Pathol. Oncol. Res. (2015) 21:1217–1221
DOI 10.1007/s12253-015-9955-2

RESEARCH

Bone Metastases and the EGFR and KRAS Mutation Status in Lung Adenocarcinoma - The Results of Three Year Retrospective Analysis

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Received: 16 January 2015 / Accepted: 22 May 2015 / Published online: 9 June 2015
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Abstract Lung cancer is a heterogeneous group of disease and mutational profiling of lung adenocarcinomas is a routine practice in thoracic oncology. Kirsten-RAS (KRAS) and EGFR mutations play an important role in the carcinogenesis of a subset of lung adenocarcinomas. Our aim was to investigate the correlation between bone metastases and EGFR and KRAS mutation status in lung adenocarcinoma patients. Retrospectively we analysed 224 patients with recurrent or metastatic lung adenocarcinomas. Patients were treated with standard chemotherapy as first line therapy and with EGFR-TK inhibitors as a second or third line therapy. 72 of 224 patients (32 %) had verified bone metastases. Bone metastases and Skeletal Related Events (SRE) were more frequent in men, heavy smokers and without treatment of EGFR TK inhibitors. We have found that EGFR and KRAS mutation status are both predictive factors for the treatment efficacy and prognostic factors for the disease progression. However there were no significant correlation between mutation status and the presence of bone metastases (P=0, 59). In our study the presence of bone metastases proved to be an independent prognostic factor related to poor performance status and worse Quality of Life (QL).

Keywords Lung adenocarcinoma · Skeletal related events · Tyrosine kinase inhibitors · EGFR mutation · KRAS mutation

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Introduction

Lung cancer is the leading cause of cancer related mortality all over the world in both men and women [1]. Non-small cell carcinoma (NSCLC) includes three main cell types (squamous cell carcinoma, adenocarcinoma and large cell carcinoma) which can be further divided into various subtypes revealed in the new pathologic classification (IASLC/ATS/ERS International Multidisciplinary Lung Adenocarcinoma Classification) [2]. The development of molecular pathology methods has become increasingly important in the prediction of chemotherapy sensitivity and to identify driver mutations as important targets of new therapeutic agents. Lung adenocarcinomas show various numbers of driver mutations such as EGFR and KRAS mutations which are mutually exclusive [3]. The most significant change in the treatment of NSCLC is the introduction of molecularly targeted therapies, which include monoclonal antibodies and small molecule tyrosine kinase inhibitors.

Some years ago the median lung cancer survival rate was 10–12 months [4–6]. Now days with the specific targeted therapies, a significant increase has been achieved. The median survival rate is between 24 and 36 months, [7–9]. These agents give an opportunity to provide a new standard of care [10]. Therefore testing of EGFR and KRAS mutations in patients with advanced lung adenocarcinoma should be incorporated into routine clinical practice [9–12].

Bone is the most frequent type of distant metastases of advanced Non- Small Cell Lung Cancer (NSCLC); it is developing in 30–40 % of cases. Relevant clinical studies summarised that, bone metastases and Skeletal Related Events (SRE) are frequently observed in men, heavy smokers, with non-adenocarcinoma histology and without treatment of EGFR TK inhibitors [13, 14]. According to the NCCN and ESMO guidelines the first line therapy for the advanced Stage III/B- IV disease is the platinum based chemotherapy

combined with other second or third generation cytotoxic drugs. When the therapy reached at least Stable Disease (SD) we follow the treatment as a maintenance therapy giving the patients longer survival time [4, 5]. EGFR mutations are detected in 10–15 % of pulmonary adenocarcinomas of Caucasians and 20–30 % of pulmonary adenocarcinomas of East-Asians. The most common mutations are exon 19 and exon 21 mutations. The meta-analysis showed a therapeutic response over 70 % in cases of EGFR mutation of adenocarcinomas (exon 19, 21 mutations). EGFR mutation status is a significant predictive factor of the effectivity of EGFR TK inhibitors. KRAS mutation is more frequent among smokers. KRAS mutation is detected in 25–35 % among the TKI treatment resistant patients. This mutation is found 17 %- in Afro-American patients and 26 % -in Caucasian patients [15]. KRAS mutation is a poor prognostic factor but not an independent predictor of treatment result of EGFR TKI therapy. Up to now the EGFR mutation status is the only significant predictor of EGFR TKI treatment [16].

In this 3 years retrospective study we have analysed the EGFR and KRAS mutation status related to smoking and the correlation between EGFR and KRAS mutation status and the appearance of bone metastases. We investigated whether mutation status has a predictive or prognostic value.

