



Concomitant ALK/KRAS and ALK/EGFR mutations in non small cell lung cancer: different profile of response to target therapies

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The development of therapeutic agents targeting products of epidermal growth factor receptor (*EGFR*) gene mutation and anaplastic lymphoma kinase (ALK) rearrangements has significantly improved survival in patients with non small cell lung cancer (NSCLC). Thus, the patients eligible for the treatment with EGFR or ALK inhibitors should be selected through appropriate molecular tests (1). On the other hand, although representing the most frequent genic alteration in NSCLC patients, Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation-specific therapy has not been validated in clinical practice (2). Indeed KRAS mutations are described in approximately 20–30% of NSCLC, commonly observed in smokers and associated with a poor prognosis (2).

Although driver genes mutations were reported to be mutually exclusive in NSCLC (3,4), however in several series driver genes mutations seem to occur particularly associated to EGFR mutations (5,6). In wide series of NSCLC, rare cases of concomitant mutations were reported with different frequency, however the TKI response data were conflicting (6–8). In particular, the frequency of concurrent EGFR/ALK mutations was reported in a range of 0.0% to 6% (6,9). Recently, in a large series of Chinese NSCLC patients, the concomitant EGFR and ALK mutations was observed in 1.9% of the cases (6). In a total of 977 NSCLCs, EGFR mutations was found in 336 (32.7%), ALK rearrangements in 70 (6.8%), KRAS mutation in 40 (3.9%) patients and concomitant EGFR and ALK aberrations were observed in 13 patients (1.3%). Although the overall rate of EGFR/ALK co-alterations was only of 1.3% (13/977), however the prevalence of co-alterations was 3.9% (13/336) in EGFR mutant patients and 18.6% (13/70) in ALK-positive patients (6). These results suggested that

driver mutations of EGFR and ALK genes could occur in a small group of NSCLC, but more frequently in ALK-positive tumors. In literature, the concomitant EGFR/KRAS mutations were described mainly in case reports, but lately in a large cohort of 5,125 Chinese NSCLCs 30 cases harboring concomitant aberrations were reported (5).

Besides ALK and KRAS alterations, several mutations in various oncogenes were described as concomitant with EGFR mutations. Rarely occurrence of other driver genes mutations were reported associated to EGFR mutations, such as *HER2*, *RET*, *KRAS* and *ROS1* genes mutations, while no *BRAF* and *NRAS* were found coexisting with *EGFR* mutations (6). Furthermore, the phosphatidylinositol 3-kinase (PI3K), playing a critical role in cancer cell proliferation, is mutated in approximately 2–4% of NSCLCs (10), often associated to KRAS mutations and less frequently with EGFR and ALK mutations (11).

The concomitant *EGFR* mutations and other driver genes might decrease substantially the efficacy of EGFR-TKIs (6). The median PFS of patients with concurrent *EGFR/ALK* mutations treated with EGFR-TKI ranged from 5.0 to 11.2 months, relatively lower than patients harboring only EGFR mutation (6,7). Ulivi *et al.* observed disease control rate (DCR) in 67% of co-altered patients, that is lower than the 81.7% in patients with an EGFR-mutation only (12). Particularly Yang *et al.* attributed the efficacy of EGFR-TKI to relative levels of phospho-EGFR in patients with concomitant *EGFR/ALK* mutations (7). Indeed of the ten patients receiving first-line EGFR-TKIs, eight achieved objective response with a median PFS of 11.2 months. Of the four patients treated with crizotinib, three cases were previously treated with EGFR-TKI, particularly one case was not responsive to EGFR-TKI,

but sensitive to crizotinib, whilst two cases were responsive to EGFR-TKI, but not to crizotinib. Finally, one case showed partial response to crizotinib, but no response to subsequent treatment with EGFR-TKI. Immunohistochemistry showed in all examined cases co-expression of EGFR mutant protein and ALK fusion proteins in the same cancer cells, indicating that different driver oncogenes could act in the same cell population. Moreover, different levels of receptors phosphorylation were observed using specific antibodies. Thus, three patterns of phosphorylated proteins were documented: high p-EGFR and high p-ALK, high p-EGFR and low p-ALK, and low p-EGFR and high p-ALK. High levels of p-EGFR correspond to partial responses to EGFR-TKI, while two patients with low levels of p-EGFR had progressive or stable disease. Of the four cases treated with crizotinib, two had low p-EGFR and high p-ALK; one of them was not responder to EGFR-TKI, but sensitive to crizotinib, and the other was highly responsive to crizotinib, but resistant to subsequent EGFR-TKI. On the other hand, two cases had high p-EGFR levels and low p-ALK levels, corresponding to partial responsiveness to EGFR-TKI, but with poor results when treated with crizotinib (7).

