Author’s response to reviews

Title: Mechanisms of Acquired Resistance to EGFR-tyrosine kinase inhibitor in Korean Patients with Lung Cancer

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Author’s response to reviews: see over
Dear Dr. Eng Huat Tan,

Thank you very much for the kind and considerate review of our manuscript. The reviewer’s comments are quite informative and educational for us. As the reviewers suggested, we have carefully made modifications in the manuscript and now submit this revised form for the publication.

Our revisions are as follows;

**Reviewer #1**

1. Under my point of view, reference 1 is not correct because cancer statistics is from USA. GLOBOCAN 2008 (Ferlay et al) would be more correct.
   - According to this reviewer’s recommendation, we replaced the reference 1.

2. The biopsy site after acquiring resistance to EGFR TKI was as the initial site in 15 patients. Regarding T790M mutation and based on the possibility that T790M is also a primary resistance mechanism; did you check if this mutations was present in the primary biopsy?
   - Yes, we checked T790M mutation in initial samples by direct sequencing. But we could not find T790M mutation.

3. A percentage of patients had a shift from EGFR mutant to wild type EGFR. In the manuscript should there be some reference to the heterogeneity in EGFR mutant patients.
- We described this phenomenon in discussion with reference 22 and 23.

4. EGFR mutant, should be expressed such as EGFR mutant.(it means EGFR in italics in all the manuscript. Also, in page 12, reference 6 should be [6].
- We revised EGFR and others in italics if they would indicate genes. Reference 6 was changed.

5. In the results section / discussion, I would recommend to include the percentage of rebiopsies from the entire population. In the last IASLC 2013 Congress, a mini-oral session from France about rebiopsies and its feasibility in daily clinical practice and its clinical impact was presented. This reference should be included in your discussion. As well as, the percentage of patients with specific treatment based on the rebiopsy (included in clinical trial…) should be reported.
- Although the re-biopsy after resistance is recommended to guide following treatment, it is not easy to perform in clinical practice because of patient’ condition and potential risk. So, the re-biopsy has not been routinely done in our hospital except clinical trials requiring the re-biopsy. Actually, there are no clinically available therapeutic options to overcome resistance other than new drugs on clinical trials. Because the re-biopsy has been randomly done only in a few patients, we could not define the exact percentage.

6. Seven percent of patients had CD56 overexpression, was there any morphologic feature in the biopsy which could suggest SCLC transformation?
- As we showed the morphologic features in Fig 2, there were no significant changes despite increase of CD56 expression. We added this description in results.

7. Some comment about liquid biopsies would be appreciated.
- We added comments about liquid biopsies in discussion according to this recommendation as follows;
“As the data about resistant mechanisms have been accumulated, the procurement of resistant samples to guide following treatments is becoming more important. However, the performing the re-biopsy is not so easy in clinical practice. Attempts to use circulating tumor cells or circulating free DNAs in bloods or other body fluids (“so-called liquid biopsy”) are currently in progress because those are non-invasive, convenient and can be performed repeatedly.
Technical advances in tests and processing samples would help this liquid biopsy to have broad clinical applications, especially in elucidation of resistant mechanisms.”

**Reviewer #2**

Authors showed that the mechanisms and frequency of acquired EGFR-TKI resistance in Koreans are comparable to those observed in Western populations in spite of the small number of patients.

A major point: Although this study seems to have no new findings in this area, Asian lung cancer patients with resistance to EGFR-TKI were similar to Western patients in terms of resistant mechanisms. It is important to accumulate these findings.

A minor point: Which is before or after use of EGFR-TKI in Figures 2C and 2D? All cells in Figure 2C were negative and some cells in D were positive on their cell membranes. Is this right? Please describe them.

- Fig. 2C showed negative expression of CD56 in initial sample while CD56 expression in some cells of tissues obtained after resistance was increased (positive) in Fig. 2D. We changed the figure legend to describe it more clearly.

**Reviewer #3**

Major compulsory revisions

1. The authors did not make clear whether the methods used to analyze the initial tumor and define the baseline EGFR status was the same as that done for the resistance biopsy. If it was different e.g. Sanger sequencing to define baseline and Sequenom resequencing to define the resistance biopsy, then the resistance biopsy readout may not be completely reflective of the evolution of resistance mechanisms. It is well described that EGFR mutant cases can have co-existent T790M mutations at baseline.

- We agree to this comment. Because we used the Sanger sequencing to analyze initial samples, there might be a chance to miss the pre-existing T790M. But, we don’t think that the final results for resistant mechanisms would be changed by lower sensitivity of the initial test. If we would miss some mutations in resistant samples due to sensitivity problem, that could be a problem.
2. In addition, the disappearance of L858R mutation on subsequent analysis may reflect sampling bias due to tumor heterogeneity rather than actual development of resistance. Would the authors comment on this?

- EGFR mutation such as L858R is one of driver mutations. So, most of tumor cells usually contain this mutation even though very small proportions of tumor cells can be different (harboring T790M or WT-EGFR etc). As Fig 3 shows that the biopsied tissue has enough portions of tumor, it is unlikely that disappearance of L858R might be caused by tumor heterogeneity.

3. The superior prognosis in the tumors that developed T790M as a resistance mechanism is intriguing and counter-intuitive. Could the authors indicate what salvage therapy was used in these patients as opposed to those who did not carry T790M mutation?

- Some papers already have showed the favorable prognosis of T790M-mediated resistance compared to other mechanisms. It might be caused by the indolent growth of T790M-containing tumor cells. So, many experts recommend the continued administration of EGFR-TKI in patients with T790M-mediated resistance. These patients would benefit from slow-growing nature of tumor without more aggressive treatment if only rapid-growing and TKI-sensitive clones can be suppressed by continued EGFR-TKI. However, because the characteristics of tumor cells with acquired resistance by other mechanisms are different, adding other chemotherapeutic agents or change to other drugs should be considered.

Sincerely yours,

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