Patients and Methods

Eligibility Criteria

Two hundred twenty four patients were enrolled retrospectively between 2008 and 2010. Inclusion criteria were as follows: initially diagnosed disease which was locally advanced or metastatic disease or recurrent disease, ECOG performance status≤2, cytological or histological diagnosis of lung adenocarcinoma, measurable primary and measurable metastases by imaging.

EGFR and KRAS mutation status were analysed. We also investigated the patients' smoking habit.

Patients were treated by chemotherapy as a first or second line and EGFR TK inhibitors as second or third line. Patients were also required to have adequate hematologic, renal and hepatic function, to eligible for the complex oncotherapeutic procedures. The closing date of the study was 31.Dec.2013. The study was approved by the Institutional Ethics Committee.

EGFR and KRAS Mutation Status Analysis

EGFR and KRAS mutation status were analysed with Cobas EGFR mutation test and Cobas KRAS mutation test (IVD test, Roche).

The Cobas EGFR Mutation Test detects 41 mutations in Exons 18, 19, 20 and 21 of the EGFR gene. In formalin-fixed, paraffin-embedded tissue (FFPET) specimens, the cobas EGFR test can detect <5 % mutant sequence copies in a background of wild-type DNA. The Cobas KRAS Mutation Test detects all of the reported mutations in codons 12, 13 and 61. In formalin-fixed, paraffin-embedded tissue (FFPET), the Cobas KRAS Mutation Test can detect <5 % mutant sequence copies in a background of wild-type DNA.

Statistical Analysis

Statistical data were obtained using an SPSS software package (SPSS 20.0, IBM, New York). Continuous variables were summarized by descriptive statistics. The OS analyses were estimated using the Kaplan-Meier method. Correlation of different features was examined by χ^2 (Chi-Square) test. The comparison between survival functions for different strata was assessed with the long-rank test. Differences were considered significant at $P<0.05$.

Results

Characteristics of Study Population

All patients were evaluated and treated from 1st of January 2008 to 31st of December 2010. 113 of 224 patients were enrolled from the Faculty of Medicine, Department of Pulmonology, Pecs and 111 patients from the National Institute of Oncology, Budapest.

The clinical characteristics of the 224 enrolled patients with advanced lung adenocarcinoma are listed in Table 1.

The median age was 60.54 year (range 39–85 years old), there were 116 males and 108 females.

In case of 83 patients (37 %) the diagnosis was done on cytology and in case of 141 patients (62 %) on biopsy samples. 156 of 224 patients were smokers. 87 of 116 men, and 69 of 108 women were smokers. 174 of 224 patients (78 %) had distant metastases. Contralateral lung (75), bone (72), brain (42) and liver (28) were the most common site of distant metastases.

EGFR and KRAS Mutation Status

The EGFR mutation status was analysed in 117 of 224 patients. Fifty six patients' adenocarcinoma showed EGFR mutation and 61 patients' tumour were wild type (wt).

Among the 72 patients with verified bone metastases we could detect EGFR mutation in 18 patients, and 54 patients' EGFR gene was wild type. (Table 2).

Table 1 Patients' characteristics

Characteristics	Number of patients 224 (%)
Gender	
Male	116 (52 %)
Female	108 (48 %)
Age(years) median	60,54 (39–85)
ECOG performance status	
0	70 (31 %)
1	96 (43 %)
2	58 (26 %)
Histology (Adenocarcinoma)	
Cytology	83 (37 %)
Histology	141 (63 %)
Smoking history	
Never	68 (30 %)
Current	156 (70 %)
Concomittant disease	154 (69 %)
Hypertensio	62 (28 %)
COPD	50 (22 %)
Ischemic Heart Disease (IHD)	34 (15 %)
Diabetes	8 (4 %)
Clinical stage	
III/B	93 (42 %)
IV	131 (58 %)
Metastatic site	
Controlateral lung	75 (35 %)
Bone	72 (32 %)
Brain	42 (19 %)
Liver	28 (13 %)
Treatment of bone metastases	
Bisphosphonates	70 (31 %)
Irradiation	36 (16 %)
BSC	58 (26 %)

KRAS exon 2 analysis was done in case of 187 of 224 lung adenocarcinoma. 104 of 187 patients' adenocarcinomas showed mutation of KRAS gene. 83 of 187 tumours showed wt KRAS exon 2. 19 of 72 patients' tumour with detected bone metastases were KRAS mutant (26, 38 %) and 53 patients' tumour were KRAS wt. 18 of 72 patients' tumour showed EGFR mutation. 35 of 72 patients' tumour with bone metastases did not show EGFR or KRAS mutation.