Generally, the results of subsequent treatment with crizotinib in NSCLC patients with concomitant EGFR/ALK mutations after failure of EGFR-TKI treatment are conflicting (6). Lee *et al.* observed that two ALK-positive/EGFR-mutant NSCLC patients non-responder to EGFR-TKI showed a durable partial response to ALK inhibitors (13). Therefore, in non-responded to EGFR-TKI patients, ALK gene status test should be investigated, since it might be responsible for unsuccessful treatment. In parallel, acquired *EGFR* mutations are also described as a mechanism of resistance to ALK inhibitor (14). However, in a series of 1,683 NSCLCs, all 25 ALK-positive patients crizotinib-resistant were both *KRAS* and *EGFR* wild type (3).

Finally, in NSCLC patients harboring ALK/EGFR co-alterations, EGFR-TKIs seem to be more active compared to ALK-TKIs. Schmid *et al.* identified five patients with EGFR/ALK co-alteration, four out of five received one or more lines of EGFR-TKIs and three patients received one or more lines of ALK-TKI. In particular, patients showed different response to TKI: one out of three patients responding to ALK-TKI and three out of four patients responding to EGFR-TKI. Median PFS were slightly better in patients treated with EGFR-TKIs than in patients treated with ALK-TKIs (8).

Different response rate might be explained considering intratumor heterogeneity of both genes, strictly related to gene mutation tumor burden (9,15). Therefore, the mutation tumor burden of each mutation could affect targeted therapy response. Won *et al.* detected in a series of 1,458 NSCLC

14 EGFR/ALK co-altered cases, eight patients treated with crizotinib showed DCR and three patients who received EGFR-TKI showed poor response. These results could be explained considering that most patients were studied for EGFR mutations through targeted NGS or mutant-enriched NGS, suggesting that relative lower EGFR-mutation burden could cause lack of response to EGFR-TKIs in these patients (16). Thus, since highly sensitive *EGFR* tests have widely been introducing in practical diagnosis of NSCLC, an increasingly high number of cases with concomitant alterations in different oncogenes could be identified in the future. It is calculated a significant increased rate of concomitant *EGFR* and *ALK* mutations in NSCLC—from 4.4% to 15.4%—using targeted next-generation sequencing (NGS) (16).

As regards *KRAS* mutations, since they are responsible for secondary resistance to ALK TKIs such as crizotinib (17), the concomitant ALK and *KRAS* co-alteration may be associated with primary resistance to ALK-TKI treatment. Indeed, for the first time Schmid *et al.* reported that six out of seven patients treated with crizotinib were non-responder patients (8). In the clinical setting of concomitant EGFR/*KRAS* mutations, *KRAS* mutations seems to be related to a reduced response to EGFR TKI (2). On the contrary Lee *et al.* reported that response rate to EGFR-TKI in concomitant EGFR/*KRAS* mutation patients is superimposable to only EGFR mutant patients. This observation might be attributable to *EGFR* mutation as driver dominant role, even if the tumor cells harbored an additional *KRAS* mutation (14).

In conclusion, most NSCLC patients harboring concomitant EGFR/*KRAS* mutations partially responded to EGFR TKI, while NSCLC patients harboring concomitant EGFR/ALK mutations slightly responded to specific ALK or EGFR TKI. EGFR and ALK alterations play an important role in the oncogenesis of NSCLC, however their interaction in terms of synergism versus the possible dominance of one rather than the other oncogene are currently not completely clarified. The dominance of one oncogenic alteration over the other could be explained essentially through two mechanisms, a different mutation tumor burden of each driver gene (*Figure 1*) and differential phosphorylation of the single mutated proteins (*Figure 2*). Different mutation tumor burden could justify the inconsistency of TKI response in patients investigated through cytology or small biopsies, clearly representing only a small portion of the entire tumor. On the other hand the presence of concomitant EGFR/ALK mutation could have a little value, if not associated to the evaluation of altered protein phosphorylation. Finally, the alternative over-phosphorylation of altered EGFR and ALK proteins needs more studies of validation in order to address the

