutation since good and poor prognostic mutations were evaluated together for TTP and OS analysis. Two third of these patients [EGFR mutation (n= 2), KRAS(n= 1), BRAF (n= 1)] had locally advanced disease.

Epidermal growth factor receptor tyrosine kinase inhibitors are effective even as first line or following treatment modalities in NSCLC, especially in non-smoker female patients. It seems to have safety even in older patients. Erlotinib was given to our patients as a median second line therapy, especially after first line platinum based chemotherapy regimens. None of them had serious erlotinib toxicity and it was well-tolerated. The survival of our patients who had erlotinib seemed to be better (28 + 3 months vs 15 + 2 months, p= 0.068). It was reported that EGFR overexpression and high gene copy number might have-significance in prediction of erlotinib efficacy.[17] It was reported that gefitinib was not inferior than docetaxel in advanced stage NSCLC patients who had prior platinum based chemotherapy in INTEREST trial. However, the investigators failed to demonstrate superiority of gefitinib in the patients with higher EGFR gene copy number.[21] We did not evaluate EGFR gene copy number in any of our patients since the role of EGFR gene copy number is controversial.

The efficacy of sequential chemotherapy and TKIs was reported with survival advantage in some trials, such as BR-21 trial.[19] However, Rosell et al reported that first or second line erlotinib treatment had no survival difference (16). Advanced stage NSCLC patients with EGFR activating somatic mutation had significantly better PFS with first line gefitinib when compared with carboplatin and paclitaxel combination.[22,23] In our study, only an 81-year old woman had erlotinib as first line therapy for relapse. She was given erlotinib as first line therapy without evaluating mutation status. She had progression after a month of erlotinib treatment without any toxicity. It was determined that she had no EGFR mutation when she was evaluated for mutation analysis with other patients.

Tyrosine kinase inhibitors impair ras/raf/MAPK and PI3K/Akt pathways in carcinogenesis.[2] Inhibition of a pathway might lead compensatory activation of other pathways in tumor cells.KRAS mutation might decrease gefitinib efficacy probably by inhibiting MAPK pathway independent from EGFR.[24] So, KRAS or BRAF mutations might contribute to less TKI efficacy. It was reported that KRAS mutation might have a role in prediction of 'de novo' resistance to EGFR-TKIs in a meta-analysis.[2,25] In our study, a 53-year old non-smoker woman with stage IV adenocarcinoma and KRAS exon 2 codon 61 deletion had 3 months of disease stabilization with second line erlotinib, however she had progression after 7 months. The effect of BRAF mutation on gefitinib response could not have been evaluated in ISEL study since none of the patients had BRAF mutation.[17] In our study, we could not also comment on the relationship between BRAF mutation and erlotinib response as none of our patients on erlotinib treatment had BRAF mutation. Two patients had cetuximab with platinum based chemotherapy and none them had any mutation in our study.

It was emphasized that EML-4/ALK ('echinoderm microtubule associated protein like-4 / anaplastic lymphoma kinase') fusion gene rearrangement (2-7%) might have had significance in NSCLC.[26] The NSCLC patients with EML4/ALK tend to be younger with no smoking history. It was shown that these patients had favorable outcomes with crizotinib, an inhibitor of ALK and MET tyrosine kinases. We consider that NSCLC patients should also be evaluated for MET amplification and ALK gene rearrangement since these patients may have benefit from crizotinib. It is a well-known process that MET amplification is one of EGFR-TKIs resistance mechanisms. So, crizotinib in NSCLC patients with MET amplification seems to be a better therapeutic option. Recently, it was reported that an actionable driver mutation rate was 64% in lung adenocarcinomas and coincidence of more than one mutation was 3%.[27] It was also emphasized that the patients with oncogenic driver mutations had longer survival with genotype-targeted therapy. Accelerations in the research of genotyping led more effective tailored therapy, especially in lung adenocarcinomas in the last decade.

We evaluated our patients for other factors which might have prognostic significance such as anemia, leukocytosis, thrombocytosis, hypercalcemia and hypoalbuminemia. It was difficult to comment on these parameters in our patients since mean and median values were all in normal ranges.

In conclusion, this is the first study evaluating EGFR, KRAS and BRAF mutation rates in advanced stage lung cancer in our country. Mutation rates were similar to western countries. One forth (23.8%) of the patients had erlotinib. Two of these patients had EGFR exon 19 deletion, one patient had KRAS codon 61 deletions. However, we need further prospective trials with large number of patients to demonstrate the significance of these mutations in our country, since mutations might have ethnicity differences and these differences might contribute to various clinical outcomes besides "tailored-therapy".

