Author's response to reviews

Title: Novel c-Met inhibitor suppresses the growth of c-Met-addicted gastric cancer cells.

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Author's response to reviews: see over
Cover Letter

Point by point response to the reviewer’s concerns.

**Concerns by Reviewer T Mashima**

- Major Compulsory Revisions

In the manuscript, it is not clear how series of the compounds were designed and developed. In the Result section, they mentioned the previously reported c-Met inhibitors (triazolopyrazine scaffold in Cui et al.). However, it is not clear in what concept they developed candidate compounds based on the previous information. In addition, it is not clear what are the characteristics of the identified compounds compared to the previously reported ones (for instance, in their selectiveness to c-Met).

- We have anticipated and endeavored to introduce new hinge binders instead of quinoline of PF-04217903 which binds in a mono binding manner. For this purpose, we designed and synthesized pyridoxazine as a dual-binder to hinge region of c-Met. Due to the introduction of pyridoxazine, there is no possibility of patent conflict with PF-04217903. In addition, KRC-00715 maintains the specificity to c-Met like PF-04217903. Although KRC-00715 has structural similarity with PF-04217903, PF-04217903 has been suspended in clinical trial several years ago. Therefore, in this paper, we mainly compared our compound with crizotinib, which is an excellent c-Met inhibitor and is under clinical trial for c-Met positive cancer. I explained these in the Result and Discussion part. I also referred to the patent number which describes the synthesis of these compounds in materials and methods part.

- Minor Essential Revisions

1) The method of kinase panel assay (by Millipore) which are mentioned in Abstract and in Result should be written in Materials and methods more in detail.

- In materials and methods, I added the kinase panel assay and explained how Millipore performed the assay.

2) The information on all the cell lines used in the study should be described in the Materials and methods more in detail.

- These cell lines are from gastric adenocarcinoma. They can be divided into two categories according to the c-Met expression level. I explained it in the methods.

3) Background section should be written in more focused manner.

- I corrected Background as reviewer suggested. The followings are the background outline;

  First, I mentioned oncogene-addiction as a rationale for the current targeted cancer therapy, and suggested c-Met as a prominent target. After that, I explained c-Met in detail both in normal cell and cancer cell, and the importance of c-Met activity in gastric cancer. I explained
gastric cancer briefly, and the reason why c-Met inhibitor should be developed. I think this outline is reasonable for this manuscript.

4) In the manuscript, many typographical or grammatical errors are also found.
   łuż I corrected them as possible as I can.

5) In Figure 2B, MKN-45 data is missing.
   łuż When I did experiment with KRC-00509, I didn’t have MKN-45 cell line. That’s why MKN-45 data is missing in figure 2B.

6) Layout of Figure 3 should be re-organized. A and B should be combined and C and D should be combined. E and F could be moved to Supplementary data (size markers should be included).
   łuż I corrected it.

7) Figure 4 should be indicated in more easily understandable manner (several data are small).
   łuż I corrected it.

8) In Figure 5, statistical significance (p value) should be indicated.
   łuż I indicated the p value.
Concerns by Reviewer Flavio maina

- Major Compulsory Revisions

1) The authors used crizotinib as a reference Met inhibitor. It would be more relevant to compare the effects of identified compounds with PF04217903 for their structure similarities.

Although our compounds have triazolopyrazine like PF04217903, they have different moiety, pyridoxazine, for hinge binding, from PF-04217903 which has quinoline. For this reason, KRC0-00715 has no possibility of patent conflict with PF04217903. Because of the structure similarities, PF04217903 would be best compound to compare our compound with as reviewer suggests. However, PF-04217903 is suspended at phase I clinical trial several years ago. When we develop the c-Met inhibitor, crizotinib was the most comparable and nominated compound among c-Met inhibitors. That’s the reason we intentionally used crizotinib as a reference compound in our study.

2) Concerning xenograft experiments, the authors should perform histological analysis during treatment to assess cell dead and proliferation index. Moreover, they should extend these studies by following tumour evolution overtime after KRC-715 treatment. This would clarify whether and to which extent cancer cells die, and establish a possible relapse kinetic mainly due to cytostatic versus cytotoxic effects exerted by KRC-715.

We failed to get the successful histological image due to the lack of scientific resources. I hope you understand my situation. I focused on the development of the new compound which has pyridoxazine and studied its efficacy in vitro and in vivo. Therefore I didn’t think too much whether it acts as a cytostatic agent or cytotoxic agent.

3) The author showed that KRC-715 does not cause loss of weight of mice during 10 days of treatments. Possible toxic effects should be analysed by following standard parameters and during a longer time of treatment. This is particularly important taking into account that mice die after administration of the other compound (KRC-509).

We couldn’t do this experiment. However, I am sure that KRC-00715 is very safe compound in mouse. Although mice administered with KRC-00509 were dead 3-4 days after administration, as you can see the graph of body weight in Figure 5B, there is no loss of weight for as long as 10 days in KRC-00715 administered mice. In addition, in our experiment, no mice administered with KRC-00715 were dead after 10 days. Therefore, I think you don’t have to worry about KRC-00715 regarding safety.

4) The quality of written English must be significantly improved, with extensive editing (besides several typing mistakes).

I corrected several typing mistakes and also tried to improve the English.

5) The discussion lacks of structure and in the present form the authors summarize the overall findings rather than discuss key points. For example: 1) similarities versus differences in chemical, physical, and biological properties of KRC-509 and KRC-715 versus compounds like PF04217903; 2) advantages and limits compared to other Met inhibitors (particularly PF04217903).
I totally agree with reviewer. According to the reviewer’s advice, I added the explanation of the characteristics of our compounds structure to Result and in Discussion part. Like PF04217903, our compounds have triazolopyrazine. However, our compounds have pyridoxazine instead of quinoline of PF04217903 to bind hinge region. We didn’t compare our compounds with PF04217903 for it had been in suspended status for a long time in clinical trial. In discussion part, I mainly focused the advantage of KRC-00715 over crizotinib.

Minor Essential Revisions

1) I would include the kinase panel assay data in the manuscript rather than as a Supplementary Table.

I included the kinase panel assay data in the manuscript.

2) Toxicity data reported in Supplementary Figure 3, together with additional studies I recommended above, should be included in Figure 5.

I included the toxicity data in figure 5.