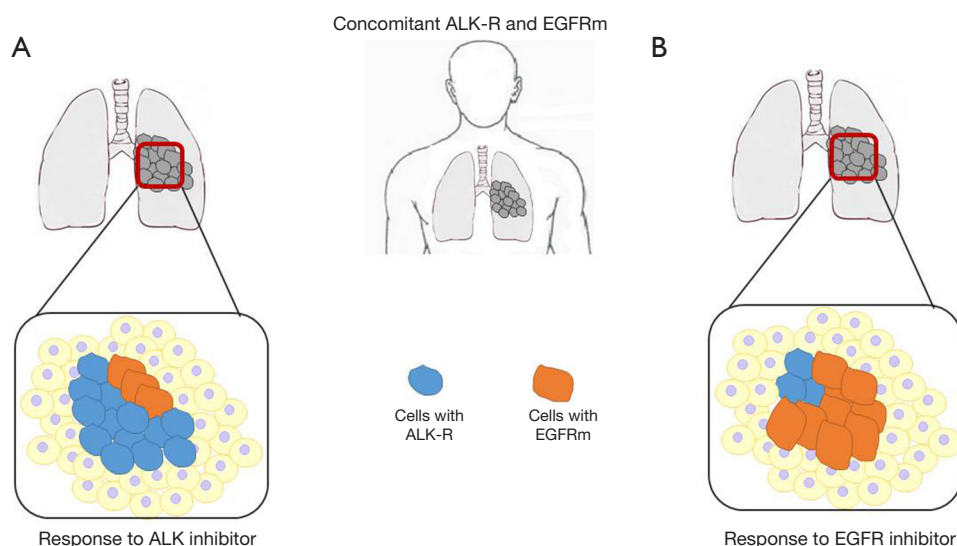


Figure 1 Different tumor mutations burden in concomitant EGFR/ALK mutations NSCLC related to the response to target therapies. In (A) most tumoral cells harboring ALK rearrangement (ALK-R) with better response to ALK-TKI with respect to EGFR-TKI and in (B) most tumoral cells harboring EGFR mutation (EGFRm) with better response to EGFR-TKI with respect to ALK-TKI. EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; NSCLC, non small cell lung cancer.

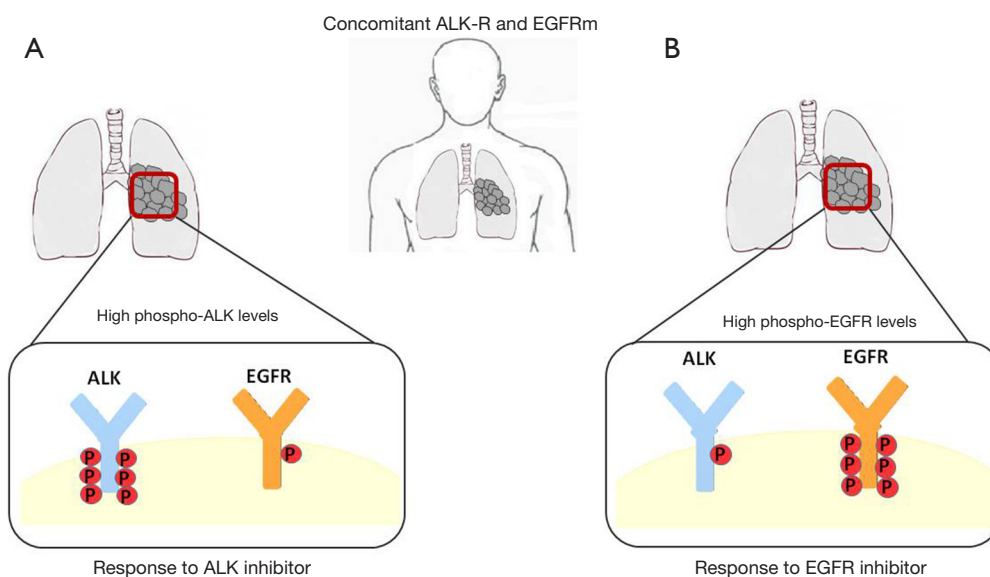


Figure 2 Different level of phosphorylation of ALK and EGFR in concomitant EGFR/ALK mutations NSCLC related to the response to target therapies. In (A) higher level of phosphorylation of ALK than EGFR, with better response to EGFR-TKI with respect to ALK-TKI; in (B) higher level of phosphorylation of ALK with better response to ALK-TKI with respect to EGFR-TKI. EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; NSCLC, non small cell lung cancer.

patients to the better treatment.

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Footnote

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