Table 2 KRAS and EGFR mutation status of patients with bone metastases

	KRAS mut	EGFR mut	KRAS and EGFR wt
Bone metastases 72 patients	19/72 26,38 %	18/72 25 %	35/72 48,6 %

Among never smokers KRAS mutation was rare, 7 of 68 (10 %) never smokers' adenocarcinoma showed KRAS mutation (Table 3). KRAS mutation status showed significant correlation with smoking habit ($p<0.02$).

60 %(109) of patients who died at the closing time harboured KRAS mutation, and 40 %(73) were KRAS wt. From the all 42 survivors 31 patients were KRAS mutant and 11 were wt.

In this study we have found synchronic bone and brain metastases in case of 32 patients (14 %). Duplex metastases were documented in 68 cases (30 %) and they were verified as KRAS mutant patients.

Treatment

During the investigated period in the everyday practice we used different combinations of cytotoxic drugs for Stage IIIB/IV patients as a first line therapy: bevacizumab+paclitaxel, gemcitabin+cisplatin, gemcitabin+carboplatin, paclitaxel+carboplatin, pemetrexed+cisplatin, docetaxel+cisplatin. The first line therapy was given for all 224 patients; they all were in good general condition, ECOG 0–2. The second line therapy was used for 85 patients (38 %). EGFR TKI erlotinib was given as second line therapy for 38 patients whose KRAS status was wild type. The KRAS mutant patients were treated by pemetrexed. Third line therapy was given only 18 % of patients. The reasons were disease progression, death, decrease of performance status (ECOG ≥2), and sometimes the decision of patients. In this third line group 19 patients were treated with erlotinib, and 14 patients with pemetrexed and for eight patients docetaxel was the therapeutic choice (Table 4). Those patients who suffered from bone metastases were treated by complex oncotherapy. Bisphosphonates were used for 70 of 224 (31 %) patients, irradiation was given only for 36 of 224 (16 %) patients, while Best Supportive Care (BSC) was given for 58 (25 %) patients.

Survival Data

Median OS was longer in patients receiving erlotinib second or third line in case of bone metastases versus those who received chemotherapy. Survival analysis demonstrated that OS was 340 weeks with chemotherapy without bone metastases and 550 weeks with erlotinib in case of bone metastases.

Table 3 Correlations between KRAS mutation status and smoking habit

KRAS (187)	Never smokers (68)	Smokers (119)
Mutant	7 (10 %)	76 (64 %)
Wild type	61 (90 %)	43 (36 %)

Table 4 Treatment choices. (TKI: tyrosine kinase inhibitor)

Treatment	Chemotherapy	TKI (Erlotinib)
1st line *N*=224	224	0
2nd line *N*=85/224 (38 %)	47	38
3rd line *N*=33/224 (15 %)	14	19

The different was significant (95 % CI, 2.78–9.95, $P<0.003$) (Fig. 1).

Discussion

With constant improvement in the quality of life, modern society, people's life span has been prolonged. In the Hungarian population the incidence of Lung Cancer is still increasing, and the majority of these patients have advanced disease when they are diagnosed. In this study we have retrospectively analyzed clinical data of 224 patients with metastatic lung adenocarcinoma. From this group 57 patients received EGFR TK inhibitor treatment as 2nd and 3rd line therapy. The treatment choice was based on the erlotinib label and the Hungarian Health Insurance rules. Our data validated the fact that ratio of EGFR mutation is decreasing within heavy smokers and the ratio of KRAS mutation is higher among heavy smokers.

In our study population more than 70 % of patients were smokers. The majority of smokers were not eligible for EGFR TK inhibitor therapy because of higher presence of KRAS mutation. Cytotoxic chemotherapy was given to all these patients as a 1st line therapy, so there is some overlap in these two types of therapy.

The presence of bone metastases at the time of diagnosis suggests a faster disease progression. Considering the EGFR mutation status as a well-known prognostic and predictive factor in case of lung adenocarcinoma our aim was to find correlation between the prevalence of bone metastases and EGFR mutation status. We have investigated the survival time of patients treated with cytotoxic chemotherapy and EGFR TKI during bone metastases. These data confirmed the hypothesis that treatment results with EGFR TK inhibitors for selected patients (EGFR mutant or KRAS wild type) gives survival benefit even in case of bone metastases [17]. There are different reasons: one hand, according to the Hungarian Health Insurance Rules EGFR TK Inhibitors treatment is allowed only for good performance patients (ECOG: 0–2) therefore the survival chance is longer. On the other hand these good results are coming from not only the result of signal transduction blockade pathways in nucleus, but also from the new recognition that EGFR TK inhibitors are able to change osteolytic metastases into osteoblastic metastases.

