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Molecular pathological predictive diagnostics in a patient with non-small cell lung cancer treated with crizotinib therapy: A case report

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Abstract. Lung cancer is one of the most common malignant cancers in the Czech Republic in men, with the highest mortality rate of all the malignant diseases. The development of biological treatment enables study into novel personalized treatment options. This type of treatment is usually of high quality, and is often demanding of predictive and biopsy diagnostics, which is dependent on the quality of the collected material and close cooperation among particular departments. The present study describes the complete biopsy and predictive examinations performed in a male patient with lung adenocarcinoma, with an emphasis on the logistics of the whole process and the application of the tyrosine kinase inhibitors, crizotinib and LDK378. The patient experienced a long overall survival time of 28 months from diagnosis.

Introduction

Lung cancer is one of the most common malignant cancers in the Czech Republic in men, with the highest mortality rate of all the malignant diseases (1). This type of treatment is usually of high quality, and is often demanding of predictive and biopsy diagnostics (2). The development of biological treatment enables use of novel personalized treatment options for clinical oncologists and pulmonologists.

Molecular investigation in patients with lung carcinoma includes molecular-biological [epidermal growth factor receptor (*EGFR*) exons 18, 19, 20 and 21, Kirsten rat sarcoma viral oncogene homolog (*KRAS*), B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) or phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α mutations] and cytogenetic [anaplastic lymphoma receptor tyrosine kinase (*ALK*), *ALK*/echinoderm microtubule-associated protein-like 4 (*ALK/EML-4)* fusion, and ROS proto-oncogene 1, receptor tyrosine kinase (*ROS-1)* or human epidermal growth factor receptor 2 (*HER2)*] analysis. When considering biological treatment these analyses form an inseparable part of a diagnostic process, and the predictive and prognostic character of the response to a treatment is derived from them (3). The treatment logistics begin in a clinical department, where a sample representative enough for biopsy diagnostics is obtained [recommended procedure for histological lung cancer testing by the Society of Czech Pathologists, 2013 (4)], and particularly in cancer cases, a range of immunohistochemical tests is performed. Appropriate sample fixing is also extremely important, otherwise the material may be devalued for pathological testing.

Laboratory testing includes DNA isolation and subsequent mutational analysis of signaling pathway genes, such as *EGFR*. This receptor participates in non-small cell lung carcinoma (NSCLC) carcinogenesis (5), and due to this, has become the target of novel cancer treatment methods. EGFR belongs to a family of transmembrane receptors for growth factors erB/HER and has an intracellular domain mediating signal transfer into a cell via tyrosine kinase activity. This domain is a target for an inhibitor. *EGFR* mutations that cause permanent tyrosine kinase activity without the receptor being occupied by

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a ligand are examined, and detection of these specific mutations indicates the possibility of using tyrosine kinase inhibitor (TKI) treatment. Other analyzed EGFR signaling pathway transmitters are the Raf and Ras proteins, and mitogen-activated protein kinase (MAPK), which regulate cell proliferation. In the case of the Ras protein, mutations at codons 12, 13 and 61 may be observed. The *KRAS* gene product is a protein with GTPase activity that is found in the inner region of a cell membrane. *KRAS* gene point mutations play a significant role in carcinogenesis, since they are responsible for the protein remaining in an active form with the GTP bound. The result is a permanent signaling state and uncontrollable cell proliferation. In the case of the Raf protein, *BRAF* gene mutations are studied. The *BRAF* gene is located in the 7q24 region and consists of 15 exons. This gene encodes a protein that is significant in the regulation of the MAPK/ERK signaling cascade, affecting cell division, differentiation and secretion. In total, 80% of mutations are caused by T1799A transversion leading to V600E amino acid substitution. V600E mutation supports cell transformation activity, as it simulates T599 and/or S602 phosphorylation in the BRAF protein activation domain. As a result, BRAF protein is permanently in an active state, despite RAS signaling.

Laboratory testing also includes cytogenetic diagnostics of *ALK* via fluorescence *in situ* hybridization (FISH). The *ALK* gene encodes a membrane tyrosine kinase receptor that is, in humans, located on chromosome 2p23. ALK break leads to the formation of the ALK/EML-4 fusion protein, against which inhibitors are available.

