Letters to the Editor

EGFR-tyrosine kinase inhibitor (TKI) sensitivity of these rare mutations differ from classic EGFR mutant. As a matter of fact, they are similar to EGFR wild-type patients. Lohinai et al.'s results are in line with previous report. Rare EGFR mutations had a high incidence, almost the same as classic and synonymous (5%, 6%, and 4%; respectively). They were also associated with smoking and poor response to EGFR-TKI as opposed to classic mutations. In addition, of the 49 rare mutations, 20 were never described before (not registered in the Catalogue of Somatic Mutations in Cancer [COSMIC] database).

We must be aware that the diagnostic of advanced non–small-cell lung cancer is often made by biopsy rather than resected specimen, and these rare mutations were all noted from samples of DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissue. Artifacts can easily be observed when sequencing multiple polymerase chain reaction (PCR) amplification products of very small amounts of DNA, particularly if the DNA is isolated from paraffin-embedded tissue. Therefore, the hypothesis that several of these rare mutations might be actually PCR-amplification artifacts must be discussed.

Akbari et al. performed direct sequencing of the PCR-amplified coding region of the uracil-DNA glycosylase gene using DNA isolated from FFPE tissue specimens from patients with gastric cancer (only one mutation in the uracil-DNA glycosylase gene in human cancer had been previously reported in a sporadic human glioblastoma). In nearly 35% of the samples, they detected base substitutions, which, after further investigation, proved to be PCR artifacts. They also demonstrated that very low concentration of DNA template in PCR mix can give rise to false base substitutions. Marchetti et al. identified 45 rare EGFR mutations in 70 samples of lung tumor DNA extracted from FFPE tissue, and they were all found to be artifacts. This was confirmed because they also found the same mutations in multiple amplifications of DNA extracted from FFPE of normal tissue (50 patients’ lymph nodes without neoplasm). In addition, series whose fresh-frozen tissue was used do not observed rare EGFR mutations.

There are some hypotheses for the occurrence of artificial mutations. For instance, base damages and large-scale DNA fragmentation caused by the chemical preparation of FFPE samples might result in cytosine deamination. Thereby the tag DNA polymerase would insert an adenosome instead of a guanosine resulting in C → T and G → A transitions (so-called “a-rule”). Moreover, degraded PCR products allowed the tag DNA polymerase performs a “jump” from a damaged template to another to continue the extension.

Some strategies were reported to prevent artificial mutations. Routine application of microdissection and use of fresh-frozen tissue to enrich tumor-cell DNA are one option. Also, if small amounts of DNA extract from FFPE were inevitable, addition of uracil-N-glycosylase to the DNA template before PCR amplification and the examination of multiple amplifications are imperative.

Because analysis of EGFR gene is mandatory for decision regarding EGFR-TKI use, the correct interpretation of EGFR exons 18 to 21 sequencing and its genetic alterations is crucial to select patients whom would benefit from treatment. Therefore, these data preclude the indiscriminate use of uracil-DNA glycosylase in the DNA template before PCR amplification and the examination of multiple amplifications are imperative.

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REFERENCES


Reply to Rare Versus Artifactual EGFR Mutations

In Response:

We thank Domingues et al. for their comment on our study and the opportunity to further discuss the clinical relevance of rare epidermal growth factor receptor (EGFR) mutations, especially in an era where there is an urgent, unmet need to increase the number of lung cancer patients who can benefit from targeted therapies.

Their comment on the possibility that some of the rare mutations identified during the routine EGFR testing might be artifactual is well taken. While the demonstration of EGFR mutation is required to prescribe EGFR-TKI treatment for lung adenocarcinoma patients, there are no definite requirements for the sample preparation, molecular diagnostic procedures or the type of EGFR mutations that needs to be identified. Our study was retrospective using the mutational data generated during the routine molecular pathological diagnosis.
of formalin-fixed paraffin embedded tumor tissues or cytological samples, and the majority of cases were analyzed from bronchoscopic biopsy specimens, where the amount of DNA available for analysis was limited. Indeed, throughout the world, the overwhelming majority of EGFR molecular testing is performed on formalin-fixed surgical tumor specimens or biopsies, or even on cytological preparations. We fully agree with Domingues et al. that the EGFR mutation analysis should be very carefully performed and interpreted, especially when rare or novel mutations are found. Therefore, we not only pointed out the possible clinical relevance of rare EGFR mutations but also stated clearly in the interpretation of our results that our data needs to be validated in additional studies. However, it is now evident that certain rare mutations are sensitizing to anti-EGFR therapy and that these patients need to be identified and treated accordingly.1,2

It is important to discuss the possibility of formalin-fixation-related polymerase chain reaction artifacts in our study. First, the majority of the nonsynonymous rare EGFR mutations identified in our cohort have already been described in the COSMIC database. Among the 20 previously not published rare EGFR mutations, there were three microdeletions and five point mutations which were not C->T or G->A transitions that often appear as formalin induced artifacts. Of note, five patients with novel rare EGFR mutations responded to therapy and had a survival benefit which would not be expected in the case of artifact mutations (i.e., in wild-type EGFR patients). Nevertheless, we hope that because an increasing number of EGFR mutation analyses are being performed on non-formalin-fixed specimens the spectra of validated somatic EGFR mutations will eventually be established. Novel approaches—for instance using circulating tumor DNA in “liquid biopsy”—may overcome some of the limitations in the future.3

In summary, on the one hand, the harmonization of tissue processing and molecular pathological methods can further improve the reliability of EGFR mutational tests. On the other hand—as we concluded in our study—reporting the clinical response to EGFR-TKI treatment in each patient with a rare EGFR mutation is of utmost importance to identify every patient who can benefit from this therapeutic modality.

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Maintenance Treatment with Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor after First-Line Chemotherapy in Mutation-Positive Non–Small-Cell Lung Cancer

To the Editor:

The Chinese Thoracic Oncology Group has recently reported final overall survival (OS) results of a phase III study examining the effectiveness of

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Disclosure: The authors declare no conflicts of interest.

DOI: 10.1097/JTO.0000000000000597

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ISSN: 1556-0864/15/1008-e81