in elderly patients treated with bev and erlotinib or gefitinib. Furthermore, even elderly patients with epidermal growth factor receptor (EGFR)-mutated lung cancers had greater benefit when treated with bev in combination with erlotinib.2 We agree that elderly patients should be followed very carefully during treatment, even when bev is combined with a molecular targeted agent such as gefitinib or erlotinib, because of their slower metabolism, poorer performance status, and increased comorbidities, potentially leading to greater toxicity.

Regarding cost-effectiveness, we agree that economic evaluation is essential when developing a new cancer treatment as the cost of pharmacological cancer therapy is increasing at an incredible pace. As Dr. Zhang mentioned, an incremental cost-effectiveness ratio (ICER) per quality-adjusted life-year (QALY) gained is often used to evaluate cost efficacy and an ICER per QALY of less than a certain value ($20,000 in the United Kingdom and $50,000 in the United States) is generally considered as cost effective. However, calculating the ICER itself has potential problems: difficulty in determining such a threshold based on evidence; uncertainty in measuring utility values; and a tendency to obtain a lower QALY in elderly or sicker patients with lower expectation of life. We should not only include a cost-effectiveness parameter in clinical trials but also need to have a broad discussion about how it should be evaluated.

Finally, we agree with Dr. Zhang’s suggestion that it is necessary to identify predictive biomarkers of acquired resistance to bev and gefitinib. In addition, we need to know which mechanisms cause resistance to a combination of bev and gefitinib, those of bev resistance (angiogenic pathways other than the vascular epidermal growth factor pathway), those of gefitinib resistance (secondary EGFR T790M mutation, MET amplification, etc.), or other yet undefined mechanisms. Given that gefitinib itself can inhibit EGFR-mutated lung cancers, it is unlikely that just bev-resistant mechanisms will induce resistance to a combination of bev and gefitinib. If bev and gefitinib resistance is caused by the same mechanisms as those of gefitinib resistance, it might be potentially beneficial to continue bev in subsequent therapies because the tumors might not be resistant to bev itself.

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Rare versus Artifactual EGFR Mutations

To the Editor:

Rare epidermal growth factor receptor (EGFR) mutations in non–small-cell lung cancer have been frequently reported in the last decade. A high incidence and large variety have been identified in several cohorts.1 Notably, clinical characteristics and
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. Lohiaini 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.7 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.8 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.9

There are some hypotheses for the occurrence of artifactual 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 adenosine 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.6

Some strategies were reported to prevent artifactual 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 EGFR-TKI in patients harboring EGFR rare mutations and drive us to a more careful molecular analysis to identify molecular artifacts.

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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

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