Possible amplification of the *HER2/neu* gene can also be examined. The product of this gene is a membrane receptor with tyrosine kinase activity, with a weight of 186 kDa, belonging to the HER gene family. The gene is located on the long arm of chromosome 17 in region q11.2-q12. *HER2/neu* amplification initiates dimerization and autophosphorylation of tyrosine kinase residues in the HER2 cytoplasmic domain, which activates complex signaling cascades leading to cell proliferation and thus, to the tumor formation process (6). Genetic aberrations of the EGFR signaling pathway in NSCLC are associated with a different sensitivity to EGFR TKI inhibitors in association with activating mutations on exons 19 and 21 (deletion and mutation L858R). It has been shown that *HER2/neu* amplification can result in acquired EGFR TKI resistance in EGFR-mutant tumors (7).

ROS-1 gene aberrations can be cytogenetically detected. ROS-1 is a protein located in the inner region of a cell membrane. This protein is important for cell growth and proliferation. *ROS-1* gene mutations can lead to the formation of tumors, including NSCLC. Simultaneously, these mutations increase sensitivity to TKI (e.g., crizotinib) treatment.

The present study describes the complete biopsy and predictive examinations of a patient with lung adenocarcinoma who experienced a long survival time, with emphasis on the logistics of the whole process and the application of the TKIs, crizotinib and LDK378.

Case report

At the beginning of March 2012, a male patient (aged 67 years) who presented to the Department of Oncology (First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic) with skeletal pain was examined. Large metastases were confirmed by scintigraphy. A computed tomography (CT) scan showed a large tumor in the left lung, affecting the hilar and mediastinal nodes (pT4N3M1) (8). A bronchoscopic biopsy of the left lower bronchus was performed and the presence of a tumor was confirmed. Microscopic examination showed that the bronchial mucosa was infiltrated by solidly structured adenocarcinoma with intracellular mucin production, passing to areas with regions of signet-ring cells. The tumor grew in an infiltrative manner, with evident carcinomatous lymphangiopathy. Immunohistochemical examination revealed that the tumor cells expressed cytokeratin 7, but thyroid transcription factor 1 (TTF1) and caudal-type homeobox 2 protein (cdx2) were not detected. Molecular pathological examination of EGFR was negative, mutations were not detected. When the *KRAS* gene was tested, a Gly12Ala (GGT≥GCT) mutation at codon 12 was found. Mutational analysis the of *BRAF* gene did not detect V600E mutation. For cytogenetic analysis (FISH), the Vysis ALK Break FISH Probe kit (Abbott Pharmaceutical Co. Ltd., Lake Bluff, IL, USA) fluorescence probe was hybridized to the tissue section (2-3 μm). Duplex formation (regions 2p23.1p23.2) is directly detected by fluorescence-labeled polynucleotide labels. The tissue was denatured at 75˚C (±2˚C) for 10 min. The probe was then hybridized to ThermoBrite (Abbott Pharmaceutical Co. Ltd.) overnight at 37˚C. The changes were detected using a fluorescence microscope (Olympus, Tokyo, Japan). Cytogenetic examination by FISH did not detect *HER2/neu* amplification. Examination of the *ALK* (2p23) gene proved the break in the relevant area; a 15-20% incidence of individual red signals showed evidence of the loss of an interstitial area of the *ALK* gene (9).

In July 2012, the patient underwent two stabilizing surgical interventions in the area of the thoracic and cervical spine in the Department of Neurosurgery. The patient was then transferred to the care of the Department of Oncology. As the first-line treatment, the patient received four cycles of cisplatin (150 mg) and pemetrexed (1,000 mg), with a partial response after the second cycle, as confirmed by a CT scan. However, primary tumor progression occurred after the fourth cycle. Alimta was administered in monotherapy until progression occurred in July 2012.

With regard to *ALK* gene break detection, crizotinib therapy (250 mg twice a day) was prescribed. The therapy commenced in July 2012 and lasted for 1 year. After 1 month, the medication had to be stopped due to grade 3 liver toxicity [alanine aminotransferase (ALT), 15.79 μkat/l; aspartate aminotransferase (AST), 4.67 μkat/l; γ-glutamyl transpeptidase (GGT), 1.78 μkat/l; and alkaline phosphatase (ALP), 3.18 μkat/l]. After 14 days, the liver enzyme levels were lower (grade 1) (ALT, 2.67 μkat/l; AST, 0.66 μkat/l; GGT, 1.62 μkat/l; and ALP, 1.84 μkat/l) and the patient continued therapy, which was reduced to a 50% dose for 1 month. As the treatment was well tolerated, the dose was increased back to the original level in November 2012. The patient received the full dose of crizotinib until January 2014, when primary tumor progression and bone metastases were detected on PET/CT, according to Response Evaluation Criteria In Solid Tumors (version 1.1) (10). Due to crizotinib therapy, the generalized lung tumor had been

