Format for ANSWERING REVIEWERS

July 20 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 20018-Review.doc).

Title: JAK3 inhibitor VI is a mutant specific inhibitor for epidermal growth factor receptor with the gatekeeper mutation T790M

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Name of Journal: *World Journal of Biological Chemistry*

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We are pleased that the editors would be willing to reconsider our revised manuscript. The comments from the reviewers are constructive and helped improve the manuscript. We have been able to address to all of the reviewers' comments. We believe that the revised manuscript has now been improved. Responses to the reviewers' comments are as follows.

Reviewer #1:

In current manuscript entitled "JAK3 inhibitor VI is a mutant specific inhibitor for EGFR with gatekeeper mutation T790M", the authors demonstrated that JAK3 inhibitor VI could specifically inhibit EGFR gatekeeper mutation (T790M) in non-small cell lung cancers. There are some major concerns regarding the manuscript:

Response to Reviewer #1

We appreciate the reviewer's constructive comments. We have added new graphs, and believe that the revised version of our manuscript is an improved one.

Reviewer #1 comment 1

The authors indicated that the JAK3 inhibitor VI was selected by screening a kinase inhibitor library, but didn't give the further details about the library, for example, the size and composition of the library as well as the screening method.

Response to the Reviewer #1 comment 1

Thank you for the comment. Our original manuscript included a list of kinase inhibitors used in the screening (Table 1). The first sentence of the RESULTS also mentions the library size and Table 1. Because the screening method was the same as the "*in vitro* kinase assay" in the MATERIALS AND METHODS, the statement "screening for kinase inhibitors" was added in the section.

Reviewer #1 comment 2.

The authors didn't provide the fundamental pharmacology information, the IC50 of the inhibitor. Without it, the readers cannot tell the inhibitor's efficacy and potential.

Response to the Reviewer #1 comment 2

We agree with the reviewer. The IC50 value of EGFR T780M/R858R was added in the text (the last sentence of the first paragraph in the RESULTS).

Reviewer #1 comment 3

In Figure 2 A and B, the variation of total EGFR is too big, the total protein of each sample should be measured and the same amount of protein should be loaded to each lane. Second, the phospho-EGFR should be normalized against control and the results should be shown in a separate bar graph with statistic analysis. Third, there is no indication that how many independent repeats were performed for these experiments.

Response to the Reviewer #1 comment 3

The band intensities were measured and quantified. Relative phosphorylation levels were calculated by dividing phosphorylated EGFR intensities with corresponding total EGFR intensities. Relative phosphorylation levels are indicated in Figures 2A and B. According to the reviewer's comment, bar graphs were newly added to the original Figures 2A and B. Number of repeats is also indicated in the FIGURE LEGENDS for Figure 2.

Reviewer #2:

In this manuscript the author demonstrated that of JAK3 inhibitor VI could specifically inhibit EGFR gatekeeper mutation (T790M/L858R) in non-small cell lung cancers and this is the main contribution of actually paper.

Response to Reviewer #2

We appreciate the reviewer's constructive comments and have responded to each of them below.

Reviewer #2 comment 1

In the other hand, is necessary included more information and references about of inhibitor-resistant, see for example: 1.J?nne PA, Yang JC, Kim DW, Planchard D, Ohe Y, Ramalingam SS, Ahn MJ, Kim SW, Su WC, Horn L, Haggstrom D, Felip E, Kim JH, Frewer P, Cantarini M, Brown KH, Dickinson PA, Ghiorghiu S, Ranson M. AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. N Engl J Med. 2015 Apr 30;372(18):1689-99. 2.Jiang T, Zhou C. Clinical activity of the mutant-selective EGFR inhibitor AZD9291 in patients with EGFR inhibitor-resistant non-small cell lung cancer. Transl Lung Cancer Res. 2014 Dec; 3(6):370-2.

Response to the Reviewer #2 comment 1

Thank you very much for the suggestions. The suggested references have been cited as references 11 and 12.

Reviewer #2 comment 2

The authors didn't provide pharmacology information, example the IC50 of the inhibitor, please include.

Response to the Reviewer #2 comment 2

The IC50 value of EGFR T780M/R858R was added in the text (the last sentence of the first paragraph in the RESULTS).

Reviewer #2 comment 3

Finally, the author will be considerate change the statistical analysis to U Mann-Whitney for not parametric data.

Response to the Reviewer #2 comment 3

According to the reviewer's comment, the statistical analysis was redone using the Mann–Whitney U-test. Use of this test is mentioned in the MATERIALS AND METHODS.

Reviewer #3:

The authors suggest that JAK3 inhibitor VI can selectively inhibit in-vitro the kinase activation of EGFR with the gatekeeper mutation T790M, which is resistant to EGFR TKIs in NSCLC and suppress cellular proliferation. Their finding indicate that JAK3 inhibitor VI is a mutant selective reversible TKI for EGFR T790M. Although I am not an expert in the field, the experimental approach seems sound and the results appear solid. The writing of the manuscript is solid, as well and explanatory. I tentatively suggest that it can be published to the World Journal of Biological Chemistry.

Response to Reviewer #3

We are pleased that the comments from the reviewer are overall positive and appreciate them.