Abstract. Background: Concurrent presence of EGFR and KRAS mutations in non-small cell lung cancer (NSCLC) patients is relatively rare and their appearance is believed to be mutually exclusive. Tumours harbouring KRAS mutation are perceived as not being capable of response to tyrosine kinase inhibitor (TKI) therapy. Patients and Methods: This paper presents 5 case reports of patients with tumours harbouring multiple EGFR and/or KRAS mutations. There were 3 patients with EGFR mutations (2 × exon 19 deletions, 1 × L858R) combined with KRAS mutations (2 × Gly12Asp, 1 × Gly12Val), 1 patient with two EGFR mutations (exon 19 deletion + L858R) and 1 patient with two KRAS mutations (Ala11Pro + Gly12Val). Results: All EGFR+/KRAS+ patients had initially showed positive response to TKI treatment. The EGFR+/EGFR+ patient has exhibited strong rash and good response with the best survival, while the KRAS+/KRAS+ patient did not respond to TKI therapy. Conclusion: EGFR+/EGFR+ patient has exhibited strong rash and good response with the best survival, while the KRAS+/KRAS+ patient did not respond to TKI therapy. Conclusion: EGFR+/EGFR+ combination does not necessarily pose a negative prediction. This is probably due to the multiclonal character of the tumour displaying partial response in the EGFR+ subpopulation.

Since its introduction in 2004, screening for somatic EGFR mutations has become best practice prior to administering biological targeted therapy of advance stages of non-small cell lung cancer (NSCLC) (1). A presence of activating exon 19 deletions or exon 21 point substitutions results in uncontrolled tumour proliferation due to permanent growth stimulation. Hence, the class of EGFR-mutated tumours shows a good response towards targeted blockade of EGFR signalling (2). For such tumour types, a biological therapy by small tyrosine kinase inhibitors (TKI) such as gefitinib and erlotinib has proved to be effective (3, 4). Furthermore, phenotypic distinction of patients with EGFR-positive tumours is expressed by specific side-effects such as skin acniform exanthema known as rash and itching, as well as mild gastrointestinal symptoms (5).

Aside from predictive EGFR testing, many recent postudies have been focused on detecting KRAS mutations (6). KRAS-positive patients are expected to have a poor prognosis with respect to overall survival and a limited response to targeted treatment with TKI (7, 8). Results of our recent study somewhat question such universally negative prognostic value of KRAS mutations, narrowing it to only cases of smokers with squamous cell tumour subtype (9). Howly, a preferential KRAS testing of smokers remains speculative (10).

The aim of the present work was to investigate each of the mutation types in several clinical cases where multiple mutations were detected in a single NSCLC tumour tissue. With the mutation multiplicity, this study focused on the mutual relation between EGFR and KRAS mutations for cases with EGFR+/KRAS+ combination. In addition, a possible intensification of the clinical manifestation was examined in cases with multiple mutations in either EGFR or KRAS.

Patients and Methods

Clinical data for each patient are listed in the results section. Experimental setup of molecular testing is described in detail elsewhere (9). In brief, formaline-fixed paraffin embedded sections or cytological slides were used for mutation testing. When the total tumour mass was low, laser microdissection by P.A.L.M. microlaser instrument (Carl Zeiss MicroImaging GmbH, Bernried, Germany) was used. Genomic DNA was extracted by JetQuick Tissue DNA Isolation Kit (GENOMED GmbH, Loehne, Germany) and then the mutations were detected by fragment analysis using cycling-gradient capillary electrophoresis techniques in an automated 96-capillary array sequencer (MegaBACE 1000, GE Biosciences, Piscataway, NJ, USA).
Results

An overview of the presented cases is given in Table I. Five case reports are presented including NSCLC patients with multiplicity in either EGFR or KRAS (or in a combination of both). There were 3 patients with concurrent KRAS and EGFR mutations, 1 patient with two EGFR mutations and 1 patient with two KRAS mutations.