stabilized for a total of 19 months. The patient was offered a place in the German clinical trial for LDK378, an ALK inhibitor developed by Novartis International AG (Basel, Switzerland), but this was declined and treatment was continued in the Czech Republic. Erlotinib therapy was started in February 2014, however, the treatment was ended in April 2014 due to fast disease progression. Subsequently, the patient participated in the formerly considered LDK378 study in Frankfurt on the Main, Germany. Nevertheless, the disease progressed and the patient succumbed in May 2014. The patient was treated using the TKI crizotinib until disease progression, which occurred after 19 months, an unusually long time compared to the commonly reported 8-10 months. The overall survival time following diagnosis was 28 months.

Discussion

In the present study, the tumor morphology and results of immunohistochemical examinations (TTF1, CK7 and cdx2) were not unambiguous, and from a differential diagnostic point of view, the metastatic tumor origin (11-13), e.g., the stomach, was also considered. *HER2/neu* gene amplification was assessed by FISH during the diagnostic process. Metastases from stomach cancer would have shown increased protein expression (relevant gene amplification) and monoclonal antibody Herceptin® (transtuzumab) therapy would have been possible (14) Moreover, *HER2/neu* amplification correlates with possible resistance to EGFR TKI.

EGFR ras/raf/MAPK signaling pathway analysis was interpreted to the patient's disadvantage. Existing studies have not confirmed a direct correlation between activating mutations of the *EGRF* gene and inhibiting mutations of the *KRAS* gene, however, it has been shown that these mutations can occur simultaneously. If *KRAS* gene mutation occurs together with the activating mutations of EGFR, the response to TKI inhibitor treatment could be affected (15) and carriers of these mutations would not respond to TKI treatment (16). *BRAF* gene mutation was not detected in the present study. This mutation could have an activating character with a positive response to MAPK kinase (MEK) inhibitor (Vemurafenib) treatment, nevertheless, it decreases response to TKI inhibitor treatment in lung carcinomas with *EGFR* gene mutations (17), and according to certain studies, BRAF mutation (V600E) represents an unfavorable prognostic factor (18,19). Due to these reasons, it is necessary to observe mutational changes in the whole EGFR ras/raf/MAPK signaling pathway (20). In the present study, cytogenetic examination detected ALK break and ALK/EML-4 fusion protein. This oncogenic rearrangement of the *ALK* gene has also been described in NSCLC. The inhibitor Xalkori® (crizotinib; Pfizer, Tadworth, UK) has demonstrated hopeful results in lung carcinoma cases with ALK rearrangements (21,22). ALK is an oncogene that is activated by a fusion with other genes. Other changes in the *ALK* gene, such as mutations or amplifications, lead to the early resistance to crizotinib. This resistance, however, was not confirmed in the present case. According to previous research, the occurrence of *ALK* gene break together with activating mutations of the *EGFR* gene is not possible (21).

The present study on a 67-year-old man elucidated the importance of comprehensive and complex diagnostics as the most important precondition for achieving an adequate response to currently available biological treatments. In predictive diagnostics, it is not appropriate to focus on the analysis of just one marker. A tumor cell is complex, and thus all aspects participating in its increased proliferative activity must be observed.

