China Lung Cancer Research Highlight

EGFR mutation heterogeneity and mixed response to EGFR tyrosine kinase inhibitors of non small cell lung cancer: a clue to overcoming resistance

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Abstract: The presence of an *EGFR* activating mutation is predictive of benefit from reversible and irreversible EGFR tyrosine kinase inhibitor (EGFR-TKI) allowing personalized medicine in lung cancer. However, intratumoral heterogeneity in *EGFR* mutation status has recently been described and ranged from 13.9% to 27% in some studies. Intratumor heterogeneity may have important consequences for personalized-medicine approaches that commonly rely on a single tumor-biopsy to portray tumor mutational landscape. *EGFR* mutation heterogeneity could also explain the mixed responses phenomenon and act as a mechanism of acquired resistance to EGFR-TKI. In order to a better tailored treatment in advanced non-small cell lung cancer (NSCLC), it is extremely important to elucidate the relevance and degree of heterogeneous distribution of the targeted biomarker regarding the metastasis localisation, previous systemic treatments and interval between primary tumor and metastasis. Additionally, these findings would also help us to design new strategies for patients with lung cancer harboring heterogeneous *EGFR* mutations.

Keywords: *EGFR* mutation; heterogeneity; mixed responses; lung cancer

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Personalised medicine in non-small cell lung cancer (NSCLC) is a reality in our days and tumor genomic landscape is based on a single tumor biopsy results. Several studies have demonstrated that the presence of an *EGFR* activating mutation is predictive of benefit from reversible and irreversible EGFR tyrosine kinase inhibitor (TKI) in non-small cell lung cancers, with significant advantage compared to chemotherapy in progression free survival and response rate (RR) in first line. Among those *EGFR* mutant patients the tumor RR to first-line EGFR TKI is in the range of 58-84%, indicating that there are additional factors mediating the sensitivity of tumors to EGFR TKI (1-8). This phenomenon may be explained by heterogeneity in *EGFR* mutation status within an individual tumor. On the contrary to this theory, because the driver mutation is acquired in an early step of progression, subsequent clonal expansion distributes the mutation through the tumor. However, Gerlinger *et al.* (9) map out the remarkable intratumoral heterogeneity within a single renal cell cancer respect to somatic mutations in driver and passenger genes, which may foster tumor adaption and therapeutic failure via Darwinian selection. Intratumor heterogeneity may have important consequences for personalized-medicine approaches that commonly rely on single tumor-biopsy samples to portray tumor mutational landscape. This heterogeneity has been investigated regarding *EGFR* mutation in NSCLC.

Chen *et al.* (10) studied discordance in *EGFR* mutation status using direct DNA sequencing in paired samples of lung adenocarcinoma and regional lymph nodes or distant metastases in 180 Asian patients. In case of discordance between the primary tumor and the metastasis, results were confirmed using the high-resolution melting method (HRM). The overall discordance rate was 13.9%.

Heterogeneity was significantly higher in patients with multiple pulmonary nodules (24.4%) than in patients with distant metastasis (14.3%), lymph nodes metastases (10.2%) or metachronous primary tumors (9.1%). Additionally, the discordance also was higher between paired samples from metachronous tumors (15.7%) than samples from synchronous tumors (7.5%). These results are in contrast to a study by Yatabe *et al.* (11) who did not find *EGFR* mutation heterogeneity by reverse transcriptase polymerase chain reaction among 77 *EGFR* mutant patients with paired primary and metastatic site samples or among 54 primary and recurrent tumor pairs. The authors also performed a transactional analysis of 50 lung adenocarcinomas carrying *EGFR* mutation. Three parts of each individual tumor were selected and examined for their *EGFR* mutation status and all three parts demonstrated identical mutations. Also, five tumors were dissected into more than 100 pieces and examined for EGFR status and again no *EGFR* mutation heterogeneity was found. The authors concluded that heterogeneous distribution of *EGFR* mutations is extremely rare and that pseudoheterogeneity is observed as a result of the use of less sensitive methods of detection. Other studies using heteroduplex analysis or Scorpion Amplification Refractory Mutation System (ARMS) method have reported *EGFR* mutation heterogeneity in the range of 16.8% to 27%, respectively (12). Tomonaga *et al.* (13) described intratumor heterogeneity of *EGFR* mutation by PCR in nine out of 38 patients with resected mixed-type lung adenocarcinoma and it was significantly associated with smoking history.