Case 1: A 74-year-old male iron-founder, with smoking history of 15 pack-years was diagnosed with stage IIIA (T2N2M0) squamous cell carcinoma in the left lung with involvement of major bronchus and metastases to the left hilar and mediastinal lymph nodes. The patient initially received 4 cycles of gemcitabine and carboplatin chemotherapy resulting in partial regression on chest CT and skiagram and also in bronchological regression. Treatment was followed by irradiation of tumour and surrounding mediastinum (46 Gy) and left hilus (60 Gy). Persistence of the tumour was then confirmed bronchologically. Consequently, the patient received 3 cycles of docetaxel in monochemotherapy, which was followed by bronchologically confirmed tumour progression; The skiagram showed atelectasis of the left upper lobe, and treatment with gefitinib was therefore initiated. A partial regression was detected on a chest scan 3 months later. This was followed by abrupt disease progression, with the patient dying 21 months after diagnosis. The mutation analysis revealed exon 19 deletion in the EGFR gene along with a codon 12 substitution in the KRAS gene (Gly12Val).

Case 2: A 65-year-old male manual worker (reported inhalation of cast-iron dust), with non-smoking history was diagnosed with stage IV (T2N3M1) adenocarcinoma in the right upper lobe with multiple metastases to both lungs and supraclavicular lymphadenopathy. Performance status was 75%. After the start of erlotinib treatment, there was occurrence of intense rash (stage 3-4) along with gastrointestinal toxicity, prompting a dose reduction (100 mg) with partial regression. Five months into the treatment, the disease relapsed and the patient died 5½ months from the start of biological therapy. Mutation analysis recorded a point substitution in EGFR exon 19 (L858R) along with KRAS codon 12 substitution (Gly12Asp).

Case 3: A 71-year-old female, non-smoker was diagnosed with stage IV (T2N3M1) adenocarcinoma in the right upper lobe with multiple metastases to both lungs and supraclavicular lymphadenopathy. Performance status was 75%. After a later disease progression, the patient died 19 months from the initial diagnosis. Bronchoscopy samples revealed the presence of EGFR exon 19 deletion together with KRAS codon 12 substitution (Gly12Asp).

Case 4: A 53-year female nurse, with non-smoking history was diagnosed with stage IV (T2N2M1) adenocarcinoma in the right lung with metastases into the right hilar and mediastinal lymph nodes and multiple metastases in the lung. The patient received a single cycle of chemotherapy (gemcitabine/cisplatin) with haematological toxicity and occurrence of malignant pleural effusion on the right. The patient was then treated with 1 cycle of gemcitabine/cisplatin, which also resulted in haematological toxicity and progression of malignant pleural effusion requiring talc pleurodesis. Following disease progression, treatment with gefitinib was started. Following 12 months of TKI therapy, there was partial regression on chest scan and bronchoscopy. Chest CT confirmed the disappearance of pleural effusion along with regression of the tumour and mediastinal lymphadenopathy. Following subsequent disease progression, the patient died 23 months from the time of diagnosis. Genetic examination confirmed concurrent presence of deletion in exon 19 and point substitution in exon 21 of the EGFR gene.

Table I. Overview of presented clinical cases of NSCLC patients with confirmed occurrence of multiple mutations in EGFR and/or KRAS.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Smoking status</th>
<th>Mutation</th>
<th>Tumour histological type</th>
<th>Gefitinib/Erlotinib treatment (months)</th>
<th>Survival from diagnosis (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Smoker</td>
<td>EGFR exon 19 del + KRAS Gly12Val</td>
<td>Squamous cell carcinoma</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>Non-smoker</td>
<td>EGFR exon 19 del + KRAS Gly12Asp</td>
<td>Adenocarcinoma</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>Non-smoker</td>
<td>EGFR exon 21 L858R + KRAS Gly12Asp</td>
<td>Adenocarcinoma</td>
<td>5</td>
<td>5½</td>
</tr>
<tr>
<td>4</td>
<td>Non-smoker</td>
<td>EGFR exon 19 del + EGFR exon 21 L858R</td>
<td>Adenocarcinoma</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>5</td>
<td>Smoker</td>
<td>KRAS Ala11Pro + KRAS Gly12Val</td>
<td>Adenocarcinoma</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
Case 5: A 51-year-old male forger, with 30 pack-years smoking history was diagnosed with stage IIIB (T2N3M0) adenocarcinoma in the left lung, with metastases in bilateral supraclavicular lymph nodes. Initial treatment included one cycle of chemotherapy (gemcitabine/cisplatin) which resulted in haematological toxicity and allergic reaction. The patient then received erlotinib with disease progression and occurrence of malignant pleural effusion after 1 month of TKI therapy. In addition, progression in the supraclavicular lymph nodes was confirmed by ultrasonographic examination and the patient died prior to planned radiotherapy, 2 months after the diagnosis. Genetic examination of the tissue sample revealed the presence of two KRAS mutations, in codon 11 (Ala11Pro) and in codon 12 (Gly12Val).

