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Extraordinary Response to Erlotinib Therapy in a Patient with Lung Adenocarcinoma Exhibiting *KRAS* Mutation and *EGFR* Amplification

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Abstract. *Case reports on the co-incidence of Kirsten rat sarcoma (KRAS) mutation and epidermal growth factor receptor (EGFR) amplification in patients with NSCLC are very rare. This combination is usually considered a negative prognostic factor, despite EGFR amplification alone having positive predictive value. The whole course of treatment of a patient with both EGFR amplification and KRAS mutation present is decribed. The patient in question was a smoker for whom both first- and second-line chemotherapy had been unsuccessful. In stage IV disease biological therapy was administered and proved highly beneficial. Today, 38 months since commencing the treatment, the patient still has no signs of progression and the therapy is still in progress.*

Lung cancer is one of the most common types of cancer worldwide. The majority of the cases are histologically classified as non cmall cell lung cancer (NSCLC) and are usually found in smokers (1). Although it has slightly better prognosis compared to small cell lung cancer (SCLC), the hope of lasting complete regression is minimal mainly due to the early development of chemoresistance.

Biological therapy focused on epidermal growth factor (EGF) signaling pathway inhibition constitutes a new treatment opportunity that increases patients' life expectancy while maintaining a good quality of life (1).

Epidermal growth factor receptor (EGFR) is a transmembranous protein whose extracellular activation by binding of the EGF ligand sets off several intracellular signaling cascades which cause cellular growth and, in case of tumor tissue, malignant proliferation.

Many papers have indicated that in a certain proportion of NSCLC tumors, the EGFR cascade is permanently activated due to mutation or amplification of the *EGFR* gene even without the presence of the EGF ligand. Such tumors are highly sensitive to treatment by the low-molecular EGFR inhibitors (gefitinib, erlotinib). In the Caucasian population, the frequency of carcinomas exhibiting *EGFR* mutation is about 7-15%. This is in agreement with recent findings in the Czech population. Interestingly such EGFR-defective tumors are mostly observed in lifelong non-smokers (2).

It should be noted that *EGFR* amplification only occurs during the tumor aging and as such is not detectable in the early stages of the disease, contrary to *EGFR* mutation which, if present, can be demonstrated in all stages of tumor development. The oncogene *KRAS* (Kirsten rat sarcoma) produces a protein with GTPase activity which acts as a mediator of EGFR-induced signals in the so-called mitogen-activated protein (MAP)-kinase pathway. About 10-20% of lung cancer patients have a *KRAS* mutation. In smokers, this mutation is more frequent (1, 3, 4). *KRAS* mutation causes permanent activation, resulting in continual transfer of growth signals regardless of the regulation otherwise managed by EGFR. Such permanent activation has an impact on tumor proliferation and influences resistance to biological therapy aiming at EGFR inactivation (3). *KRAS* mutation is therefore considered a negative predictor of treatment response and overall survival, as it is in other solid tumors (3, 4).

The co-incidence of *KRAS* mutation and *EGFR* amplification in NSCLC is rarely reported. The negative role of KRAS is usually considered to be indicative of probable treatment failur regardless of the positive predictive contribution of EGFR amplification (2). Such a case was encountered during a clinical project focused on influences of *EGFR* mutations and amplifications (2). A thorough analysis of this particular clinical case was conducted.

