Reviewer's report

Title: Next-generation sequencing of tyrosine kinase inhibitor-resistant non-small-cell lung cancers in patients harboring epidermal growth factor-activating mutations

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The manuscript by Masago et al. reported the feasibility and the result of gene testing for re-biopsy samples from NSCLC patients with EGFR-TKI treatment failure by Ion Torrent PGM NGS system. Specifically, they utilized 13 samples from NSCLC patients to perform target sequencing and found all of them had EGFR-activating mutation. In addition, 53.8% of them had EGFR T790M and other gene mutations including TP53 (92.3%), KDR (30.8%), KIT (15.3%). This study provided an experience in NGS application in clinical practice in JAPAN. It is valuable for related researcher to develop similar NGS platform. However, in the view of publication, there are several weaknesses should to be faced including:

General issue:

1. The application of NGS in clinical practice is the trend of worldwide. It almost became a powerful tool in routine testing in some countries. Several studies had similar reports1-4. Therefore, the result of this study was anticipated and the novelty was very restricted!

2. Although the author mentioned about the issue of sample size, it is a fundamental consideration for a study majorly focused on evaluation of feasibility by a well-established platform. In this study, the author only tested 13 clinical samples and the result was anticipated. A large cohort with clinical information for a comprehensive analysis to provide clinical correlation as well as epidemiological reference is necessary.

3. To empathize the power of NGS in clinical practice, the author should try to develop its applications and usages such as challenging specimens or testing processes. For example, whether mutations detected in re-biopsy also can be identified in peripheral blood.

Specific issue:

1. In the Background paragraph of Abstract, the author mentioned about: The aim of this study was to evaluate “the effects” of epidermal growth factor receptor (EGFR)-activating mutations and other oncogene alterations in patients with non-small-cell lung cancer (NSCLC) who experienced a treatment failure in response to EGFR-tyrosine kinase inhibitors (TKIs). I am curious to know the “effects” between EGFR mutations and other oncogene alterations. The results seemed not to meet this specific aim.
2. I am confused about the statistical wording in the manuscript. The author mentioned about all 13 patients had EGFR-activating mutation. However, according to the author’s data, there were only 12 patients with EGFR-activating mutation (6 cases of exon 19 deletion, 5 cases of L858R and 1 case of L861Q). The author should check it.

3. According to general investigation, EGFR T790M mutation conferred to ~60% primary EGFR-TKI resistance. It is not surprising that the author’s result was consistent with this finding. However, in the mention about EGFR T790M, the author should discuss more comprehensively in methodological issue (the second paragraph of Discussion in page 13). There were several suggested references for the author6-12, although NGS still had its strength in this field.

4. Some references should be updated and some typos should be checked.


**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.