Discussion

Studies of genetic alterations (mutations, amplifications, overexpressions etc.) used as molecular predictors and prognostic factors often relate each marker to its respective function within the cellular signal transduction network (11). Figure 1 illustrates the oncogenic pathway involving EGFR and KRAS. There, the signalling is passed from EGFR either to the KRAS-mediated MAP-kinase (Ras/Raf/MEK/ERK) pathway or to alternative PI3 kinase or JAK-STAT pathways. Although patients with concurrent EGFR and KRAS mutations are relatively rare, there is no a priori reason for the two mutation types to be inversely linked. With KRAS mutation occurring in approximately 20-35% and EGFR mutation occurring in 5-10% of NSCLC, the independent occurrence model puts the combined mutation genotype at a level of 1-3% (12). The presented 3 EGFR+/KRAS+ cases in an entire cohort of 360 NSCLC patients are in agreement with such expectation. Naturally, cases of patients with tumours harbouring both EGFR+ and KRAS mutations have already been reported in the literature (13-15).

From the signalling systems depicted in Figure 1, it is clear that activation in KRAS renders the concurrent upstream activation of EGFR unimportant. Therefore, a cell with activated KRAS is not expected to respond to TKI-based therapy designed to block EGFR signalling. Similar to other types of cancer, KRAS positivity is usually a negative factor for TKI therapy response to NSCLC (12, 16, 17). The EGFR/KRAS+ patients, described in this study, who clearly responded to TKI
therapy, suggest that the situation may be more complex. A recent study directed at the development of resistance against gefitinib has confirmed the polyclonal nature of lung carcinomas (18). It has been demonstrated that genotypically heterogenous cancerous cell subpopulations indeed exist within the tissue of a single tumour. While the TKI-sensitive subpopulation (usually cells bearing EGFR mutation) is eliminated by the targeted therapy, growth of the remaining cells is largely unaffected, resulting in therapy resistance. Similarly to the presented cases, it can be speculated that subpopulations of cells exist, each bearing different mutation types. A partial response to TKI therapy could, therefore, be observed in EGFR+/KRAS+ tumours with only the EGFR+ cells responding (18). This is in agreement with the presented cases, where all 3 EGFR+/KRAS+ patients have shown positive initial response to TKI therapy. On the contrary, other literature sources describe occurrence of EGFR+/KRAS- tumours without an objective response to the TKI therapy (15). Apparently, in these cases, it is highly likely that both mutations affect the same cells and KRAS mutations dictate the overall tumour behaviour.

Finally, since a single (heterozygous) mutation is sufficient for oncogenic activation, there was no amplification effect of multiple mutations within the same gene. However, in some double somatic EGFR mutations, sensitivity to TKIs could vary (7). The positive response of the patient with a double EGFR mutation as well as a negative response of the patient with double KRAS mutation was in accordance with this theory.

Contrary to an original notion of mutual exclusivity of EGFR and KRAS mutations, we have confirmed recent reports of others by discovering multiple NSCLC patients harboring mutations of EGFR along with those of KRAS. The data suggest an ambiguity in clinical outcome for such tumours. We contemplate the effect of clonal origin of mutated cells on the mechanism of their response to the biological therapy. Cells concurrently affected by both mutations will likely present resistance due to KRAS activation acting independently on EGFR status. As suggested by our data, in cases of mixed populations of cells bearing only a single mutation at a time, a partial response may be observed as a result of the EGFR+ cellular fraction responding alongside unaffected KRAS+ fraction.

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References


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