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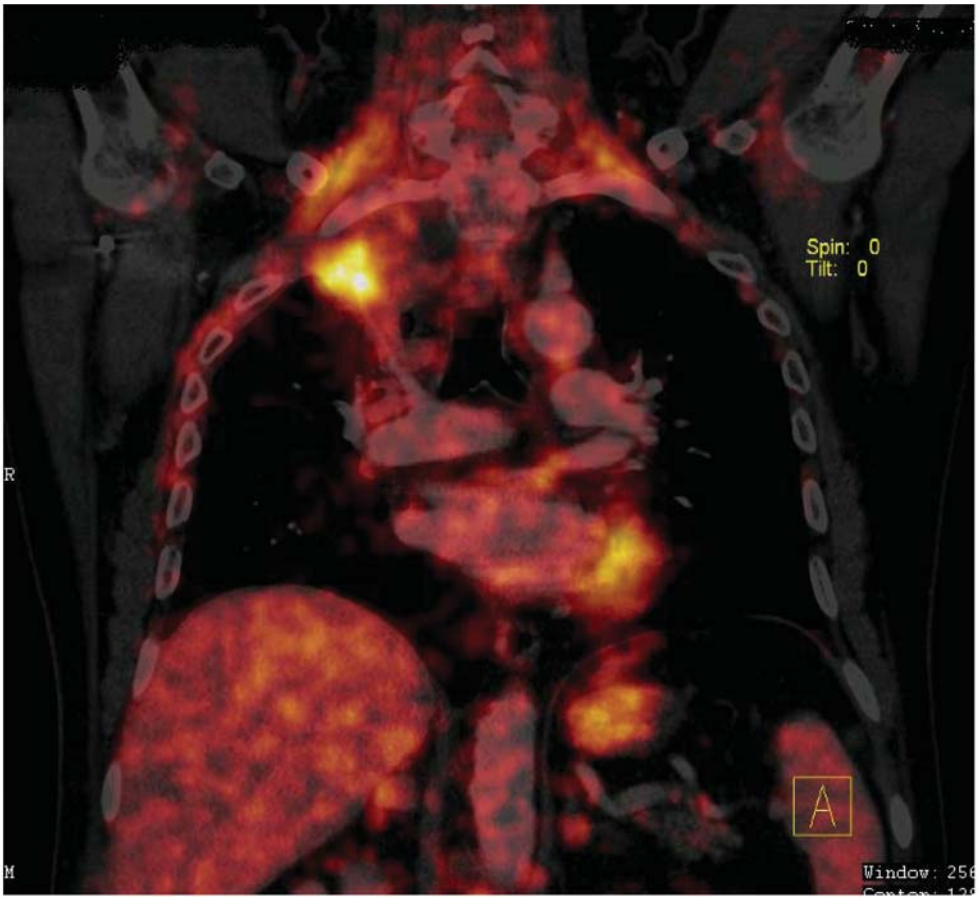

Figure 1. *PET-CT 02/2007*.

Case Report

In November 2006, a 58-year-old male smoker (10 cigarettes daily), a mechanic by occupation, visited the Department of Tuberculosis and Respiratory Diseases. His subjective symptoms included pleural pain, exercise-induced dyspnea, progressive fatigue lasting three months and 10 kg weight loss. He had never been seriously ill, nor had he ever taken any medications for prolonged periods and there was no family history of cancer.

Complex examination revealed a low-grade adenocarcinoma located in the upper lobe of the right lung. The tumor infiltrated the mediastinum deeply and was in contact with the trachea and vertebrae. Furthermore, metastases in the lymphatic nodes of the right pulmonary hilus and the mediastinum were found. According to the CT scan, the tumor was inoperable and was diagnosed as T4N3M0, IIIB, Karnofsky score (KS) 70-80%.

The patient underwent a first-line of chemotherapy by taxotere+cis/carboplatin (11/2006-01/2007). At the same time, concomitant radiotherapy was administered: a total dosage of 51.4 Gy in 23 fractions, 5 sessions per week (12/2006-01/2007). Post-radiation pneumonitis was present.

Table I. *Oncomarkers*.

Oncomarkers	11/2006	01/2008	12/2008	12/2009
CEA (ng/mL)	247.7	1.4	2.0	1.3
CYFRA 21-1 (ng/mL)	0.7	0.7	0.5	0.5
NSE (ng/mL)	6.8	7.0	5.0	6.0
TK (IU/L)	9.1	11.7	5.8	5.4
TPA (IU/L)	10	19	10	10

CEA: Carcinoembryonic antigen; CYFRA 21-1: cytokeratin 19 fragments; NSE: neuron-specific enolase; TK: thymidine kinase; TPA: tissue polypeptide antigen.