REFERENCES

1. Kawaguchi T, Takada M, Kubo A, et al. Performance status and smoking status are independent favorable prognostic factors for survival in non small cell lung cancer. A comprehensive analysis of 26,957 patients with NSCLC. J Thorac Oncol 5: 620-630, 2010.
2. Linardou H, Dahabreh IJ, Kanaloupiti D, et al. Assessment of somatic k-ras mutations as a mechanism associated with resistance to EGFR-targetted agents: a systematic review and meta-analysis of studies in advanced non-small cell lung cancer and metastatic colorectal cancer. Lancet Oncol 9 : 962-972, 2008.
3. Zhu JQ, Hong W, Zhang G, et al. Better survival with EGFR exon 19 than exon 21 mutations in gefitinib-treated non-small cell lung cancer patients is due to differential inhibition of downstream signals. Cancer Letters 265: 307-317, 2008.
4. Carey KD, Garton AJ, Romero MS, et al. Kinetic analysis of epidermal growth factor receptor somatic mutant proteins shows increased sensitivity to the epidermal growth factor receptor tyrosine kinase inhibitor erlotinib. Cancer Res 66: 8163-8171, 2006.

5. Rodenhuis S, Slebos RJ, Boot AJ, et al. Incidence and possible clinical significance of k-ras oncogene activation in adenocarcinoma of the human lung. Cancer Res 48 : 5738-5741, 1988.
6. Mascaux C, Iannino N, Martin B, et al. The role of RAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis. British J Cancer 92: 131-139, 2005.
7. Youn CK, Kim MH, Cho HJ, et al. Oncogenic H-ras up-regulates expression of ERCC1 to protect cells from platinum-based anticancer agents. Cancer Res 64: 4849-4857, 2004.
8. Sharma SG, Gulley ML. BRAF mutation testing in colorectal cancer. Arch Pathol Lab Med 134: 1225-1228, 2010.
9. Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med 363: 809-819, 2010.
10. Cortes-Funès H, Gomez C, Rosell R, et al. Epidermal growth factor receptor activating mutations in Spanish gefitinib-treated non-small cell lung cancer patients. Ann Oncol 16: 1081-1086, 2005.
11. Bai F, Nakanishi Y, Takayama K, et al. Codon 64 of k-ras gene mutation pattern in hepatocellular carcinomas induced by bleomycin and 1-nitropyrene in A/J mice. Teratog Carcinog Mutagen suppl. 1: 161-170, 2003.
12. Mitsiades CS, Negri J, McMullan C, et al. Targeting BRAF V600E in thyroid carcinoma: therapeutic implications. Mol Cancer Ther 6: 1070-1078, 2007.
13. Tsao MS, Sakurada A, Cutz JC, et al. Erlotinib in lung cancer-molecular and clinical predictors of outcome. N Engl J Med 353: 133-144, 2005.
14. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small cell lung cancer to gefitinib. N Engl J Med 350: 2129-2139, 2004.
15. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response with gefitinib therapy. Science 304: 1497-500, 2004.
16. Rosell R, Moran T, Queralt C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 361: 958- 967, 2009.
17. Hirsch FR, Varella-Garcia M, Bunn PA, et al. Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non-small cell lung cancer. J Clin Oncol 24: 5034-5042, 2006.
18. Ready N, Janne PA, Bogart J, et al. Chemoradiotherapy and gefitinib in stage III non small cell lung cancer with epidermal growth factor receptor and k-ras mutation analysis. Cancer and leukemia group B (CALEB) 30106, a CALGB-stratified phase II trial. J Thorac Oncol 5: 1382-1390, 2010.
19. Shepherd FA, Pereira JR, Ciuleanu T, et al. Erlotinib in previously treated non small cell lung cancer. N Engl J Med 353: 123-132, 2005.
20. Lee YJ, Cho CC, Jee SH, et al. Impact of environmental tobacco smoke on the incidence of mutations in epidermal growth factor receptor gene in never smoker patients with non-small cell lung cancer. J Clin Oncol 28: 487-492, 2009.
21. Kim ES, Hirsh V, Mok T, et al. Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomized phase III trial. Lancet 372: 1809-1818, 2008.
22. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non small cell lung cancer with mutated EGFR. N Engl J Med 2010; 362: 2380-2388, 2010.
23. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 361: 947-957, 2009.
24. Johnson DH, Arteaga CL . Gefitinib in recurrent non-small cell lung cancer: An IDEAL trial? J Clin Oncol 21: 2227-2229, 2003.
25. Zucali PA, Ruiz MG, Giovannetti E, et al. Role of cMET expression in non small cell lung cancer patients treated with EGFR tyrosine kinase inhibitors. Ann Oncol 19: 1605-1612, 2008.
26. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small cell lung cancer. N Engl J Med 363: 1693-1703, 2010.
27. Kris MG, Johnson B, Berry L, et al. Treatment with therapies matched to oncogenic drivers improves survival in patients with lung cancers: Results from The Lung Cancer Mutation Consortium (LCMC), 15th World Conference on Lung Cancer (27-30 Oct 2013, Sydney, Australia). J Thor Oncol 8: S3-S4, 2013.

Correspondence

Dr. Mutlu DOĞAN

Ankara Üniversitesi Tıp Fakültesi

Medikal Onkoloji Anabilim Dalı

Cebeci Hastanesi

06590 Dikimevi

ANKARA / TURKEY

Tel: (+90.312) 595 71 12

Fax: (+90.312) 319 22 83

e-mail: mutludogan1@yahoo.com