EGFR inhibitors have been examined as treatments for unselected NSCLC patients until the discovery that activating EGFR mutations render the tumors highly sensitive to these drugs (1,2). Since then, several studies have demonstrated that the presence of an EGFR activating mutation identifies a different population of NSCLC patients, with a significantly better prognosis, provided EGFR inhibitors are one of the treatment lines. Thus, testing for such mutations is recommended as part of the initial workup of advanced NSCLC patients.

EGFR mutation status is predictive of benefit from EGFR tyrosine kinase inhibitors (TKIs) in NSCLC, with significant advantages compared to chemotherapy in PFS and response rates in both first and second line. Conversely, patients with wild type (wt) EGFR tumors derive a greater benefit from chemotherapy in the first line setting. However, there is conflicting data regarding whether chemotherapy or EGFR TKIs is most beneficial in the treatment of wt EGFR after first line therapy. Specifically, identification of an EGFR mutation in tumors was not required for overall survival benefit from erlotinib or gefitinib as second- or third-line treatment (3,4). Erlotinib given as maintenance treatment improved OS also in patients with a wt EGFR (HR, 0.77; P=0.0243) (5). Chemotherapy-induced changes may partially explain the different results regarding first-line treatment or later treatment lines, as the EGFR mutation status is usually defined by the pre-chemotherapy tumor sample. In addition, only 66-83% of EGFR mutation positive patients respond to first line EGFR inhibitors, indicating that additional factors determine the sensitivity of tumors to EGFR inhibitors. Among many causes tested as modifying the EGFR inhibitors sensitivity, EGFR mutation abundance was found to be predictive for benefit from gefitinib (6). In the study by Zhou et al. (6) high abundance was defined as identification of a sensitizing mutation by sequencing, while low abundance as identification only by the use of an ultra-sensitive technique, Amplification Refractory Mutation System (ARMS). Difference in mutation abundance can be explained by any combination of several factors: variations in the copy numbers of mutation positive EGFR gene in cancer cells, variability in the proportion of tumor cells to stroma cells in the tested specimen, and tumor heterogeneity.

The presence of tumor heterogeneity has recently been highlighted regarding driver mutations in renal cell cancer (7) and has been investigated regarding EGFR mutations in NSCLC, with conflicting results (8,9). An extensive recent study by Bai et al. (10) describes a three-pronged approach to the question of EGFR tumor heterogeneity, mostly looking at systemic chemotherapy-induced changes. The tests reported include examining EGFR mutation status in plasma free DNA and in tumors before and after chemotherapy, and EGFR mutation testing of multiple fragments of the same tumor. Each of these approaches demonstrates evidence that can be interpreted as an indication of tumor heterogeneity and variations in the prominent clones following anti-neoplastic treatments. EGFR mutation testing in plasma free DNA as used by Bai et al. has been previously demonstrated to identify about 80% of tumor EGFR mutations (11). In 20% of patients EGFR mutation was detected before any treatment but not following first-line chemotherapy, while in 9% an EGFR mutation was detected in the plasma only after...
chemotherapy treatment. As could be expected, the loss of EGFR mutation detection was associated with tumoral response to treatment, probably associated with reduced tumor cells exposure to the circulation. The opposite change is more difficult to comprehend, but could indicate that one mechanism of chemotherapy resistance is emergence of an EGFR-mutation-bearing clone, thus the correlation reported with tumor progression. An obvious caveat of this set of results is the 20% rate of false negative EGFR mutation detection by the method used to examine plasma DNA.

The EGFR mutation status was determined in biopsy specimens taken at diagnosis and in pathology specimens resected following two cycles of neo-adjuvant chemotherapy treatment. The results reveal a 22% rate of discordance, the vast majority of cases involving an EGFR mutation at presentation and no mutation found following systemic neo-adjuvant chemotherapy. No method of mutation detection is 100% accurate, and variability in mutant EGFR allele amounts due to any of the above explanations could explain the discordance. Yet, the authors have used highly sensitive and reliable methods, thus highlighting the possibility that in a significant proportion of lung cancers harboring an EGFR mutation, a clone that lacks this driver mutation can appear. The authors suggest such phenomena would cause reduced benefit from EGFR TKI if given following chemotherapy treatment; however, several reports indicate no reduced OS for EGFR mutation-bearing patients not treated with EGFR TKIs at first line (12-14). Tumors responding to neo-adjuvant chemotherapy demonstrate in many cases fibrotic changes, reducing the percentage of tumor cells in the samples taken and potentially the chance of mutation detection.

The third set of evidence Bai et al. present include a large-scale analysis of EGFR mutation status in different parts of 79 tumors. The authors report a staggering 38% rate of tumor heterogeneity in terms of EGFR mutation. This corroborates a few additional smaller studies (15), but contrasts with others (8). In cases where EGFR mutation positive tumors were found to have foci with no EGFR mutation, an obvious potential explanation is that some of the foci were taken of stromal normal cells or of fibrotic areas. The authors do not detail the procedure of micro-dissection chosen nor how did they verify that only tumor cells were examined. Tumors identified initially as wt EGFR where low abundance of mutant EGFR was found in some foci might actually represent multi-clonal tumor evolution, although at a rate lower than suggested by the authors. EGFR inhibitors secondary resistance that develops in tumors found initially to harbor a sensitizing EGFR mutation does not involve overgrowth of a wt EGFR clone in most cases (16,17). This fact itself suggests that the presence of such clones is not as common as can be expected from the results reported by Bai et al.

EGFR mutations were found in the normal epithelium in EGFR mutation positive tumors’ vicinity (18), indicating this genetic abnormality is one of the earliest events of malignant transformation for such tumors and that all tumor cells that arise in such cases would harbor this driver mutation. However, the possibility of an EGFR mutation evolving at different stages in some tumors, giving rise to EGFR mutation heterogeneity cannot be ruled out. A convincing way to demonstrate this requires positive identification of alternative clones, through the presence of different driver mutations in different parts of the same tumor, either in the EGFR gene or in other oncogenes or tumor suppressor genes. Such events were found to be rare by one report (8). Performing micro-dissection of small tumor foci, and using molecular biology tools currently available, deep sequencing of small amounts of tissue samples would allow a definite answer to the important question of lung cancer intra-tumoral heterogeneity in general, and specifically to the question of EGFR heterogeneity.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References