They decrease the risk of SRE and increase the Quality of Life. As a result of all this inhibition tumour progression has become more effective. Our data verify that the presence of bone metastases are not independent predictive markers of EGFR TKI treatment, but very strong prognostic factor of disease progression. In the absence of EGFR mutations and treatment without of EGFR TK inhibitors the prevalence of SRE is significant higher ($P=0.02$) therefore it is a predictive factor. Normanno et al. [18]) detected relatively new correlations in the pathophysiology of bone involvement within EGFR mutation positive patients. In those patients who were treated with cytotoxic chemotherapy because of advanced lung cancer the ratio of SRE was much higher than in those who were treated by EGFR TK inhibitors. The theory behind

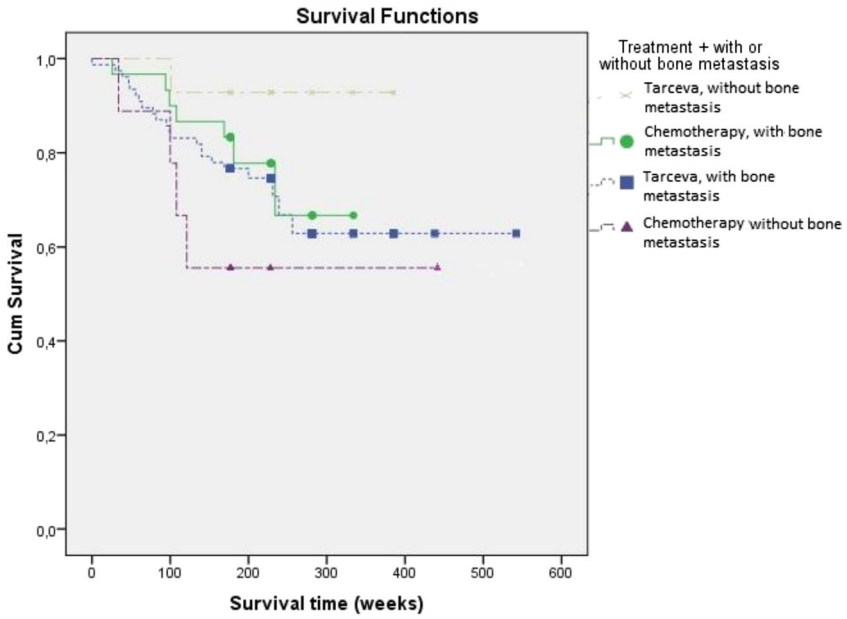

Fig. 1 Survival time in weeks

that, loss of bone density is related with cytotoxic chemotherapy [14, 19]. Nagata observed that the incidence of osteoblastic bone lesion is much higher in the EGFR mutation positive patients than the osteolytic bone metastases. Nowadays the prevalence of osteoblastic metastases is more frequent mostly in lung adenocarcinoma ([18, 20]. The presence of osteoblastic metastases or the evolution to osteoblastosis from previous osteolytic metastases should always be noted since it might represent and important predictive factor of response to EGFR TKI treatment. The EGFR mutation status is a predictive marker for the efficacy of treatment, and is a prognostic marker also for the disease progression [21]. The presence of bone metastases is an independent prognostic marker which correlates with the poor performance and worse Quality of Life (QL). In our study we were not able to differentiate the osteolytic or osteoblastic metastases because there were no clinical data on it. Those patients lived longer who were never smokers, who were KRAS wild type and who were treated with EGFR TK inhibitors. Good performance status (ECOG 0–2) which was a selection criterion by Hungarian Health Insurance has a great influence on these results.

In this retrospective study we have investigated the correlations between the EGFR, KRAS mutations status and prevalence of bone metastases and survival. We have found that EGFR and KRAS mutation status are both predictive factors for the treatment efficacy and are prognostic factors for the disease progression but there was no significant difference between KRAS and EGFR mutation status in metastatic patients. We have found that smoking is not only the most important risk factor of lung cancer but an independent risk factor of SRE also.

Acknowledgments Many thanks for the National Institute of Oncology Budapest, and the Medical University Pecs, approved this retrospective analysis.

Conflict of Interest The Authors declare that they have no known conflict of interest with respect to the authorship and/or publication of this article.

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