# Multiplicity of *EGFR* and *KRAS* Mutations in Non-small Cell Lung Cancer (NSCLC) Patients Treated with Tyrosine Kinase Inhibitors

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**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$^+$/KRAS$^+$ 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 acneiform exanthema known as rash and itching, as well as mild gastrointestinal symptoms (5).

Aside from predictive EGFR testing, many recent pstudies 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).

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Table I. *Overview of presented clinical cases of NSCLC patients with confirmed occurence of mutliple mutations in EGFR and/or KRAS.*

| Patient | Smoking status | Mutation | Tumour histological type | Gefitinib/Erlotinib treatment (months) | Survival from diagnosis (months) |
|---|---|---|---|---|---|
| 1 | Smoker | *EGFR* exon 19 del + *KRAS* Gly12Val | Squamous cell carcinoma | 3 | 21 |
| 2 | Non-smoker | *EGFR* exon 19 del + *KRAS* Gly12Asp | Adenocarcinoma | 7 | 19 |
| 3 | Non-smoker | *EGFR* exon 21 L858R + *KRAS* Gly12Asp | Adenocarcinoma | 5 | 5 1/2 |
| 4 | Non-smoker | *EGFR* exon 19 del + *EGFR* exon 21 L858R | Adenocarcinoma | 12 | 23 |
| 5 | Smoker | *KRAS* Ala11Pro + *KRAS* Gly12Val | Adenocarcinoma | 1 | 2 |

## 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 left lung with further metastases into the right supracavicular and mediastinal lymph nodes, as well as into the right lower lobe. The patient initially received 6 cycles of chemotherapy (gemcitabine/carboplatin). A complete remission was detected by bronchoscopy with a regression also evident on chest skiagram and scintigraphy. After 6 months, a single cycle of docetaxel was given in monochemotherapy with intolerance followed by tumour progression on the skiagram and the development of multiple metastases in the lung, the hepar and retroperitoneum. Gefitinib treatment was started with partial regression 7 months later. 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 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 the start of erlotinib treatment, there was occurence 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 21 (L858R) along 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 occurence of malignant pleural effusion on the right. The patient was then treated with 1 cycle of gemcitabine/carboplatin, 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.

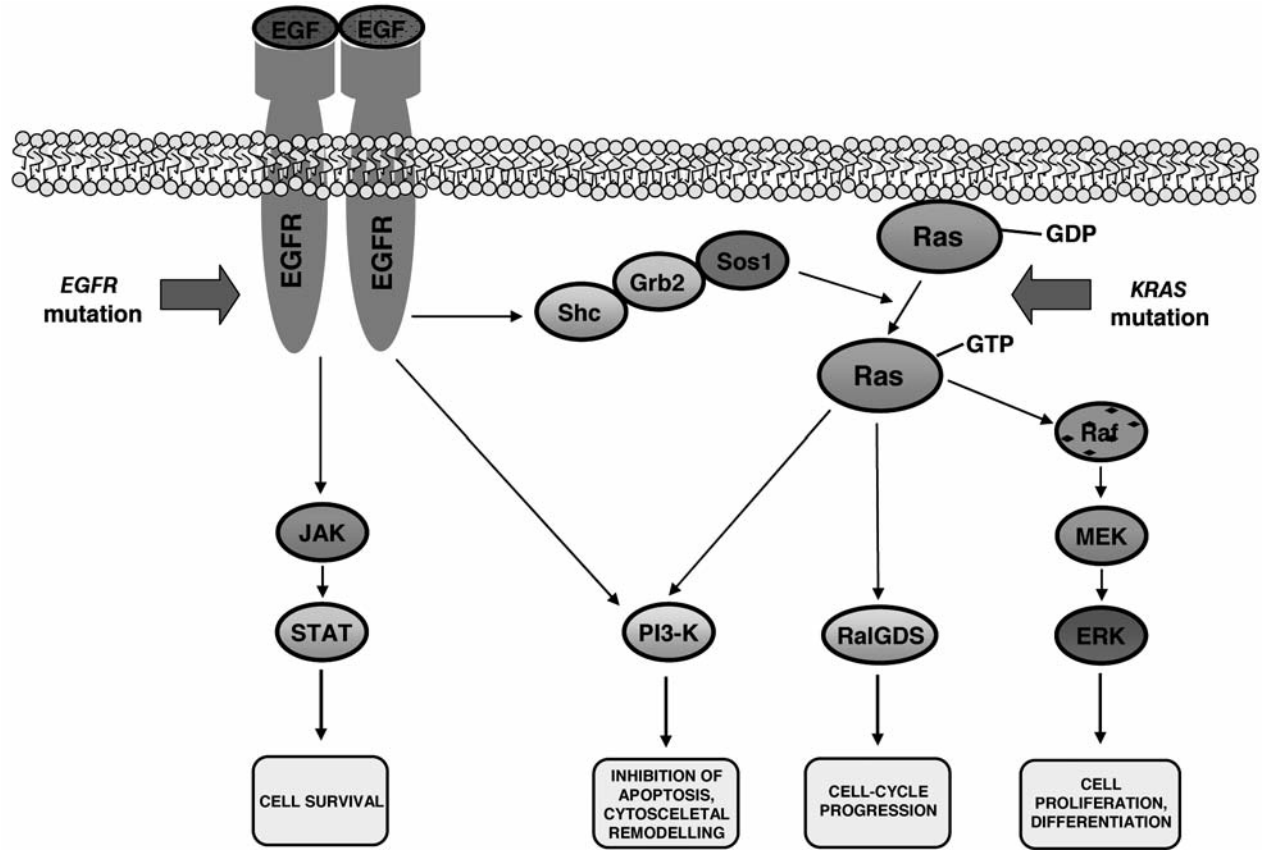


Figure 1. *Oncogenic signalling pathways involving EGFR and KRAS genes.*

*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 occurence 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