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References

1. Zdenek A, Krejčí M and Vorlíček J: Special Oncology. 1st edition. Galen, Prague, p38, 2010.
2. Thomas R and Wolf J: Personalized therapy of lung cancer. Onkologie 35 (Suppl 1): S14-S19, 2012.
3. Li C, Hao L, Li Y, Wang S, Chen H, Zhang L, Ke B, Yin Y, Suo H, Sun B, *et al*: Prognostic value analysis of mutational and clinicopathological factors in non-small cell lung cancer. PLoS One 9: e107276, 2014.
4. Dundr P, Hornychová H, Matěj R, Ryška A, Staněk L and Tichý T: Guidelines for histological examination of lung carcinoma. Society of Czech Pathologists ČLS JEP: 12-14, 2013 (In Czech).
5. Li Y and Song L: Research progress on resistance mechanisms of epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Zhongguo Fei Ai Za Zhi 15: 106-111, 2012 (In Chinese).
6. Iqbal N and Iqbal N: Human epidermal growth factor receptor 2 (HER2) in cancers: Overexpression and therapeutic implications. Mol Biol Int 2014: 852748, 2014.
7. Ricciardi GR, Russo A, Franchina T, Ferraro G, Zanghì M, Picone A, Scimone A and Adamo V: NSCLC and HER2: Between lights and shadows. J Thorac Oncol 9: 1750-1762, 2014.
8. Edge SB, Bryd DR, Compton CC, Fritz AG, Greene FL and Trotti A, III (eds): AJCC Cancer Staging Manual. 7th edition. Springer, New York, NY, 2009.
9. Camidge DR, Theodoro M, Maxson DA, Skokan M, O'Brien T, Lu X, Doebele RC, Barón AE and Varella-Garcia M: Correlations between the percentage of tumor cells showing an anaplastic lymphoma kinase (ALK) gene rearrangement, ALK signal copy number and response to crizotinib therapy in ALK fluorescence in situ hybridization-positive non small cell lung cancer. Cancer 118: 4486-4494, 2012.
10. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, *et al*: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45: 228-47, 2009.
11. Compérat E, Zhang F, Perrotin C, Molina T, Magdeleinat P, Marmey B, Régnard JF, Audouin J and Camilleri-Broët S: Variable sensitivity and specificity of TTF-1 antibodies in lung metastatic adenocarcinoma of colorectal origin. Mod Pathol 18: 1371-1376, 2005.
12. Su YC, Hsu YC and Chai CY: Role of TTF-1, CK20 and CK7 immunohistochemistry for diagnosis of primary and secondary lung adenocarcinoma. Kaohsiung J Med Sci 22: 14-19, 2006.
13. Yatabe Y, Mitsudomi T and Takahashi T: TTF-1 expression in pulmonary adenocarcinomas. Am J Surg Pathol 26: 767-773, 2002.
14. Bunn PA Jr, Helfrich B, Soriano AF, Franklin WA, Varella-Garcia M, Hirsch FR, Baron A, Zeng C and Chan DC: Expression of Her-2/neu in human lung cancer cell lines by immunohistochemistry and fluorescence in situ hybridization and its relationship to in vitro cytotoxicity by trastuzumab and chemotherapeutic agents. Clin Cancer Res 7: 3239-3250, 2001.
15. Roberts PJ, Stinchcombe TE, Der CJ and Socinski MA: Personalized medicine in non-small-cell lung cancer: Is KRAS a useful marker in selecting patients for epidermal growth factor receptor-targeted therapy? J Clin Oncol 28: 4769-4777, 2010.
16. Riely GJ, Marks J and Pao W: KRAS mutations in non-small cell lung cancer. Proc Am Thorac Soc 6: 201-205, 2009.

17. Pratilas CA, Hanrahan AJ, Halilovic E, Persaud Y, Soh J, Chitale D, Shigematsu H, Yamamoto H, Sawai A, Janakiraman M, *et al*: Genetic predictors of MEK dependence in non-small cell lung cancer. Cancer Res 68: 9375-9383, 2008.
18. Marchetti A, Felicioni L, Malatesta S, Grazia Sciarrotta M, Guetti L, Chella A, Viola P, Pullara C, Mucilli F and Buttitta F: Clinical features and outcome of patients with non-small-cell lung cancer harboring BRAF mutations. J Clin Oncol 29: 3574-3579, 2011.
19. Villaruz LC, Socinski MA, Abberbock S, Berry LD, Johnson BE, Kwiatkowski DJ, Iafrate AJ, Varella-Garcia M, Franklin WA, Camidge DR, *et al*: Clinicopathologic features and outcomes of patients with lung adenocarcinomas harboring BRAF mutations in the lung cancer mutation consortium. Cancer 121: 448-456, 2015.
20. Schmid K, Oehl N, Wrba F, Pirker R, Pirker C and Filipits M: EGFR/KRAS/BRAF mutations in primary lung adenocarcinomas and corresponding locoregional lymph node metastases. Clin Cancer Res 15: 4554-4560, 2009.
21. Katayama R, Shaw AT, Khan TM, Mino-Kenudson M, Solomon BJ, Halmos B, Jessop NA, Wain JC, Yeo AT, Benes C, *et al*: Mechanisms of acquired crizotinib resistance in ALK-rearranged lung cancers. Sci Transl Med 4: 120ra17, 2012.
22. Solomon BJ, Mok T, Kim DW, Wu YL, Nakagawa K, Mekhail T, Felip E, Cappuzzo F, Paolini J, Usari T, *et al*: First-line crizotinib versus chemotherapy in ALK-positive lung cancer. N Engl J Med 371: 2167-2177, 2014.