The overall condition stabilized for a short time with no further progression and no change to KS, however, the PET/CT examination (see Figure 1) performed in February 2007 revealed systemic spread (stage IV, metastases were

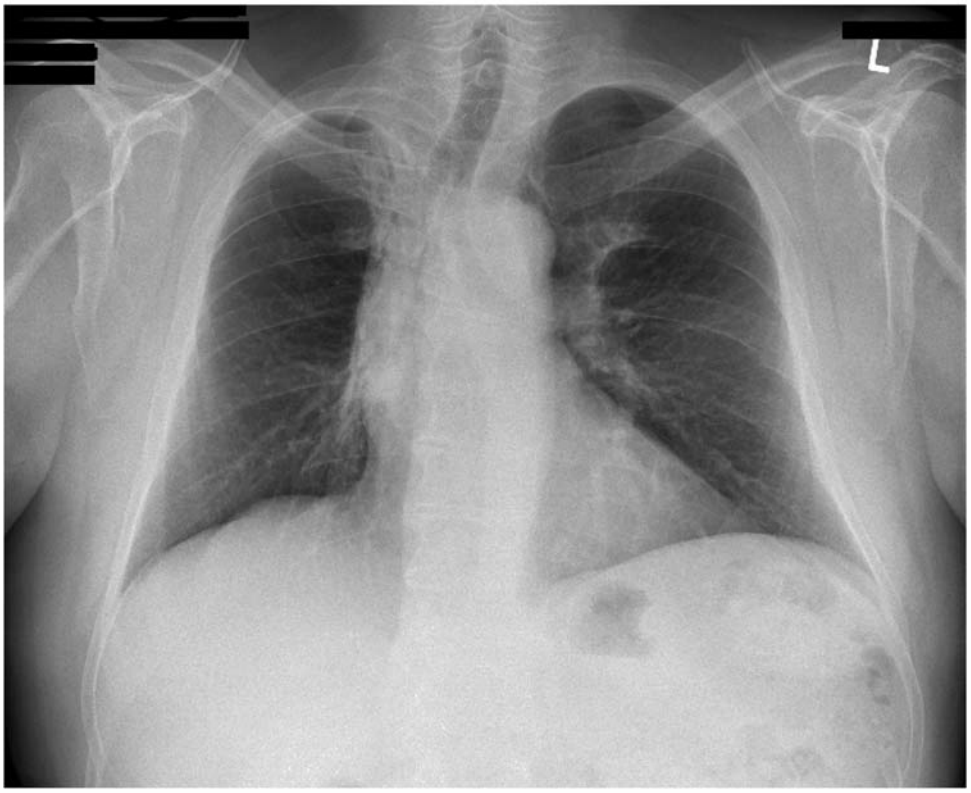

Figure 2. *RTG 04/2010*.