1 Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, Majem M, Lopez-Vivanco G, Isla D, Provencio M, Insa A, Massuti B, Gonzalez-Larriba JL, Paz-Ares L, Bover I, Garcia-Campelo R, Moreno MA, Catot S, Rolfo C, Reguart N, Palmero R, Sanchez JM, Bastus R, Mayo C, Bertran-Alamillo J, Molina MA, Sanchez JJ and Taron M: Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med *361*: 958-967, 2009.

2 Sequist LV, Bell DW, Lynch TJ and Haber DA: Molecular predictors of response to epidermal growth factor receptor antagonists in non-small cell lung cancer. J Clin Oncol *25*: 587-595, 2007.

3 Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J and Haber DA: 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.

4 Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE and Meyerson M: *EGFR* mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science *304*: 1497-1500, 2004.

5 Li T and Perez-Soler R: Skin toxicities associated with epidermal growth factor receptor inhibitors. Target Oncol *4*: 107-119, 2009.

6 Riely GJ and Ladanyi M: *KRAS* mutations: an old oncogene becomes a new predictive biomarker. J Mol Diagn *10*: 493-495, 2008.

7 Choong NW, Dietrich S, Seiwert TY, Tretiakova MS, Nallasura V, Davies GC, Lipkowitz S, Husain AN, Salgia R and Ma PC: Gefitinib response of erlotinib-refractory lung cancer involving meninges role of *EGFR* mutation. Nat Clin Pract Oncol *3*: 50-57, 2006.

8 Riely GJ, Kris MG, Rosenbaum D, Marks J, Li A, Chitale DA, Nafa K, Riedel ER, Hsu M, Pao W, Miller VA and Ladanyi M: Frequency and distinctive spectrum of *KRAS* mutations in never smokers with lung adenocarcinoma. Clin Cancer Res *14*: 5731-5734, 2008.

9 Pesek M, Benesova L, Belsanova B, Mukensnabl P, Bruha F and Minarik M: Dominance of *EGFR* and insignificant *KRAS* mutations in prediction of tyrosine-kinase therapy for NSCLC patients stratified by tumor subtype and smoking status. Anticancer Res 29: 2767-2773, 2009.

10 Girard N, Deshpande C, Azzoli CG, Rusch VW, Travis WD, Ladanyi M and Pao W: Use of epidermal growth factor receptor/Kirsten rat sarcoma 2 viral oncogene homolog mutation testing to define clonal relationships among multiple lung adenocarcinomas: comparison with clinical guidelines. Chest *137*: 46-52, 2010.

11 Kamath S and Buolamwini JK: Targeting EGFR and HER-2 receptor tyrosine kinases for cancer drug discovery and development. Med Res Rev *26*: 569-594, 2006.

12 Greenman C, Wooster R, Futreal PA, Stratton MR and Easton DF: Statistical analysis of pathogenicity of somatic mutations in cancer. Genetics *173*: 2187-2198, 2006.

13 Zhu CQ, da Cunha SG, Ding K, Sakurada A, Cutz JC, Liu N, Zhang T, Marrano P, Whitehead M, Squire JA, Kamel-Reid S, Seymour L, Shepherd FA and Tsao MS: Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR.21. J Clin Oncol *26*: 4268-4275, 2008.

14 Pallis AG, Voutsina A, Kalikaki A, Souglakos J, Briasoulis E, Murray S, Koutsopoulos A, Tripaki M, Stathopoulos E, Mavroudis D and Georgoulias V: 'Classical' but not 'other' mutations of *EGFR* kinase domain are associated with clinical outcome in gefitinib-treated patients with non-small cell lung cancer. Br J Cancer *97*: 1560-1566, 2007.

15 Han SW, Kim TY, Jeon YK, Hwang PG, Im SA, Lee KH, Kim JH, Kim DW, Heo DS, Kim NK, Chung DH and Bang YJ: Optimization of patient selection for gefitinib in non-small cell lung cancer by combined analysis of epidermal growth factor receptor mutation, *K-ras* mutation, and Akt phosphorylation. Clin Cancer Res *12*: 2538-2544, 2006.

16 Rosell R, Cuello M, Cecere F, Santarpia M, Reguart N, Felip E and Taron M: Treatment of non-small cell lung cancer and pharmacogenomics: where we are and where we are going. Curr Opin Oncol *18*: 135-143, 2006.

17 Riely GJ, Pao W, Pham D, Li AR, Rizvi N, Venkatraman ES, Zakowski MF, Kris MG, Ladanyi M and Miller VA: Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. Clin Cancer Res *12*: 839-844, 2006.

18 Jiang SX, Yamashita K, Yamamoto M, Piao CJ, Umezawa A, Saegusa M, Yoshida T, Katagiri M, Masuda N, Hayakawa K and Okayasu I: EGFR genetic heterogeneity of non-small cell lung cancers contributing to acquired gefitinib resistance. Int J Cancer *123*: 2480-2486, 2008.

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