Recently, 45 tumors of patients with *EGFR* mutant stage IIIA-IV NSCLC with palliative surgery in which *EGFR* mutations were determined using Denaturing High Performance Liquid Chromotography and ARMS revealed 30% of intratumoral *EGFR* mutational heterogeneity, accompanying with low EGFR copy number. The prognosis of the patients was also related to the *EGFR* mutation heterogeneous status (14). These findings suggest that patients with advanced lung cancer harbor *EGFR* mutational heterogeneity and this heterogeneity might have clinical consequences in the efficacy of EGFR-TKI, and it could be a mechanism of resistance to EGFR TKI. Taniguchi *et al.* (15) demonstrated that those patients harboring heterogeneous tumors had a statistically significant decreased survival compared with those patients harboring mutation-positive tumors cells only after gefitinib treatment.

It is not well understood if systemic therapy may influence the expression of different biomarkers such as *EGFR* mutation in the tumor. In the Chen *et al.* (10) study, those patients that had received systemic therapy had a higher *EGFR* mutation discordance than those without exposure to any systemic therapy, suggesting potential mutagenic effects of chemotherapy. In a cohort of 264 advanced NSCLC patients, chemotherapy significantly decreased frequency of *EGFR* mutations from 34.5% in the prechemotherapy plasma samples to 23.1% in the postchemotherapy plasma samples (P<0.001). It is interesting to underline that the majority of *EGFR* mutation changes after chemotherapy were from mutant state to wild type (16). Notwithstanding these results, Rosell *et al.* (17) demonstrated no statistically significant differences in RR, PFS and OS in *EGFR* mutant patients receiving EGFR TKI in either first- or second-line setting. Also, data from the SATURN trial showed a compelling PFS HR of 0.10 in patients positive for *EGFR* mutation who received erlotinib as a maintenance treatment after standard chemotherapy (18). Chen *et al.* (10) also reported in a multivariable analysis that heterogeneity was significantly higher in patients with EGFR TKI exposure. The *EGFR* mutation heterogeneity accounted 8.9% of TKI-resistant cases. It is difficult to estimate whether discordance biomarker expression between pre and post treatment samples is due to a change in a biomarker status or simply a reflection of the pre-existing tumor genetic heterogeneity that can influence tumor phenotype after EGFR TKI treatment. Taniguchi *et al.* (15) tested EGFR mutation in multiples areas in 21 resected tumors, and six of them had both *EGFR*-mutated and wild type NSCLC cells. This fact could explain why patients with multiple pulmonary nodules had a higher heterogeneity in *EGFR* mutation status in the Chen *et al.* study (10).

EGFR mutant heterogeneity could explain mixed responses to EGFR TKI, suggesting that EGFR TKIs should be continued beyond progression in combination with other therapies such as chemotherapy to act in all cell clones that are part of the tumor in those patients. The IMPRESS study (NCT01544179) is currently evaluating the role of gefitinib combined with chemotherapy in patients with EGFR mutations that have progressed to gefitinib.

Tailored treatment in advanced NSCLC is going to improve in the next years based on new research on druggable biomarkers. Treatment of patients with advanced NSCLC and a positive biomarker requires that all tumor clones are eradicated. The question is if a single biopsy might represent the mutation status of the entire tumor,

and the answer would probably be no. Then, it gets increasingly important to elucidate the relevance and degree of heterogeneous distribution of the targeted biomarker regarding the metastasis localisation, previous systemic antineoplastic treatments and interval between primary tumor and metastasis (synchronous or metachronous). Furthermore, and based on these results, to perform a rebiopsy when the treatment fails to offer an individually tailored treatment would be crucial to determine the status of the druggable biomarker. Since not all patients are suitable for a rebiopsy of all tumor lesions, new techniques such as liquid biopsies might help us to distinguish those patients who could have higher *EGFR* mutation heterogeneity (19,20). These findings would also help us to design new strategies for patients with lung cancer harboring heterogeneous *EGFR* mutations.

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