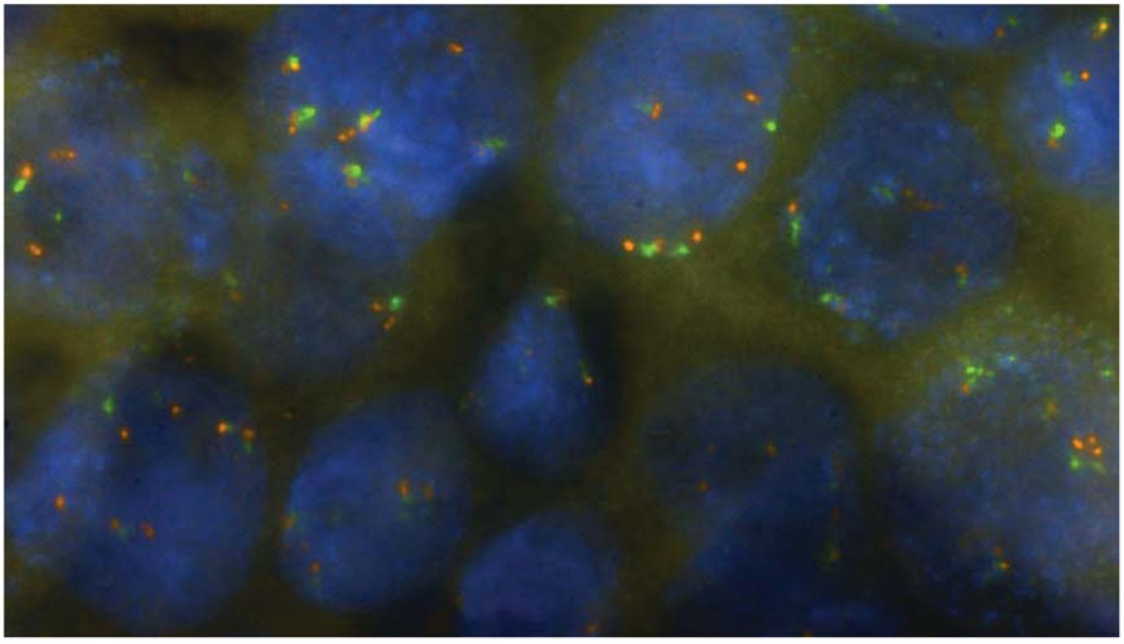

Figure 3. *FISH methods using EGFR probe*.

found in both lungs, right adrenal gland and an additional mediastinal lymphatic node). A second line of treatment with pemetrexed was administered (03/2007-04/2007), 2 cycles in total. The therapy was terminated due to observed tumor progression.

Samples for genetic tests were taken and on 07/2007 erlotinib treatment was commenced.

The patient developed characteristic erlotinib adverse-effects: within two weeks, a grade 2 papulopustular rash appeared in seborrheic areas (face, scalp, neck, upper trunk and shoulders). Within one month, the patient also started suffering from mild diarrhea. These adverse effects responded well to therapy and since both diarrhea and rash severity had lessened, there was no necessity for erlotinib discontinuation or dosage reduction.

Treatment response, however, was promising, with fast tumor regression. A PET/CT examination (09/2007) confirmed regression, absence of viable tumor and decreased metabolic activity of the adrenal metastasis. During cytological sample analysis, *EGFR* amplification and *KRAS* mutation were found.

Cutaneous late adverse-effects were observed during the course of erlotinib therapy and evaluated eight months later. The patient still had a mild, intermittent papulopustular rash, along with telangiectasia, and he had thicker and longer eyebrows and long, curly, rigid eyelashes that needed shortening in order to prevent eye irritation. Substantial worsening of more-existing androgenetic alopecia was also observed. The patient complained of cutaneous dryness, fingertips fissure and conjunctivitis sicca. During a dermatological examination of the patient complained, painful paronychia of both hands and feet.

Nevertheless, there was no change to KS. The patient was free of serious subjective symptoms and his weight had stabilized.

At the time fo writing (08/2010), the erlotinib treatment has been administered for 38 months and since the regression persists, the therapy with its parameters unchanged (see Figure 2).

The values of key treatment response markers during the course of the therapy are shown in Table I.

Methods

KRAS mutation (GGT->TGT) was detected by the denaturing capillary electrophoresis technique following a previously published protocol (5). *EGFR* amplification was detected using fluorescence in situ hybridization (FISH) (Figure 3). The specimen was examined with factory premixed probe Vysis® LSI® EGFR SpectrumOrange/ CEP® 7 SpectrumGreen TM Probe (VYSIS/Abbott, IL, USA). Scoring of amplification was based on the observed number of fluorescent signals in 40 randomly selected non-overlapping tumor cell nuclei. The slide was enumerated and interpreted using the classification of Pugh *et al*. (6).

Discussion

The patient has been treated with erlotinib for 38 months and still shows no sign of progression. Previous chemotherapy regimens proved to be ineffective soon after commencement. No complications which would force the cessation of the biological therapy have been encountered so far. The patient has a good quality of life, with KS 80-90%, *i.e*., PS=1.

Papers describing such coincidence of *KRAS* mutation and *EGFR* amplification are extremely rare. Based on these findings, we propose that the generally accepted opinion that biological therapy is ineffective under these conditions should be reconsidered.

The presence of *KRAS* mutation was assumed to be responsible for the early failure of both the first- and second-line chemotherapies. The question of why the biological therapy is so successful in this patient is yet to be answered.

The results of genetic testing were received during the first line of treatment. Although it might be argued that it would have been better to wait for the results before starting the treatment, we believe that our decision was correct and that waiting any longer would have led to certain and fast tumor progression.

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