Author's response to reviews

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

Authors:

Chi Hoon Park (chpark@kRICT.re.kr)
Sung Yun Cho (sycho@kRICT.re.kr)
Jae Du Ha (jdha@kRICT.re.kr)
Heejung Jung (heejung@kRICT.re.kr)
Hyung Rae Kim (hyungrk@kRICT.re.kr)
Chong Ock Lee (chongol@kRICT.re.kr)
In-Young Jang (scentofgod@naver.com)
Heung Kyoung Lee (craet@kRICT.re.kr)
Sang Un Choi (suchoi@kRICT.re.kr)
Chong Hak Chae (chchae@kRICT.re.kr)

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Editors comments:

1.) Please add the email address for Chong Hak Chae on the title page
   # E-mail address was added.

2.) Please include an abbreviations section
   # It was included.

Major Compulsory Revisions

The authors have improved the quality of the text and the discussion is better structured in the revised version of the manuscript. There are still several sentences that are not correct (either in English or as a concept). Five examples, among others:

- Introduction: “Oncogene addiction refers to the phenomenon which the tumorigenesis results from the abrogation of specific one or two normal signaling pathway”. Oncogene addiction refers to the dependency of cancer cells to a specific signal (cells are addicted to this signal), and it is not a phenomenon by which tumorigenesis results from the abrogation of pathways.
   # I agree with reviewer. I corrected the sentence as reviewer suggested.

- Introduction: “In adults, the expression of these two proteins are normally very low and confined to the epithelial or mesenchymal origin cells”. Expression of Met is restricted to some epithelial cell types whereas the expression of HGF is predominantly confined to some mesenchymal-like cells.
# I agree with reviewer. I corrected the sentence.

- Results: “Hs746T cell line has constitutively activated c-Met signaling by the amplified c-Met gene and the truncated form of c-Met protein”. In Hs746T cells, a splice site mutation in c-Met causes deletion of a specific exon located in the juxtamembrane domain, leading to a form of Met protein that lack only this exon (and it is not a truncated form). This can be also appreciated in Figure 2A of the present manuscript.

# I agree with reviewer. I corrected the sentence.

- Results: “Doxorubicin, …, was treated with 18 cancer cell lines for 72 hrs”. It is rather the cells that are treated with doxorubicin for 72 hrs.

# I corrected the sentence.

- Discussion: First sentence in the discussion section.
Experimentally, the authors did not address any concern I previously highlighted, which, in my opinion, were meant to improve the quality of their study and strengthen the outcomes.

- 1° point: The fact that “PF04217903 is suspended at phase I clinical trial” is not a reason for not using it as a reference compound; the authors may gather further evidences that KRC compounds permit overcoming limits of PF04217903, thus supporting the relevance to further explore them in clinics. The issue of patent conflicts is irrelevant for the point I underlined.

# We started c-Met project to develop compound superior to crizotinib, for it was most advanced compound when we started c-Met project. While performing project, we got some idea from PF04217093 in structure. However, we didn’t compare our compounds with PF04217093 for it failed in clinical trial. That’s why we used crizotinib as reference compound.

- 2° point: Additional in vivo studies together with histological analyses would further clarify the benefit (and limitations) of KRC compounds. The “lack of scientific resources” is not a scientific reason for not doing studies that would improve the quality of a research work (beside the fact that it is not clear what the authors intend to state with “lack of scientific resources”).

# I agree with the reviewer’s opinion. However, we primarily focused on the development of new c-Met inhibitor and investigated its efficacy in vitro and in vivo. We had limitation on studying the mechanism of our compound in detail in vivo. I hope you understand our situation.

- 3° point: In vivo side effects should be explored through a series of basic parameters related to: a) tolerance of a drug overtime; b) effects on different organs, c) dose effects (using increasing compound doses). The authors have only explored KRC-00715 toxicity by following body weight, which is a first indication and could be sufficient for this type of reports. However, based on 10 days of treatment, the authors cannot state that KRC-00715 is very safe in mice (considering also that mice treated with KRC-00509 die after 3-4 days of
treatment). It is therefore not an issue that “I have to worry” or not about KRC-00715 regarding safety.

# I agree with reviewer’s opinion. As his advice, we can’t say assertively that our compound is safe. However, I think, we can say that our compound was well tolerated at 50mpk, for there was no loss of weight.

Although I maintain my overall positive evaluation on the discovery of these compounds and on the results reported in the present manuscript, I leave the editor to take an editorial decision concerning these experimental issues.

Minor Essential Revisions:
- Results: How was the reduction on Met phosphorylation quantified (number of experiments performed, statistics, …)?
  # We performed twice, and we quantified the Met phosphorylation using imageJ program. We added the quantified data to Figure 3.

- Results: “This means that the inhibition of the c-Met activity is the direct reason for the suppression of the Hs746T cell proliferation”. The authors should reformulate this sentence as such strong statement must be supported by rescue experiments.
  # I agree with reviewer. I reformulated the sentence.

- The authors claim that SNU5 cells treated with KRC drugs were arrested at G1/S phase. Whereas I can see an increase in the percentage of cells in G phase, I rather see a decrease in the percentage of cells in S phase (Results and Figure 4).
  # It is natural that the population at S phase is decreased when cells are arrested at G1/S phase, for the cells arrested at G1/S phase can’t enter S phase.

In Figure 4A and B, M1, M2, M3, and M4 must be replaced with subG1, G1, S, G2/M (I suppose).
  # As reviewer suggested, I explained it on figure legend.